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Abstracts of the 30th Annual Meeting of the European Society of Human Reproduction and Embryology

Munich

Germany

29 June to 2 July 2014

Abstracts

30th Annual Meeting of the
European Society of
Human Reproduction and Embryology
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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 30 June 2014 **08:30 - 09:30**

O-001 The Human Reproduction Keynote Lecture - The clinical significance of calcium-signalling pathways mediating human sperm hyperactivation

C. Barratt[1]
[1]*Ninewells Hospital and Medical School, The University of Dundee, Reproductive Medicine, Dundee Scotland, United Kingdom*

Ca^{2+} signalling pathways mediating sperm motility among sub fertile patients.
Study question: To determine the incidence and causes of defects in Ca^{2+} signalling pathways (calcium influx and store mobilisation) mediating sperm motility among sub fertile patients.
Summary answer: Ca^{2+}store and CatSper defects adversely affecting fertilisation competence were identified and the characteristics of these were determined using electrophysiology.
What is known already: Sperm motility and hyperactivation are important for fertility and KO studies in mice have shown a key role of calcium regulation in these events with the identification of several key channels (primarily Slo1/3 and CatSper). However, there is no data on abnormalities in men and their effect on fertility.
Study design, size, duration: A series of functional assays on motility and $[Ca^{2+}]_i$ signalling on spermatozoa from sub-fertile men attending for ICSI/IVF identified those with phenotypic abnormalities (Alasmari et al., 2013a) and patch clamping was utilised in patients with specified abnormalities and those with apparently unexplained total fertilisation failure following ART.
Participants/materials, setting, methods: Men undergoing ART at Ninewells Assisted Conception Unit, Dundee. Surplus semen samples at time of treatment, or produced specifically for research purposes and subjected to analysis including patch clamping under quasiphysiological conditions (Mansell et al., 2014; Alasmari et al., 2013ab).
Main results and role of chance: A screen of 181 subfertile men showed that defects in Ca^{2+} signalling pathways are significantly associated with fertilisation potential. Electrophysiological studies of men with abnormalities and fertilisation defects identified (1) 2 patients with minimal outward potassium conductance compared to controls (2) a further two patients with no effective CatSper current or a rapidly inactivating Cation conductance (I_{Tail}).
Limitations, reasons for caution: The electrophysiological analysis is, by definition, based on a limited numbers of cells.
Wider implications of findings: The study documents the key role played by Ca^{2+} signalling pathways in sperm function and identified key patients to clearly determine the impact of CatSper and Slo1/3 channels on human sperm function. The possibility to identify targets for therapeutic manipulation with an effective drug screening program is now a reality.
Study funding/competing interest: Funded by Chief Scientist Office [Scotland], Infertility Research Trust, NHS Tayside, Wellcome Trust and MRC, Ethical approval granted by East of Scotland Research Ethics Service (EoSRES) REC 1: 12/ES/0091.
References:
Alasmari W, et al., (2013a) Hum Reprod. 2013 28:866-76.
Alasmari W, et al., (2013b) JBC, 288:6248-58.
Mansell SA, et al., (2014) Mol Hum Reprod. Jan 16. [Epub ahead of print].

O-002 Bipedalism: the genesis of uterine fibroids

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[1]*London Health Sciences Centre- Victoria Campus, The Fertility Clinic, Obstetrics and Gynaecology, London Ontario, Canada*

Bipedalism: In 1974, Donald Johanson's team discovered approximately 40% of a hominid skeleton who walked upright 3.2 million years ago in Northern Ethiopia. That evening, the radio was playing the Beatles' song *"Lucy in the sky with diamonds"* and they named their new found female skeleton ***Lucy***. In 1978, at Laetoli, Tanzania, Mary Leakey's team found 3 australopithecine footprints, likely parents and a child, frozen in wet ash dated 3.5 million years ago. In 1994, Tim White's team found a partial skeleton of a 110 lb, 4-foot female hominid who lived 4.4 million years ago in Ethiopia. They named their find *Ardipithecus ramidus* – root of the ground ape subsequently nicknamed her ***Ardi.***
Narrow Pelvis: Why our ancestors came down from the trees and started walking upright, >5 millions years ago, is still a matter of debate. However bipedalism required re-alignment of the pelvic structures which resulted in narrowing of the birth canal and transformation from a simple straight cylindrical pipe to a complex convoluted structure in which the planes of the pelvic inlet, mid-pelvis, and outlet are all misaligned.
Encephalization: Use of tools appeared approximately 2.5 millions years ago and soon after the brain of the *Homo sapiens* evolved and grew very rapidly. In the last 500,000 years our brain increased from 750cc to 1800cc.
Evolutionary conflict: The evolutionary events of bipedalism and encephalization caused an 'Evolutionary Conflict' or obstetrical dilemma. The need to walk with a narrow pelvis and the need to think with a larger head would certainly eliminate natural childbirth which would lead to a dead end or extinction of our species!
Counter-evolutionary adaptations: To avoid extinction, *Lucy's* offspring countered the conflict of childbirth by several additional evolutionary adaptations including delivering smaller babies by shortening gestation and/or restrict fetal growth at term, both of which have been proven to be true. In addition the uterus adapted to pushing the baby through the pelvis by moulding the baby using excessive force over lengthier parturition.
Neo-uterus: As the human head grew in volume, additional myometrial force was required to push a large-headed baby through a narrow pelvis. Recent evidence indicates that the outer two thirds (approximately 90% of the total uterine musculature) is a later acquisition of the uterus from the mesenchyme and brought with it its own blood supply - the uterine arteries.
Genesis of fibroids: Angiography has shown that 94% of all uterine fibroids are supplied by the uterine arteries indicating that the majority of fibroids arise from the outer two thirds of the myometrium. The genesis of fibroids then is a direct consequence of the evolutionary adaptation of the uterus to deliver the ever enlarging human head through a narrow birth canal directly related to bipedalism.

SELECTED ORAL COMMUNICATION SESSION

SESSION 02: REVISITING EMBRYO CULTURE

Monday 30 June 2014 **10:00 - 11:30**

O-003 Molecular mouse embryo assay: a functional bioassay for studies involving assisted reproductive technologies (ART)

R. Gilbert[1], S. Es-slami[1], H.T. Ni[1]
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Study question: Can transgenic mouse embryos carrying endogenous early developmental genes linked to green fluorescence protein (GFP) provide a superior molecular mouse embryo assay (MoMEA™) compared to morphological assessment alone for the screening and detection of detrimental conditions?
Summary answer: Early expression of selected pluripotent markers (e.g. Oct-4) in transgenic mouse embryos was correlated with development to blastocysts and provided earlier and more sensitive detection of suboptimal conditions.
What is known already: The one-cell MEA is the most widely used for quality testing of human ART products, however concerns exist due to the insensitivity/variability of this bioassay which lacks standardization and involves subjective analysis by morphology alone. Growing concerns about safety of ART on

human gametes, embryos, clinical outcomes and long-term health of offspring require improved methods of risk assessment to provide scientifically robust and functionally relevant assays for pre-clinical studies prior to clinical implementation.
Study design, size, duration: Cross sectional- control versus treatment. Transgenic mouse embryos were cultured to blastocysts in varied test and control conditions to compare assessment by standard morphology alone versus the added dynamic expression of GFP for screening of critical raw materials and detection of suboptimal culture conditions.
Participants/materials, setting, methods: Fresh one-cell transgenic mouse embryos carrying GFP linked to Oct-4 were harvested and cultured (4 embryos/20 µL media) for 96 h in CSCM with 5 mg/mL HSA (Irvine Scientific) in control and test conditions (5 lots HSA, n = 43; 2 lots oil, n = 90) and assessed blinded for GFP-Oct-4 fluorescence intensity and morphology.
Main results and the role of chance: Early fluorescence intensity (EFI) at 48 h was correlated with progression to blastocysts: EFI was 13%, 48% and 67% for early blastocysts, expanded and hatching blastocysts, respectively. Double blind assessment of morphology and EFI at 48 h showed 63% of embryos at ≥8 cells with 33% EFI in suboptimal oil conditions versus control with 90% of embryos ≥8 cells and 66% EFI; subsequent blastocyst rates were 27% versus 81%, respectively. Embryos cultured 48 h in 5 lots of HSA all showed >95% reaching ≥8 cells with 2 lots showing lower EFI (<90%), and corresponding 96 h blastocyst rates of 72.7% (failing) and 83.7% (borderline), demonstrating the predictive value and sensitivity with EFI over morphology alone. Replicate experiments show similar results.
Limitations, reason for caution: The currently accepted MEA may not be sufficiently sensitive to detect toxicity. However, improvements to MEA by the use of functional molecular biomarkers could enhance sensitivity and improve detection of suboptimal materials/conditions. Further testing is required before extrapolation to human preimplantation development.
Wider implications of the findings: Transgenic mouse embryos expressing functionally relevant biomarkers of normal early embryo development can be used to monitor the developmental impact of culture conditions. This novel approach provides a superior MEA that is more meaningful and sensitive for detection of embryotoxicity than morphological assessment alone. Further studies are required with additional pluripotency markers to define more comprehensive molecular indicators of early development which may benefit reproductive studies including ART, stem cell research and epigenetic effects.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Irvine Scientific.
Trial registration number: N/A.

O-004 In vitro development of donated frozen-thawed human embryos in microfluidic chips: a randomized controlled trial

D.C. Kieslinger[1], Z. Hao[2], C.G. Vergouw[1], E.H. Kostelijk[1], C.B. Lambalk[1], S. Le Gac[2]
[1]VU University Medical Center, Department of Obstetrics and Gynaecology - IVF Center, Amsterdam, The Netherlands
[2]University of Twente, MESA + Institute for Nanotechnology - BIOS Lab-on-a-chip, Twente, The Netherlands

Study question: Does a novel microfluidic platform provide equal or better conditions for the *in vitro* culture of frozen-thawed human Day 4 embryos in terms of blastocyst formation rate, compared to embryo culture in standard microdrop culture dishes?
Summary answer: This study shows that human embryos cultured in microfluidic chips develop to blastocysts, hatching and hatched embryos at a rate comparable to standard microdrop culture dishes.
What is known already: Microfluidic technologies may provide more optimal and natural conditions for the culture of mammalian embryos *in vitro*. A prototype of the presented microfluidic platform supported full-term mouse embryo development in an earlier study. Several research groups have studied the application of microfluidic embryo culture for a number of animal species. However, attempts to employ microfluidics for assisted reproduction in humans are rare and have not fully explored the potential of this technology yet.
Study design, size, duration: This randomized controlled trial included 118 donated frozen-thawed human Day 4 embryos that were either cultured in standard microdrop dishes or in microfluidic platforms between August 2012 and August 2013. Embryos were assigned to either the microdrop control or microfluidics group using computerized randomization and sealed envelopes.
Participants/materials, setting, methods: Embryos that fulfilled the inclusion criteria were randomly allocated to the microdrop (n = 60) or microfluidics group (n = 58). Routine embryo grading was performed at four different time points using the Gardner & Schoolcraft system for blastocysts. The study was conducted in an IVF laboratory of an academic hospital in The Netherlands.
Main results and the role of chance: The percentage of frozen-thawed Day 4 embryos that developed to the blastocyst stage did not differ in the control dishes and microfluidic chips after 28 hours of culture (53.3% vs. 58.6%; p = 0.583) or at any of the other time points. The proportion of embryos that would have been suitable for embryo transfer was comparable after 28 hours of culture in the control dishes and microfluidic chips (90.0% vs. 93.1%; p = 0.743). Furthermore, blastocyst quality was similar in the two study groups. A potentially positive effect of microfluidic embryo culture on blastocyst quality may remain unrevealed as a consequence of the use of Day 4 embryos since many important steps in the development of human embryos already take place by Day 2/3.
Limitations, reason for caution: A possible limitation of this study is the use of frozen-thawed Day 4 embryos. While two other studies with earlier stage human embryos found superior blastocyst quality after microfluidic culture, our data did not reveal an improvement.
Wider implications of the findings: Microfluidics could revolutionize assisted reproductive technology in the future by combining a more natural culture environment with an advanced tool for embryo characterization. The results of this study show that a microfluidic device can successfully support human blastocyst development *in vitro*. Further research is needed to determine if medium refreshment in microfluidic chips will improve blastocyst quality and built-in sensors facilitate embryo selection resulting in increased pregnancy rates.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Grant for Fertility Innovation by Merck Serono.
Trial registration number: Dutch Trial Register (NTR), registry number NTR3867.

O-005 Blastocyst development in single-step versus sequential culture media: a prospective randomized study with sibling oocytes

I.A. Sfontouris[1], E.M. Kolibianakis[2], G.T. Lainas[1], I.Z. Zorzovilis[1], G.K. Petsas[1], T.G. Lainas[1]
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[2]Aristotle University of Thessaloniki, 1st Department of Obstetrics & Gynecology, Thessaloniki, Greece

Study question: Is embryo development to the blastocyst stage different between single-step media (Global) and sequential media (Origio)?
Summary answer: Culture of embryos in single-step media (Global) is associated with significantly higher blastocyst formation rates, utilization rates and overall quality compared to culture in sequential media (Origio).
What is known already: Sequential culture media were designed to meet the changing requirements of the developing embryo in vitro. However, there has been interest in the use of single-step media, aiming to allow the embryo to choose the necessary nutrients while maintaining a more stable culture environment. Previous studies suggest that sequential media do not appear superior to single-step media.
Study design, size, duration: Prospective randomized study of sibling oocytes, performed at Eugonia Assisted Reproduction Unit, Athens, from October 2013 to January 2014.
Participants/materials, setting, methods: A total of 371 metaphase-II oocytes from 21 women (aged ≤39 years) were randomly allocated to be fertilized (ICSI) and cultured to the blastocyst stage in either Origio (ISM1/BlastAssist) media (n = 187) or Global medium (n = 184). Media were changed on Day 3 and blastocyst transfers were performed on Day 5.
Main results and the role of chance: Fertilization [155 (84.2%) vs. 153 (81.8%)] and cleavage rate [153 (83.2%) vs. 150 (80.2%)] were similar in Global and Origio groups. On Day 3, embryos with good morphology [122 (66.3%) vs. 117 (62.6%)], ≥6-cells [133 (72.3%) vs. 136 (72.7%)] and ≤20% fragmentation [135 (73.4%) vs. 126 (67.4%)] did not differ. On Day 5, culture in Global yielded significantly more blastocysts [94 (51.1%) vs. 67 (35.8%), p = 0.03], more full-to-hatched blastocysts [74 (40.2%) vs. 48 (25.7%), p = 0.04], more

good quality blastocysts [57 (31%) vs. 32 (17.1%), p = 0.02], and more blastocysts with Grade-A trophectoderm [44 (23.9%) vs. 22 (11.8%), p = 0.01] than Origio. Blastocyst transfer rate [29 (15.8%) vs. 14 (7.5%), p = 0.02] and total utilization (transfer + cryopreservation) rate [95 (51.6%) vs. 69 (36.9%), p = 005] were higher in Global than Origio.
Limitations, reason for caution: This is a prospective randomized study with sibling oocytes. In order to firmly establish a beneficial effect of the single-step medium (Global) over sequential media (Origio) it is necessary to evaluate pregnancy rates in a prospective RCT with randomization of patients.
Wider implications of the findings: Culture in a single-step medium is associated with higher blastocyst formation rates, better blastocyst quality and higher blastocyst utilization rates compared to sequential media. Therefore, the sequential approach may not be necessary for embryo culture, and a single medium may provide adequate support to the developing embryo. Future RCTs reporting on pregnancy and live birth rates are necessary.
Study funding/competing interest(s): Funding by hospital/clinic(s), The study was self-funded by Eugonia Assisted Reproduction Unit.
Trial registration number: NCT02048527.

O-006 Comparisons between different types of culture medium for human embryo development

M. Shimomura[1], N. Fukunaga[2], H. Kitasaka[2], T. Yoshimura[1], F. Tamura[2], N. Hasegawa[1], K. Nakayama[2], M. Kato[1], E. Kojima[1], N. Aoyagi[1], H. Ohno[2], K. Yasue[2], H. Watanabe[1], Y. Hashiba[3], Y. Asada[4]
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Study question: Do Single step and Sequential media affect the rate of blastocyst formation?
Summary answer: The rate of blastocyst formation measured at Day 5 and 6 was significantly higher when embryos were cultured in a single step medium compared to embryos cultured in Sequential medium.
What is known already: Currently in ART lab procedures, changing the culture medium according to the developmental stage of embryos is common practise. This however includes an extra step requiring handling of the culture dish outside of the incubator. Recently, it has been suggested that changing the medium is not necessary and may lead to improvements in the rate of blastocyst formation. Additionally it has been reported that different culture media may have an impact on embryo development parameters.
Study design, size, duration: From January to March in 2013, 927 embryos were obtained by IVF from 112 cycles and cultured with a Single step medium (Irvine Scientific : Group A). From October to December 2012, 817 embryos were obtained by IVF from 111 cycles and cultured with a Sequential medium (SAGE : Group B).
Participants/materials, setting, methods: Medium changes were performed at Day 1, Day 3 and Day 5, in both groups and embryos were cultured in K-systems G185 up to Day 7. We compared the rate of blastocyst or good-quality blastocyst formation in Group A and Group B.
Main results and the role of chance: The rate of blastocyst formation at Day 5 and Day 6 was 55.3% (513/927) in Group A and 46.6% (381/817) in Group B. The rate of blastocyst formation was significantly higher in Group A compared to Group B (P < 0.01). The rate of blastocyst formation at Day 5 was 41.1% (381/927) in Group A and 29.5% (241/817) in Group B. The rate of good-quality blastocyst formation at Day 5 was 36.6% (339/927) and 26.9% (220/817) in Group B. The rate of blastocyst formation at Day 5 and good-quality blastocyst formation at Day 5 was significantly higher in Group A compared to Group B (P < 0.01).
Limitations, reason for caution: The study was a prospective cohort study and not an RCT. These are from analysis with a limited sample size.
Wider implications of the findings: These results suggest that changing the culture medium is not necessary for blastocyst culture.
Study funding/competing interest(s): Funding by hospital/clinic(s). None.
Trial registration number: None.

O-007 Protein-free culture medium has a negative effect on embryo quality: data analysis of a cancelled randomized trial

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Study question: To test the hypothesis that the proportion of top quality embryos (TQEs) on day 3 is 10% higher in a study group (SG) with protein-free medium (PF Protein Free Culture medium, CellCura ASA, Norway), than in a control group (CG) with a HSA supplemented medium (Gynemed, Lensahn, Germany).
Summary answer: Both the number of top quality embryos (TQE) on day 3 as well as the embryo utilization rate = ((N embryos frozen + N embryos transferred)/total N embryos) were significantly lower in the SG compared to the CG.
What is known already: During culture of gametes and embryos HSA is added to the culture medium. Quality control assessment at the Leuven University Fertility Centre showed a significant difference in embryo quality between batches of culture medium, probably due to differences in composition of HSA. Shahata *et al.* (2000) reported that a protein-free medium is as efficient as a HSA supplemented culture medium (using sibling oocytes). These data require confirmation in randomized trials.
Study design, size, duration: Between 2012 and 2013, 40 patients were randomized during their second IVF/ICSI cycle. The primary outcome is the percentage of TQE on day 3. Secondary outcomes were utilization rate, pregnancy rate and implantation rate. The hypothesed 10% increase of TQE needed 407 embryos in each group (power 0.8).
Participants/materials, setting, methods: Embryo development and embryo quality was evaluated on day 3. An embryo with 7, 8 or 9 blastomeres, less than 10% fragmentation and equally or slightly unequally sized blastomeres was defined as a TQE. Due to external reasons the powered study was cancelled prematurely. Therefore the collected data were analysed.
Main results and the role of chance: After elimination of cycles with a day 2 embryo transfer, the SG contained 20 patients (101 embryos) and the CG 18 patients (108 embryos). Although fertilization rate was comparable in both groups (SG 62.4%; CG 65.4%), the percentage of TQE on day 3 was significantly lower in the SG (11.9% (12/101)) compared to the CG (37.0% (40/108)) (p < 0.0001). Furthermore the utilization rate was significantly lower in the SG (42.6% (43/101)) compared to the CG (62.0% (67/108)) (p = 0.0035). Secondary outcome variables, the pregnancy rate (25.0% (5/20) in SG and 33.3% (6/18) in CG) and implantation rate (15.6% (5/32 embryos transferred) in SG; 35.0% (7/20 embryos transferred) in CG) were comparable in both groups.
Limitations, reason for caution: The recruitment period was extended because of a low participation rate (44.3% (58/131)). 18 patients were excluded from the study due to external reasons. Although the study was cancelled prematurely the negative effect on embryo quality and utilisation rate was significant (power of 0.8 even despite the small sample size).
Wider implications of the findings: Data analysis of a prematurely cancelled RCT showed that, when compared to a HSA supplemented medium, culture in protein-free medium had a significantly negative effect on embryo quality and embryo utilisation rate, with an expected negative effect on the cumulative delivery rate per patient based on reproductive outcome after embryo transfer from fresh ART cycles and subsequent frozen-thawed cycles. Additional research is needed to optimize embryo culture in protein-free medium before clinical application is acceptable.
Study funding/competing interest(s): Funding by hospital/clinic(s), University Hospital Leuven.
Trial registration number: ClinicalTrials.gov Protocol Registration System. Unique Protocol ID: s 53398

O-008 Developmental evaluation of human embryos cultured in two different systems: sequential versus single medium

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Study question: Which is the best approach to perform embryo culture, sequential or single culture medium?

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 and grow faster as compared to embryos cultured in sequential media.
What is known already: The quality of the embryo culture is an important factor that determine IVF success. Two kinds of 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 composition of culture medium, which is constant and 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: A prospective cohort study was conduced from September 2013 to January 2014. Embryo development in terms of blastocyst formation and timings of cleavage was compared between cultures in sequential (Group A) or single step medium (Group B) where no media refresh was performed on day 3. Consecutive patients treated were randomly assigned to Group A (SAGE, Cooper Surgical, USA), or Group B (IrvineScientific, CA, USA). A total of 1.255 embryos were analyzed of which 247 with time-lapse cinematography.
Participants/materials, setting, methods: All embryos analysed were cultured in same conditions (bench-top incubators, 37°C, 6% CO_2 and 5% O_2). Logistic regression analysis was used to control for possible confounding factors. Morphokynetic parameters were used to analyse timing of PN appearance, syngamy, 1st to 7th cell divisions and blastocyst formation.
Main results and the role of chance: Blastocyst developmental rate at day 5 or day 6 was 322/781 (41.2%) for group A, and 238/474 (50.2%) for group B (p = 0.0019). Logistic regression analysis adjusted for female age, infertility factor, and sperm quality confirmed this association (OR 1.4; 95% CI 1.11–1.78). Faster timings of development were recorded for group B embryos analyses by time-lapse cinematography, in particular timing of 5 cells formation (54.4 ± 8.2 h vs. 48.4 ± 7.4 h for group A and group B, respectively), 8 cells (72.1 ± 9.2 h vs. 66.1 ± 8.8 h) and Morula formation (94.7 ± 10.1 vs. 89.1 ± 7.6) were lower in group B ($p < 0.05$).
Limitations, reason for caution: Time-lapse evaluation should be continued on a larger population of embryos to confirm these preliminary data. From this study design it can not be excluded that the advantages observed in the use of single media system is also due to the reduced manipulation of embryos. This study is restricted to in vitro condition, clinical data are necessary to understand the possible relation-ship between culture conditions, implantation and perinatal outcomes.
Wider implications of the findings: Optimal in vitro conditions to support blastocyst development are crucial in the modern embryology. Most of the new approaches (i.e. technologies for embryo selection, single embryo transfer strategy, pre-implantation genetic diagnosis) require extending the culture to day 5/day 6. The possibility to increase the relative blastocyst formation rate up to 40% by adjusting some simply laboratory protocols is thus crucial to continue the progression of embryology.
Study funding/competing interest(s): Funding by hospital/clinic(s). None.
Trial registration number: None

SELECTED ORAL COMMUNICATION SESSION

SESSION 03: RECENT ADVANCES IN PCOS

Monday 30 June 2014 **10:00 - 11:30**

O-009 Metastin levels in relation with hormonal and metabolic profile in patients with polycystic ovary syndrome

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Study question: Is there a correlation between metastin and polycystic ovary syndrome (PCOS) related reproductive and metabolic disturbances.
Summary answer: Metastin levels were higher in women with PCOS as compared to controls regardless of body mass index (BMI).
What is known already: Three studies assessed metastin in patients with PCOS and their results are inconsistent. Two previous studies reported higher metastin levels in women with PCOS however, one study reported lower levels in women with PCOS as compared to controls. There are also controversial data regarding the association between metastin levels with BMI, insulin resistance, LH and androgenic profile.
Study design, size, duration: One hundred forty nine women participated in this prospective study between March 1st 2012 and September 31st 2013.
Participants/materials, setting, methods: A total of 83 women with PCOS and 66 BMI matched controls participated in the study in our university hospital setting. Women with PCOS and controls were divided in to two groups, based on BMI: overweight and normal weight. Serum metastin levels were assessed by an enzyme-immunoassay kit.
Main results and the role of chance: Metastin levels were significantly higher in the PCOS group compared to controls (2.02 ng/mlversus 1.16 ng/ml, $p < 0.001$). Metastin levels correlated significantly positively with luteinizing hormone (LH), total testosterone (T), dehydroepiandrosteronesulphate (DHEA-SO4) levels, modified Ferriman Gallwey (mFG) scores and free androgen index (FAI); however correlated negatively with sex hormone binding globulin (SHBG) levels ($p < 0.05$). When overweight (BMI ≥ 25 kg/m^2 and <30 kg/m^2) and normal weight (BMI < 25 kg/m^2) women with PCOS were compared to body mass index (BMI) matched controls, higher metastin levels were also found in PCOS groups (1.94 ng/ml versus 1.18 ng/ml, and 2.06 ng/ml versus 1.08 ng/ml, $p < 0.05$, respectively).
Limitations, reason for caution: The limitation of this study is the lack of an obese PCOS group with a BMI ≥ 30 kg/m^2.
Wider implications of the findings: These findings suggest that metastin levels were higher in women with PCOS as compared to controls regardless of BMI. Furthermore, metastin levels can be used as a specific marker for androgenic profile and this marker might play a role in the pathogenesis of PCOS.
Study funding/competing interest(s): Funding by hospital/clinic(s), The work was funded by the Department of Obstetrics and Gynaecology, Selcuk University, Konya, Turkey. The authors declare no conflicts of interest.
Trial registration number: N/A.

O-010 miRNA expression profile and bioinformatics analysis in polycystic ovary syndrome patients with insulin resistance

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Study question: What is the unique expression signature of microRNAs (miRNAs) in the ovary of polycystic ovary syndrome patients and whether the miRNAs play a pivotal role in the pathophysiology of PCOS?
Summary answer: Microarray analysis demonstrated expression of miRNAs altered in the PCOS patients with insulin resistance. Because differential expression miRNAs target gene involve in 59 pathways, including VEGF pathway, MAPK pathway, and GnRH signalling pathway, we hypothesize that miRNAs may participate in the pathophysiology of PCOS.
What is known already: Polycystic ovary syndrome (PCOS), a complex genetic condition, is a highly prevalent heterogeneous syndrome with reproductive abnormalities and metabolic dysfunction.

MiRNAs have recently emerged as key regulators of metabolism. Other reports have confirmed that correct expression of miRNAs are necessary for follicular development and hormone synthesis of ovary. Those data highlight the potential pathological roles of aberrant miRNA expression in PCOS.
Study design, size, duration: This is a laboratory-based, cross-sectional study comparing miRNAs expression in archived ovarian tissue from 9 normal ovulatory women (controls), 12 women with PCOS with insulin resistance (PCOS-IR).
Participants/materials, setting, methods: The miRNAs expression of ovary were detected using Agilent Human miRNA Microarray V16.0. Differential expression miRNAs were analyzed the functions and interactive networks using integrated analysis of cross-platform microarray and pathway data (InCroMap)

tool. Microarray results for selected miRNAs were confirmed by real-time quantitative PCR.
Main results and the role of chance: There were 517 (42.9%) miRNAs detected in ovary by microarray. 84 miRNAs were up-regulated, and 4 miRNAs were down-regulated in PCOS patients (fold change >2; $P < 0.05$). Using principal components analysis, it is showed samples from the same conditions have similar patterns of miRNAs expression. The first three components could present 94% variables. Using prediction analysis of microarrays, it successfully discriminate the PCOS and control patients by a cluster of 29 miRNAs. The pathway analysis identified the differential expression miRNA target gene involve in 59 pathways, including VEGF pathway, MAPK pathway, and GnRH signalling pathway, which may participate in the pathophysiology of PCOS.
Limitations, reason for caution: Due to the difficulties in collecting tissues sample, we couldn't detect the differential miRNAs expression in different phenotypes of PCOS.
Wider implications of the findings: Studies identifying the unique expression signature of miRNAs in certain ovarian pathological conditions such as polycystic ovarian syndrome, might offer an additional diagnostic tool to assess this disorder, and eventually provide insight for novel treatments of the disease.
Study funding/competing interest(s): Funding by national/international organization(s), This study was supported by National Natural Science Foundation of China, (Grant No. 81070466; NO. 81370680). The authors declare that they have no competing interests.
Trial registration number: None.

O-011 The role of subcutaneous adipose tissue in the development of hyperandrogenism in PCOS

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Study question: Is there any increase in peripheral adipose tissue steroidogenesis in women with PCOS? This was investigated by comparing the expression of the genes encoding the main steroidogenic enzymes including 17-alpha hydroxylase/17.20-lyase (CYP 17A1) and 17-beta hydroxysteroid dehydrogenase type 5 (AKR1C3) in adipose tissue of women with and without PCOS.
Summary answer: The study findings suggest an increased steroidogenic activity in peripheral adipose tissue with possible subsequent increase in androgen production in women with PCOS.
What is known already: Current evidence suggests that excess androgen production is central in the pathogenesis of PCOS. Although, ovarian theca cells are thought to be the main source of this androgen excess, recent research has provided evidence of androgen synthesis in peripheral adipose tissue, which could be another potential source of hyperandrogenism in PCOS. The role of peripheral adipose tissue steroidogenesis in PCOS remains to be investigated.
Study design, size, duration: This was a laboratory based study involving subcutaneous adipose tissue biopsies obtained during elective gynaecological surgery from women with PCOS ($n = 8$) and from an age and BMI matched group of healthy women ($n = 8$). All participants were of reproductive age (20–45) with a BMI of 20–35 kg/m^2.
Participants/materials, setting, methods: Adipose biopsies were stored at –80°C. Total RNA was isolated from the frozen adipose tissue (using Trizol extraction) followed by reveres transcription. Quantitative RT-PCR was performed to determine the expression of a panel of reference genes, CYP17A1 and AKR1C3. Data were analysed with GenEx and compared using ΔΔCt method.
Main results and the role of chance: Of the reference genes tested, GAPDH, ACTB, and LPR10 were shown to be consistently expressed across the PCOS and the control group ($P > 0.05$). The mean ± sem relative expression level of AKR1C3 mRNA in PCOS adipose tissue was 15.1 ± 2.0, which was significantly ($P = 0.0003$) greater than that (3.3 ± 1.1) of the control samples. The expression level of CYP17A1 mRNA was not significantly ($P = 0.56$) different between the two groups.
Limitations, reason for caution: These findings should be supported by other laboratory methods e.g. WB.
Wider implications of the findings: The data of this study reveled a 5-fold increase in the expression level of AKR1C3 mRNA in subcutaneous adipose tissue of PCOS women. This suggests an increased adipose tissue steroidogenic activity with subsequent increased androgen production. It is therefore possible to postulate that peripheral adipose tissue plays an important role as a source of excess androgen production in women with PCOS. This could potentially pave the way for the development of innovative therapeutic targets for the management of this very common syndrome.
Study funding/competing interest(s): Funding by national/international organization(s), The Libyan Government.
Trial registration number: N/A.

O-012 Metabolic response to folate supplementation in overweight women with polycystic ovary syndrome: a randomized double-blind placebo-controlled clinical trial

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Study question: The current study was, therefore, done to investigate the effects of folate supplementation on glucose metabolism and lipid concentrations in overweight and obese women with PCOS.
Summary answer: In conclusion, five milligram per day folate supplementation for 8 weeks among women with PCOS had beneficial effects on Hcy, serum insulin, total-, LDL-, and non-HDL-cholesterol levels, total- to HDL-cholesterol ratio and HOMA-IR.
What is known already: Some studies have reported the high prevalence of folate deficiency among women with PCOS. Treatment of folate deficiency via supplementation has been resulted in a better function of the vascular endothelium and led to the reduced serum homocysteine (Hcy) among women. We are aware of no study examining the effects of folate supplementation on glucose metabolism and lipid concentrations in patients with PCOS.
Study design, size, duration: This randomized double-blind placebo-controlled clinical trial was conducted among 81 women diagnosed with PCOS, aged 18–40 year old. Duration of the intervention was for 8 weeks.
Participants/materials, setting, methods: Methods: This randomized double-blind placebo-controlled clinical trial was conducted among 81 women diagnosed with PCOS, aged 18–40 year old. Participants were randomly assigned to three groups receiving: 1) Folate-1: 1 mg/d folate supplements ($n = 27$); 2) Folate-5: 5 mg/d folate supplements ($n = 27$) and 3) placebo ($n = 27$) for 8 weeks. Fasting blood samples were taken at baseline and after 8 weeks' intervention to quantify glucose metabolism and lipid concentrations.
Main results and the role of chance: Results: Folate supplementation (5 mg), compared with folate-1 and placebo, resulted in reduced plasma Hcy (–2.2 ± 3.6 vs. –1.7 ± 4.0 and 1.0 ± 4.3 µmol/L, respectively, P-interaction = 0.009), serum insulin levels (–1.7 ± 3.7 vs. –0.4 ± 3.5 and 2.6 ± 6.5 µIU/mL, respectively, P-interaction = 0.006), HOMA-IR score (–0.3 ± 1.0 vs. –0.2 ± 0.9 and 0.7 ± 2.0, respectively, P-interaction = 0.01) and total cholesterol/HDL-C ratio (–0.7 ± 1.3 vs. –0.1 ± 0.6 and 0.1 ± 1.1, respectively, P-interaction = 0.01). Furthermore, we found a significant difference in mean change of serum total cholesterol (–27.8 ± 36.5 vs. –4.0 ± 35.2 and –0.3 ± 37.3 mg/dL, respectively, P-interaction = 0.01), LDL- (–29.7 ± 39.5 vs. –3.0 ± 27.3 and –5.1 ± 33.2 mg/dL, respectively, P-interaction = 0.007) and Non-HDL-cholesterol levels (–27.4 ± 37.3 vs. –3.5 ± 29.2 and –1.4 ± 38.4 mg/dL, respectively, $P = 0.01$) in the folate-5 group compared with folate-1 and placebo.
Limitations, reason for caution: We were unable to assess the effect of folate supplementations on biomarkers of systemic inflammation and biomarkers of oxidative stress. Furthermore, due to budget limitation we could not examine the effect of folate supplementations on serum levels of folate and vitamin B12.
Wider implications of the findings: As insulin resistance is the cornerstone of metabolic disorders in PCOS, folate supplementation through influencing insulin resistance might be useful in the management of these patients.

Study funding/competing interest(s): Funding by University(ies), The study was supported by a grant (no. 92147) from Arak University of Medical Sciences.
Trial registration number: www.irct.ir: IRCT201306085623N8.

O-013 Polycystic ovary syndrome (PCOS) is associated with depression and anxiety, and decreases quality of life independently of BMI – a population based cohort analysis

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[5]*University of Oulu and Oulu University Hospital, Department of Psychiatry, Oulu, Finland*

Study question: Do women with polycystic ovary syndrome (PCOS) or PCOS-related symptoms [oligo/amenorrhea and/or excessive hair growth (hirsutism)] present with depression and anxiety, and decreased quality of life (QoL) independently of obesity already at the age of 31 in population based cohort analysis of Northern Finland Birth Cohort 1966 (NFBC66)?
Summary answer: The analysis revealed that coexistence of both PCOS-related symptoms or hirsutism alone were related to increased anxiety and depression (Hopkins Symptom Checklist-25, HSCL-25) and decreased QoL (15D) independently of body mass index (BMI), although BMI as well as history of infertility were also linked to these psychoemotional variables.
What is known already: Infertility and metabolic-related factors have been associated to psychoemotional distress in women with PCOS. However, data on larger population based studies with adequate adjustments are still missing.
Study design, size, duration: A postal questionnaire including questions on oligo-amenorrhea, hirsutism, depression, anxiety, QoL, life satisfaction and temperament at the age of 31 were sent to 5608 women in the NFBC66. The data were available from 4427 women and after excluding pregnant women and hormonal contraceptive users 2967 women were left for the final analysis.
Participants/materials, setting, methods: The women were divided in four groups; 2188 asymptomatic, 331 with oligo/amenorrhea, 323 with excessive hair growth, 125 with both symptoms (PCOS). The associations were calculated using ANOVA or Pearson's Chi-square test or Fisher's exact test when appropriate. The results were adjusted for BMI, history of infertility and social and marital status.
Main results and the role of chance: The mean (standard deviation) score of HSCL was increased in women with PCOS when compared to asymptomatic women [1.50 (0.44) vs. 1.36 (0.32), $P < 0.001$] and sub-analysis with cut-off score 1.75 supported this (anxiety 16.1% vs. 8.2%, $P < 0.001$; depression 19.4% vs. 12.7%, $P = 0.015$) also implying that hirsutism was associated more strongly to mental stress than oligo/amenorrhea. The results remained statistically significant after adjusting for BMI, history of infertility, and social and marital status. The QoL was decreased ($P < 0.001$) and the PCOS women were less satisfied with their life situation when compared to asymptomatic women (not content 16.5% vs. 7%, $P < 0.001$). These findings were strongly but not independently associated to infertility. PCOS women presented with social anhedonia, which was related to BMI. No differences were observed in temperament traits between the groups.
Limitations, reason for caution: The diagnosis of PCOS at the age of 31 was based on self-reporting, and an information bias in reporting the symptoms is possible. Ovarian ultrasonography was not available for the diagnosis of PCOS.
Wider implications of the findings: This study provides the first large population based data showing strong association between depression, anxiety and decreased QoL in women with PCOS symptoms. Body mass index and infertility status were also associated with psychoemotional distress in PCOS. Given that the findings were present already at the age of 31 follow-up studies are warranted as well as acknowledgement of the mental distress related to PCOS when treating this pleiotropic syndrome.

Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Funding by national/international organization(s), The Academy of Finland (project grants 104781, 120315, 129269, 1114194, SALVE), Sigrid Juselius Foundation, the North Ostrabothnia Regional Fund, University Hospital Oulu, Biocenter, University of Oulu, Finland (75617), the European Commission (EURO-BLCS, Framework 5 award QLG1-CT-2000-01643) and the Medical Research Council, UK (PrevMetSyn/SALVE).
Trial registration number: None.

O-014 The adverse effect of obesity/high fat diet on oocyte quality and metabolism is not reversible with resumption of regular diet in mice

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Study question: Are the effects of an obesogenic diet on oocyte spindle formation, mitochondrial metabolism and lipid accumulation reversible with 8 week washout period when the mice are placed back on a regular chow and resume normal metabolic parameters? Does reversing the metabolic phenotype seen in the high-fat fed rodent diet reverse the reproductive phenotype?
Summary answer: Despite normalization of weight, glucose utilization and cholesterol levels 8 weeks after switching from a high fat to a regular chow, oocytes from these washout mice exhibited significantly higher rates of meiotic spindles defects. The mitochondrial metabolism and distribution also remained abnormal.
What is known already: Obese women experience subfertility, increased miscarriage rates and poor pregnancy outcomes. Obese women and high fat fed mice experience higher rates of abnormal spindle formation. Obese mice demonstrate abnormal mitochondrial metabolism, distribution and brain morphology. Reversibility of these effects by diet has not been explored in rodents or women.
Study design, size, duration: Sixty female C57BL6/J mice were placed on either a high fat diet (HFD) containing 35.8% fat, and 20.2% protein by nutritional content ($n = 30$) or an isocaloric control diet (CD) containing 13% fat and 25% protein ($n = 30$) for a total of 6 weeks.
Participants/materials, setting, methods: Following the 6 week feeding period, metabolic parameters including weight and serum lipids/cholesterol and glucose tolerance were assessed. All mice were placed on control diet for 8 weeks. Following this "washout" period, metabolic parameters were repeated, and mice were sacrificed. MII and GV oocytes were collected and the following parameters measured by confocal immunofluorescent microscopy: meiotic spindles formation, mitochondrial intracellular distribution using Mitotracker, mitochondrial membrane potential by JC-1 assay, and lipid droplet accumulation by Bodipy staining. Images were evaluated and analyzed by a blind observer. Oocyte metabolism was assayed using microanalytic enzyme cycling assays on individual oocytes for ATP and citrate concentrations.
Main results and the role of chance: The metabolic phenotype of the HFD including weight, lipid parameters, and serum glucose were fully reversible after the washout period; however, the negative effects of the HFD on oocytes were not fully reversible. HFD washout oocytes demonstrated significantly more abnormal meiotic spindles (55% vs. 17%) and abnormal meiotic progression (19% vs. 5%) compared to control mice. The HFD washout GV oocytes also had decreased mitochondrial membrane potential (1.489 ± 0.46 HFD washout vs. 1.83 ± 0.79 CD, $p < 0.05$), decreased ATP (5.524 ± 0.177 mm/Kw washout, *vs.* 7.770 ± 0.141 mm/Kw control), and citrate levels (0.239 ± 0.016 mm/Kw washout *vs.* 0.929 ± 0.045 mm/Kw control), as well as abnormal distribution of cytoplasmic mitochondria. Furthermore, oocytes from the washout arm demonstrated a unique blebbing pattern in 55% of the oocytes when stained with BODIPY, whereas this pattern was not seen in the control oocytes.
Limitations, reason for caution: This study was conducted using mice, however the similarity between this model and human obesity has been established previously. Although the abnormalities persist, the pregnancy outcomes of the oocytes after fertilization and gestation have not been examined.
Wider implications of the findings: Even with reversal of the metabolic effects of the HFD in mice, an altered reproductive phenotype persists. These findings could have implications when counseling and treating obese, subfertile women. It is possible that with a longer washout period, some aspects of the phenotype may be reversed. It is also possible that exercise plus dietary changes are needed to see complete reversal.
Study funding/competing interest(s): Funding by national/international organization(s), American Diabetes Association.
Trial registration number: N/A.

SELECTED ORAL COMMUNICATION SESSION

SESSION 04: MALE INFERTILITY

Monday 30 June 2014 **10:00 - 11:30**

O-015 Functional characterization of gpr55 receptor in human spermatozoa reveals a novel biological role for the phospholipid lysophosphatidylinositol in sperm motility and viability

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Study question: Does human spermatozoa express the orphan G-protein coupled receptor GPR55 and what are the physiological effects of stimulation of these receptors with endogenous ligand Lysophosphatidylinositol?
Summary answer: GPR55 mRNA transcripts in spermatozoa were shown to correlate with better sperm quality. A potent activator of GPR55 receptor, lysophosphatidylinositol, promoted sperm motility and maintained viability but did not evoke changes in intracellular calcium mobilization. The stimulatory effect of Lysophosphatidylinositol was inhibited by co-incubation with cannabidiol.
What is known already: Endocannabinoid signalling, through the cannabinoid receptors (CB1 and CB2), triggers a series of secondary messenger pathways which modulate mammalian spermatogenesis, epididymal maturation and post ejaculatory maturation. The orphan cannabinoid-like G protein-coupled receptor (GPR55) has recently been proposed as a novel cannabinoid receptor that may engage some lipid ligands, such as lysophosphatidylinositol and several cannabinoid-like compounds, including the endocannabinoids. The functional significance of GPR55 in human spermatozoa and male reproductive physiology remains unexplored.
Study design, size, duration: Molecular and physiological studies of human spermatozoa in an Academic tertiary-care medical centre.
Participants/materials, setting, methods: Using qRT-PCR, GPR55 transcript levels were determined in spermatozoa from men with normozoospermia, asthenozoospermia, oligoasthenoteratozoospermia and teratozoospermia. Normal spermatozoa were exposed in-vitro to lysophosphatidylinositol to determine its effect on sperm motility, viability and intracellular calcium mobilisation.
Main results and the role of chance: Analysis of the GPR55 transcript expression patterns showed a significant decrease in GPR55 transcripts in spermatozoa from men with oligoasthenoteratozoospermia (0.27 ± 0.12, $P < 0.0001$), asthenozoospermia (0.77 ± 0.23, $P < 0.0001$) and teratozoospermia (0.77 ± 0.11, $P < 0.0001$) when compared to the normozoospermic controls. After incubation, lysophosphatidylinositol exerted a significant time-dependent increase in progressive sperm motility when compared with untreated control. When sperm were treated with increasing lysophosphatidylinositol concentrations, a dose-dependent increase in progressive sperm motility and maintenance of viability up to 6 hours was observed. The lysophosphatidylinositol-mediated stimulation of progressive sperm motility and viability was completely inhibited when sperm were pre-treated with cannabidiol. Up to a dose of 100 µM, lysophosphatidylinositol did not induce changes in sperm intracellular Ca^{2+} concentration.
Limitations, reason for caution: The stimulatory effect of lysophosphatidylinositol was only shown in-vitro and may not reflect what happens in-vivo. Differences in mRNA transcript levels in men with normal and abnormal sperm parameters may not reflect protein synthesis.
Wider implications of the findings: These novel findings reveal a role for GPR55 receptor and lysophosphatidylinositol signalling in the maintenance of normal human sperm functions, and could constitute a new biomarker and therapeutic target for male reproductive failure. These findings have important public health ramifications as delta-9-tetrahydrocannabinol and cannabidiol found in cannabis, could compete with these endocannabinoid and lysophosphatidylinositol for the cannabinoid receptors including GPR55, upsetting their finely balanced, normal functioning and resulting in male reproductive failure.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), This work was funded in part by miscellaneous educational funds from the University Hospitals of Leicester National Health Services Trust to support the Endocannabinoid Research Laboratory of University of Leicester.
Trial registration number: Not Applicable.

O-016 Artificial insemination with donor sperm (AID): heterogeneity in sperm banking facilities in a single country

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Study question: This study aimed to present an overview of the sperm banking facilities in Belgium, with special attention to the methods and criteria used for recruitment, screening and selection of potential sperm donors, procedures for sperm washing and freezing and costs associated with sperm donation.
Summary answer: According to our results, a wide variation in methods associated with sperm banking is observed in Belgian centres. Donor recruitment methods, screening of donors, acceptance rates, thresholds for acceptable sperm quality, sperm preparation techniques, freezing methods and even the financial reimbursement per sperm sample differ substantially between all centres.
What is known already: The number of AID cycles in Belgium has increased from 4706 in 2007 to 13048 in 2011. Centres face a shortage in donor sperm due to the high inflow of patients from neighbouring countries and an increased number of lesbian couples/single women calling for sperm donation. Advertising for potential donors is difficult due to legal restrictions and the number of pregnancies from a single donor is limited. Therefore, most Belgian centres import donor sperm.
Study design, size, duration: A short questionnaire was sent by e-mail to all Belgian Centres of Reproductive Medicine with laboratory facilities ($n = 18$) in order to determine which centres have their own sperm bank. Subsequently, a more elaborate questionnaire was sent to inform specifically on many different aspects used in their sperm banking facility.
Participants/materials, setting, methods: By law, Belgium counts eighteen centres for reproductive medicine with full laboratory facilities for assisted reproduction. Thirteen centres have their own sperm bank. They all received a questionnaire designed to obtain information on different aspects of recruitment and screening of the donors, laboratory methods used for sperm preparation and cryopreservation.
Main results and the role of chance: Results showed that 82% of the centres rely partially or completely on the import of foreign donor sperm. In 60% of the Belgian AID cycles foreign donor sperm is used. Donor recruitment is mainly performed through the centre's website (64%) and by using flyers and posters (45%). Centres reported a minimum of 10 to a maximum of 180 new potential donors to be recruited each year. Eventually, 15–70% of these candidate donors were accepted. This huge difference can be explained by different criteria handled by the centres: donor age limits range from 18–25 to 40–46 years old, thresholds for sperm normality to be accepted differ considerably. Anonymous sperm donation is more popular than non-anonymous sperm donation. Donors are reimbursed 50–85€ per donated sperm sample.
Limitations, reason for caution: Belgium is a small country, with only thirteen sperm banking facilities. It is well known that country regulations concerning all different aspects of gamete donation vary a lot because of political, ethical, socio-cultural and religious differences. The results we obtained only describe the Belgian situation.
Wider implications of the findings: The acquired data from this questionnaire helped us to get a better overview of the situation on sperm banking in Belgium. Moreover, this study is part of a PhD in which ethical aspects of donor recruitment and a cost- analysis on sperm banking will be performed. We aim to find enough data and evidence to initiate a negotiation with the government to change their attitude against sperm banks.
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, Hasselt University, Ziekenhuis Oost-Limburg and Jessa Hospital.
Trial registration number: Not applicable.

O-017 Does body mass index influence sperm parameters in infertile men

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Study question: The aim of our study was to investigate the eventual correlation between body mass index (BMI) and semen parameters in a population of unselected subfertile men.
Summary answer: Contrary to what has already been described in other studies, we did not find any significant difference in terms of conventional sperm parameters and CASA parameters between obese, overweight and normoweight infertile men. However, progressive sperm motility was significantly lower in underweight men compared to other categories.
What is known already: Extreme BMI categories, i.e. underweight and obese categories, are known to negatively impact female fertility and ART success rates. Concerning men, things are not so clear, even if male obesity has been shown by some authors to lead to altered semen parameters and lower success rates in ART cycles.
Study design, size, duration: This prospective monocentric study based on a questionnaire was conducted between January 2011 and June 2012. Participants were classified into four groups according to their BMI (<18.5, 18.5–25, 25–30 and >30 kg/m^2).
Participants/materials, setting, methods: All men who came for a first routine fertility evaluation at the andrology laboratory received a questionnaire dealing with various demographic characteristics. Sperm analysis was performed with strict adherence to WHO 5th edition recommendations. Kinetic parameters were assessed with a CASA system. Statistical evaluation was performed with SPSS software.
Main results and the role of chance: A total of 1226 men were included, 18 men being underweight, 769 normoweight, 385 overweight and 94 obese. Men were significantly younger in underweight category than in other categories (29.2 ± 5.9 years vs. 33.9 ± 5.9; 34.6 ± 5.9 and 35.8 ± 7.1 years respectively). Abstinence delay was comparable in all groups. Concerning semen parameters, mean volume of the ejaculate, sperm concentration, total sperm numeration, CASA kinetic parameters (VCL, VSL, ALH) and morphology were not significantly different among the 4 BMI groups. Mean progressive motility was significantly lower in underweight men than in other categories (29.3 ± 18.6% vs. 39.5 ± 15.7; 41.4 ± 15.9 and 39.1 ± 14.7% respectively).
Limitations, reason for caution: Only 18 underweight men were included in this study (1.4% of our population), which is probably too low to draw relevant conclusions.
Wider implications of the findings: The issue of the correlation between BMI and sperm parameters in infertile men remains to be addressed. Further studies might benefit from using CASA systems, as they allow better standardization. A specific focus should be put on underweight infertile men, as they might constitute a particular subgroup.
Study funding/competing interest(s): Funding by hospital/clinic(s), University hospital of Nantes, France.
Trial registration number: None.

O-018 In male factor infertility, the level of DNA fragmentation in sperm correlates with polymorphism of follicle-stimulating hormone receptor

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Study question: Chromatin compaction failures due to high level of sperm DNA fragmentation have been studied depending on stochastic variables as the genetic polymorphisms of the follicle-stimulating hormone receptor (FSHR) Thr307Ala and Asp680Ser.
Summary answer: Sperm DNA fragmentation level was identifiably higher in carriers of FSHR polymorphisms. Comparison of normal-genotype patients and ones with polymorphisms demonstrates a statistically significant difference with respect to a 12%-level of DNA fragmentation ($p < 0.01$). If the 20% fragmentation is considered as a "damage threshold" representing certain risk in medical conditions.
What is known already: The influence of variants of FSH receptor (FSHR) on male infertility is not completely understood. Proper interaction between FSH and its receptor FSHR is needed for normal spermatogenesis. In males, FSH is responsible for Sertoli cell function and, by means of specific receptor FSHR, participates in induction and maintenance of spermatogenesis. However, any results allowing demonstration of influence of FSHR genotype on semen DNA fragmentation level have not been reported to date.
Study design, size, duration: The study population consists of 30 severe oligozoospermic (sperm count 5 mln/mL) infertile men with normal FSHR gene genotype**s**, and 30 severe oligozoospermic infertile men with genetic polymorphism in FSHR gene. In addition the karyotype abnormalities and Y chromosome long arm micro-deletions have been observed in both groups.
Participants/materials, setting, methods: Totally, for 60 infertile male patients, the genotype distribution and allele frequency of FSHR Thr307Ala and FSHR Asp680Ser polymorphisms were analyzed by Taqman assays on the ABI PRISM 7500 system. In both two 30-patient groups, the sperm DNA fragmentation level was analyzed exploiting the method of sperm chromatin dispersion (SCD).
Main results and the role of chance: Average DNA fragmentation among patients with normal genotype is 12%, while for patients with polymorphism the average fragmentation is 26.8%. Comparison of normal-genotype patients and ones with polymorphism demonstrates a statistically significant difference with respect to a 12%-barrier (the Chi-square statistic is 7.39, the *P* value is 0.006, $p < 0.01$). DNA fragmentation of 20% might be considered as a "damage threshold" for infertile patients. Patients with DNA fragmentation over 20% represent 10% of all population with normal genotype. DNA fragmentation over 20% is observed among 67% of the patients having any form of FSHR gene polymorphism. For two (of possible four) forms of this polymorphism, the DNA fragmentation over 20%-threshold has been found in sperm of 82% of such polymorphism carriers.
Limitations, reason for caution: Present technique allows evaluation of DNA fragmentation level in cases when the sperm count excides 2 mln/mL.
Wider implications of the findings: Our findings suggest that genetic variants of FSHR might have some impact on the process of chromatin compaction in spermatozoa. Sperm DNA damage is worse among patients with FSHR polymorphic genotypes. Results are statistically significant in a general comparison "normal genotype vs. all polymorphic ones", particularly severe fragmentation observed among patients having one of two specific forms of polymorphism, while two other forms require further studies. Additional genetic screening is important.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Center of Human Reproduction, Kharkiw, Ukraine.
Trial registration number: No number.

O-019 Cerium dioxide nanoparticles induce DNA damage in human spermatozoa

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Study question: Do cerium dioxide nanoparticles induce DNA damage in human spermatozoa?

Summary answer: *In vitro* exposure of human spermatozoa to cerium dioxide nanoparticles (CeO_2NPs) significantly increased DNA damage assessed by comet assay, compared to unexposed spermatozoa. A wide range of concentrations was studied, the mechanisms of genotoxicity varied depending on the CeO_2NPs concentration.

What is known already: Cerium dioxide nanoparticles (CeO_2NPs) are widely used for industrial purposes, as diesel additive, and have potential therapeutic applications. The Organization for Economic Cooperation and Development included CeO_2NPs in the priority list of nanomaterials requiring urgent evaluation. Metal NPs can cross the blood-testis barrier and could interact with spermatozoa. The genotoxicity of CeO_2NPs was demonstrated *in vitro* on human cell lines and mouse oocytes. However, the effects of CeO_2NPs on human spermatozoa DNA are unknown.

Study design, size, duration: Comet Assay was performed on spermatozoa exposed to 3 different conditions: 1) CeO_2NPs solutions in Ferticult© (NP); 2) supernatant of the same solutions (containing dissolved Ce without CeO_2NPs) (D); or 3) CeO_2NPs solutions with L-ergothionein (anti-oxidant) (L-erg). DNA damage was quantified by percentage Tail DNA (% Tail DNA).

Participants/materials, setting, methods: Human spermatozoa from fertile donors were obtained from GERMETHEQUE biobank; motile sperm were selected by swim-up and exposed *in vitro* (1 hour, 37°C, 5% CO_2) to 5 CeO_2NPs concentrations: 0 (negative control), 0.01, 0.1, 1 and 10 mg/l, under the 3 conditions described above. Positive control: 110 µMol H_2O_2.

Main results and the role of chance: Significant increases in % Tail DNA (mean ± SE) were detected in: i) spermatozoa exposed to NP, D and L-erg 4 concentrations, versus negative control (25.4 ± 0.5) ($p < 0.0001$); ii) spermatozoa exposed to NP 0.01 and 0.1 mg/l (67.4 ± 1.2 and 52.48 ± 0.7, respectively) versus NP 1 and 10 mg/l (46.8 ± 0.6 and 44.9 ± 0.8, respectively) ($p < 0.0001$); iii) spermatozoa exposed to NP 0.01 mg/l versus D 0.01 mg/l (51.7 ± 2.6) ($p < 0.0001$); iv) spermatozoa exposed to NP 0.01 mg/l versus L-erg 0.01 mg/l (63.06 ± 0.5 = 8) ($p = 0.002$). We showed that: i) DNA damage increased when CeO_2NPs concentrations decreased; ii) at 0.01 mg/l, the genotoxicity mechanisms probably involve oxydative stress and interactions between spermatozoa and CeO_2NPs. At higher concentrations, no statistical differences were assessed between the 3 conditions, suggesting that the genotoxicity mechanisms were different.

Limitations, reason for caution: These results cannot be extrapolated to *in vivo* toxicity of CeO_2NPs after inhalation, but demonstrate that interactions between CeO_2NPs and human spermatozoa induce statistically significant genotoxicity. Additional data should be needed to assess DNA damage in spermatozoa after *in vitro* exposure to very low CeO_2NPs concentrations.

Wider implications of the findings: To our knowledge, this is the first study examining the *in vitro* genotoxicity of CeO_2NPs on human spermatozoa. DNA breaks observed in spermatozoa exposed to the lowest concentration could result from oxidative stress and from interaction between spermatozoa and CeO_2NPs. These results add new and important insights regarding the reproductive toxicity of priority nanomaterial requiring urgent evaluation, and warrant further *in vivo* studies examining exposures to low concentrations of CeO_2NPs.

Study funding/competing interest(s): Funding by national/international organization(s), This work is a contribution to the LABEX SERENADE (no ANR-11-LABX-0064) funded by the « Investissements d'Avenir », French Government program of the French National Research Agency (ANR) through the A*Midex project (no ANR-11-IDEX-0001-02)".

Trial registration number: no ANR-11-LABX-0064 and no ANR-11-IDEX-0001-02

O-020 Comparisons of pregnancy rates at insemination based on total motile sperm counts from the 1999 and 2010 World Health Organization (WHO) semen analysis norms

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Study question: Are the 2010 semen analysis parameters better predictors of pregnancy than were the 1999 limits when intra uterine inseminations (IUI) are performed?

Summary answer: Normal total motile sperm counts (TMSC) based on the 1999 parameters correlate better with likelihood of pregnancy, than do abnormal TMSC by the 1999 or 2010 limits when performing IUI. Interestingly, pregnancy rates did not differ when specimens with abnormal TMSC, by the 1999 or 2010 limits were compared.

What is known already: For the last 50-years semen analysis norms in the fertile population have been calculated by the WHO and have decreased at each reassessment. The 95-percentile is used to calculate the lower limits of normal. It could be assumed that this reassessment of limits should have clinical implications, translating into lower pregnancy rates. Particularly, if they are to be used for treatment planning in an infertile population. No previous study has assessed this relationship.

Study design, size, duration: Retrospective study, approved by the Human Subjects Committee at Stanford University. All semen analysis performed on the day of IUI were enrolled for evaluation ($N = 2229$) for a period of 2.5 years. Data was compared using chi-squared, correlation coefficients and T-tests. Data presented as mean ± standard deviation.

Participants/materials, setting, methods: Fresh semen for IUI was evaluated using a count slide at 37celcius. It was processed by density gradient, in couples with ≥1-year of infertility and ≥one patent fallopian tube. Preprocessing specimens were categorized by the TMSC and the 1999 normal (≥20 million/ml), 1999 abnormal (19.9–7.2 million/ml) (but normal by 2010), 2010 abnormal (<7.2 million/ml).

Main results and the role of chance: Mean TMSC were greater in the ($N = 1604$) 1999 norms (114 ± 107 mil per ml) than in the ($N = 362$) 1999 abnormal (13 ± 3.7 mil per ml) which were greater than the ($N = 265$) 2010 abnormal (3.5 ± 2.0 mil ml) ($p < 0.0001$ in all 3 cases). As expected, when comparing pregnancy rates based on the 1999 norms, 1999 abnormal and 2010 abnormal there was a negative correlation ($r = -0.42$, $p = 0.045$). However, pregnancy rates did not differ at IUI when sperm was abnormal based on 1999 or 2010 parameters ($p = 0.77$). Pregnancy rates per IUI were for the 1999 norms (21%), 1999 abnormal (18%) and 2010 abnormal (17%).

Limitations, reason for caution: Retrospective study, with excellent pregnancy rates per IUI at irrelevant of the category in which the specimen fell. (Note: patients with strict morphology under 4% were treated with in-vitro fertilization and intra cytoplasmic sperm injection). (Patients received IUI combined with ovulation induction with clomiphene citrate, letrozole, or gonadotropins)

Wider implications of the findings: Abnormal patients by the 2010 guide lines are no less likely to conceive than abnormal by the 1999 limits. Therefore, changing these limits has little clinical implications, since it is only values above the 1999 limits that correlate with a higher pregnancy rate. Importantly, pregnancy rates remain excellent below the 1999 and 2010 parameters of normal for TMSC. Therefore controlled ovarian stimulation combined with IUI remains a viable option for patients with abnormal sperm.

Study funding/competing interest(s): Funding by national/international organization(s), National Institutes of Health.

Trial registration number: Retrospective, not applicable.

SELECTED ORAL COMMUNICATION SESSION

SESSION 05: MANAGING ENDOMETRIOSIS

Monday 30 June 2014 **10:00 - 11:30**

O-021 Magnetic resonance enema versus rectal water contrast transvaginal ultrasonography in the diagnosis of rectosigmoid endometriosis

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Study question: To compare the accuracy of magnetic resonance enema (MR-e) and transvaginal ultrasonography combined with water-contrast in the rectum (RWC-TVS) in diagnosing sigmoid and rectal endometriotic nodules.

Summary answer: RWC-TVS is as accurate as MR-e in the diagnosis of recto-sigmoid endometriosis.
What is known already: Previous investigations showed that MR and TVS are accurate in the diagnosis of rectosigmoid endometriosis. However, no previous study compared these techniques combined with the distension of the rectosigmoid that may facilitate the identification of the endometriotic nodules.
Study design, size, duration: Prospective cross-sectional study performed in a referral centre for the treatment of endometriosis between November 2008 and December 2013. The radiologist performing MR-e and the ultrasonographer performing RWC-TVS were blinded to the results of the other investigation.
Participants/materials, setting, methods: Inclusion criteria for the study were: reproductive age, suspicion of deep pelvic endometriosis on the basis of gynaecological symptoms and vaginal examination, presence of gastrointestinal symptoms that might be caused by rectosigmoid endometriosis. All patients underwent laparoscopy performed within 3 months from the diagnostic procedures.
Main results and the role of chance: Out of 286 women included in the study, 151 had rectosigmoid endometriotic nodules (52.8%). The sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratio positive and likelihood ratio negative of MR-e and RWC-TVS in the diagnosis of recto-sigmoid endometriosis were 95.36% (95% CI, 90.68%–99.11%), 97.78% (93.63%–99.51%), 97.96% (94.14%–99.55%), 94.96% (89.89%–97.94%), 42.91 (14.01–131.46), 0.05 (0.02–0.10) and 92.72% (87.34%–96.30%), 97.04% (92.58%–99.17%), 97.22% (93.03%–99.22%), 92.25% (86.56%–96.06%), 31.29 (11.90–82.25), 0.08 (0.04–0.13), respectively. There was no significant difference in the accuracy of the two techniques in the diagnosis of rectosigmoid endometriosis (p = 0.063; McNemar's test). There was no significant difference in the intensity of pain experienced by the patients during the two exams.
Limitations, reason for caution: The experience of the ultrasonographer in colon study may influence the accuracy of RWC-TVS in diagnosing rectosigmoid endometriosis. In contrast, the MR pattern of endometriosis, the availability of different sequences, the complementary pattern in T1W and T2W sequences may facilitate all radiologists in diagnosing of bowel endometriosis.
Wider implications of the findings: TVS should be the first line investigation in patients with clinical suspicion of rectosigmoid endometriosis and physicians should be trained in performing this exam. Considering that MRI is more expensive that TVS, it should be used only when the findings of TVS are unclear.
Study funding/competing interest(s): Funding by University(ies), University of Genoa, Italy.
Trial registration number: None.

O-022 Virtual salpingoscopy and hysteroscopy with fly thru: a new approach for ultrasonographic study of fallopian tubes and uterine morphology in infertile patients

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Study question: The investigation of Fallopian tubes patency and uterine morphology in infertile women is fundamental.

The aim of the present study was to report the first experience with a new novel approach to infertile patients using Fly Thru virtual reconstruction of tubal and uterine morphology.
Summary answer: The virtual salpingoscopy and hysteroscopy is a feasible and well-tolerated exam which offers possibility of tubal, uterine and ovarian study in infertile patients. It allows intraluminar virtual navigation inside of Fallopian tubes and uterine cavity similar to fertiloscopic and hysteroscopic study with a non-invasive approach.
What is known already: In the past hysterosalpingography (HSG) was the most common first-line diagnostic test for tubal patency. Hysterosalpingosonography (HyCoSy) has the advantage of reduced invasivity. The use of a contrast agent during HyCoSy improves the image quality but is associated to higher costs. Another approach, more invasive, is fertiloscopy as it allows the study of tubaric lumen. For uterine cavity a glod-standard exam is hysteroscopy.
Study design, size, duration: All consecutive patients who underwent Fly Thru study of uterus and fallopian tubes compared to traditional HyCoSy in Infertility Centre of Sacro Cuore Hospital during a period of 3 months. All patients were asked to describe their discomfort and pain using a VAS score scale during and after procedure.
Participants/materials, setting, methods: All 80 infertile patients participated to study. After introduction of Ringer Lactate solution into uterine cavity and fallopian tubes, a 3D volume acquisition and than a traditional HyCoSy was performed. Manipulation of images included: Multi-View, surface rendered coronar sections and Fly Thru using the Toshiba's Aplio 500 ultrasound system.
Main results and the role of chance: The present study reports first experience of this innovative approach to first-line infertility study.

Uterine evaluation included images 3D of uterine wall and a reconstruction with Fly Thru of uterine cavity comparable to hysteroscopic visualization. Both patency and aspect was examined with virtual reconstruction of fallopian tubes lumen similar to fertiloscopic evaluation (virtual salpingoscopy).

Fly Thru is a Toshiba technology which allows to 'fly through' cavities of human body giving a perspective of 4D imaging looking from the inside out. The obtained image is that of a perspective projection, with the impression of performing an endoscopic procedure.

All patients reported good tolerability and low pain perception. The exam was compared to traditional HyCoSy in terms of time, liquid volume and obtained images.
Limitations, reason for caution: Time of exam is not longer than of a traditional HyCoSy but the successive manipulation of images requires about 15 minutes more.

Although in comparison to hysteroscopy, the virtual hysteroscopy allows to visualize not only the cavity but also the uterine wall, it doesn't allow to perform endometrial biopsy.
Wider implications of the findings: According to modern approach, the first-line exam of tubal patency should be as less painful as possible and the presented exam is a non-invasive good-tolerated procedure. The ultrasonographic approach has the advantage of not-explousure to X-rays and the use of Ringer lactate is related to low costs and less adverse affects. The other important advantage is the possibility to integrate the exam in infertility clinic.
Study funding/competing interest(s): Funding by hospital/clinic(s), Centro per lo Studio della Fertilità di Coppia, Ospedale Sacro Cuore di Negrar (Verona), Italy.
Trial registration number: No.

O-023 High prevalence of adenomyosis in recurrent pregnancy loss and previous ART failure

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Study question: To evaluate the prevalence associated with adenomyosis in infertile patients undergoing assisted reproductive techniques (ART).
Summary answer: The prevalence of adenomyosis is high in infertile patients, 24.4% specially in those cases of recurrent pregnancy loss (38.2%) and previous ART failure (34.7%). Adenomyosis was diagnosed "de novo" in 80.6% of cases. The assessment of both the "junction zone" and coronal plane of the uterine cavity by 3D ultrasound scan improve the diagnostic sensitivity of the technique. The reduction of the uterine cavity size secondary to adenomyosis may account for infertility.
What is known already: The prevalence of adenomyosis in infertile women is nearly 20%. The 3D ultrasound scan has emerged as a useful tool to diagnose adenomyosis over the past decade, due to the strong correlation existing between the sonographic features and either histological or imaging findings observed by magnetic resonance imaging (MRI).
Study design, size, duration: Cross-sectional study conducted on 1015 patients undergoing assisted reproductive techniques from January 2009 to December 2013 And referred for 3D scan after multiple IVF failures (≥3) or recurrent miscarriage (≥2).
Participants/materials, setting, methods: The diagnostic criteria for adenomyosis are globular uterine configuration, myometrial anterior-posterior asymmetry, heterogeneous myometrial echotexture, poor definition of the endometrial-myometrial interface ("junction zone") and the presence of subendometrial cysts.

Data was obtained from sonographic results collected from our hospital database. The degree of affectation of the endometrial cavity was classified into three categories (1 = mild, 2 = moderate, 3 = severe).
Main results and the role of chance: The prevalence of adenomyosis was 24.4% (n = 248) [29.7% (94/222) in women aged ≥40 y.o and 22% (154/545)

in women aged <40 y.o ($p < 0.01$)]. Its prevalence was higher in those cases of recurrent pregnancy loss [38.2% ($n = 26$), $p < 0.005$] and previous ART failure [34.7% ($n = 107$), $p < 0.0001$]. The presence of adenomyosis has been shown to be associated to endometriosis [35.1% ($n = 34$)], uterine fibroids [18% ($n = 48$)] and endometrial polyps [3.1% ($n = 2$)]. Adenomyosis was diagnosed as a primary finding "de novo" in 80.6% ($n = 200$) of the infertile patients.

Regarding to the uterine assessment, the affectation of the uterine cavity was mild, moderate and severe in 67.3%, 22.6% and 10.1% of the cases, respectively. In this study, adenomyosis was the primary cause for uterine cavity reduction in 73.3% of patients diagnosed with recurrent pregnancy loss and 62.5% of the patients with previous ART failure.

Limitations, reason for caution: A histological assessment is lacking in the present study. The relationship between adenomyosis and infertility still remains controversial nowadays.

Wider implications of the findings: In summary, our results indicate that adenomyosis is a clinical condition with a high prevalence that may affect the reproductive results by reducing the uterine cavity size. The present findings may help to better understand the origin of unexplained infertility and, thus, develop new therapeutic strategies aimed to improve the uterine cavity capacity.

Study funding/competing interest(s): Funding by hospital/clinic(s), Instituto Valenciano de Infertilidad IVI Madrid.

Trial registration number: no.

O-024 Impact on ovarian reserve of second laparoscopic surgery for recurrent unilateral endometriotic cyst: case-control study

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Study question: Which is the impact on ovarian reserve of second laparoscopic surgery for recurrent unilateral endometriomas?

Summary answer: Laparoscopic stripping of recurrent endometriomas is associated with high risk of ovarian reserve damage and ovary loss.

What is known already: Several studies showed that laparoscopic excision of ovarian endometriomas is associated with a reduction in ovarian reserve. However, no data is available on the impact on ovarian reserve of second surgery for recurrent unilateral endometriomas.

Study design, size, duration: This case-control study was based on a retrospective analysis of a prospectively collected database including all consecutive patients who underwent surgery for endometriomas at our institutions between January 2007 and November 2013.

Participants/materials, setting, methods: This study included patients who underwent stripping of endometriomas (diameter >4 cm) and had either second surgery for recurrent unilateral endometriomas ($n = 18$, cases) or no recurrence ($n = 18$, controls). AMH level was the primary outcome. Secondary outcomes were basal FSH level, antral follicle count (AFC) and ovarian volume.

Main results and the role of chance: In cases, the median time between the two surgical procedures was 47 months (range, 22–74 months); in controls the median time between first surgery and ovarian reserve assessment was 46 months (range, 24–78 months). The mean (±SEM) volume of the recurrent endometriomas was 70.9 (±12.6) cm^3. The mean (±SEM) AMH levels were lower in cases (1.2 ± 0.3 ng/mL) than in controls (3.1 ± 1.5 ng/mL; $p < 0.001$). Basal FSH levels were higher in cases than in controls ($p < 0.001$). The mean AFC was lower in cases than in controls ($p < 0.001$); notably, AFC was 0 in 8 ovaries operated twice (44.4%; 95% CI, 21.5%–69.2). In cases, the volume of the operated ovary was lower than the contralateral ovary ($p < 0.001$).

Limitations, reason for caution: The major limitation of this study is that it was retrospective; however, data were prospectively collected. All the procedures were performed by ovarian stripping and, therefore, the results of this study cannot be applied to other surgical techniques.

Wider implications of the findings: Recurrent ovarian endometriomas should not be operated unless there is a risk of malignancy or the patient complains severe endometriosis-related pain. Patients undergoing surgery should be informed of the high risk of ovarian function damage and ovary loss.

Study funding/competing interest(s): Funding by University(ies), University of Genoa, Italy, PRA 2012.

Trial registration number: NCT02047838

O-025 Aromatase Inhibitor plus GnRH analogue in the treatment of patient with ovarian endometriosis recurrence after surgery: a controlled trial

F. Scarpellini[1], M. Sbracia[1]

[1]*Hungaria, Private Center, Rome, Italy*

Study question: May the treatment with the aromatase inhibitor Anastrazole plus GnRH analogue (Leuprolide) be an effective therapy in women with recurrence of ovarian endometriosis after previous surgery and progestin treatment?

Summary answer: Aromatase inhibitor plus GnRH agonist may be useful in the treatment of women with recurrence of ovarian endometriomas in order to avoid further surgery and preserve, to much it is possible, ovarian reserve and improving IVF outcome.

What is known already: Endometriosis is a chronic condition affecting around 10% of women in reproductive age, and after surgery it recur in about 50% of cases. It is recognized that surgical treatment of ovarian endometriomas affects ovarian reserve. For this reason in case of ovarian endometriomas recurrence we tried a medical approach instead of the surgery to preserve ovarian reserve using a combined treatment to maximize the hypoestrogenic effect on endometriotic lesions.

Study design, size, duration: This randomized controlled study was conducted from the April 2012 to January 2014 on 59 women with recurrence of ovarian endometriomas, previously diagnosed by operative laparoscopy.

Participants/materials, setting, methods: All patients did not undergo surgery for the recurrence of ovarian lesions. The mean age of women was 35.1 ± 2.0 (range 25/39). The women were assigned to the study group or to the control group by a computer generated sequence. The 31 women of the study group were treated for 6 months with Anastrazole 1 mg/day plus Leuprolide 3.75 mg/month. The 28 women of the control group were treated for 6 months with Leuprolide 3.75 mg/month alone. The women of the two groups were followed up during the treatment and after the end of treatment for at least 1 year. Primary outcomes were time of endometriomas disappearance and IVF outcome.

Main results and the role of chance: The two groups of patients did not show statistically significant differences for epidemiological data. The time of endometriomas disappearance was shorter in the group treated with the combined therapy than in the control group (2.8 ± 0.9 months vs. 4.1 ± 1.2 months, $P < 0.01$). The pregnancy rate for IVF in the women treated with the combined therapy was significantly higher than in the control group (45.1% vs. 21.4%, $P = 0.0485$).

Limitations, reason for caution: These data need to be validated in larger group of patients.

Wider implications of the findings: Aromatase inhibitor plus GnRH agonist therapy seems to be a promising second line treatment in patients with endometriosis recurrence, especially for ovarian lesions, to avoid ovarian reserve reduction.

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

Trial registration number: NCT01769781

O-026 The ESHRE's endometriosis app, based on ESHRE's 2013 guideline on the management of women with endometriosis

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Title: THE ESHRE ENDOMETRIOSIS GUIDELINE APP

Authors: Gerard Dunselman, Nathalie Vermeulen, Annemiek Nap, and the Guideline Development Group

Introduction: In 2014 ESHRE has published a guideline on the management of women with endometriosis containing 83 recommendations in 8 chapters. Guidelines as extensive as the current guideline on endometriosis might benefit from using specific tools and applications to improve usability and

implementation. Therefore, the guideline will not only be available as a full guideline, an open-access scientific publication and a patient version, but also as an App for consultation on Smartphone, Tablet and Internet (web-based).
Methodology: An evidence-based App was created containing the full text of the guideline, separated in chapters, with links to background information and instructions. Furthermore, the App includes a decision-aid based on the recommendations within the guideline. For reasons of transparency and validity, a rigorous methodology was applied on defining the decision-aid: recommendations are copied to the letter, links to the full text of the guideline describing the reasoning behind the recommendations are provided and pathways are clarified to the users by visualising the flow charts.
Results: This new endometriosis App provides evidence-based advice for the diagnosis and treatment of endometriosis. The decision-aid is subdivided in 6 topics including recognition of symptoms, clinical examination and establishing diagnosis, and treatment of endometriosis-associated pain and endometriosis-associated infertility. This division and a clear overall structure enable different entry points into the decision aid. Doctors are guided through questions, answers of which will conveniently lead them to the appropriate evidence-based recommendations of the guideline. Additional background information, including instructions for performing clinical examination, and educational pictures were added to increase the usefulness of the App. The availability of hormonal treatments per country was added to bridge significant differences that exist across Europe.
Conclusion: As clinicians use smartphones and internet more frequently, rather than textbooks, in search for information and assistance, ESHRE has developed an evidence-based App on management of endometriosis with the aim of improving implementation of the guideline and hence, improving the quality of care and the quality of life of women with endometriosis.

SELECTED ORAL COMMUNICATION SESSION

SESSION 06: OVARIAN RESERVE AND AGEING

Monday 30 June 2014 **10:00 - 11:30**

O-027 Is the prevalence of impaired ovarian reserve overrepresented among infertile women below the age of 40 – an age-adjusted cohort study

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Study question: To what extent does impaired ovarian reserve estimated by the antral follicle count (AFC) and anti-Müllerian hormone (AMH) level contribute to infertility among an unselected population of infertile women below the age of 40?
Summary answer: Impaired ovarian reserve was found not to contribute significantly to decreased fertility among infertile women aged 20–40 years when compared with a control population of the same age with no history of infertility. The prevalence of AMH <5 pmol/l in the infertile population was comparable to the control group.
What is known already: It is well-established that AMH and AFC are sensitive parameters of the ovarian reserve and fairly good predictors of the ovarian response to exogenous gonotrophins. In contrast, there is a broad variability in the level AMH and AFC compatible with conception. Ovarian reserve declines with female age, but the inter-individual variation is considerable. Delayed childbearing may cause more women to experience infertility and low ovarian reserve is often considered to be overrepresented among infertile women.
Study design, size, duration: A cohort study including 508 cohabiting women who were referred to fertility treatment at our centre during 2011–2013. The control group has previously been described [Bentzen, JCEM 2013] and included 407 non-users of hormonal contraception with no history of infertility recruited during 2008–2010.
Participants/materials, setting, methods: All included women were aged 20–40 years and resident within the referral area of our centre. On cycle Days 2–5, AFC and ovarian volume were measured by transvaginal sonography and endocrine parameters including AMH, FSH and LH were assessed. Data were analysed by multivariate logistic analysis adjusted for age.
Main results and the role of chance: Mean age (±SD) was 32.5 ± 3.7 and 32.4 ± 4.0 years in the infertile and the control group, respectively. Median cycle length (IQR) was 28 (26–33) in both cohorts, but a larger proportion of the infertile women were anovulatory (15 vs. 5%, $p < 0.001$). Age-adjusted odds ratios for AMH and AFC were 1.011 (95% CI: 1.005–1.017) and 1.013 (95% CI 1.004–1.022) when comparing infertile women with controls. The difference became insignificant when adjusting for anovulation (aOR 1.000; 95% CI 0.997–1.012 and OR 0.995; 95% CI 0.984–1.008). The prevalence of AMH < 5.0 pmol/l was 4.9 vs. 4.7% ($p = 0.859$). When excluding male factor and anovulation, the prevalence of low AMH increased in the infertile population (9.3 vs. 4.7%, $p = 0.022$). However, the difference was insignificant when adjusting for age (aOR 0.619, 95% CI –0.051–1.289).
Limitations, reason for caution: The inclusion of patients at a tertiary centre may introduce bias. However, only residents from the referral area were included.
Wider implications of the findings: This study indicates that early age-related depletion of ovarian reserve is a minor contributor to infertility in couples with female age below 40 referred to fertility treatment. Thus, the frequent observation of poor responders in assisted reproduction may not be due to an overrepresentation of an early age-related decline in ovarian reserve compared to the background population, but rather a result of the expected age-related decline in fertility.
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by commercial/corporate company(ies), The study was funded by MSD and the Fertility Clinic at Rigshospitalet, Copenhagen, Denmark. The authors have no conflict of interest.
Trial registration number: Data were collected as part of the daily practice at the fertility clinic. Therefore, no trail registration number.

O-028 BRCA1/2 mutation carriers do not have earlier natural menopause compared to proven non-carriers: report from the Dutch hereditary breast and ovarian cancer study group (HEBON)

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[10]*Academic Medical Center, Department of Clinical Genetics, Amsterdam, The Netherlands*
[11]*University Medical Center Utrecht, Department of Medical Genetics, Utrecht, The Netherlands*

Study question: Can we confirm an earlier report that claims a significant and large effect of the BRCA mutation status on the age of natural menopause (ANM) in women carrying a BRCA1 or 2 mutation?
Summary answer: In the multivariate model we could not confirm that carrying a BRCA1/2 mutation leads to an earlier age of natural menopause compared to proven non-carriers.

What is known already: BRCA genes are involved in many essential processes like DNA damage repair. It is recently suggested that DNA double stranded break repair efficiency is an important determinant of ovarian ageing. Previous studies have shown inconsistent results regarding the association between the timing of ovarian ageing and BRCA carrier status. Hence, it is yet unclear whether BRCA1/2 mutation carriers have an increased risk of advanced ovarian depletion accompanied by an earlier ANM.
Study design, size, duration: Self-reported data were obtained by a standardized questionnaire at baseline in a nationwide prospective cohort study [HEreditary Breast and Ovarian cancer study, the Netherlands (HEBON study)] among members of BRCA1/2 families in the Netherlands (2012–2013). In total, 1236 proven BRCA1/2 mutation carriers and 2253 proven non-carriers were eligible for analyses.
Participants/materials, setting, methods: ANM was defined as age at which women experience 12 consecutive months of amenorrhoea due to no other reason than natural cessation of menses. Proven non-carriers were members of BRCA1/2 families. We used Kaplan- Meier approach and Cox proportional hazard regression analyses stratified for birth cohort and clustered on family.
Main results and the role of chance: Women in the BRCA1/2 mutation carrier group who completed the questionnaire were significantly younger than women in the proven non-carrier group (47.13 ± 11.76 vs. 48.73 ± 11.02; $p < 0.001$, respectively). Natural menopause was reached by a significantly lower proportion of BRCA1/2 mutation carriers compared with proven non-carriers (10% vs. 18%; $p < 0.001$, respectively). The mean ANM was significantly lower in BRCA1/2 mutation carriers than in proven non-carriers (49.11 ± 5.08 vs. 51.14 ± 3.96; $p < 0.001$, respectively). From the multivariate analyses adjusted for smoking status, it appeared that carrying a BRCA mutation was not associated with an earlier menopause (HR = 1.04, 95% CI = 0.81–1.34).
Limitations, reason for caution: If the uptake of a prophylactic bilateral salpingo-oophorectomy is associated with the occurrence of signs of ovarian ageing, like irregular menstrual cycles, a real difference in ANM between BRCA1/2 carriers and proven non-carriers may be masked. Furthermore, we were unable to control for other genetic factors that could influence ANM.
Wider implications of the findings: Although we did not find an earlier ANM in BRCA1/2 mutation carriers in this large study at baseline, this finding should be confirmed during follow-up of this cohort. Nevertheless, BRCA1/2 mutation carriers can still be at risk for a reduced ovarian reserve capacity earlier in life. Therefore, research into ovarian reserve status in reproductive years, using established markers such as anti-Müllerian hormone, is still advocated.
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by national/international organization(s), Financially supported by the Dutch Cancer Society, Foundation Pink Ribbon, the Netherlands Organisation for Health Research and Development, the department of Medical Genetics at the University Medical Center Utrecht, and the department of Reproductive Medicine at the University Medical Center Utrecht. The authors declare no conflicts of interest.
Trial registration number: Not applicable.

O-029 Poor responders (PR): androgens concentration in plasma and follicular fluid at the day of follicular aspiration

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Study question: Is the reported hypoandrogenemia in poor responders (PR) associated with decreased androgens levels in the follicular fluid at the day of follicular aspiration in Assisted Reproductive Technology (ART) cycles?
Summary answer: Women obtaining three or less oocytes in ART have significantly lower plasmatic levels of total testosterone and androstenedione that are not associated to decreased concentrations of these androgens in the follicular fluid at the day of follicular aspiration. No differences in concentrations of dehydroepiandrosterone sulfate (DHEA-S) were detected.
What is known already: Women with diminished ovarian reserve often respond poorly to controlled ovarian hiperstimulation (COH) resulting in retrieval of fewer oocytes and reduced pregnancy rates. Identifying potential PR is of critical clinical importance. Hypoandrogenemia has been advocated as a fundamental finding in PR. However, the follicular fluid androgen concentrations in PR ongoing an ART cycle have not been explored so far.
Study design, size, duration: A prospective study was conducted from March to December 2013 to investigate concentrations of total testosterone, androstenedione, and dehydroepiandrosterone sulfate (DHEA-S) in plasma and follicular fluid of consecutive cases of women responding poorly to COH compared to normal responders without PCOS, ovarian surgery or ovarian endometriosis.
Participants/materials, setting, methods: The first aspirated follicular fluid and a blood sample were collected in a university center in Santiago from 20 women ongoing ART and resulting in 3 or less retrieved oocytes. Twenty women obtaining 8 or more oocytes served as controls. Total testosterone (TT), androstenedione and DHEA-S were measured by routine immunochemical methods. Mann Whitney test and analysis of covariance and Receiver Operating Curve (ROC) were used for statistical analysis.
Main results and the role of chance: No significant difference in age between PR and controls was found (35.80 ± 1.15 and 34.35 ± 1.14 years respectively). Plasmatic TT was significantly decreased in PR (0.43 ± 0.05 and 0.79 ± 0.11 ng/mL respectively $P = 0.01$). The plasmatic concentration of androstenedione was also significantly decreased in PR (3.62 ± 0.37 and 5.22 ± 0.43 ng/ml respectively $P = 0.008$). A marginally not significant difference in DHEA-S plasmatic concentration was found ($P = 0.08$). No significant differences in these androgens concentrations were found in the follicular fluid. The analysis of covariance showed no statistical interaction with age and BMI when plasmatic TT and androstenedione were used as the dependent variable. The plasmatic cut off value at ROC analysis were 0.5 ng/mL for TT and 4.5 ng/mL for androstenedione (area under the curve 0.70 and 0.75 respectively).
Limitations, reason for caution: This is a preliminary report of a prospective study planning the recruitment of fifty subjects per group. However, the two groups were comparable regarding to basal characteristics such as age and BMI. Furthermore, consecutive cases of both groups were included in order to avoid the selection bias.
Wider implications of the findings: These findings suggest that in spite of decreased levels of androgens in plasma the granulose cells of the follicles recruited from PR manage to preserve levels of steroids comparable to those of normal responders. Thus, the local steroidal environment in the ovary may not be directly responsible for the decreased pregnancy rate observed in poor responders. Additional data is needed to establish the real value of plasmatic androgens concentration to predict ovarian response to COH.
Study funding/competing interest(s): Funding by University(ies), Mother and Child Research Institute. University of Chile.
Trial registration number: no.

O-030 Anti-müllerian hormone in prediction of menopause: results from a large prospective cohort study

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Study question: What is the added value of anti-müllerian hormone (AMH) on top of patient characteristics in the prediction of time to menopause (TTM) in a population-based setting?
Summary answer: This study justifies AMH as an additive predictor of TTM. The added value of AMH differs per subgroup of women and is largest in young women with regular menstrual cycles.
What is known already: Prediction of age at menopause may be useful in predicting end of female fertility. Current evidence on the role of AMH in such prediction is based on small studies or specific subgroups.
Study design, size, duration: 1163 premenopausal women participating in the second follow-up round of the Doetinchem Cohort Study were included. Menopausal status was assessed at follow-up after 5 and 10 years. Multivariable Cox' proportional hazards regression analysis provided associations between potential predictors and TTM.
Participants/materials, setting, methods: A model without AMH was fit using variables selected through Aikike's information criterion. Performance

of the prediction rule was assessed with C-statistics and compared to a model including AMH and one with age only. Added value of AMH was assessed with Net Reclassification Index and change in absolute predicted risk. Performance of these 3 models was compared in subgroups based on age and reproductive characteristics.

Main results and the role of chance: The final model included age, BMI, pack-years of smoking, and menstrual cycle status (regular, irregular, pregnant or taking oral contraceptives). This model had a C-statistic of 0.89, compared to 0.88 of age only. Addition of AMH increased it to 0.91. In a subgroup of 25–43 year olds with regular menstrual cycles, age only had a C-statistic of 0.79 and models without and with AMH of 0.79 and 0.87, respectively. In the entire cohort the risk to enter menopause within 10 years assigned by the model with AMH was on average 3% higher than that assigned by the model without AMH for women who did enter menopause. In the subgroup of young women with regular cycles this increase was 11%.

Limitations, reason for caution: The AMH Gen II assay (Beckman Coulter Ltd) was used which is currently the most reliable assay of AMH. Samples were determined by a single experienced laboratory technician and assay result precision was validated using linearity of dilution assessment, which supports homogenous specimen sampling.

Wider implications of the findings: This study has, with up to date statistical methods, justified AMH as an additive predictor of both TTM and the occurrence of menopause on top of female age and other reproductive and lifestyle factors. However, the added value differs per subgroup of women and is largest in women who are young when AMH is measured and who are still regularly cycling.

Study funding/competing interest(s): Funding by hospital/clinic(s), University Medical Center Utrecht.

Trial registration number: not applicable.

O-031 A prospective randomised controlled study (RCT) on role of dehydroepiandrosterone (DHEA) on improving the ovarian response in known poor responders in previous failed IVF-ICSI cycle

A. Jindal[1], R. Singh[1]
[1]*Bhopal Test Tube Baby Centre, Infertility, Bhopal, India*

Study question: Does DHEA administration improve poor ovarian reserve in known poor responders in previous failed IVF cycles?

Summary answer: Yes DHEA improves ovarian response in known poor responders.

What is known already: Ovarian ageing is one of the major determinants of outcome following in-vitro fertilization (IVF-ICSI).

Patients with reduced ovarian reserve often respond poorly to controlled ovarian stimulation resulting in retrieval of fewer oocytes, producing poorer quality embryos and reduced implantation rates and pregnancy rates. Androgens may, however, influence ovarian follicular growth not only by acting as metabolic precursors for steroid production, but also by serving as ligands for androgen receptors 11 or by other, non-classical mechanisms. During ovulation induction with exogenous gonadotropins, DHEA is the prehormone for up to 48% of follicular fluid testosterone, which is, in turn, the prehormone for estradiol. There is evidence that androgens act, together with FSH, to stimulate follicular differentiation. Androgens are also known DHEA may enhance the follicular environment through: augmentation of the growth promoting and survival enhancing effect of IGF-I; LH-stimulated follicular androgen and oestrogen production; and the augmentation of granulosa cell FSH-receptor (FSH-r) expression and associated increase in the number of growing preantral and small antral follicles. DHEA could potentially improve oocyte quality via the GH axis through the promotion of DNA repair in oocytes. An effect of DHEA on mitochondrial activity in both follicular cells and oocytes is also possible since androgens have been shown to beneficially affect mitochondrial function.

Study design, size, duration: This study involved 406 infertile patients above 32 years old, who were treated at our hospital between 2006 to 2013. The diagnosis was considered confirmed if patients either qualified for a sub-diagnosis of premature ovarian aging (POA) or diminished ovarian reserve (DOR). POA was, in turn, defined as baseline follicle stimulating hormone (b-FSH), on cycle 2/3 of cycle as <12 mIU/ml, concern for their impending loss of ovarian function, these women were treated with IVF-ICSI as soon as possible and all failed cycles on ground of poor ovarian response were included in this study.

Participants/materials, setting, methods: This is a prospective randomised control study (RCT) of 406 women with diminished ovarian function and failed previous IVF-ICSI cycles. The study group includes 203 patients who used supplementation with 75 mg daily of oral, micronized DHEA for up to 6 months prior to entry into another repeat in vitro fertilization (IVF-ICSI) cycles. The control group is composed of 203 women who received infertility treatment, but did not use DHEA. The primary outcome was clinical pregnancy after repeat IVF-ICSI cycles.

Main results and the role of chance: Cumulative clinical pregnancy rates were significantly higher in the study group (19.4% vs. 10.1%)

Limitations, reason for caution: The positive effect of pre-treatment DHEA on IVF outcome in women with diminished ovarian reserve suggests that DHEA improve the quantitative ovarian response and pregnancy outcome. Based on these data, DHEA adjuvant therapy can be recommended in diminished ovarian reserve for improving IVF outcome. However, as the sample size in this analysis was small and the effect of DHEA on pregnancy rates was statistically significant, further large scale multicentre randomised controlled studies are required to clarify the benefits of DHEA adjuvant therapy in routine clinical management of predicted poor responders.

Wider implications of the findings: It has been proposed that oral administration of Dehydroepiandrosterone (DHEA), an adrenal androgen, may have anti-ageing effects and may improve ovarian response and pregnancy rates in women with reduced ovarian reserve during IVF-ICSI.

Study funding/competing interest(s): Funding by hospital/clinic(s), BTTB CENTRE.

Trial registration number: BTTB/2006/21

O-032 450 IU vs. 600 IU of gonadotropins for controlled ovarian stimulation in poor responders: a randomized controlled trial

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Study question: Assess if the number of mature (metaphase II) oocytes retrieved in women at risk of poor ovarian response undergoing a microdose agonist flare-up IVF/ICSI cycle is equivalent between those receiving 450 IU and 600 IU of gonadotropins divided between of Menopur® and Bravelle®.

Summary answer: In a population of poor ovarian responders undergoing controlled ovarian stimulation for IVF, administration of a daily dose of 600 IU of gonadotropins does not significantly increase the number of mature (metaphase II (MII)) oocytes retrieved compared to those receiving 450 IU.

What is known already: To our knowledge, no other prospective randomized trial has proved that, in poor ovarian responders, the use of very high doses of gonadotropins (600 IU) is more effective than lower doses (450 IU) in terms of mature oocytes, fertilization rates, number of embryos, and pregnancy rates. Furthermore, some authors have discussed about a potential detrimental effect of very high doses of gonadotropins on embryo quality.

Study design, size, duration: In a prospective randomized (by sequential number) controlled non-blinded non-inferiority study in a university-affiliated private IVF clinic, 356 women were recruited from October 2009 to September 2013. Sample size was calculated by started cycle with a tolerance margin of 1,6 mature oocytes, with 90% power and alpha error of 0.025.

Participants/materials, setting, methods: Female <41 years at risk of poor ovarian response: <5 oocytes or <8 follicles or a cancellation in a previous IVF cycle with ≥300 IU gonadotropins/day, basal FSH > 10 IU/L or AMH <1 ng/ml or antral follicle count ≤8.

Main results and the role of chance: 176 patients were randomized into the 450 IU group and 180 into the 600 IU group. The two groups were similar in terms of age, ovarian reserve, rate of cycle cancellation and duration and cause of infertility. There were no statistically significant differences in the number of MII oocytes (4.1 vs. 4.5; $p = 0.17$), implantation rate (29.8% vs. 30.4%; $p = 1$), biochemical pregnancy rate per cycle (20.5% vs. 22.9%; $p = 0.68$) or per transfer (36.1% vs. 38.1%; $p = 0.88$), and clinical pregnancy rate per cycle (16.4% vs. 18.3%; $p = 0.74$) or per transfer (28.9% vs. 30.5%; $p = 0.92$) between the 450 IU and 600 IU groups respectively. In addition, there was no significant difference in the fertilization rate between the two groups (62.4% vs. 57.0%; $p = 0.22$).

Limitations, reason for caution: One issue in our study was that our inclusion criteria for poor ovarian responders are different from the 2011 ESHRE

consensus Bologna Criteria because the study was started in 2009. Our results include the cancelled cycles as the analysis was done by an intention to treat per started cycle.
Wider implications of the findings: Poor ovarian responders have reduced success rates in IVF. Increasing gonadotropins dosage above 450 IU does not seem to significantly increase the number of oocytes nor pregnancy rates in this population. In agreement with previously published data, this study seems to indicate a lower embryo quality in cycles with high dosage stimulation, and therefore a dose higher than 450 IU should not be used even in poor responders.
Study funding/competing interest(s): Funding by hospital/clinic(s), The study was funded by OVO Fertility clinic. Gonadotropins were partially provided by Ferring®. We declare no financial conflict of interest with any commercial entity.
Trial registration number: The registration number is NCT00971152 on clinicaltrials.gov.

SELECTED ORAL COMMUNICATION SESSION

SESSION 07: DEVELOPMENTS IN GENETIC ANALYSIS

Monday 30 June 2014 **10:00 - 11:30**

O-033 Development, validation and clinical application of a next-generation sequencing (ngs)-based protocol for 24-chromosome aneuploidy screening of embryos

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[2]*BlueGnome Ltd, Cambridge, United Kingdom*
[3]*European Hospital, Reproductive Medicine, Rome, Italy*

Study question: Can next generation sequencing (NGS) techniques be reliably used for comprehensive aneuploidy screening of human embryos produced from patients undergoing in-vitro fertilization (IVF) treatments, with the purpose of identifying and selecting for transfer chromosomally normal embryos?
Summary answer: Evidence of accuracy demonstrates that NGS represents a reliable high-throughput methodology for 24-chromosome aneuploidy screening, capable of detecting whole chromosome aneuploidies and segmental changes. The protocol was clinically applied in preimplantation genetic screening (PGS) cycles, allowing identification and transfer of euploid embryos resulting in healthy pregnancies.
What is known already: The rapid development of NGS technologies has generated an increasing interest in determining whether NGS could be reliably applied for PGS purposes and if the technique may offer any improvements for the detection of chromosomal aneuploidy in preimplantation embryos compared to current comprehensive aneuploidy screening technologies. NGS may ultimately provide a number of advantages, including reduced costs and enhanced precision as well as parallel and customizable analysis of multiple embryos in a single sequencing run.
Study design, size, duration: The NGS method was validated using 18 karyotypically defined chromosomally abnormal single cells. Additionally, 244 embryos previously analyzed by array-comparative genomic hybridization (aCGH), were reanalyzed in a blinded fashion. Subsequent clinical application involved a parallel evaluation of 192 blastocysts from 55 consecutive PGS cycles with both NGS and aCGH techniques.
Participants/materials, setting, methods: Fifty-five patients undergoing PGS were enrolled in the study; 45 (mean age 39.8 ± 1.8 years) were with indication of advanced maternal age and 10 (mean age 35.5 ± 1.3 years) with repeated implantation failure. The method involved whole-genome amplification followed by NGS on either HiSeq2000 and MiSeq sequencers. Bioinformatics analysis was accomplished with BlueFuse software.
Main results and the role of chance: The NGS methodology was robust, with 454/454 (100%) samples yielding results. Aneuploidy diagnoses were concordant with those obtained using the well-established aCGH technique in all cases (100%). NGS was also able to detect chromosomal mosaicism in 54/54 (100%) of mosaic embryos assessed. Mosaicism detection limits were also determined for both chromosome gains and losses. Clinical application of the NGS-based 24-chromosome aneuploidy screening protocol revealed 76 (39.6%) euploid blastocysts. Following transfer of 50 embryos, 34 women had positive hCG levels: 30 pregnancies continued, confirmed by at least one fetal sac and heart beat (63.8% clinical pregnancy rate/ET), 3 were biochemical and 1 miscarried. A total of 32 embryos implanted and led to the presence of a fetal sac (64.0% implantation rate).
Limitations, reason for caution: The NGS method developed provides high-throughput with parallel analysis of multiple embryos and high resolution data for chromosomal analysis, with the potential to dramatically reduce the costs of PGS and enhance precision of testing. However, before recommending widespread application, a randomized clinical trial confirming its clinical efficacy, is advisable.
Wider implications of the findings: This is the first study reporting extensive preclinical validation and accuracy assessment of NGS-based comprehensive aneuploidy screening. The feasibility of applying NGS in a clinical setting was also confirmed. NGS technique has the advantage of screening, at reduced costs and enhanced precision, not only for aneuploidies. It may also allow for simultaneous evaluation of single-gene disorders, translocations and abnormalities of the mitochondrial genome, from the same biopsy without the need for multiple unique technological platforms, with the potential to revolutionize preimplantation diagnosis.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), BlueGnome Ltd.
Trial registration number: None.

O-034 Trophectoderm biopsy for aneuploidy screening using different platforms and conflicting test results

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Study question: New technologies are now available using several different platforms to determine embryo chromosomal number (PGS) to facilitate single normal embryo transfer. However it is unclear how much discrepancy may occur in the test results. The objective of this study was to validate three different PGS techniques offered by commercial laboratories.
Summary answer: the conflicting results obtained from pieces of the same blastocyst, both within tests and between tests in the present study reveal a concern about the accuracy and reliability of TE testing for PGS.
What is known already: It is known for years that embryo morphology cannot predict aneuploidy and that aneuploidy rate increases with maternal age. The TE biopsy, in contrast to cellular stage blastomere biopsy or polar body biopsy, provides more genetic material for testing, and therefore may be more accurate. In addition, TE biopsy does not seem to negatively affect embryo implantation potential.
Study design, size, duration: The study was designed to validate the PGS results on TE biopsies using three different PGS techniques [array CGH (Center A), SNP microarray (Center B), and qPCR (Center C)] offered by three commercial laboratories.
Participants/materials, setting, methods: Ten couples donated 27 vitrified blastocysts that had been identified as aneuploid by one of 3 testing Centers. The thawed blastocysts were cut into 3 pieces, one sample was sent back to original testing lab and another two to validating lab. Neither centre knew which pieces were from which embryo.
Main results and the role of chance: Results of CGH, SNP and qPCR were compared. All samples provided clear signals. Results from Center A and B revealed that in only 2 out of 10 embryos, all pieces provided the same results and 3 embryos that were initially reported abnormal by Centre "A" were reported normal by Center "B". One also tested normal on the repeat CGH. The PGS results of pieces of the same embryo were in agreement in 29% of the paired samples by CGH and in 63% by SNP. When comparing Center B and C, only 3 out of 16 embryos, provided same results for both pieces. Eleven out of 24 abnormal embryos were retested as normal (6 out of 13 by Centre B and 8 /14 by Centre C).
Limitations, reason for caution: The possible explanations for the discrepancy between results of pieces of the same embryo in each center or between centers could be related to methodological failure or mosaicism in the trophectoderm. There is an immediate need for a large properly designed study to provide

insight into validation of each of the commercial platforms to ensure that abnormal embryos are accurately identified.
Wider implications of the findings: The TE biopsy, in contrast to cellular stage blastomere biopsy, provides more genetic material and should be more accurate. However, the conflicting results obtained from pieces of the same blastocyst, both within tests and between tests in the present study, raise concern about the accuracy and reliability of TE testing.
Study funding/competing interest(s): Funding by hospital/clinic(s), Toronto Center for Advanced Reproductive Technology.
Trial registration number: N/A.

O-035 RCT study in advanced maternal age patients using array-CGH: interim analysis

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Study question: Is comprehensive chromosome screening (CCS) by array CGH of clinical value in women of advanced maternal age between 38–41 years?
Summary answer: Significantly higher ongoing implantation and pregnancy rates per transfer were observed in the group of CCS compared to the group of blastocyst transfer without aneuploidy screening. Ongoing pregnancy rates per cycle did not reach statistical significance, although this was just the Interim analysis.
What is known already: Aneuploidy in human embryos increases with advanced maternal age. CCS has been applied for the selection of euploid embryos aiming to improve clinical outcome. However, the clinical benefit of this approach in cleavage stage embryos was controversial when aneuploidy screening was performed by FISH for a limited number of chromosomes.
Study design, size, duration: RCT Interim analysis performed from June 2012 to October 2013. Patient allocation through computer-generated randomization into two groups: conventional blastocyst transfer or CCS cycle. Sample size for the endpoint of ongoing pregnancy rates per cycle and delivery rates was 120 patients per arm with ovum pick-up (α 5%, β 20%).
Participants/materials, setting, methods: Inclusion criteria were women between 38–41 years, <3 implantation failures, <3 previous miscarriages, and ≥5 MII oocytes retrieved. In the CCS group, cleavage stage embryo biopsy and array CGH were carried out. Main outcomes were ongoing pregnancy rates per transfer, per cycle and implantation rates (>12 weeks of pregnancy).
Main results and the role of chance: In the CCS group, 68 cycles were completed with 41 transfers and 25 ongoing pregnancies (61.0% ongoing pregnancy rate per transfer and 36.8% per cycle). In the group of blastocyst transfer alone, 80 cycles were completed with 77 transfers and 21 ongoing pregnancies (27.3% ongoing pregnancy rate per transfer and 26.2% per cycle). Ongoing implantation rates were 57.4% and 18.6% with versus without CCS. Two-sided Fisher's exact test showed significant differences for ongoing pregnancy rates per transfer (p = 0.0006; OR 0.2400; CI [0.2400–0.5360]) and for ongoing implantation rates (p<0.0001; OR 0.1692; CI [0.08509–03365]). The percentage of abnormal embryos in the CCS group was 81.0%. In the blastocyst transfer group, miscarriage rate was 41.7%, whereas no miscarriage was observed in the CCS group.
Limitations, reason for caution: This is an Interim analysis and the study needs to be completed to drawn stronger evidence. This is not a blinded study. The results are valid for this specific population.
Wider implications of the findings: Embryo aneuploidy testing could be considered as a valuable clinical tool to assess embryo viability in advanced maternal age patients since clinical outcomes and time to pregnancy is improved by CCS.
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by commercial/corporate company(ies), BlueGnome (Illumina), IVI, IVIOMICS.
Trial registration number: ClinicalTrials.gov NCT01571076

O-036 A direct comparison of next generation sequencing (NGS) and array-CGH for PGD for structural chromosomal abnormalities

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Study question: Can NGS be used for preimplantation genetic diagnosis (PGD) for structural abnormalities? Are there any advantages in using NGS over array-CGH?
Summary answer: Next generation sequencing can be used for PGD for structural abnormalities and offers a small increase in the accuracy and precision of the diagnosis compared to array-CGH.
What is known already: Carriers of structural chromosomal abnormalities are at a high risk of meiotic malsegregation leading to the production of unbalanced gametes and embryos. Carriers are phenotypically normal but experience infertility, recurrent miscarriages or unbalanced offspring. PGD involves the biopsy and testing of embryonic material from IVF generated embryos in order to select balanced/normal embryos for transfer. PGD for structural chromosomal abnormalities is routinely performed using array-CGH. NGS is a novel approach to PGD for structural aberrations.
Study design, size, duration: Overall 191 embryos from carriers of structural abnormalities (27 reciprocal translocations, 2 Robertsonian translocations and 2 intrachromosomal inversions) were tested using NGS. Sequencing was performed using the Illumina workflow on a HiSeq 2500.
Participants/materials, setting, methods: A total of 200 blastomeres were biopsied from 191 day 3 embryos. PGD was performed using 24 sure + array-CGH from January 2011 to September 2013. Balanced/normal embryos were selected for transfer. Whole genome amplified material from these blastomeres was used to validate the use of NGS for PGD for structural abnormalities.
Main results and the role of chance: The results obtained from the NGS analysis were concordant to the array-CGH results. In 2 cases where one of the translocated segments was very small, in the array-CGH analysis only one of the derivative chromosomes was seen, but NGS results were more accurate since both derivative imbalances were observed. Aberration breakpoints were mapped in finer detail by NGS, with a great dynamic range, therefore providing more precision in the diagnosis.
Limitations, reason for caution: This study compared array-CGH which is a well-established method for testing structural abnormalities with NGS. The results between the two different methods were concordant.
Wider implications of the findings: NGS can provide an alternative method for PGD, for embryo testing for structural chromosomal abnormalities. Embryonic samples are not compared to a reference sample, rather each chromosome is compared to the rest of the chromosomes within that sample, allowing small gains in precision and accuracy.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Illumina.
Trial registration number: N/A.

O-037 Use of single nucleotide polymorphism (SNP) arrays and karyomapping in a clinical setting for preimplantation genetic diagnosis (PGD) of single gene disorders

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Study question: Could a new universally applicable method be used in a clinical setting to perform PGD of single gene disorders (SGD) and how does this method compare to the gold-standard polymerase chain reaction (PCR) method in clinical practise?
Summary answer: Results and diagnosis obtained from clinical application of a new method for performing PGD of SGD (karyomapping) were 100% concordant with diagnosis made after application of conventional PGD test methods on the same set of biopsied samples.
What is known already: PGD for single gene disorders is currently performed using PCR methodologies to target specific genes and involves the development and validation of patient-specific tests; a procedure that often requires several

months to complete. A method that offers universal applicability through utilisation of the same validated protocol across a wide range of single gene disorders is highly desired and would be beneficial to the patients and practitioners of preimplantation genetics.
Study design, size, duration: Eighty-five blastocysts derived from 17 couples were clinically assessed for PGD of SGD using conventional test methods and karyomapping analysis. Fourteen different disorders were tested in total. Ten of the couples also opted to have comprehensive chromosome screening (CCS) of their embryos in parallel to the single gene test.
Participants/materials, setting, methods: Multiple displacement amplification was used to amplify all biopsied samples. Aliquots from each amplified sample were used to perform in parallel the clinically validated PCR protocol for each disorder, karyomapping analysis and CCS through array comparative genomic hybridisation (aCGH) - when indicated.
Main results and the role of chance: Fourteen cases were initially carried out using both, the conventional PGD test and karyomapping to validate the second for clinical application. Karyomapping analysis agreed with the conventional PGD test diagnosis in all 66 embryos that amplified successfully (100% concordance). Subsequently, 3 clinical cases (14 embryos), were performed with karyomapping analysis alone coupled with direct mutation detection via PCR. For these 3 cases, time of test preparation was estimated to be 1/3 of the time usually needed for test development when using conventional methods. Furthermore, karyomapping readily identified all monosomies detected by aCGH after assessment of 60 embryos, while it only detected 2/21 trisomies detected by aCGH indicating these to be of meiotic (maternal) origin. Two haploid and one triploid embryo were also identified.
Limitations, reason for caution: Approximately 10% of the genome is not covered adequately by the SNP array used for the karyomapping test. Karyomapping therefore is expected to be applicable to the great majority of single gene disorders but a small percentage of disorders will still need to be processed using conventional test methods.
Wider implications of the findings: Karyomapping uses polymorphic loci located across the entire human genome, to perform PGD for SGD. This study proved the accuracy and efficiency of the method by assessing it under real clinical conditions. The method uses a universal protocol for each single gene disorder and therefore, reduces significantly the time that patients have to wait to start their *in vitro* fertilisation cycle. Moreover, karyomapping is found to provide additional data of clinical and biological importance.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Part of the study was funded by Illumina, Inc.
Trial registration number: N/A.

O-038 Comparison of next generation sequencing (NGS) and aCGH for PGS

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Study question: Can a massively parallel genome sequencing method accurately assess embryos previously analyzed using aCGH.
Summary answer: NGS using the Ion Torrent PGM successfully detects aneuploidy in cells biopsied from human embryos, providing results equivalent to those obtained using the most widely used preimplantation genetic screening (PGS) method. The possibility of increased throughput, reduced costs and additional genetic information promised by NGS may revolutionize preimplantation embryo testing.
What is known already: Aneuploidy has a dramatic negative effect on reproductive outcome and represents one of the principal causes of implantation failure and miscarriage. PGS using a combination of blastocyst biopsy and comprehensive chromosome screening (CCS) has been shown in several randomized clinical trials to improve pregnancy outcome. Currently, the most widely used technique for CCS is aCGH, but NGS could become a superior alternative, detecting polyploidy, mtDNA and other aspects of genetics.
Study design, size, duration: 78 embryos, previously analyzed using aCGH, were donated for research and re-biopsied. A protocol for NGS using Ion Torrent PGM for aneuploidy analysis was optimized and then applied to the biopsy specimens. The first 59 samples were analyzed in a blinded fashion, allowing evaluation of NGS accuracy.
Participants/materials, setting, methods: The samples came from either blastomere, trophectoderm biopsy or cell lines and were previously diagnosed using aCGH or G-banding. The first 43 samples were analyzed in one PGD lab, and the remaining 35 samples were tested in another PGD Lab. Multiple samples were bar-coded and tested simultaneously, ranging from 16–32.
Main results and the role of chance: Not counting a polyploid embryo detected by NGS (not detectable by aCGH), there was a 76/77 (98.73%) concordance between aCGH or G-banding results and those obtained using NGS. A single sample had a false negative result. Of the 78 samples, 21/21 (100%) euploid samples were confirmed, 55/56 (98.21%) aneuploid samples were entirely concordant, and 3/3 (100%) chaotic profiles (degraded DNA) were confirmed.
Limitations, reason for caution: Seven abnormal embryos identified using aCGH showed some discordance with NGS. However, the discrepancies were confined to embryos with multiple aneuploidies. Such embryos typically display mosaicsm explaining the karyotypic divergence. The single false negative result was obtained after using multiple displacement amplification. Sureplex amplification was not associated with any errors.
Wider implications of the findings: NGS was shown to be highly accurate. Unlike aCGH, polyploidy could be detected as well as aneuploidy. The NGS method developed is rapid, cost-effective, scalable and has the ability to reveal additional aspects of embryo genetics. These results suggest that NGS offers significant advantages over current methods of embryo testing.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Reprogenetics.
Trial registration number: None.

INVITED SESSION

SESSION 08: FERTILITY PRESERVATION AND THE ROLE OF SURGERY

Monday 30 June 2014 **11:45 - 12:45**

O-039 Uterine transplantation

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One of last frontiers to conquer in treatment of female infertility is absolute uterine infertility (AUFI). It is estimated that around 1:500 of women of reproductive age suffer from AUFI, with gestational surrogacy and biological adoption being the only means to acquire motherhood today. The patients with AUFI are those that lack a uterus from birth (MRKH-syndrome) or through hysterectomy (cervical cancer, myoma, peripartum emergency hysterectomy) in addition to those that have a non-functional uterus (Ashermans's syndrome, myoma, uterine malformation). Uterine transplantation (UTx) may become a treatment options for these women.

Our research group initiated animal-based research on UTx in 1999 after the concept of UTx, was introduced to us by a young patient with cervical cancer that would undergo a radical hysterectomy. Extensive research has been conducted in rodents, pig, sheep and non-human primates. We then recently performed the first clinical trial of human UTx, receiving the uterus from live donors. This trial comes after two single cases (Saudi Arabia, 2000; Turkey, 2011), that were not preceded by any internal research or extensive animal-based training.

In 2013 our team completed the surgeries of a series of totally 9 human UTx, with live uterus donors (ClinicalTrials.gov NCT01844362). Most recipients were MRKH patients and a majority of the donors were mothers to the recipient. All patients had undergone IVF treatments before UTx and embryos had been cryopreserved. Altogether 10 surgeons (4 gyne-oncology surgeons, 3 gynecologists, 3 transplant surgeons) took part in the human UTx surgery in two adjacent ORs. The surgery of the recipients involved isolation of the uterus with long vascular pedicles of the uterine arteries/veins and parts of the internal iliac arteries/veins. This pårocedure laste 10–12 h. After procurement from the

donor the uterus was flushed with cold Custodiol and kept at cold ischemia until the recipient was fully prepared in an adjoining OR. Through a sub-umbilical midline incision, the external iliac artery and vein were mobilized bilaterally. The vaginal vault was separated from the bladder and ureters. The uterus was then brought into the pelvis of the recipient. Bilateral end-to-side anastomosis was accomplished between the vessels of the graft and the external iliacs. After blood flow had been re-established, the vaginal rim of the grafted uterus was sutured to the vaginal vault. The graft was fixed bilaterally to the round, cardinal and sacrouterine ligaments and an extensive leaf of bladder peritoneum of the graft was sutured on top of the bladder for extra structural support. The recipient surgeries lasted 5–6 h. The recipients received two ATG treatments iv and was then put on standard triple immunosuppression with tacrolimus, prednisolone and mycophenolate. After 6 months the patients are only on low dose tacrolimus. One uterus donor has a major complication, with a uretero-vaginal fistula diagnosed 10 days after surgery. This was repaired by ureteric reimplantation 3 months after uterus donation. Two of the 9 transplanted uteri have been removed during the initial 12 months. In one case, bilateral thrombosis of the uterine vessels occurred 3 days after transplantation and in another case persistant, and antibiotics resistant, intrauterine infection necessitated hysterectomy 3.5 months after transplantation. Seven of 9 transplanted patients have regular menstruations and ET have now started in some of these.

In conclusion, we have shown the feasibility of human UTx from live donors with a low dose immunosuppression protocol. Pregnancy results from this cohort is expected in late 2014 and 2015.

O-040 Ovarian transplantation

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Different cryopreservation options for fertility preservation in cancer patients include embryo cryopreservation, oocyte cryopreservation and ovarian tissue cryopreservation.

Cryopreservation of ovarian tissue is the only option available for prepubertal girls, and for woman who cannot delay the start of chemotherapy.

More than 60 cases of orthotopic reimplantation of frozen-thawed ovarian tissue have been reported to date and 30 live births have been published, yielding a pregnancy rate of more than 22%. After orthotopic reimplantation of cryopreserved tissue, restoration of ovarian function, proved by follicular development and estradiol secretion, occurred in all cases but two (absence of follicles in the frozen-thawed tissue). A time interval of 3.5 to 5 months was observed before estradiol secretion. In the literature, pregnancies were naturally obtained in 50% of cases. Graft activity was found to persist for 2.5 to 4 years, and transplantation can be performed twice or more if still frozen tissue left. In non-pregnant patients, IVF was performed, but the quality of oocytes and embryos was not optimal.

Prognostic factors (age, previous chemotherapy) are therefore discussed.

In conclusion, fertility preservation by ovarian tissue cryopreservation is now a real possibility for patients whose gonadal function is threatened by radiotherapy or chemotherapy.

INVITED SESSION

SESSION 09: FERTILITY SOCIETY OF AUSTRALIA EXCHANGE LECTURE

Monday 30 June 2014 **11:45 - 12:15**

O-041 Paternal obesity impairs the reproductive health and ovarian molecular profile of their female offspring

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Recent evidence has demonstrated paternal transmission of adiposity and impaired reproductive health to their female offspring. However, the impact on embryo development, embryo quality and the ovarian molecular profile in these female offspring remain unknown.

This study fed founder male mice either a control diet (CD) or a high-fat diet (HFD) for 12 weeks, which induced obesity but not diabetes, and were mated to CD fed female mice. Female offspring were maintained on a CD, super-ovulated, mated and the resultant zygotes were cultured to the blastocyst stage. Ovaries and cumulus cells from offspring were collected for gene expression analysis. Offspring measures that were assessed included embryo morphology, cell number, apoptosis; and ovarian and cumulus cell expression of selected genes that maintain endoplasmic reticulum (ER), metabolic, inflammatory and epigenetic homeostasis.

Our results showed that sperm from HFD fed males had reduced motility ($P < 0.01$) and increased levels of intracellular reactive oxygen species ($P < 0.001$) compared to sperm from CD fed males. Our results also showed that female offspring sired by HFD fed fathers produced embryos with delayed pre-implantation development ($P < 0.05$) and impaired quality, displayed increases in ovarian expression of *Glut1, Glut3* and *Glut4* ($P < 0.05$), and an increase in cumulus cell expression of *Glut4* compared to offspring sired by CD fathers ($P \leq 0.05$).

This study demonstrates that paternal obesity is associated with subfertility in female offspring despite the offspring being fed a CD and this paternal transmission of subfertility may relate to the observed ovarian and cumulus cell molecular alterations.

INVITED SESSION

SESSION 10: DATA REPORTING SESSION

Monday 30 June 2014 **11:45 - 12:15**

O-042 Data from the ESHRE PGD Consortium

J. Traeger-Synodinos[1], E.C. Coonen[2], M. De Rycke[3], C. Moutou[4], S. SenGupta[5], V. Goossens[6]
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[5]*University College London, UCL Centre for PG & D Institute for Women's Health, London, United Kingdom*
[6]*ESHRE, PGD Consortium, Grimbergen, Belgium*

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 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: The latest data collection (XV) was for cycles performed during 2012, with babies born by October 2013. As all recent data collections, it was 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: The Consortium has analyzed 15 sets of data including overall 58485 cycles. The indications include inherited chromosomal abnormalities (9182 cycles), monogenic disorders (13044 cycles), sexing for X-linked diseases (1668 cycles) or for social reasons (860 cycles), and aneuploidy screening for infertility (PGS) (33731 cycles). In addition 11913 clinical pregnancies and 9106 deliveries have been analysed in detail. Evaluating the data over the 15 years various trends are noted. The methods used for biopsy have changed, with lately a small, but gradual increase in the number of cycles with polar body or blastocyst biopsy (data set XV shows 15.1% polar body, 78.3% cleavage and 6.0%

blastocyst biopsy). The methodologies used for the diagnosis are also evolving, with data set XV showing around 1910/6782 (28%) cases using array-CGH. Between 2006 and 2011, the number of PGS cycles dropped from 66% of total cases to 51%, but the data from 2012 indicates a slight increase again (55.8% of total cases in 2012). Besides data collection other WGs have been active. The WG developing the on-line database is progressing with the aim to go live for next data collection (XVI). The WG to monitor new technologies in PGD distributed a questionnaire to all Consortium member centres in the autumn of 2013. The analysis of replies (from about 50 responding PGD centres) indicates interesting trends (in preparation). For the WG to evaluate outcomes and clinical utility of HLA-PGD a database is currently being developed for retrospective and prospective collection of data. The WG which aims to look at collaborative working practices between genetics and IVF teams when delivering a PGD service has a planned launch in the Spring of 2014, as does the first of the interactive webinars. Finally, in collaboration with the SIG in Reproductive Genetics, some introductory talks on PGD were recorded in the autumn of 2013 and will soon be available for viewing through the ESHRE website.

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.

INVITED SESSION

SESSION 11: PARAMEDICAL INVITED SESSION - LABORATORY

Monday 30 June 2014 **11:45 - 12:45**

O-043 Isolation and culture of human preantral follicles for fertility preservation

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Nowadays, the available methods for fertility preservation in women have different advantages and limitations. Cryopreservation of ovarian cortical tissue is the only available option for young patients who cannot undergo ovarian stimulation for oocyte/embryo cryopreservation, and with this technique menstrual cyclicity can be restored in the patients following grafting of thawed tissue which enables spontaneous conception in most patients. However, for some patients there may be a risk of reintroducing the original cancer in connection with transplantation of ovarian tissue. To overcome this issue follicles could potentially be isolated to their basal membrane in order to exclude malignant cells and subsequently grown and matured *in vitro* or *in vivo*.

Recently, successful animal experiments have shown that isolation and culture of preantral stage follicles holds huge potential as a new method for preserving fertility. The use of follicle culture systems that can efficiently use all classes of ovarian follicles would maximize reproductive potential for future fertility. Moreover, advances in biomaterial engineering have resulted in the development of an alginate-based 3D follicle culture system to maintain the cell–cell and cell–matrix connections important in regulating follicle development *in vivo*. This approach has so far produced live offspring in mice and maturation to MII stage in non-human primates. However, the development of follicle culture systems with the aim of growing oocytes from the earliest stage of follicle through to maturity has proven difficult in humans, first of all due to the scarcity of ovarian tissue, and major hurdles remain for improving culture systems to produce live human offspring as the ultimate clinical endpoint.

O-044 Thinking out of the box to bring innovations to the IVF lab

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Many things have changed since John Rock and Miriam Menkin were the first to successfully fertilize a human egg in vitro in 1944. Initially developments were slow as it took nearly 35 years for this initial success to translate into the birth of the first IVF-child. In the next 35 years, up until today, Medically Assisted Reproduction has undergone rapid development. We can now freeze gametes and embryos indefinitely, analyze every single base pair of a single human embryo, track every second of preimplantation embryo development, all within modern day clean room facilities. So where will the next 35 years bring us? Stem cell based therapies for male and female infertility, reproduction without gametes, individualized custom in vitro cultures, and presumably much more. Or are we bringing reproduction again where it belongs, at home in bed? This out of the box lecture will shed light on future developments in human reproduction, how to bring them to your clinic and, most importantly, how to determine whether they are truly of benefit to your patient.

INVITED SESSION

SESSION 12: WHEN THE FALLOPIAN TUBE FAILS

Monday 30 June 2014 **14:00 - 15:00**

O-045 Why chlamydia and smoking are bad for your Fallopian tubes - lifestyle factors and other aetiologies contributing to the risk of ectopic pregnancy

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An ectopic pregnancy is a pregnancy that occurs outside of the normal uterine cavity, and over 98% implant in the Fallopian tube. Tubal ectopic pregnancy remains the most common cause of maternal mortality in the first trimester of pregnancy and is a significant cause of maternal morbidity. The epidemiological risk factors for tubal ectopic pregnancy are well established and include: tubal damage as a result of surgery or infection (particularly *Chlamydia trachomatis*), and smoking. The aetiology of tubal ectopic pregnancy is less certain but current evidence supports the hypothesis that it is caused by a combination of retention of the embryo within the Fallopian tube due to impaired embryo-tubal transport and alterations in the tubal environment allowing early implantation to occur. This lecture aims to appraise the data to date explaining the link between the above risk factors and tubal ectopic pregnancy, focusing on the functional consequences of infection and smoking on Fallopian tube physiology. A greater understanding of the aetiology of tubal ectopic pregnancy is critical for the development of improved preventative measures, the advancement of diagnostic screening methods and the development of novel treatments.

References:

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Shaw JL, Horne AW. The paracrinology of tubal ectopic pregnancy. Mol Cell Endocrinol. 2012 358(2):216–22.

O-046 Translating molecular diagnostics and therapeutics for ectopic pregnancy from the bench top to the clinic

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Ectopic pregnancies complicate 1–2% of all pregnancies and remains a leading cause of maternal mortality in the first trimester. Our team has been exploring new diagnostic and therapeutic approaches to improve clinical management of this life-threatening condition.

The current protocol for medical management gauges treatment response by noting whether serum human chorionic gonadotrophin (hCG) levels falls ≥15% between days 4 and 7 after treatment. The drawback of this protocol is that patients have to wait 7 days before receiving the first indication whether there is a response to methotrexate. We have undertaken two studies in separate populations showing an early decline in hCG levels between days 0 (day of

treatment) and day 4 is associated with an 85% probability the ectopic pregnancy will resolve without need for further treatment.

While methotrexate can medical resolve smaller ectopic pregnancies, its efficacy and cost effectiveness drops with larger ectopic pregnancies. Thus, many are still treated surgically. A more effective medical therapy could reduce the need for operative intervention (and its inherent risks).

Given the placenta is heavily reliant on Epidermal Growth Factor Receptor (EGFR) signaling, we are investigating whether Gefitinib (Iressa, EGFR inhibitor) could be used to medically ectopic pregnancy.

We first performed preclinical studies showing combination gefitinib and methotrexate potently regressed placental tissue in vitro and in vivo. We translated this concept to the clinic, undertaking a phase I study of 12 women with ectopic pregnancies, administering methotrexate and gefitinib (pre-treatment hCG <3000 IU/L). The treatment appeared efficacious in resolving ectopic pregnancies. Importantly, the combination resulted in far steeper declines in the serum hCG levels compared to hCG trends observed in a historic cohort administered methotrexate only. The median time to cure with combination therapy was 34% shorter than methotrexate alone.

We have also treated eight women with extra-tubal ectopic pregnancies using combination gefitinib and methotrexate (interstitial ectopic pregnancies n = 5, scar ectopic pregnancies n = 3). The pre-treatment hCG levels ranged between 2458–48550 IUL, and six participants had pre-treatment hCG levels >5000 IU/L. All women were successfully treated, promptly resuming menstruation. Three have subsequently conceived an intrauterine pregnancy.

We are now finalising a phase II study of 28 women with larger ectopic pregnancies (pre-treatment hCG 1000–10,000 IU/L). It is a single arm trial, powered to show combination methotrexate and gefitinib is 90% effective (and at least over 70% effective) in resolving these ectopic pregnancies.

We believe combination gefitinib and methotrexate shows promise for improving the care of women diagnosed with stable ectopic pregnancies. It possible this drug combination could result in faster resolution of smaller ectopic pregnancy, and be used to treat large ectopic pregnancies that are currently being offered surgery. However, a large phase III randomised controlled trial will be required before gefitinib and methotrexate could be integrated into clinical care.

References:

Skubisz M et al. Using a decline in serum hCG between days 0–4 to predict ectopic pregnancy treatment success after single-dose methotrexate: a retrospective cohort study. *BMC Pregnancy and Childbirth* 2013; Feb1;13:30. doi: 10.1186/1471-2393-13-30.

Skubisz MM et al. Combination gefitinib and methotrexate compared to methotrexate alone to treat ectopic pregnancy. *Obstetrics and Gynecology* 2013; 122(4):745–51.

Nilsson UW et al. Effects of gefitinib, an epidermal growth factor receptor inhibitor, on human placental cell growth. *Obstetrics and Gynecology* 2013;122(4):737–44.

Horne AW et al. Phase II single arm open label multicentre clinical trial to evaluate the efficacy and side effects of combination gefitinib and methotrexate to treat tubal ectopic pregnancies (GEM II): study protocol. *BMJ Open* 2013; Jul 19;3(7) e002902

INVITED SESSION

SESSION 13: FROM PLURIPOTENT STEM CELLS TO GAMETES AND BACK

Monday 30 June 2014 **14:00 - 15:00**

O-047 Gametes from pluripotent stem cells: why does it work in the mouse and not in the human?

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The possibility to generate patient-specific artificial gametes is a topic that has wide importance both societal and scientific. On the one hand it concerns the optimization of protocols to make the generation of patient-specific artificial gametes feasible, a fast evolving research area worldwide, and on the other hand this topic is often covered in the media as it has a very direct interest from all layers of society with direct visible applications. However, most knowledge gathered so far on gametogenesis is based on animal models and not readily translated to humans. Gametogenesis is a complex niche-dependent multistep process that shows some similarities, but also a lot of differences between mice and humans. Studying the whole process of human gametogenesis is therefore paramount if we want to understand how to generate functional human gametes from pluripotent human stem cells. Here, I will explore the scientific possibilities and limitations of this exciting field.

O-048 Genome exchange in human oocytes

D. Egli[1], M. Yamada[1], B. Johannesson[1], D. Paull[1], M. Hirano[2], V. Emmanuele[2], R. Goland[3], R. Leibel[3], M. Sauer[4]

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We have explored the question whether the genome in the human oocyte can be exchanged while maintaining developmental competence. We found that the genome of the egg can be exchanged with either the genome of another oocyte, or with that of a somatic cell, and result in development to the blastocyst stage, allowing stem cell derivation. Though similar techniques are used to transfer either the genome of another oocyte or of a somatic cell, the aims and applications are different.

The aim to exchange the genome between oocytes is to prevent the inheritance of mutant mitochondria. Mutations in mitochondrial DNA can cause mitochondrial diseases by alteration of respiratory chain function and energy production, resulting in severe and lethal multisystemic disorders. Such mutations are transmitted from mother to child with the mitochondria contained in the cytoplasm of the oocyte. We have determined whether the transfer of the nuclear genome between oocytes of two different donors can substitute one mitochondrial genotype for the other. Using parthenogenesis and stem cell derivation, we found that during preimplantation development, less than 0.5% of mitochondrial DNA is derived from the donor of the nuclear DNA, decreasing to undetectable levels in stem cell lines, resulting in a complete substitution of the mitochondrial genotype. Therefore, the transfer of the genome from an oocyte affected by mitochondrial DNA mutations to an oocyte of an egg donor without these mutations should allow affected women to have a genetically related child without also inheriting the mutant mitochondrial DNA. While concerns have been raised regarding genetic changes in the human germ line, the changes made alter the pattern of inheritance, and not the genetic material itself.

The transfer of somatic cell nuclei into human oocytes serves the purpose of generating stem cell lines with the genotype of subjects with degenerative diseases, such as diabetes or Parkinson's. Such cells may be useful for the development of cell replacement therapies. Even though induced pluripotent stem (iPS) cells can now be derived from somatic cells by overexpression of transcription factors, several studies have described differences between iPS cells and embryonic stem (ES) cells. We have previously reported that human oocytes can reprogram a somatic cell to a pluripotent state. And recently, diploid embryonic stem cells have been derived after nuclear transfer of embryonic fibroblasts. Because of the therapeutic potential of developing diploid embryonic stem cell lines from adult cells of normal and diseased human subjects, we have systematically investigated the parameters affecting efficiency and developmental potential in their derivation. Using modifications to the artificial activation protocol and addition of histone deacetylase inhibitors, we successfully derived diploid pluripotent stem cell lines from skin cells of a newborn and from adult somatic cells of a type 1 diabetic subject. This is a proof-of principle experiment; further optimizations are required to generate cell lines that will be therapeutically relevant. In summary, we consider these findings an important step towards the use of reprogrammed cells for cell replacement.

SELECTED ORAL COMMUNICATION SESSION

SESSION 14: NOVEL TECHNIQUES IN THE LABORATORY AND IVF OUTCOME

Monday 30 June 2014 **14:00 - 15:00**

O-049 In-vitro maturation of cumulus enclosed oocytes collected from the remaining medulla tissue at the time of ovarian tissue cryopreservation

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Study question: Is it possible to obtain *in-vitro* matured metaphase-II oocytes isolated from remaining medulla tissue at the time of ovarian tissue cryopreservation, and do they show a normal spindle structure? What is the optimal *in-vitro* maturation time to achieve a high yield of healthy metaphase-II oocytes?
Summary answer: Maximum *in-vitro* maturation (IVM) of cumulus surrounded immature oocytes derived from remaining medulla tissue yielded a percentage of 30.4% matured metaphase-II (MII) oocytes and 61.3% of these *in-vitro* matured oocytes showed a normal spindle structure.
What is known already: Standard cryopreservation of ovarian tissue consists of freezing of ovarian cortical strips or biopsies and discarding the medulla. This tissue contains antral follicles and could therefore harbour immature oocytes (Kristensen, *et al*. 2011). IVM of primordial follicles has not been yet successfully achieved in human. If immature oocytes are present in the medulla, then IVM could yield mature oocytes to be preserved for future use. Proof of principle was recently published (Wilken-Jensen, *et al.* 2013).
Study design, size, duration: I: Split IVM of sibling cumulus enclosed oocytes for 24 hrs (n = 57) and 48 hrs (n = 56). II: Consecutive IVM (n = 157): 24 hrs IVM with analysis of the matured MII oocytes, delayed immature oocytes underwent an extra 24 hrs IVM. III: Maximum IVM for 48 hrs (n = 102). Statistical analysis was performed by Fisher's exact test.
Participants/materials, setting, methods: 14 female-to-male transgender persons were included with a mean age of 25.4 ± 7.4 years. A total of 372 cumulus enclosed oocytes (CEO) were collected from medulla tissue during standard ovarian tissue cryopreservation. After IVM culture, matured MII oocytes were further analysed for spindle structure by immunostaining and confocal imaging.
Main results and the role of chance: I: Split IVM resulted in 15.8% (9/57) matured MII-oocytes after 24 hrs and, 30.4% (17/56) (P = 0.07) in the 48 hrs group. Spindle structures were comparable (P = 0.54) in both groups, 88.9% (8/9, 24 hrs)) versus 58.8% (10/17, 48 hrs).

II: Consecutive IVM revealed 6.4% (10/157) after 24 hrs IVM, where 22.3% (30/130) of the remaining immature oocytes reached maturity after another 24 hrs. The total IVM rate was 25.5% (40/157). Normal spindle status was comparable after 24 hrs or 48 hrs, 50.0% (5/10) versus 51.7% (15/29) (P = 1.00)

III: Maximum IVM revealed 30.4% (31/102) of matured MII oocytes after 48 hrs and 61.3% (19/31) showed normal spindle structures.
Limitations, reason for caution: The results are based on transgender persons who donated their ovaries and were treated with androgen hormones. Other patient cohorts might have less immature oocytes to start with and pathology or treatment could influence the maturation rate.
Wider implications of the findings: Immature oocytes can be collected from spare medulla and can reach the MII-stage after 48 hrs of IVM with a normal spindle structure.

This approach has wide implications for cancer patients who freeze ovarian tissue and where spare medulla is discarded. It is clear that this tissue contains potentially healthy oocytes and although even in small numbers, they should be preserved for future fertility treatment of patients.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), University Hospital Ghent - University Ghent.
Trial registration number: This research is conducted with the approval of the local ethics committee (2012/780).

O-050 Follow-up of child growth regarding new technologies: testicular sperm extraction (TESE), in vitro maturation (IVM) and assisted oocyte activation (AOA)

H. Hattori[1], T. Kyoya[1], Y. Nakamura[1], N. Matsukawa[1], T. Miyagawa[1], S. Suzuki[1], M. Doshida[1], M. Toya[1], K. Kyono[1]
[1]Kyono ART Clinic, Obstetrics and Gynecology, Sendai, Japan

Study question: In this study, we evaluated the outcome of infants using relatively new technologies – Testicular Sperm Extraction (TESE), In Vitro Maturation (IVM), and Assisted Oocyte Activation (AOA) – in Assisted Reproductive Technology (ART).
Summary answer: There were no significant differences between Standard IVF, Conventional ICSI and advanced technology such as TESE, IVM and AOA.
What is known already: In recent years, ART has been performed widely and its treatment methods have become complicated. However, there are only a few reports on surveys after child birth; therefore an ongoing follow-up, regarding the safety of ART, is needed. At our clinic, children's physical and mental growth is monitored until the age of 6 (Reprod Med Biol 2004) and patients are informed of the results to highlight the safety of the treatment.
Study design, size, duration: The subjects were 2063 children who were born after fresh or frozen embryo transfer at Kyono ART Clinic in Japan, from 1995 to 2012. The subjects were separated into 5 groups: Group 1, Standard IVF; Group 2, ICSI; Group 3, TESE; Group 4, IVM; and Group 5, AOA.
Participants/materials, setting, methods: Among 2063 children {Group 1 (322 singletons, 106 twins, 42 triplets), Group 2 (1048 singletons, 281 twins, 30 triplets) Group 3 (128 singletons, 42 twins), Group 4 (36 singletons, 4 twins) and Group 5 (17 singletons, 6 twins)}, congenital abnormality, low birth weight, and premature birth rates were compared.
Main results and the role of chance: From Groups 1 to 5, abnormality rates were 3.6%, 3.6%, 1.8%, 2.5% and 4.2%, respectively. Premature birth rates were 24.3%, 23.1%, 20.0%, 17.4% and 26.1%, respectively. Low birth weights rates were 30.2%, 33.2%, 22.4%, 20.0% and 30.4%. There were no significant differences between standard IVF and the other groups. The congenital abnormalities found included inguinal hernia, mild antral septal defect and ventricular septal defect due to low birth weight and the majority of children have healed naturally. A few cases of Hyperdactylia, 18 trisomy, 21 trisomy and patent ductus arteriosus were also found. Moreover, physical growth up to 6 years old, including weight and height, was within the normal range overall.
Limitations, reason for caution: None.
Wider implications of the findings: We concluded that advanced technology such as TESE, IVM, and AOA do not have negative effects on child birth and development. However, we need to continue long-term follow-up to evaluate more cases.
Study funding/competing interest(s): Funding by hospital/clinic(s), Kyono ART Clinic.
Trial registration number: None.

O-051 Outcome of ICSI after removal of apoptotic sperm by magnetic activating cell sorting

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[1]Skånes Universitetssjukhus, Reproduktionsmedicinskt Centrum, Malmö, Sweden

Study question: Can magnetic activating cell sorting (MACS) improve clinical outcomes after ICSI?
Summary answer: MACS-preparation as a complement to DGC should be used on sperm samples with poor morphology prior to ICSI. This needs however further investigation before being set as a clinical recommendation.
What is known already: The success of assisted reproduction depends to some degree on the quality of the sperm sample. It is known that elevated DNA fragmentation can occur in a spermatozoon despite normal morphology and motility. Using sperm with high DNA fragmentation may have adverse effects on the outcome of ART. Current techniques used to isolate the most motile and morphological normal sperm cells do not efficiently remove all spermatozoa with damaged DNA.
Study design, size, duration: Randomized sibling oocyte study. Seven hundred and two oocytes from 105 couples were included in the study under period of 6 months.
Participants/materials, setting, methods: One hundred and five couples undergoing ICSI treatment. The study was performed in a university hospital based IVF clinic.
Main results and the role of chance: No significant differences were seen between the oocytes injected with DGC-prepared and those injected with

DGC + MACS-prepared sperm in regard to fertilisation, embryo transfer, embryo transfer/ cryopreservation frequency, biochemical pregnancy rate and clinical pregnancy rate. However, a significant positive correlation was seen between sperm morphology and the use of MACS preparation on fertilisation rate. In average DFI decreased with 2.9% due to MACS-preparation.
Limitations, reason for caution: Limitations of the study was the sample size.
Wider implications of the findings: MACS preparation can be used as a complement to density gradient centrifugation or swim up with sperm samples with poor morphology prior to ICSI.
Study funding/competing interest(s): Funding by hospital/clinic(s), Centre of Reproductive Medicine, Skane University Hospital, Sweden.
Trial registration number: Not applicable.

O-052 Morphokinetics of embryos derived from cryopreserved human oocytes assessed by time-lapse cinematography (tlc)

C. Shitara[1], Y. Nakamura[1], H. Hattori[1], Y. Nakajo[1], Y. Sato[1], T. Kyoya[1], N. Matsukawa[1], T. Oyama[2], T. Takeuchi[2], K. Kyono[1]
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Study question: What should we expect for blastocyst formation in an embryo derived from vitrified oocytes, and how does oocyte vitrification affect embryonic development?
Summary answer: Cell division time can be a predictor of blastocyst formation in embryos derived from vitrified oocytes. We have observed that embryos derived from vitrified oocytes tend to develop more slowly than those from fresh oocytes in terms of completing division into 2 or following cleavages.
What is known already: As assisted reproductive technology (ART) is rapidly becoming more well-known, a larger number of cancer and blood vessel disease patients want to cryopreserve their oocytes aiming for fertility preservation. It may be expected that more healthy single women are also going to have their oocytes cryopreserved for fertility preservation in the near future.
Study design, size, duration: Morphological attribute and assessment study. We used 87 vitrified mature oocytes from 12 consenting patients from 1996 to 2013. Intracytoplasmic sperm injection (ICSI) was performed for 78 warmed oocytes, 50 embryos at two pronuclear (2PN) were obtained and incubated individually in a Promo vision time-lapse embryo monitoring system for 144 h.
Participants/materials, setting, methods: We compared cultured embryos (scored by Gardner's classification) in groups A (developed into blastocysts) and B (others): times (t) of PN appearance/disappearance, each completing divisions (t2–t8), first to subsequent cleavages (cc2) and between divisions to 3-cell and to 4-cell (s2). We also compared vitrified with fresh oocytes (cultured after ICSI).
Main results and the role of chance: Significant differences were found in t3, t5, t8, and cc2 between groups A and B: t3 (37.4 ± 5.5 h vs. 31.7 ± 6.8 h), t5 (52.6 ± 8.5 h vs. 39.7 ± 9.4 h), t8 (67.9 ± 12.2 h vs. 46.2 ± 15.1 h), cc2 (10.8 ± 3.6 h vs. 4.9 ± 5.9 h). There was no significant difference between vitrified and fresh oocytes when both included blastocysts and non-blastocysts. However, there were significant differences in t4, t8 and s2 between vitrified and fresh oocytes which included only blastocysts: t4 (41.2 ± 5.2 h vs. 38.9 ± 4.6 h), t8 (67.9 ± 12.2 h vs. 59.7 ± 10.8 h), s2 (3.8 ± 4.5 h vs. 1.8 ± 3.1 h, $p < 0.05$). Embryos from vitrified oocytes tend to develop more slowly regarding completion of division to 2 or following cleavages.
Limitations, reason for caution: Only limited cases were used to produce these results; therefore, a larger number of cases are needed to make this result a reliable one.
Wider implications of the findings: In vitrified embryos, times of cleavage cells are useful to estimate the formation of blastocysts. The appearance, first cleavage cells and the subsequent cleavage are slower than fresh oocyte embryos among blastocysts, and it may be the effect of cryopreservation. We should definitely continue analyzing development of embryos using TLC, and standardizing the embryo screening for vitrified oocyte embryos.
Study funding/competing interest(s): Funding by hospital/clinic(s), Kyono ART Clinic.
Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATION SESSION

SESSION 15: IVF FROM A VITRIFIED PERSPECTIVE

Monday 30 June 2014 **15:15 - 16:30**

O-053 Is time now for single frozen embryo transfer? A retrospective analysis of 2146 frozen cycles

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Study question: The aim of the present study was to compare pregnancy and multiple pregnancy rates after single frozen embryo transfer (SFET) and double frozen embryo transfer (DFET) over a period of 5 years in order to establish a systematic SFEF policy similar to that used for fresh transfer in Belgium.
Summary answer: Multiple pregnancy rates are higher than 20% with DFET of vitrified blastocysts as opposed to less than 2% with SFET. The pregnancy rate does not significantly differ between SFET and DFET. These findings led us to introduce a SFET policy for all patients younger than 36 who have vitrified blastocysts.
What is known already: Since July 2003, the reimbursement system in Belgium imposes single embryo transfer (SET) for fresh cycles in patients with favorable prognosis (younger than 36, first and second IVF attempts). This regulation is proven to be effective in reducing multiple pregnancy rates without affecting the pregnancy/transfer rate particularly when elective single embryo transfer is used. In all cases of frozen/thawed embryos, it is legal to transfer two.
Study design, size, duration: We realized a retrospective study including 2146 FET cycles from January 2008 to October 2013.
Participants/materials, setting, methods: All patients, whatever their age, indication or number of attempts were included. We took into account 1608 cycles with slow frozen embryos (day 2–3) and 538 cycles with vitrified blastocysts (day 5–6) Pregnancy and multiple pregnancy rates per transfer were compared between single and double embryo transfers. Statistical analysis was obtained by χ^2 test.
Main results and the role of chance: For slow frozen embryos, the survival rate was 56%. The pregnancy rates/SFET and DFET were respectively 27.8% and 39.3% ($p < 0.001$). The multiple pregnancy rates represented respectively 3.8% and 9.8% ($p < 0.01$) of the clinical pregnancies. For vitrified blastocysts, the survival rate was significantly higher compared to slow frozen embryos 90.5% vs. 56% ($p < 0.0001$). The pregnancy rates/SFET and /DFET were 35.0% and 43.9% (NS). The multiple pregnancy rates were respectively 1.5% and 20.7% ($p < 0.01$). There was no difference between compared groups for patient ages, infertility duration and etiology nor number and rank of trials.
Limitations, reason for caution: This is a retrospective analysis and a selection bias may exists although the characteristics of the two groups were not different. The choice between SFET and DFET was based on the number of embryos available, their quality and the couple wishes, aspects which were not randomized.
Wider implications of the findings: The benefit of transferring two frozen embryos is less significant with vitrified blastocysts than with slow frozen segmented embryos. This is probably due to the fact that we rarely take the risk of transferring two top quality blastocysts to young patients. However, multiple pregnancy rates doubled with DFET of vitrified blastocysts compared to slow frozen segmented embryos. These findings led us to introduce a SFET policy for all the patients younger than 36 who have vitrified blastocysts. We share information and decision-making in other situations.
Study funding/competing interest(s): Funding by hospital/clinic(s), CHC Saint-Vincent, Rocourt, Belgium.
Trial registration number: None.

O-054 Vitrification of germinal vesicle (GV) stage oocytes physically disrupts contact with the surrounding cumulus granulosa cells

J. Suzuki[1], E. Garcia Cerrudo[1], S. El-Hayek[1], H. Clarke[1], H. Holzer[1], W.Y. Son[1]
[1]*McGill University Health Center, Obstetrics and Gynecology, Montreal, Canada*

Study question: Immediately following oocyte retrieval, what should be done first in order to best preserve immature oocytes, vitrification or *in vitro* maturation (IVM)? Does vitrification of mouse immature (GV-stage) oocytes, or their exposure to vitrification solution, alter contact between the oocyte and the cumulus granulosa cells that surround it?
Summary answer: Exposure GV-oocytes to vitrification solution, with or without subsequent vitrification, causes loss of the cumulus cell processes, termed transzonal projections (TZPs) that physically link the cumulus cells to the oocyte. This disruption might be the cause of inferior embryonic development observed when oocytes are vitrified prior to IVM.
What is known already: It has been well documented that communication between the oocyte and surrounding cumulus cells are crucial during the early stages of meiotic maturation both *in vivo* and *in vitro*. Actin-rich TZPs are the contact sites for gap-junctions between oocyte and cumulus cells, and disruption of this communication drastically compromises oocyte cytoplasmic maturation and embryo development. However, it is unknown whether vitrification is able to preserve these communication networks.
Study design, size, duration: (1) IVM prior to vitrification versus vitrification prior to IVM was compared, with non-vitrified oocytes matured *in vitro* as control; (2) GV-stage oocytes exposed to vitrification solutions versus GV-oocytes that were vitrified and then warmed, with non-vitrified immature oocytes as control. Three replicates were performed with $n = 25$ and $n = 38$, respectively.
Participants/materials, setting, methods: GV-stage oocytes were collected from CD1 mice injected with 5IU of equine chorionic gonadotropin 48 h previously. TZP density was assessed using a fluorescent actin-binding probe and confocal microscopy. Maturation, fertilization rate and embryo development were recorded. Data was analyzed using one-way ANOVA and $p < 0.05$ were considered statistically significant.
Main results and the role of chance: In experiment 1, there was no difference in maturation and fertilization rates when vitrification of GV-stage oocytes was performed before IVM (88.8% and 72.6%, respectively) $p = 0.341$ or after IVM (86.8% and 82%, respectively) $p = 0.178$. However, blastocyst development was significantly lower when vitrification was performed before (4%) versus after IVM (30%) with $p < 0.05$. in experiment 2, exposure of GV-stage oocytes to vitrification medium significantly reduced TZP density to 40% of control and, when vitrification was performed, TZP density was reduced to 14% of control ($p < 0.0001$).
Limitations, reason for caution: The study was done in a mouse model. A larger data set, including detailed pre-implantation and post-implantation development and clinical trials are needed to validate these initial findings.
Wider implications of the findings: Retrieval and vitrification of GV-stage oocytes has significant clinical advantages for fertility preservation, especially when stimulation is not recommended. However, GV-stage oocytes require IVM and most fail to reach blastocysts. Our results suggest that TZP may partially underlie the poor results obtained using oocytes vitrified at the GV-stage. Improving the conditions for cryopreservation and IVM is imperative to make this technique attractive for clinical purposes.
Study funding/competing interest(s): Funding by national/international organization(s), Canadian Institutes of Health Research (CIHR).
Trial registration number: None.

O-055 Clinical outcomes following cryopreservation of blastocysts by vitrification and slow freezing: a population-based cohort study

Z. Li[1], Y.A. Wang[1], W. Ledger[2], D.H. Edgar[3], E.A. Sullivan[1]
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[3]*Royal Women's Hospital, Melbourne IVF/Reproductive Services, Melbourne, Australia*

Study question: What are the clinical efficacy and neonatal outcomes following vitrification of blastocysts compared with fresh blastocyst transfer and slow freezing methods?
Summary answer: Compared with slow freezing of blastocysts, vitrification of blastocysts resulted in a significantly higher clinical pregnancy rate and live delivery rate as well as comparable neonatal outcomes at population level.
What is known already: Although vitrification has been reported to be associated with significantly increased post-thaw survival rates compared with slow freezing, there has been a lack of general consensus over which method of cryopreservation (vitrification versus slow freezing) is most appropriate for blastocysts.
Study design, size, duration: A population-based cohort of autologous fresh and thaw cycles performed between 2009 and 2011 in Australia and New Zealand was evaluated retrospectively. A total of 46,890 fresh blastocyst transfer cycles, 11,644 slow frozen blastocysts transfers cycles, and 19,978 vitrified blastocysts transfers cycles were included in the data analysis.
Participants/materials, setting, methods: Cox regression was used to examine the pregnancy outcomes (clinical pregnancy rate, miscarriage rate, and live delivery rate) and neonatal outcomes (preterm delivery, low birthweight births, small for gestational age (SGA) births, large for gestational age (LGA) births, and perinatal mortality) following transfer of fresh, slow frozen and vitrified blastocysts.
Main results and the role of chance: The 46,890 fresh blastocysts transfers, 11,644 slow frozen blastocysts transfers, and 19,978 vitrified blastocysts transfers resulted in 16,845, 2,766, and 6,537 clinical pregnancies, which led to 13,049, 2,065 and 4,955 live deliveries, respectively. Compared with slow frozen blastocyst transfer cycles, vitrified blastocyst transfer cycles resulted in a significantly higher clinical pregnancy rate (Adjusted rate ratio (ARR): 1.47, 95% confidence intervals (CI): 1.39–1.55) and live delivery rate (ARR: 1.41, 95% CI: 1.34–1.49). Compared with singletons born after transfer of fresh blastocysts, singletons born after transfer of vitrified blastocysts were at 14% less risk of being born preterm (ARR: 0.86, 95% CI: 0.77–0.96), 33% less risk of being low birthweight (ARR: 0.67, 95% CI: 0.58–0.78), and 40% less risk of being SGA (ARR: 0.60, 95% CI: 0.53–0.68).
Limitations, reason for caution: A limitation of this population-based study is the lack of control for factors associated with the cryopreservation processes. The protocols and outcomes for slow freezing-thaw and vitrification-warm of blastocysts may vary between clinics.
Wider implications of the findings: This study presents the population-based evidence on the clinical efficacy and neonatal outcomes after transfer of fresh, slow freezing and vitrification of blastocysts. The higher clinical pregnancy rate and live delivery rate as well as comparable neonatal outcomes at population level suggested that vitrification should be considered as an alternative embryo transfer strategy to achieve better clinical outcomes following Assisted Reproduction Technology (ART) treatment.
Study funding/competing interest(s): Funding by University(ies), No specific funding was obtained. The authors have no conflicts of interest to declare.
Trial registration number: Not applicable.

O-056 Freeze All Embryos: a cycle rescue or a novel strategy

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Study question: Elective cryopreservation of blastocysts followed by frozen-thawed transfer in a subsequent cycle may be a valuable option to circumvent the problem of the various degree of asynchrony observed between the development of the embryo and endometrial receptivity following controlled ovarian stimulation for ART.
Summary answer: Implantation and pregnancy rates following ART cycles may be improved by performing FET (Frozen Embryo Transfer) after "freeze all embryos" in all those clinical situations in which fresh embryo transfer is contraindicated.
What is known already: In good-responder patients, extended culture to blastocyst stage is associated with higher rates of implantation, clinical pregnancy and delivery compared to transfer of embryos on day 3. Cryotop vitrification is being applied with increasingly high efficiency to blastocyst-stage embryos. The availability of blastocyst culture and vitrification and the growing knowledge on endometrial receptivity suggest that postponing embryo transfer to a better "implantation window" might be a novel strategy for optimizing ART outcomes.
Study design, size, duration: Retrospective case-control study on 252 IVF/ICSI cycles was performed from January 2012 to December 2013. The outcome

of 63 postponed FET cycles for clinical contraindications (cases) was compared to outcome of 189 fresh ET cycles (controls).

Patients were matched for age, ovarian reserve, cause of infertility, collected oocytes. (ratio 1:3).

Participants/materials, setting, methods: The study was performed at the Ospedale San Raffaele IVF Unit, Milano. Pregnancy rate was defined as positive β-hCG test. Implantation rate is the percentage of embryos which are implanted compared to the number of embryos transferred in a given period.

Main results and the role of chance: Mean number of transferred blastocysts in fresh and in "freeze all" FET cycles was 1.76 ± 0.44 and 1.57 ± 0.50, respectively (p = 0.004). A pregnancy rate of 55.0% was reported for fresh cycles, while "freeze all" FET cycles showed an pregnancy rate of 71.4% (104/189 vs. 45/63, p = 0.026, Fisher's Exact Test; OR 2.04, 95% CI 1.10–3.79, Chi Square test). Implantation rate was also significantly higher in "freeze all" cycles compared to fresh cycles 45.4% vs 34.2%, respectively, p = 0.044 Fisher's Exact Test; OR 1.63, 95% CI 1.03–2.58, Chi Square test).

Limitations, reason for caution: This is a retrospective, case-control study. Prospective RCTs are needed and results should be confirmed in different infertile populations.

Wider implications of the findings: The results of this study suggest that implantation and pregnancy rates may be superior when FET is performed in all those clinical situations in which fresh embryo transfer is contraindicated. These results may be explained by improved embryo-endometrium synchrony in frozen-thawed transfer. Furthermore, FET could be an elective strategy due to non inferior neonatal outcomes in terms of prematurity, low birth weight, stillbirth, neonatal death and major malformation, as reported, if compared with fresh ET.

Study funding/competing interest(s): Funding by hospital/clinic(s), San Raffaele Scientific Institute.

Trial registration number: Not required.

O-057 Vitrification impact on human oocyte mitochondrial activity and redox homeostasis

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Study question: Do the extreme conditions of vitrification differentially affect human oocytes depending on maternal age?

Summary answer: Our data show that vitrification seems to shift the intracellular redox potential towards oxidation, especially in those coming from young donors; but by contrast did not decrease the mitochondrial activity and did not alter the reactive oxygen species production levels, which remained invariable after thawing.

What is known already: Recent studies have reflected increased reactive oxygen species (ROS) levels in warmed young oocytes and highlighted the temporally dynamic loss of mitochondrial potential that could therefore lead to ATP production decrease, impairing embryo development.

Mitochondrial function can be evaluated *in vivo* by NADH/FAD++ autofluorescence ratio, which reflects the respiratory chain activity and it is considered as a marker of the intracellular redox state. Vitrification effect on this redox state has never been studied.

Study design, size, duration: This control versus treatment study included 550 oocytes (fresh vs vitrified) recruited between January 2013 and January 2014.

Participants/materials, setting, methods: Discarded MII oocytes donated to research from young (<26 years) and reproductively aged (>37 years) women were used.

Redox state was assessed by measuring FAD++/NADH autofluorescence ratio; reactive oxygen species levels and mitochondrial activity were reported by *in vivo* labelling (with carboxy-H2DCFDA and JC1 respectively).

Main results and the role of chance: We found that young and aged oocytes showed similar vitrification survival rates (86.35% vs 89.15%, p-value > 0.05). Confocal microscopy revealed that the FAD++/NAD(P)H ratio was higher in young vitrified than in young fresh oocytes (52,0% vs 36.8%, p-value 0.01, on a scale that runs 100% for full oxidation and 0% for full reduction), suggesting a shift towards the oxidised state in young oocytes after vitrification. Although it was not significant, this same tendency was observed in the aged group.

The mitochondrial activity and pattern of distribution did not reflect differences between fresh or vitrified oocytes depending on maternal age. Likewise, the assessment of general oxidative stress by measuring ROS levels showed similar pixel intensity in fresh and thawed oocytes from young and reproductively aged oocytes.

Limitations, reason for caution: Due to sample availability MII discarded oocytes – i) *in vitro* matured oocytes, ii) unfertilized oocytes 20 h after ICSI – were included in the study.

Wider implications of the findings: Despite vitrified oocyte yields comparable clinical outcome compared to fresh ones, lower cleavage and blastocyst rates can be observed during *in vitro* culture. Our data suggest that the redox state of human oocytes is affected by vitrification. Therefore, the importance of adding protective antioxidant molecules to the vitrification solution and to the post-thawed culture medium to improve embryo cleavage should deserve some research.

Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by national/international organization(s), Valencian Government: Val+i+D program, PhD grant. INCLIVA: Foundation for health research.

Trial registration number: None.

SELECTED ORAL COMMUNICATION SESSION

SESSION 16: OVARIAN STIMULATION

Monday 30 June 2014 **15:15 - 16:30**

O-058 Comparison of luteal-phase (LP) versus early-follicular-phase (EFP) ovarian stimulation in the same oocyte donor and pregnancy rates among corresponding recipients of vitrified oocytes

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Study question: Does starting ovarian stimulation during luteal vs early follicular phase change the characteristics of ovarian response, oocyte yield and pregnancy rates among the corresponding recipients of the vitrified oocytes?

Summary answer: No significant differences were observed regarding duration of stimulation, gonadotropin consumption and number of oocytes retrieved (total and MII). Pregnancy rate per embryo transfer in the recipients of the vitrified oocytes obtained after LP stimulation was 58.3% and 62.5% after EFP stimulation (n.s.)

What is known already: There is an increasing demand for fertility preservation in oncologic patients. To avoid delaying the cancer treatment, ovarian stimulation in the LP has been described, yielding similar results than with EFP stimulation.

No direct comparison of both types of stimulation has been described in the same cohort of women. There are no data evaluating pregnancy rates with these cryopreserved oocytes after warming, IVF and embryo transfer in recipients.

Study design, size, duration: Prospective observational comparative study of 9 oocyte donors, and 20 recipients carried out in a University affiliated fertility clinic from 2012 to 2013.

Each donor underwent two consecutive stimulation cycles (one EFP and one LP) within 3 months. Mature oocytes were vitrified. Recipients were primed with estradiol and progesterone.

Participants/materials, setting, methods: Ovarian stimulation was done with rFSH.

In the EFP cycles, GnRH antagonist was added when at least one follicle measured 14mm, in the LP cycles it was administered from the beginning of the stimulation. Ovulation was triggered with GnRH agonist. Recipients of vitrified oocytes were primed with estradiol and progesterone.

Main results and the role of chance: The mean donors' age was 26.78 ± 2.95 years, the mean antral follicle count 22.11 ± 12.53 and the mean AMH 3.56 ± 2.16 ng/ml.

Comparing LP-cycles with EFP-cycles, no significant differences were observed for: total number of oocytes retrieved (22.56 ± 10.56 vs. 16.67 ± 6.65); number of MII oocytes (16.89 ± 7.52 vs. 14 ± 6.96); gonadotropin consumption (2147 ± 535 IU vs. 2261 ± 940 IU) and duration of stimulation (9.89 ± 1.26 vs. 10.44 ± 1.74 days).

The recipients' age was 44.0.9 ± 4.66 years. Eight recipients received LP-oocytes and 12 recipients received EFP-oocytes. There were no significant differences between both groups in terms of: fertilization rate (76.5% vs. 77.3%); number of embryos transferred (1.67 ± 0.65 vs. 1.50 ± 0.53), and embryo quality. Twelve clinical pregnancies were obtained. When comparing LP-recipients vs. EFP-recipients, no significant differences were noted in pregnancy rates (58.3% vs. 62.5%) (n.s.).

Limitations, reason for caution: Our results are limited by the small sample size.

Findings of this study correspond to a sample of oocyte donors <35 years old, with good ovarian reserve, and under this particular stimulation protocol and their corresponding recipients.

Wider implications of the findings: Ovarian stimulation with gonadotropins and GnRH antagonists yielded, in the same oocyte donor, similar mature oocyte numbers regardless of the phase in which the stimulation begun (LP vs. EFP), confirming that the LP stimulation is a useful strategy not only for fertility preservation issues but perhaps also for oocyte donation programs.

Pregnancy rates obtained from vitrified oocytes after LP stimulation in the most similar situation to that of cancer patients are encouraging. Further studies are needed.

Study funding/competing interest(s): Funding by national/international organization(s), Grant from "Merck Serono de Investigación 2012".

Trial registration number: NCT 01645241.

O-059 Antral follicle priming prior to ICSI in confirmed low responders: a randomized clinical trial (FOLLPRIM)

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Study question: Does follicular priming prior to controlled ovarian hyperstimulation (COH) improve the number of mature (MII) oocytes retrieved in low responders? Which of the following is the best priming treatment: testosterone, estradiol or combined estrogens/progestins? What are the underlying molecular mechanisms mediating the effect of priming on the follicles?

Summary answer: Follicular priming with transdermal estradiol increases significantly the number of retrieved MII oocytes in previously diagnosed low responders but this phenomenon is not associated with increased expression of FSH-R and/or A-R in granulose and cumulus cells. No priming strategy was significantly superior over the others.

What is known already: Follicular asynchrony has been proposed as one of the mechanisms leading to low response after COH. Marked follicular size discrepancies imply that an important number of follicles undergo unsatisfactory maturation. Several studies suggest that follicular priming with estradiol, testosterone or progestins during the luteal phase of the previous cycle may synchronize follicular growth and improve follicular milieu, and hence, increase the amount of mature oocytes retrieved. Nevertheless there are no clinical trials comparing such approaches.

Study design, size, duration: Unicentric, randomized, parallel, open-label, controlled trial, in two phases. Potential low responders (n = 96) underwent a first ICSI cycle between 2011–2013. Confirmed low responders in phase1 (n = 66) were randomized to different priming protocols prior to a new ICSI: transdermal testosterone −20 µg/kg/day (T), transdermal estradiol −200 µg/day (E) or combined oral estradiol −4 mg/day + progestins −150 µg desogestrel + 30 µg ethinylestradiol/day (C).

Participants/materials, setting, methods: Setting: University-based IVF Unit.

Patients-phase1: (≥2 criteria) maternal age ≥38, antral follicle count ≤6, basal FSH ≥10 mUI/mL and AMH ≤5 pM.

Patients-phase2: (no pregnancy and ≥1 criteria) ≤ 4 follicles >16 mm, E2 ≤500 pg/mL or ≤4 mature oocytes.

Methods: Ovarian response parameters, expression of FSH-R, A-R and steroidogenesis enzymes in granulosa cells were compared using flow cytometry and RT-PCR.

Main results and the role of chance: Analysis was performed in an intention-to-treat basis. All intervention groups were comparable regarding demographic characteristics. The mean number of follicles >16 mm the day of ovulation induction was higher in the E group (E:3.8 ± 2.0; T:2.4 ± 1.0; C:2.3 ± 1.3; p = 0.012) although the number of retrieved MII oocytes didn't differ between groups. Pretreatment with estradiol suppressed basal FSH more intensively than other priming treatments (E:4.9 ± 4.2; T:10.3 ± 4.1; C:11.1 ± 7.5; p = 0.001) and the duration of stimulation was longer in this group (T:9.4 ± 2.1; E:11.1 ± 2.5; C:9.4 ± 2.2; p = 0.045). Primed cycles yielded higher numbers of MII oocytes retrieved in all groups as compared to previous non-primed cycles, but only pretreatment with estradiol reached the significance level (E:2.75 ± 1.69 vs 1.56 ± 1.21; p = 0.029; T:2.31 ± 2.15 vs 1.81 ± 1.56-n.s.; C: 2.08 ± 1.26 vs 1.92 ± 1.26-n.s.).

When comparing priming vs non-priming, testosterone treatment increased A-R gene expression (T:14.92 ± 2.81; non-T:13.26 ± 2.88; p = 0.047) and priming with combined estrogens and progestins increased the percentage of granulosa cell expressing FSH-R (C:123.4 ± 98.9; non-C:69.6 ± 46.1; p = 0.029) but no differences were found between different priming strategies.

Limitations, reason for caution: Despite the increased number of MII oocytes retrieved after estradiol priming, caution must be pay since such increase (about 1 oocyte) seems to be of little clinical relevance. The present study was not design to detect pregnancy or live birth rate differences.

Wider implications of the findings: The results of this randomized controlled study questions the clinical utility of follicular priming in low responders, regardless of the treatment employed. Only differences of modest clinical relevance could be found when comparing primed vs non-primed groups.

Study funding/competing interest(s): Funding by hospital/clinic(s), This study was partially founded by the Valencian Health Board (Grant number: AP-199/10).

Trial registration number: ClinicalTrial.gov Identifier: NCT01310647.

O-060 Is the current dosing guidance for corifollitropin alfa, based on body weight and age, able to optimize ovarian response to controlled ovarian stimulation?

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Study question: Can we confirm that the optimal dosing regimen for corifollitropin alfa for different age and body weight groups has been provided, based on an evaluation of drug exposure and ovarian response?

Summary answer: The recommended dose of corifollitropin alfa based on body weight and age: 100 µg for women weighing ≤60 kg aged ≤36 years; 150 µg for women weighing >60 kg aged ≤36 years or women ≥50 kg aged >36 years, is appropriate. Body weight and age should be considered for dosing.

What is known already: Body weight is the major determinant of corifollitropin alfa exposure. Exposure after a single subcutaneous injection is similar after administration of 100 µg and 150 µg to women with body weight ≤60 kg and >60 kg, respectively. Clinical data were previously available in women aged ≤36 years (phase 3 studies Ensure and Engage). Data from women aged 35–42 years are now available based on the most recent phase 3 study, Pursue.

Study design, size, duration: Three randomized, active-controlled phase 3 studies with corifollitropin alfa were conducted: Ensure (100 µg, age ≤36 years, body weight ≤60 kg), Engage (150 µg, age ≤36 years, body weight >60 kg), and Pursue (150 µg, age 35–42 years, body weight ≥50 kg).

Participants/materials, setting, methods: 1715 subjects were treated with a single subcutaneous dose of 100 µg or 150 µg corifollitropin alfa. Serum corifollitropin alfa levels, follicular development, and cycle cancellation were assessed to monitor ovarian response and were compared by age and body weight.

Main results and the role of chance: A cross-study evaluation demonstrated that subtherapeutic exposure resulted in an increased risk of cycle cancellation due to insufficient ovarian response. The lower boundary of the target

AUC range is approximately 350 ng h/mL. Corifollitropin alfa exposure was above this lower boundary after treatment with the recommended doses and resulted in adequate follicular growth. In women ≤36 years, exposure and follicular response were similar in women weighing ≤60 kg (100 µg, AUC 599 ng h/mL; follicles ≥11 mm, 14.9) or >60 kg (150 µg, AUC 678 ng h/mL; follicles ≥11 mm, 16.0). In women aged 35–42 years, weighing ≥50 kg, 150 µg resulted in an adequate follicular response. Mean AUC was 677 ng h/mL and the mean number of follicles ≥11 mm was 11.9 on the day of hCG administration.

Limitations, reason for caution: A lower boundary of the target AUC range was defined, but an upper limit of the target AUC range has not been established. Therefore, the recommended dose of corifollitropin alfa should be used and dose increases are not indicated in case of insufficient ovarian response.

Wider implications of the findings: Presented results slightly modify the basis for the recommended dosing regimen of corifollitropin alfa. Prior to the conduct of the Pursue trial, the recommended dose for corifollitropin was based on body weight only. The results from Pursue support a dosing guidance based on body weight as well as age.

Study funding/competing interest(s): Funding by commercial/corporate company(ies), Financial support for this study was provided by Merck & Co., Inc., Whitehouse Station, NJ, USA.

Trial registration number: NCT00702845, NCT00696800, NCT01144416.

O-061 Measurement of endogenous LH surge after GnRH agonist trigger in GnRH antagonist cycles to predict trigger failure

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Study question: What is the endogenous LH response to a GnRH agonist (GnRHa) trigger in antagonist cycles?

Summary answer: The LH surge obtained in response to GnRH agonist administration in GnRH antagonist cycles mimics the physiological surge observed in natural cycles.

What is known already: We have previously shown that GnRH antagonist cycles triggered by a GnRH agonist have a trigger failure rate of 1% in egg donors and 3.4% in patients (ESHRE 2013). A correlation between the LH peak and the oocyte yield has been reported (Hum Reprod 2013;28:152–159). The present study aims to investigate the characteristics of the post-GnRH agonist trigger LH surge, the percentage of failed trigger and criteria that may predict trigger failure.

Study design, size, duration: Prospective observational study of 207 consecutive oocyte donors and 73 consecutive infertility patients undergoing ovarian stimulation with an antagonist protocol and GnRHa trigger in a single private IVF program from 11/2012 to 1/2014. The trigger failure rate was determined and the role of LH and progesterone values was investigated.

Participants/materials, setting, methods: Two boluses of 4 mg leuprolide acetate were administered 36 and 26 h before egg retrieval. Serum LH and progesterone were measured before and 10 h after GnRHa administration. A subgroup of 94 oocyte donors had five LH determinations, before and 1, 10, 11, and 35 h after trigger.

Main results and the role of chance: There were 2 trigger failures in each group (rate 2.7% in patients and 1% in donors). Mean LH levels in subjects with trigger failure were significantly lower than in those who responded appropriately [patients: 3.6 (±1.8) vs 52.7 (±21.8), $p = 0.0023$; donors 4.3 (±2.5) vs 50.6 (±26.8), $p = 0.0158$]. For patients, trigger failure occurred with LH below 5 mIU/ml and all other values were above 13 mIU/ml. In donors some overlapping of values was observed: failures occurred below 6.2 mIU/ml, but one case with successful egg retrieval occurred with a 4.9 value one at 6.4 and one at 8.4, whereas all other values were above 10 mIU/ml. There were no trigger failures with LH levels above 10 mIU/ml. There were no significant differences for progesterone levels.

Limitations, reason for caution: Due to the small number of trigger failures, it was not possible to determine a clear-cut post-trigger LH cutoff level for the prediction of failed trigger.

Wider implications of the findings: GnRH agonist trigger is a good strategy for the induction of final oocyte maturation in patients at risk for ovarian hyperstimulation syndrome and oocyte donors. The LH surge after trigger mimics the endogenous LH peak. A value of LH below 10 mUI/ml at 10 h post-trigger can alert clinicians towards a possible trigger failure. Progesterone levels do not seem to be helpful.

Study funding/competing interest(s): Funding by hospital/clinic(s), Centro de Asistencia a la Reproducción Humana de Canarias. There are not conflict of interest.

Trial registration number: None.

O-062 The predictive accuracy of anti-Müllerian hormone for live birth after assisted conception: a systematic review and meta-analysis of the literature

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Study question: Can baseline serum anti-Müllerian hormone (AMH) predict live-birth after IVF/ICSI?

Summary answer: AMH, independently of age or assay used, is positively associated with live birth after assisted conception; however, its predictive accuracy is poor.

What is known already: AMH is an established marker of ovarian reserve, a good predictor of poor or excessive ovarian response after controlled hyperstimulation but is weakly associated with pregnancy following fertility treatment. However, it is unclear whether it can predict the ultimate outcome of assisted conception, live birth.

Study design, size, duration: We undertook a systematic review and meta-analysis of the literature published until August 2013. PubMed, Embase, Medline, Web of Knowledge and the Cochrane trial register and unpublished literature were searched. The study was conducted according to the PRISMA guidelines.

Participants/materials, setting, methods: Studies fulfilling the eligibility criteria were included. A summary diagnostic odds ratio (DOR) was estimated by the random effects model. A hierarchical summary receiver operator characteristic (HSROC) model provided pooled estimates before and after adjusting for age and AMH assay.

Main results and the role of chance: Out of 361 non duplicate studies, 47 were selected; 17 met the eligibility criteria and 13 had extractable data thus were included in the meta-analysis. Three out of the 13 studies included only women with expected low ovarian reserve and were analysed individually from the remaining 10 to minimise heterogeneity. The DOR for women with unknown ovarian reserve (n = 5,764 women) was 2.39 (95% CI: 1.85–3.08). After adjustment for age the DOR was little changed at 2.48 (95% CI: 1.81–3.22) and the DOR adjusted for AMH assay was almost identical at 2.42 (95% CI: 1.86–3.14). For women with expected low ovarian reserve (n = 542 women) the DOR was 4.63 (95% CI: 2.75–7.81).

Limitations, reason for caution: Although the process of systematic literature review and meta-analysis is a robust way of generating a more powerful estimate of true-effect size with less random error than individual studies, it does have limitations and the inferences assumed by the data are subject to the limitations of the primary studies.

Wider implications of the findings: The diagnostic accuracy of AMH in live birth is poor and should not be used to alter clinical decisions; however, AMH should be considered as an alternative covariate when developing future prediction models or it may be helpful when counselling couples before undergoing fertility treatment.

Study funding/competing interest(s): Funding by University(ies), No external funding received.

Trial registration number: N/A.

SELECTED ORAL COMMUNICATION SESSION

SESSION 17: ART: ECONOMICS AND EPIDEMIOLOGY

Monday 30 June 2014 **15:15 - 16:30**

O-063 Funding source and outcomes of clinical trials in reproductive medicine

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Study question: Does commercial funding influence reported outcome measures and trial results in reproductive medicine?
Summary answer: The funding source is reported in only a minority of RCTs. Commercially funded trials report more often on surrogate primary outcomes, but do not generate more positive results than non-commercially funded trials.
What is known already: Clinical research is increasingly sponsored by pharmaceutical companies and companies supplying medical devices. There are several potential ways that sponsoring by industry can influence the outcome of a study. Results of commercially funded studies in other fields, are known to report more often on surrogate outcomes and to report more positive results in favor of the product or device of the funding company, when compared with studies with other sources of – or no funding.
Study design, size, duration: Until present, the role of funding in RCTs in reproductive medicine is unknown. We conducted a systematic sample search. The search was restricted to RCTs on IVF/ICSI treatments published in 1999/2000, 2004/2005 and 2009/2010 in five specialty journals and five high impact general medicine journals. We included 208 RCTs.
Participants/materials, setting, methods: Per included RCT data on funding source, primary outcome measure (surrogate or hard), trial result (positive or indifferent), treatment topic (stimulation regimens, laboratory techniques, transfer protocols) were extracted. We used contingency table analysis and linear-by-linear association to test the association with type of funding.
Main results and the role of chance: Overall, only 31% (64/208) of the RCTs reported funding of which 45% (29/64) were commercially funded. Commercially funded RCTs more often used a surrogate primary outcome compared to non-commercially funded RCTs (72% vs 50%, OR 2.6, 95% CI 0.80 to 8.8). Commercially funded trials did not generate more often positive results than non-commercially funded trials (59% vs 63%, OR 0.85, 95% CI 0.26–2.6). Commercially funded RCTs reported more on ovarian stimulation regimens, compared to non-commercially funded RCTs (OR 3.8, 95% CI 1.0–11.4). The reporting on funding did not increase over time (*p*-value 0.25).
Limitations, reason for caution: Selection bias might be present since we conducted a sample search. Due to the low percentage of trials reporting on their funding source, it was difficult to assess the differences in outcome between commercially funded trials and non-commercial funded trials accurately.
Wider implications of the findings: Reporting on funding should be obligatory, so that the reader can assess commercial influences and biases. The consort statement on outcome measures should be adapted to better suit the aim in reproductive medicine, i.e., the birth of a healthy child.
Study funding/competing interest(s): Funding by University(ies), Academic Medical Research Center, Amsterdam.
Trial registration number: None.

O-064 Long-term relationship of ovulation-stimulating drugs to breast and gynecologic cancers

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Study question: Do fertility drugs increase the long-term risk of breast and gynecologic cancers?
Summary answer: In a large 30 years follow-up study of infertility patients we found little evidence that fertility drug use led to cancer risk increases. However, higher risks associated with clomiphene use were seen for breast cancer among heavily exposed women (12+ cycles) and for ovarian cancer among women who remained nulligravid.
What is known already: Fertility drugs stimulate ovulation induction and raise estradiol and progesterone levels, however, their effects on breast and gynecologic cancer risk remain unresolved. Most previous studies on fertility drug use involved small numbers with relatively short follow-up periods and inability to control for other cancer predictors, including indications for drug usage, which could independently affect cancer risk (e.g. anovulation, endometriosis).
Study design, size, duration: Retrospective cohort design involving 12,193 women evaluated and treated for infertility between 1965–1988 at 5 US sites, followed-up until 2010 with patient questionnaires and through linkages against the National Death Index and cancer registries.
Participants/materials, setting, methods: A total of 9,892 women were successfully followed for cancer outcomes. Cox regression determined hazard ratios (HR) and 95% confidence intervals (CI) associated with fertility drug use after adjustment for cancer risk factors and causes of infertility.
Main results and the role of chance: During almost 30 years of follow-up, we identified 749 breast, 119 endometrial and 85 ovarian cancers. Ever use of clomiphene (40% of infertile women) was not associated with breast cancer risk (adjusted HR = 1.04, 95% CI 0.90–1.21). However, patients who received 12 or more cycles of clomiphene were at increased risk, particularly for medically validated invasive breast cancers (1.69, 1.16–2.45). Clomiphene use was not significantly associated with either endometrial (1.41, 0.98–2.04) or ovarian (1.34, 0.86–2.07) cancers, even when multiple exposure cycles were involved. Clomiphene use was associated with higher ovarian cancer risks among women who remained nulligravid compared to those who conceived (respective HRs and 95% CIs = 3.63, 1.36–9.72 vs. 0.88, 0.47–1.63; *p*-interaction = 0.001). Gonadotropin use was unrelated to cancer risk, although relatively few women (10%) were exposed.
Limitations, reason for caution: Limitations of this study include inability to follow all women in the cohort and to obtain complete risk factor information, including hysterectomy/oophorectomy status. We were also limited by small sample sizes in our subgroup analyses.
Wider implications of the findings: Overall, our findings do not support a strong relationship between fertility drug use and breast or gynecologic cancers. However, the association between fertility drug use and cancers should continue to be monitored due to the relatively young age of our study population and the later peak incidence of most of these cancers.
Study funding/competing interest(s): Funding by national/international organization(s), This project was supported in part by funds from the intramural research program of the National Cancer Institute, National Institutes of Health. None of the authors has any conflicting interests to declare.
Trial registration number: Not applicable.

O-065 Ovarian response to stimulation and obstetric outcomes: an analysis of 89,202 IVF birth outcomes

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Study question: Is there a relationship between ovarian reserve, quantified as ovarian response to stimulation, and obstetric outcomes of gestational age at delivery and birth weight following IVF treatment.
Summary answer: There was a significantly increased incidence of preterm and extreme pre-term births (<37 weeks and <32 & 32–36 weeks gestation respectively) and an increased occurrence of low birth weight (<2500 g) neonates among women with a hyper response (≥15 oocytes retrieved) following ovarian stimulation.
What is known already: Pregnancies resulting from assisted reproductive techniques are associated with a higher risk of pregnancy complications compared to natural conception. Whether ovarian ageing in poor responders is associated

with an increased risk of adverse obstetric outcomes is debated. It is also unclear if ovarian dysfunction has an association with adverse obstetric outcomes.
Study design, size, duration: Observational study using anonymized data on all IVF cycles performed in the UK from April 1991 to June 2008. Data from 402,185 IVF cycles and 89,202 birth outcomes were analysed.
Participants/materials, setting, methods: Data on all women undergoing a stimulated fresh IVF cycle with at least one oocyte retrieved between the year 1991 to June 2008 were analysed for birth outcomes. Logistic regression analysis of the association between ovarian response and outcomes of gestational age at delivery and birth weight were performed which was adjusted for confounders like multiple pregnancies.
Main results and the role of chance: Live birth rates in poor responders, normal responders and hyper responders were 9%, 23% and 30% respectively. There were significantly increased risks of adverse outcomes among women with hyper response compared to normal responders (OR 1.10; 95% CI: 1.03, 1.17, $p = 0.003$) for pre-term birth, (OR 1.28, 95% CI: 1.12, 1.46; $p < 0.001$) for extreme pre-term birth and OR 1.08, 95% CI: 1.02, 1.16; $p = 0.01$ for low birth weight neonates). There was no significant difference in the risk of preterm, extreme pre-term births and low birth weight neonates in poor versus normal responders (OR 1.12; 95% CI: 0.97, 1.29; $p = 0.11$, OR 1.03, 95% CI: 0.78, 1.35; $p = 0.85$ and OR 1.10, 95% CI: 0.97, 1.25; $p = 0.14$ respectively).
Limitations, reason for caution: The dataset contained no information on confounders such as the medical history of the women to allow adjustment.
Wider implications of the findings: Analysis of this extensive dataset suggests that hyper responders have a higher risk pre-term births and low birth weight babies. These findings lead to speculation whether ovarian dysfunction and/ or an altered endometrial milieu at the time of embryo implantation resulting from supraphysiological oestradiol levels underlie the unfavourable outcomes among hyperresponders and warrant further research.
Study funding/competing interest(s): Funding by hospital/clinic(s), Not applicable.
Trial registration number: Not applicable.

O-066 Increased risk of psychiatric disorders in children born to women with fertility problems: results from a large Danish population-based cohort study

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Study question: To examine whether children born to women with fertility problems have a higher risk of psychiatric disorders compared to children born to women without fertility problems, i.e. naturally conceived children.
Summary answer: The results suggest that children born to women with fertility problems have modest, increased risks for psychiatric disorders. The risk increase was observed both among children aged 0–19 years and among children in young adulthood (≥20 years), indicating that the increased risk of psychiatric disorders persist into adulthood.
What is known already: Only a few studies have investigated the risk of psychiatric disorders among children born after fertility treatment. Although results from most of these studies do not find an increased risk of psychiatric disorders, the results show substantial variation, which may be due to limited size and follow-up time in most studies. The present study will be the first with an adequate long follow-up period that enables assessment of risk patterns also in young adulthood.
Study design, size, duration: This retrospective register-based cohort study included all 2,430,827 children born in Denmark between 1969 and 2006. A total of 124,384 children (5%) were born to women with fertility problems and 2,306,443 children (95%) were born to women without fertility problems. All children were followed up for psychiatric disorders through 2009.
Participants/materials, setting, methods: Maternal fertility status was obtained by linkage to a Danish infertility cohort including virtually all women with fertility problems in Denmark in 1969–2006. Psychiatric disorders were identified in the Danish Psychiatric Central Registry. Cox regression models were applied to estimate hazard ratios for overall and specific groups of psychiatric disorders.
Main results and the role of chance: A total of 170,311 children were hospitalized for a psychiatric disorder during a median follow-up period of 20 years (range 0–41 years). Children born to women with fertility problems had modest, but significantly increased risks of all psychiatric disorders (HR 1.30, 95% confidence interval 1.27–1.33), and for a number of specific groups of psychiatric disorders, including schizophrenia and psychoses (1.25, 1.15–1.36), affective disorders (1.27, 1.20–1.34), anxiety (1.33, 1.28–1.38), eating disorders (1.10, 1.01–1.21), mental retardation (1.26, 1.15–1.38), mental development disorders including autism spectrum disorders (1.19, 1.13–1.25), and behavioural and emotional disorders (1.37, 1.31–1.43), compared with naturally conceived children. When the analyses were performed separately for psychiatric disorders diagnosed during childhood (0–19 years) or in young adulthood (≥20 years), the risk estimates were not markedly changed, indicating that the increased risks observed for psychiatric disorders persists into adulthood.
Limitations, reason for caution: As only severe psychiatric conditions were included, the true incidence of psychiatric disorders is likely to be somewhat underestimated. Another limitation was that it could not be determined whether the observed increased risks were caused by factors related to the underlying maternal infertility or due to fertility treatment procedures.
Wider implications of the findings: Clinicians and other healthcare personnel involved in diagnosis and treatment of women with fertility problems should be aware of the small, but potentially increased risk of psychiatric disorders among the children born to women with fertility problems. Knowledge about potential adverse health effects associated with fertility treatment should always be balanced against the physical and psychological benefits of a pregnancy.
Study funding/competing interest(s): Funding by national/international organization(s), The study was supported by the Danish Cancer Society.
Trial registration number: None.

O-067 Long-term (18-year) cost-effectiveness of single and double embryo transfer strategies

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Study question: What is the short-term (1-year), intermediate-term (5-year) and long-term (18-year) cost-effectiveness of single embryo transfer (SET) versus double embryo transfer (DET) strategies from a societal perspective?
Summary answer: Short-term (1-year) results indicate that it is cost-effective to replace double embryo transfer with single embryo transfer. However, when intermediate-term (5-year) and long-term (18-years) costs and outcomes are incorporated, it is not cost-effective to replace double embryo transfer strategies with single embryo strategies.

What is known already: According to (short-term) cost-effectiveness research into embryo transfer strategies, DET is both more expensive and more effective than SET. DET is considered cost-effective if society is willing to pay around €20,000 for an extra live birth. However, interpretation of current cost-effectiveness studies comparing embryo transfer strategies is complicated, as these studies fail to incorporate long-term costs and outcomes in their studies and use live-births as outcome measure instead of quality-adjusted life years (QALYs).

Study design, size, duration: a Markov model (cycle length: 1-year; time horizon: 18-years) was developed comparing three IVF cycles of: (1) eSET in all patients; (2) standard treatment policy (STP), i.e. eSET in women <38 years with a good quality embryo, and DET in all other women; and (3) DET in all patients.

Participants/materials, setting, methods: Expected life years, QALYs and costs were estimated for all comparators. Input parameters were derived from a retrospective cohort study, in which hospital resource data were collected (n = 580) and a parental questionnaire was send out (431 respondents). Probabilistic sensitivity analysis (5000 iterations of 5000 couples starting IVF treatment) was performed.

Main results and the role of chance: With a time-horizon of 18-years, 3xDET is most effective (0.55 live births of which 19.8% multiples and 80.2% singletons, 10.3 life years, 9.9 QALYs) and expensive (€33,745) per couple starting IVF. 3xeSET is least effective (0.44 live births of which 3.8% multiples and 96.2% singletons, 7.2 life years and 6.8 QALYs) and expensive (€23,821). Each multiple child generates significantly less QALYs (?0.17, 95%CI ?0.32–?0.02) and higher costs (€37,827, 95%CI 24,024–54,417) than singletons. We assumed that society is willing to pay €20,000 per QALY gained. With a time-horizon of 1-year, 3xeSET was the most cost-effective embryo transfer strategy with a probability of being cost-effective of 100%. With a time horizon of 5- or 18-years, 3xDET was most cost-effective, with probabilities of being cost-effective of 81.9% and 95.4%.

Limitations, reason for caution: A limitation is that treatment ends when it results in live birth and that only child QALYs were considered. Additional sensitivity analyses need to confirm the model's robustness. Results were robust for applying a hospital perspective instead of a societal perspective, and for using life years gained instead of QALYs.

Wider implications of the findings: Three-cycle DET is the preferred strategy from a cost-effectiveness point of view when adopting a time horizon of 18 years. The results were robust for applying a hospital perspective instead of a societal perspective, and for using life years gained instead of QALYs as measure of effectiveness.

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

Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATION SESSION

SESSION 18: QUALITY WITHIN THE LABORATORY

Monday 30 June 2014 **15:15 - 16:30**

O-068 To err is human, even in IVF: A review of non-conformances/ errors in 31,715 *in vitro* fertilization (IVF) treatment cycles

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Study question: Over the last 35 years IVF has grown more complex with the adoption of highly complex laboratory procedures. The aim of this paper was to interrogate a large non-conformance ISO database spanning over 10 years and answer: What types of errors are made, how often and how to minimize errors?

Summary answer: This report presents novel data demonstrating that IVF laboratories do well regarding errors compared to other medical laboratories. Implementing a quality management system throughout an organization allows one to identify and track errors, and have a very tight system to resolve them when they occur.

What is known already: Medical errors are not uncommon and are an unfortunate occurrence in medicine. In IVF, the publicized mistakes are the serious events where gametes or embryos are mixed. The Human Fertilization and Embryology Authority (HFEA) from the UK has published reports on the number of incidents involving IVF clinics and some studies exist looking at Ovarian Hyperstimulation Syndrome (OHSS) cases in particular. This has led to the implementation of mandatory witnessing procedures. Although errors occur, no baseline metrics exist for the IVF laboratory.

Study design, size, duration: The non-conformances from Andrology and Embryology between March, 2003 and November, 2013 were reviewed. The total number of egg retrievals was 25,764 and the number of embryo thaw cycles was 5,951. We also estimated the total number of major procedures (N = 128,415) during the study period, including: retrievals, cryopreservation, ICSI, PGS, etc.

Participants/materials, setting, methods: Reports were graded based on the impact on an IVF cycle. Briefly, Minimal: A problem not measurably decreasing likelihood of success, Moderate: A problem negatively affecting a cycle but not to the extent that it is lost. Significant: Loss of a cycle due to loss/mishandling of gametes or embryos. Major: Systemic problems affecting multiple patients.

Main results and the role of chance: In the reports 293 non-conformances for Andrology/Embryology were reported and classified as Minimal (N = 219: 74.7%), Moderate (N = 58: 19.8%), Significant (N = 16: 5.5%) and Major (N = 0:0%). Moderate and significant errors were dominated by human error and equipment malfunctions. The most error prone procedure was cryopreservation, representing 31% of the moderate and significant errors. The overall error rates of moderate and significant errors per procedure and per cycle were 0.05% and 0.18%, respectively during the 10.66 years period. Combining moderate and significant errors, resulted in error rates of approximately 1 per 1,735 procedures and 1 per 429 cycles. Of the significant errors only, the rates were 1 per 8,026 procedures and 1 per 1,982 cycles. This compares very favorably with other areas of medicine where the risk of adverse events due to laboratory errors ranges from 2.7 to 12%.

Limitations, reason for caution: A comparison of errors with our results is difficult because the different classifications may be subject to individual interpretation. The limited reports examining errors in IVF have also included cases of OHSS, whereas ours has largely focused on laboratory procedures. This definitely highlights the need to have universal definitions specifically related to IVF cases.

Wider implications of the findings: We propose that all IVF Centers implement a system to define and track their errors no matter how trivial they may be, so that we can continually improve laboratory procedures to keep errors to the lowest possible frequency. It will never be perfect, but we should strive to get as close to perfect as we can. All human endeavors are associated with an error rate and IVF laboratories should encourage transparency and honesty with regard to collecting and analyzing their errors.

Study funding/competing interest(s): Funding by hospital/clinic(s), Self Funded.

Trial registration number: None.

O-069 Epigenetic evaluation on chemical substances contamination in IVF culture media

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Study question: Although more than 50,000,000 chemical substances exist in the global environment, the exact levels of exposure to these toxic agents and their effects on reproduction, including *in vitro* fertilization, and other physiological processes remain unknown.

Summary answer: Several environmental chemical substances at doses detectable in fetomaternal specimens and IVF media induced significant alterations in the epigenetic profile using mouse ES cells and human iPS cells system.

What is known already: Many studies have reported chemically induced birth defects, however little has been discussed concerning the reproductive hazards posed by these chemicals with regard to assisted reproductive technology (ART) medicine, especially the contamination of culture medium by these chemicals.

Study design, size, duration: Sensitive and specific quantitation methods have been developed for the measurements of several candidate chemicals. After measurements of the chemical contaminations in fetomaternal and IVF culture media specimens, health hazards in the next generation were investigated by analysing epigenetic alteration at doses detected using mouse ES and human iPS cells.

Participants/materials, setting, methods: Three hundreds and fifty fetomaternal samples, including maternal and cord bloods, amniotic fluids and 120 various IVF culture media were collected for the measurement of phthalates (DEHP, MEHP) and polybrominated diphenyl ethers. Possible health hazards of these chemicals were investigated by analyzing epigenetic profile alterations.
Main results and the role of chance: The concentrations of these 2 chemical substances in the fetomaternal environment, as detected using newly developed and highly sensitive and specific chromatographic and spectrometric assay system, were in the ranges between 1 and 10 ppb levels. Epigenetic indicators, such as DNA methylation, histone modification, and heterochromatin/euchromatin, could real dynamic changes in cellular conditions in embryonic stem cells established from the mouse inner-cell mass. One ppb of MEHP and deca-brominated diphenyl ethers (decaPBDEs) were capable causing significant epigenetic changes.

Ten to 100 times higher amounts of MEHP and PBDEs as compared to those in the fetomaternal specimens were detected in some of the IVF media.
Limitations, reason for caution: Phthalates, popular plasticizers, have some potential reproductive toxicity in both animals and human.

PBDEs are a group of flame retardants and can induce thyroid dysfunction and adverse effect on human fertility.
Wider implications of the findings: The Center for devices and Radiological Health, a branch of US FDA, has recommended considering alternatives of phthalates, when high-risk procedures, such as transfusion and hemodialysis are to be performed on pregnant women carrying a male fetus. Several PBDEs, because of their various toxicities, have been banned and restricted internationally as part of the new Persistent Organic Pollutants under the Stockholm Convention on Persistent Organic Pollutants (UNEP, 2009).
Study funding/competing interest(s): Funding by national/international organization(s), The Ministry of Health, Labour and Welfare, Japan.
Trial registration number: None.

O-070 Volatile organic compounds (VOCs) in the IVF laboratory: presence and concentrations in ambient air, sources of emission and effects of laboratory relocation to new premises.

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Study question: Which VOCs are detectable in indoor ambient air in a long-established IVF laboratory? What are the concentrations of VOCs? Is there a difference in types and concentrations between indoor ambient air and air inside the incubators? Does relocating an IVF laboratory to new facilities affect VOC presence and concentrations?
Summary answer: In ambient air a limited number of VOC was detected, mostly alcohols. In a new facility with same laboratory equipment but overpressure system the total VOC load was reduced, but the alcohol concentrations were increased. Remarkably, inside incubators more VOCs and higher concentrations were detected than in indoor ambient air.
What is known already: VOCs may be emitted from various sources in the IVF-laboratory setting. Some VOCs have been identified as potentially genotoxic or mutagenic in contexts other than *in vitro* fertilization. Exposure of human embryos *in vitro* to VOCs may have harmful consequences on both reproductive potential of the embryo as well as long term child health. However, as yet little is known about concentrations and sources of VOCs commonly detected in IVF laboratories.
Study design, size, duration: Prospective observational study with systematic VOC assessment before and after relocation of the IVF-laboratory of a University Infertility Center in Germany. Short-term measurements during laboratory operation with samples of five liters in compliance with international standards. Relocation with same equipment into new premises (new furniture, flooring, air conditioning etc.).
Participants/materials, setting, methods: VOCs in indoor ambient air were determined with Tenax® (Dräger-Germany) thermal desorption tubes using standard gas chromatography and mass spectrometry methods with lower limits of detection between 2 to 10 µg/m³. 69 different VOCs could simultaneously be determined. VOC-sources were investigated with a VOC specific probe (GrayWolf®-USA).
Main results and the role of chance: 65 of 69 VOCs tested in indoor ambient air before relocation were below detection limit. Four substances were significantly above detection limit, three alcohols [2-ethyl-1-hexanol, 2-propanol, and 1-propanol] and one aromatic VOC [m/p-xylene]. Two of these values were, according to relevant guidelines for indoor air quality for workplaces, above permitted levels. Following the relocation of the laboratory to newly built facilities with overpressure ventilation systems, two alcohols [2-propanol and 1-propanol] remained detectable above permitted levels. When examining the equipment and other appliances, in particular the interior of the incubator, 21 identifiable VOCs were present in noticeable concentrations above the ambient indoor air, such as alcohols (e.g. 1-butanol), aromatics (e.g. toluene, m/p-xylene), terpenes (e.g. alpha-pinene), aldehydes (e.g. n-hexanal), siloxanes (e.g. octamethylcyclotetrasiloxane) and esters (e.g. methyl methacrylate).
Limitations, reason for caution: The VOCs composition will differ between laboratories depending on equipment/agents, building, location and personnel turn-over. As yet, no thresholds have been defined above which a detrimental effect of individual VOCs or groups of VOCs can be expected. The present analysis serves as a basis for further investigations (embryo culture-systems).
Wider implications of the findings: Alcohols [1-propanol and 2-propanol] were identified in noticeable concentrations in all measurements. This could mainly be caused by exogenous factors, such as hygiene measures, i.e. traces of disinfectants. Since the detected concentrations are already above recommended thresholds for healthy people, it should be tested if these concentrations are also of damage for embryonic cells. The ability of alcohols to dissolve and permeate water and oil, respectively, may aggravate this problem.
Study funding/competing interest(s): Funding by University(ies), Universität zu Lübeck.
Trial registration number: N/A.

O-071 No difference in singleton birth weight according to the type of culture medium and according to the duration of *in vitro* culture

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Study question: Does the type of medium for *in vitro* culture and the duration of culture influence singleton birth weight after IVF/ICSI treatment?
Summary answer: The present study did not show any difference in mean singleton birth weight according to four different culture media. Day 5 blastocyst transfer resulted in a similar mean singleton birth weight as compared to day 3 embryo transfer.
What is known already: Previous studies have indicated that *in vitro* culture of human embryos may affect birth weight of live born singletons. Both the culture medium and the duration of culture might be implicated. However, few studies have confirmed an effect of culture medium and duration on birth weight and reports are conflicting.
Study design, size, duration: A large retrospective analysis was conducted, including all singleton live births after transfer of fresh day 3 or day 5 embryos resulting from IVF and ICSI cycles performed between March 2004 and February 2012.
Participants/materials, setting, methods: A total of 3154 singleton live births resulting from singleton pregnancies were considered for the analysis. Four different sequential embryo culture media were used: Cook (n = 158), Medicult (n = 1388), Sage (n = 898) and Vitrolife (n = 710). Maternal age, maternal and paternal BMI, maternal smoking, maternal parity, main cause of infertility, method of fertilization (IVF or ICSI), time in culture and number of embryos transferred were documented. Embryo transfers were performed either on day 3 (n = 1855) or on day 5 (n = 1299). Singleton birth weight was the primary outcome parameter. Other outcome parameters included gestational age, mode of delivery, and gender of the newborn.
Main results and the role of chance: No significant differences in mean singleton birth weight (±SE) were observed among the four culture media: Cook 3259g (±43), Medicult 3222g (±15), Sage 3281g (±19), and Vitrolife 3251g (±21) (P = 0.107). The mean singleton birth weight was not different between day 3 embryo transfers (3241 ± 13 g) and day 5 blastocyst transfers (3257 ± 16 g; P = 0.425). Multiple regression analysis controlling for maternal, paternal, treatment and newborn potential confounders confirmed the non-significant

differences in mean singleton birth weight among the four culture media. Likewise, the adjusted mean singleton birth weight was not different according to the culture duration ($P = 0.805$).

Limitations, reason for caution: Our study is limited by its retrospective design and the fact that the four different sequential culture systems were used in different time periods. However, no other significant changes were introduced in the laboratory procedures during the whole study period. One of the four culture media groups represents a rather small group. Pregnancy-associated factors possibly influencing birth weight (such as diabetes, hypertension, pre-eclampsia) were not included in the analysis.

Wider implications of the findings: The present large retrospective study does not confirm earlier concern that both the type of culture medium (smaller studies) and the duration of embryo culture could influence singleton birth weight. However, a continuous surveillance of human embryo culture procedures (medium type, culture duration and other culture conditions) seems warranted.

Study funding/competing interest(s): Funding by hospital/clinic(s), UZ Brussel.
Trial registration number: Not applicable.

O-072 Toxicity testing of decontaminating agents and cleaning products used in human IVF laboratories

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Study question: This study aimed to determine the safety of commonly used decontamination products used in an IVF centre by determining the release of volatile organic compounds (VOC) upon use and the biological effect upon clinically relevant indirect exposure to human sperm, mouse sperm and mouse embryos.

Summary answer: VOC release from commercially available cleaning and decontamination products are minimal compared to plain alcohol or ether. However, three out of the nine tested products reduced human and mouse sperm quality and one had a negative impact on embryo development.

What is known already: According to EU regulations, embryology laboratories should operate in a GMP grade A air quality (laminar flow cabinet) and D background. GMP classification is based on particle counts and microbiological load of the laboratory air and surfaces so stringent disinfection protocols are required. Disinfectants can generate VOCs who may harm gamete and embryo quality during handling, so they should be tested in a bioassay before application in an IVF clinic.

Study design, size, duration: Sixteen detergents and decontaminants were tested for VOC release. From these, nine where tested in three bioassays: human sperm survival test (SST) in two independent laboratories, mouse sperm motility assay (SMA), and mouse embryo assay (MEA).

Designs: 1. Inter-laboratory SST comparison; 2. Inter-species comparison of SMA/SST; 3. Comparison of SMA and MEA.

Participants/materials, setting, methods: VOCs were measured with a mini RAE 2000. Prepared sperm of human semen donors $n = (13)$ and mice (B6CbF1, $n = 59$), and zygotes of 26-day old mice (B6CbaF1, $n = 24$) were used for the bioassays. Motility was microscopically determined according to WHO criteria (laboratory1) and with the Autosperm analyser (laboratory 2).

Main results and the role of chance: High VOC values were measured from alcohols (1360–350 ppm), ether (5000 ppm) and an alcohol containing agent (432 ppm), while alcohol-free products showed low VOC release (3.5–0.1 ppm).

In both labs, the sperm tests revealed that 3 out of 9 tested products reduced human sperm motility below the respective test acceptance criteria upon indirect clinically relevant exposures. Mouse sperm exposure yielded the same results as human sperm in two independent test runs. As both labs and both species demonstrated comparable test results, the three positive products should be considered toxic for sperm and avoided in an IVF environment. In the mouse embryo assay, only 1 out of 9 products revealed toxicity, manifested in a decrease or complete absence of blastocyst formation on day 5.

Limitations, reason for caution: Sperm and embryos show a different sensitivity to (cleaning) products, indicating that a single assay for reprotoxicity determination is unreliable. Correct test assay selection is critical and should be directed to the application domain of the test product.

Wider implications of the findings: Considerations should be taken when selecting cleaning products and decontaminating agents for use in human IVF centres as they may have a negative impact on the outcome of the fertility treatment. This study revealed that mouse sperm has the same sensitivity as human sperm to toxic agents, and can be considered as a good alternative to human sperm in bioassays for toxicity screening.

Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by commercial/corporate company(ies), Centre for Reproductive Medicine, UZ Brussel, Brussel, Belgium, EggCentris BVBA, Anderlecht, Belgium.
Trial registration number: N/A.

SELECTED ORAL COMMUNICATION SESSION

SESSION 19: NEW EVIDENCE IN EARLY PREGNANCY OUTCOME

Monday 30 June 2014 **15:15 - 16:30**

O-073 A randomised double-blind placebo-controlled trial of intravenous immunoglobulin in the treatment of secondary recurrent miscarriage

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Study question: Do infusions of intravenous immunoglobulin (IvIg) during the first trimester of pregnancy increase the chance of live birth in women with secondary recurrent miscarriage (RM)?

Summary answer: Results regarding the efficacy of IvIg treatment of women with secondary RM will be given in June 2014 after breakage of the trial randomisation code.

What is known already: The protocols of the 8 randomised placebo-controlled of IvIg for the treatment of RM published so far have been very heterogeneous and the results very divergent. Two meta-analyses of all the published randomised trials suggest that IvIg may be efficient in secondary but not in primary RM.

Study design, size, duration: The trial was randomised, placebo-controlled and double-blinded. Fourty-one patients received up to 8 infusions of IvIg (24–36 g immunoglobulin CSL Behring® or Privigen®) and 41 patients placebo (human albumin) from gestational week 4 to the time of miscarriage or week 15. Infusions were given between 2008 and October 2013.

Participants/materials, setting, methods: Participants were women with secondary RM with ≥3 miscarriages after a live birth and a total of ≥4 miscarriages with normal uterine anatomy, normal parental chromosomes and negative for antiphospholipid antibodies referred to a tertiary RM centre. The mean number of previous first trimester miscarriages was 5.0 (range 4–10)

Main results and the role of chance: The main outcome measure is the difference between the frequency of live births with surviving offspring in the IvIg and placebo groups, the relative risk reduction of new miscarriage and the number needed to treat. Comparisons will be done by the χ^2-test. Results will be presented in June 2014 after breakage of the randomisation code in April when the last participant is expected to give birth. Ultimo January 2014, 43 (52.4%) of all 82 participants have given birth or are still pregnant in the third trimester, 38 (46.3%) have had a further miscarriage and one (1.2%) an ectopic pregnancy. No serious adverse events have been registered.

Limitations, reason for caution: Although this is the largest randomised trial of IvIg in RM conducted so far, the numbers of participants are limited and the true therapeutic effect (if any) may be over- or underestimated. Some of the miscarriages in the trial are due to embryonal aneuploidies, which are not treatable by IvIg.

Wider implications of the findings: If a significant treatment effect of IvIg can be demonstrated, IvIg may be offered to patients with secondary RM and ≥4 miscarriages. The results cannot be extrapolated to patients with fewer miscarriages, who are expected to exhibit a favourable spontaneous prognosis or patients with primary RM.

Study funding/competing interest(s): Funding by national/international organization(s), The Danish Council for Independent Research.
Trial registration number: ClinicalTrials.gov identifier NCT 00722475.

O-074 Radiotherapy and its impact on pregnancy outcomes: results from a nationwide study among female childhood cancer survivors (the DCOG LATER-VEVO study)

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Study question: Do female childhood cancer survivors (CCSs), treated with radiotherapy, have an increased risk of miscarriage, preterm delivery or low birth weight offspring and how does field, dose, and timing (before or after menarche) of radiotherapy influence this risk?

Summary answer: Overall, female CCSs are at increased risk of preterm delivery and low birth weight offspring. This risk was particularly increased for survivors treated with high dose radiotherapy to the abdomen (≥30 Gy) and those treated with radiotherapy to the uterus before menarche. No increased risk of miscarriage was found.

What is known already: Radiotherapy involving the uterus can reduce uterine volume, decrease myometrial elasticity and damage uterine vascularity. These alterations can impair uterine function and thereby restrict fetal growth and the ability to carry the foetus to term. Cranial irradiation may impair the function of the hypothalamic-pituitary-ovarian axis, thereby increasing the risk of miscarriage.

Study design, size, duration: The study, part of a nationwide multi-center retrospective cohort study on female fertility in CCSs (the DCOG LATER-VEVO-study) in the Netherlands, included 1045 survivors and 796 controls. Data on pregnancy outcomes were assessed by means of a questionnaire. Complete treatment data were retrieved from the DCOG LATER database.

Participants/materials, setting, methods: The study population consisted of adult female 5-year CCSs, born <1-1-1993, treated between 1963 and 2002, and who reported to have been pregnant at least once. The control group consisted of sisters of survivors and randomly selected females from the general population. Logistic regression was used to calculate odds ratios.

Main results and the role of chance: Overall, treatment with radiotherapy was associated with an increased risk of preterm delivery preterm and low birth weight offspring, but not miscarriage, in CCS compared with controls (OR = 2.81, 95% CI 1.53–5.16 and OR = 4.13, 95% CI 2.00–8.54, respectively). The highest OR's were found for females treated with high-dose radiotherapy (≥30 Gy) to the abdomen (OR = 7.05, 95% CI 1.22–40.85 and OR = 12.08, 95% CI 2.01–72.58, for preterm delivery and low birth weight offspring respectively). Radiotherapy involving the uterus given before menarche (OR = 2.44, 95% CI 1.00–5.92) was associated with preterm delivery.

Limitations, reason for caution: Precise estimates regarding the radiotherapy doses to the uterus are lacking, as dosimetric calculations have not yet been performed. This will be done, however, in the near future. Pregnancy outcomes were based solely on self-reported data and were not validated.

Wider implications of the findings: This study shows an increased risk of preterm delivery and offspring with low birth weight for female survivors of childhood cancer treated with radiotherapy, especially after high dose radiation to the abdomen (≥30 Gy) and when radiotherapy is given to an area involving the uterus prior to menarche. Careful management and monitoring of these women during pregnancy is warranted.

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*.

O-075 Association between embryonic genotype for a leukemia inhibitory factor (LIF) gene polymorphism and pregnancy outcomes after ART

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Study question: Is there an association between embryonic genotype for the leukemia inhibitory factor (LIF) T/G (rs929271) gene polymorphism and pregnancy outcomes after IVF/ICSI?

Summary answer: Couples with embryos carrying only the T/T LIF genotype exhibited decreased implantation and pregnancy rates after IVF/ICSI.

What is known already: LIF plays a role in both the adhesive and the invasive phases of implantation through its anchoring effect on the trophoblast and its regulation of trophoblast differentiation. LIF has been shown to modulate trophoblast invasiveness and affect immune tolerance. The literature provides evidence that the LIF T/G gene polymorphism (rs929271) is associated with female fertility. However, no previous studies have considered the genotype of the male parent or the embryo.

Study design, size, duration: A prospective cohort study was performed on 353 couples (706 individuals) undergoing IVF/ICSI who were recruited from 03/2012 to 07/2013. The couples were divided into two groups according to their LIF genotype combinations: T/T × T/T and all of the other possible genotypes combined.

Participants/materials, setting, methods: DNA was extracted from peripheral blood samples taken from each participant. The LIF single nucleotide polymorphism (SNP) T/G was genotyped by real-time PCR. Cumulative results (including fresh and frozen cycles) were analyzed. Hardy-Weinberg genotype distributions of the entire sample indicated concordance between the observed and expected frequencies.

Main results and the role of chance: Couples with embryos carrying only the T/T LIF genotype presented decreased implantation and pregnancy rates compared with couples with embryos with other genotypes (Table 1).

Table 1: Results.

General features and clinical outcomes	Couples' genotypes combinations		
	T/T × T/T	G/G × G/G, T/G × G/G, T/T × G/G, T/G × T/G, T/T × T/G	*P*
N	61	292	
Embryo genotype	Only T/T	All genotypes	
Maternal age (years)	36.2 ± 4.4	35.4 ± 4.2	0.18
Paternal age (years)	40.2 ± 7.2	38.1 ± 6.2	0.09
Transfers (*n*): total	1.5 ± 0.8	1.4 ± 0.8	0.16
Transfers (*n*): fresh/frozen	1.2 ± 0.6/0.3 ± 0.6	1.2 ± 0.6/0.2 ± 0.5	0.77/0.46
Embryos transferred (*n*): total	3.3 ± 2.0	3.0 ± 2.0	0.14
Embryos transferred (*n*): fresh/frozen	2.7 ± 1.9/0.6 ± 1.2	2.5 ± 1.60.5 ± 1.1	0.35/0.53
Implantation rate	10.5% (21/200)	20.6% (178/865)	0.0008
Clinical pregnancy rate/patient	32.8% (20/61)	47.6% (139/292)	0.04
Clinical pregnancy rate/transfer	21.7% (20/92)	34.0% (139/409)	0.02
Miscarriage rate	45.0% (9/20)	26.6% (37/139)	0.11
Ongoing pregnancy rate/patient	18.0% (11/61)	34.9% (102/292)	0.01
Ongoing pregnancy rate/transfer	12.0% (11/92)	24.9%(102/409)	0.005

Limitations, reason for caution: Additional validation of the analyzed SNP (increasing the number of cases) will be important to provide more information

about the potential use of this polymorphism. Differences in the genetic backgrounds of various ethnic populations should also be considered.
Wider implications of the findings: This SNP could be used as a susceptibility marker capable of predicting implantation efficiency. The ability to predict ongoing pregnancy rates using genetic markers during IVF/ICSI treatment can encourage patients to undergo additional cycles of ART and increase their success rates. Due to the ethical constraints of working with human embryos, this prospective, non-invasive investigation was conducted using genotype assessments of potential parents and should encourage new research based on similar principles.
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 authorized by the local ethics committee.

O-076 An evaluation of biochemical pregnancy rates (BPR) as a functional of age in women 21 to 42-years old, who underwent single embryo transfer (SET)

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Study question: Does age affect the biochemical pregnancy rate (BPR) in women who underwent *in vitro* fertilization (IVF)?
Summary answer: Among IVF subjects who underwent single embryo transfer (SET), advancing age up until the completion of the 40th year had no effect on the BPR. However the BPR did increase in 41–42 year olds compared to younger women.
What is known already: Clinical miscarriage rates increase as women age. This is associated with an increased likelihood of aneuploidy in the embryo. It would be anticipated that biochemical pregnancy rates would also rise with maternal age. However, no study has previously evaluated the effect of maternal age on biochemical pregnancy rates in women who underwent IVF or spontaneous conception. Single embryo transfer permits the evaluation of the effect of age on the BPR for the first time.
Study design, size, duration: This is a retrospective study, approved by the Human Subjects Committee. Subjects (N = 1824) who underwent single embryo transfer (SET) as part of a fresh or thawed IVF cycle were recruited from August 2008 through December 2012. Each subject is represented only once. Data was compared using chi-squared and multivariate analysis.
Participants/materials, setting, methods: A pregnancy was defined as a serum b-hCG level of >10 mIU/ml at 16 days embryo age. The BPR was absence of ultrasonographic evidence of pregnancy as a percentage of all non-ectopic pregnancies. Clinical miscarriage was pregnancy loss before 20-weeks gestational age after ultrasonographic evidence of an intrauterine pregnancy.
Main results and the role of chance: 1636 fresh and 188 frozen SET were analyzed, and the pregnancy rate per transfer were 39% and 40% respectively. The age range was 21–42 years. The BPR per pregnancy for fresh and frozen IVF cycles were 14%, and were therefore combined for analysis. As expected the pregnancy rate decreased significantly as the subject aged ($p < 0.001$). Age also had a significant effect on clinical pregnancy rates (Age 21–30 years: 41.6%, Age 31–35 years: 40.7%, Age 36–40 years: 27.0%, Age 41–42 years: 12.1%) ($p < 0.001$). The likelihood of a clinical miscarriage also increased with age ($p = 0.001$). Surprisingly, advancing age had no effect on BPR until age 40 ($p = 0.35$) (Age 21–30 years: 13.5%, Age 31–35 years: 11.6, Age 36–40 years:15.9%). However, 41–42 year olds were more likely to have biochemical pregnancies than women aged 21–40 ($p = 0.05$) (BPR 41–42 years; 25.8%).
Limitations, reason for caution: This was a retrospective study.
Wider implications of the findings: This is the first evaluation of age on BPR in the literature. Findings imply that genetic anomalies that cause BPR are unaffected by age between 21–40 years. As aneuploidy becomes more prevalent at 41 and 42 years, the BPR does increase. We previously demonstrated that compared to spontaneous pregnancies, IVF does not increase the BPR (ESHRE 2013). Therefore, it is likely this surprising result was not caused by IVF, masking an age effect on BPR.
Study funding/competing interest(s): Funding by hospital/clinic(s), McGill University Health Centre.
Trial registration number: Retrospective, not applicable.

O-077 Assisted reproduction technology treatment does not increase the total chromosome abnormality rate but increases the incidence of trisomy and decrease the incidence of polyploidy

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Study question: Is the incidence rate of chromosomal abnormalities in early miscarriages increased after assisted reproductive technology (ART) treatment?
Summary answer: The total incidence rate of chromosomal abnormalities in early miscarriages is not increased after ART treatment compared with NC. However, a statistically difference exists in the incidence of trisomy and polyploidy. ART treatment increases the incidence of trisomic miscarriages and decreases the incidence the polyploidy miscarriages.
What is known already: ART is the primary means of treating infertility. Chromosomal abnormality is the major cause of early miscarriages in both natural conceptions (NC) and ART conception.
Study design, size, duration: A retrospective analysis of 2606 early spontaneous miscarriages in our hospital from January 2006 to December 2013 was performed by our cytogenetic laboratory. Among them 1897 samples were from patients after ART treatment and 709 samples were from couples through NC.
Participants/materials, setting, methods: Genomic DNA was extracted from the chorionic villus of early miscarriages. Reference DNA was prepared from peripheral blood lymphocytes of healthy male donors. Comparative genomic hybridization (CGH) was used to analyze the chromosome copy number variants, and fluorescent in situ hybridization (FISH) was applied to detect the polyploidy.
Main results and the role of chance: The frequency of chromosomal abnormalities in the NC group (52.61%, 373/709) and the ART group (51.98%, 986/1897) was not statistically different. The major chromosome abnormality was aneuploidy, and the frequency of chromosomally abnormal abortions increased with maternal age in both groups. Trisomy 16, 22 and monosomy X were the most frequent anomalies, followed by trisomy 21, 15 and 13. There were no trisomy 1 found in two groups. However, the type of chromosomal abnormalities was significantly different between the two groups. The rate of autosomal trisomies in the NC group and the ART group was 67.56% (252/373) vs 79.21% (781/1897), and the rate of polyploidies in the NC group and the ART group was 11.53% (43/373) vs 2.82% (22/986), respectively.
Limitations, reason for caution: CGH can only detect chromosome segment abnormality with above than 5 Mb, therefore the microduplication or microdeletion which leads to early miscarriages maybe omitted. Due to the different genetic background between ART patients and normal population, the analysis of chromosome abnormality rate in this study might exist in biases.
Wider implications of the findings: ART is a safe technique in early miscarriages resulted from chromosome abnormality. It has an advantage in decreasing the incidence of polyploidy. However, our study showed that ART has an increasing trend of trisomic pregnancy.
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).
Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATION SESSION

SESSION 20: PSYCHOSOCIAL ISSUES AND CARE IN INFERTILITY

Monday 30 June 2014 **15:15 - 16:30**

O-078 ESHRE guideline: Psychosocial care in infertility and medically assisted reproduction

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[11]ESHRE, Brussels, Belgium

Introduction: A guideline development group, composed of psychologists, a nurse, a gynaecologist, a patient representative and a search specialist, have been working on writing a guideline on psychosocial care in infertility and medically assisted reproduction. Psychosocial care is all care provided as part of routine services in the clinic, by any health care professional who comes in contact with the patient before, during, or after fertility treatment.
Methodology: This guideline was written according to the 12-step process described in the ESHRE manual for guideline development. After scoping the guideline, and listing a set of 12 key questions in PICO format, evidence was collected and analysed. A summary of evidence was written in a reply to each of the key questions, and used as the base of recommendations for clinical practice and future research. In writing these recommendations, the evidence, but also the opinion of infertile patients, as reported by the patient representative, benefits versus harms analysis, and the opinion of the guideline group members was taken in consideration. Psychosocial interventions were only included when they could be implemented by any health care professional and not dependent on support from mental health professionals.
Results: The ESHRE guideline on psychosocial care in infertility and medically assisted reproduction aims at raising awareness of psychosocial health in infertility and assist health professionals in providing optimal evidence-based psychosocial care to patients dealing with infertility and medically assisted reproduction. The guideline is written in 2 sections. The first section describes which characteristics of fertility staff and clinic, and psychosocial care components (e.g., information provision) are important to patients, and which of these characteristics are associated with their wellbeing. The second section of the guideline focusses on describing the needs patients experience across the different stages of fertility treatment and on providing advice about how to assess and address these. Needs can be behavioural (e.g., compliance with treatment), relational (e.g., relationship with partner when there is one), emotional (e.g., anxiety) and cognitive (e.g., treatment concerns).
Conclusion: The final draft of the ESHRE guideline on psychosocial care in infertility and medically assisted reproduction will be presented. The next step in the process of guideline development is an extensive review by stakeholders and future users of the guideline. All infertility health care professionals are encouraged to submit comments to the guideline during the review phase. The final guideline, incorporating all comments, should be finished by the end of 2014.

O-079 Addressing psychological needs of patients treated for infertility: an international study of practice challenges reported by reproductive physicians

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Study question: What are the challenges reported by physicians who treat and manage patients with fertility issues-from differential diagnosis, to testing, to recommended treatment, to managing and addressing the patients' psychological needs?
Summary answer: This abstract focuses on reported challenges pertaining to addressing patients' psychological needs. Skills and confidence gaps were identified in: 1) assessing the patient's psychological status, 2) assessing the patients' parenting skills and 3) identifying the patient's needs for psychological and emotional support. Significant differences were observed between countries.
What is known already: Evidence of the psychological impact of infertility has been documented. A diagnosis of infertility is associated with increased stress and anxiety. Treatment is a source of emotional distress and risk of depression is higher after ART cycle failure. Lack of psychological support has been reported among the reasons to discontinue ART-treatments. As such, assessing the patients' psychological state and addressing their psychological needs should be integrated early in the care of infertile patient.
Study design, size, duration: A mixed-methods framework, that integrates semi-structured qualitative telephone interviews and a quantitative online survey, was used. An independent Review Board provided ethics approval. The study was deployed in China, Japan, Turkey, Russia, India, the United States and in the Middle East (United Arab Emirates, Saudi Arabia, and Iran).
Participants/materials, setting, methods: Reproductive physicians treating five or more patients per month using ART were included. Data were collected through qualitative interviews and quantitative surveys. Questions related to challenges experienced by healthcare providers in their practice and addressed knowledge, skills, confidence and relevance of skills for different aspects of care.
Main results and the role of chance: The final sample consisted of 174 physicians, of which 76% self-identified as Reproductive Endocrinologists. Respondents reported needing improvement (72%) in their skills to perform a psychological assessment. Participants' mean rating of confidence in their skill to perform this assessment was 2.25 (SD 0.71; Likert-type scale 1 = low confidence; 5 = optimal confidence). Participants reported needing improvement (71 %) in assessing patients' parenting skills. Mean confidence was 2.23 (SD 0.81)). Sixty-nine percent (69 %) reported needing improvement in their skills to identify patients' psychological and emotional support needs. Mean confidence was 2.36 (SD 0.80). These three skills were not considered essential in practice by 60%, 74% and 57% of participants, respectively. Significant differences between countries were observed. Data from the qualitative interviews support those findings.
Limitations, reason for caution: Assessment and management of psychological needs may be performed by other health care providers from the clinical team (e.g. nurses, psychologists), which could explain the gaps and why these skills are not considered essential by participants. Large variability was observed between countries, which may limit the generalization of these findings.
Wider implications of the findings: This study highlights the perceived challenges and gaps of reproductive physicians regarding their own ability and confidence at addressing patient's psychological needs in infertility treatments. Closing this gap could contribute to reduction in patients' anxiety and stress and maximization of ART treatment outcomes. Further studies could investigate the presence of those clinical gaps with other members of the clinical team
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Merck Serono, Darmstadt, Germany.
Trial registration number: Not applicable.

O-080 Psychological stress and moderate/severe depression are highly prevalent among women with recurrent pregnancy loss

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Study question: Is the prevalence of self-reported psychological stress and moderate/severe depression higher for women with recurrent pregnancy loss (RPL) compared with pregnancy planners without known reproductive problems?
Summary answer: Both psychological stress and major depression were significantly more common among women with RPL than in the comparison group.
What is known already: Psychological support in the form of 'tender loving care' is probably the most universally used treatment of RPL. However, few studies of the psychological consequences of RPL have been published and the conclusions are diverse, as some describe significant psychological impact and others do not.
Study design, size, duration: In this cross-sectional study, we gathered data on 292 women with RPL between 2010 and 2013. We defined RPL as three or more pregnancy losses before 12 weeks' gestation. Data on 2011 women attempting pregnancy without known sub/infertility were gathered from 2011 to 2013.

Participants/materials, setting, methods: RPL patients completed an online questionnaire before first consultation at the Danish RPL clinic. Eligible controls were retrieved from the Internet based cohort study 'snartforældre.dk'. Cohen's Perceived Stress Scale was used to assess self-reported stress and the Major Depression Index assessed symptoms of depression. Comparisons were made by Chi^2 testing.
Main results and the role of chance: We invited 438 women with RPL to participate in the study and 292 (67%) completed the questionnaire. Of these, 25 (9%) had symptoms corresponding to the ICD-10 diagnosis of "moderate-severe depression" - in DSM-IV terms "major depression". A high stress level, defined as a score of >16 on the perceived stress scale, was reported by 153 (52%) patients.

Among controls, 46 (2%) had symptoms of major depression and 675 (34%) had high stress levels. The differences between patients and controls were highly significant for both stress and depression, $p < 0.0001$ in both cases.
Limitations, reason for caution: There may be a bias in who chooses to complete both the patient questionnaire and the online survey. Furthermore, no information was available whether any of the women in the comparison group actually had RPL, but the diagnosis is seen in <1% of women in the background population.
Wider implications of the findings: We have shown that symptoms of stress and major depression are significantly more prevalent among women referred for RPL than among women without known fertility problems. Both stress and major depression have high personal and socio-economic costs and therefore need to be considered in the management of this patient group. How stress and depression and their treatment impact pregnancy prognosis are to be investigated in future studies.
Study funding/competing interest(s): Funding by University(ies), University of Copenhagen has given a PhD grant to A.M. Kolte. No specific funding was sought for this study.
Trial registration number: N/A.

O-081 Increased risk of intimate partner violence associated with infertility or subfertility: a systematic review

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Study question: The purpose of this systematic review was to assess the available evidence on the effect that a diagnosis of infertility or an inability to become pregnant (subfertility) in women of reproductive age results in an increased risk for experiencing intimate partner violence (IPV).
Summary answer: The diagnosis of infertility/presentation of subfertility have been identified as a risk factor for intimate partner violence in specific settings and countries. The potential drivers for risk of intimate partner violence associated with this disease/disability appear to be distinct from other forms of violence against women.
What is known already: A United Nations report on global and regional estimates of violence against women, documented "not only how widespread this problem is, but also how deeply women's health is affected when they experience violence." Consequences of violence against women can result in serious injury and death, as well as emotional, verbal, psychological, and economic negative outcomes. Additionally, having fewer children than desired or expected is not only a medical concern but a socially constructed problem.
Study design, size, duration: A systematic review of literature following PRISMA guidelines was completed on articles published between and inclusive of years 2000 and 2013. Multiple preliminary searches prior to 2000 did not identify quantitative studies meeting the criteria. Seven electronic global databases and experts were contacted and criteria applied independently by two investigators.
Participants/materials, setting, methods: Studies were searched /assessed to address the following question: The effect that a diagnosis of infertility or subfertility (intervention exposure) in comparison to those without a fertility problem (comparator) women of reproductive age (population) that results in an increased risk for experiencing intimate partner violence (IPV) - outcome.
Main results and the role of chance: Out of 409 studies initially identified, 314 abstracts and 63 articles were assessed. Eighteen studies analysing the relationship between infertility/subfertility (as the exposure) and intimate partner violence (as the outcome) in a quantitative manner met the final inclusion criteria, however, qualitative studies mentioning a correlation without providing quantitative data were excluded, as were three studies which investigated the outcome indicator and exposure in the reverse. All of the studies evaluated various forms of violence against women, including physical and sexual violence. Ten studies indicate that the infertility/subfertility is a risk factor for intimate partner violence; and three high-quality studies find a significant correlation between the exposure and outcomes of physical and sexual violence.
Limitations, reason for caution: Terminology for both infertility/subfertility; Terminology describing violence against women; Few quantitative prospective studies identified; Most studies cross-sectional; and, "Selection bias" from fertility clinic-based versus poor capture in population-based studies, all present limitations/caution. Proving causality of infertility/subfertility as a definitive risk factor and lack of direct comparisons between studies present limitations.
Wider implications of the findings: Gender-based violence is inextricably linked to women's lower status in many societies as compared to men. In these same settings, Infertility/subfertility can often be inappropriately assigned as fault of the woman, since fertility manifests itself through pregnancy. Assessment tools addressing risk of harm are needed when diagnosis of infertility/subfertility occurs within settings identified through this review. Quantitative prospective studies are needed in diverse global settings, in order to assess differing effects of male/female infertility/subfertility diagnosis.
Study funding/competing interest(s): Funding by national/international organization(s), Unspecified core funds from HRP: The UNDP, UNICEF, UNFPA, WHO and World Bank Special Programme of Research, Development and Research Training in Human Reproduction.
Trial registration number: Not applicable.

O-082 Male psychological adaptation to infertility: a systematic review of longitudinal studies

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[2]*University of Copenhagen, Department of Social Medicine, Copenhagen, Denmark*

Study question: What are the psychological symptoms associated with the experience of infertility in men, how do they vary over time, and which act as risk or protective factors?
Summary answer: The psychological well-being of men facing infertility seems to deteriorate significantly 1 year after the first fertility appointment. This review identified three predominant risk factors for psychological maladjustment - active-avoidance coping, catastrophizing and difficulty in partner communication – and one main protective factor - the use of meaning-based coping strategies.
What is known already: Biological, cultural and social aspects differentiate the medical and psychological circumstances related to the experience of infertility between men and women. Previous studies have suggested that facing infertility might be in its essence a different experience for men and women. Even though there is vast evidence on the emotional adjustment of women to infertility, there are no systematic reviews focusing on the male psychological adaptation to infertility.
Study design, size, duration: A systematic review aiming to identify studies on male psychological adaptation to infertility was performed by conducting a literature search from inception to August 2013 on ISI Web of Science, Medline, PsycArticles, Scielo and Scopus. Guidelines of Cochrane Collaboration and Preferred Reporting Items were followed.
Participants/materials, setting, methods: A search was conducted using combinations of MeSH terms (e.g. 'male, infertility') and keywords (e.g. 'emotional adjustment'; 'distress'; 'depression'). Studies in English, French and Spanish were considered, and had to present longitudinal data to be eligible. A narrative synthesis approach was used to conduct the review.
Main results and the role of chance: Ten studies from 3 continents were eligible from 1435 records identified in the search. Results revealed that psychological symptoms of maladjustment significantly increased in men 1 year after the first fertility appointment. Excepting for the desire to have a child, which significantly decreased, no significant differences were found two or more years after the initial consult. Evidence was found for active-avoidance coping, secrecy, difficulty in partner communication, catastrophizing, importance of a biological family, unsuccessful treatments and duration of treatment as risk factors for psychological maladjustment. Using meaning-based and active-confronting coping strategies before entering treatment were

significant protective factors against distress. Active-avoidance coping was also found to be a risk factor for marital adjustment, while meaning based coping was found to be a protective factor.

Limitations, reason for caution: The number of follow-up studies testing significant differences or predictors was limited. Because most samples were from Europe and the United States, there is a high risk of cultural and demographic bias. Although these studies constitute the best available evidence, a cautious approach to data interpretation is required.

Wider implications of the findings: This is the first systematic review on male psychological adaptation to infertility overtime. Our findings suggest that counseling infertile men should include interventions with coping skills training in order to promote adaptive coping strategies to deal with the challenge of infertility. Further prospective large studies with good quality design and power are warranted to perform a subsequent meta-analysis and compare results concerning diagnosis and treatment options.

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: N/A.

INVITED SESSION

SESSION 21: THE LATEST FINDINGS FROM THE REPROTRAIN CONSORTIA

Monday 30 June 2014 **17:00 - 18:00**

O-083 From mouse to human via flies; the latest interdisciplinary andrology research

R. Oliva[1]

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An ongoing interdisciplinary andrology research initiative in Europe is the "Reproductive Biology Early Research Training" network (Reprotrain); a UE funded project involving seven different research laboratories and two companies (www.reprotrain.eu). The overall objectives of Reprotrain are 1) to provide a comprehensive interdisciplinary training programme for early stage researchers in state-of-the-art male Reproductive Biology and Andrology, 2) to overcome historical fragmentation in the field of spermatogenesis and Andrology research by integrating and implementing different disciplines in male Reproductive Biology and Medicine, 3) to develop and implement systems biology based approaches (genomic, proteomic, transcriptomic, epigenetic and metabolomic), and 4) to develop novel clinical and industrial applications. Ten Early Stage Researchers and four Experienced Researchers are performing studies in genetics and epigenetics, molecular male reproductive medicine, molecular and structural biology, and biotechnology with hands-on training in cutting-edge technologies relevant to current molecular-genetic and medical research. The training network integrates individual research projects based on clinical samples and key selected model organisms (mouse and flies).

As an example of the development of two of the Reprotrain projects, the research lines developed on the proteomics and epigenetics of the sperm cell is presented. Previous knowledge established that the mammalian sperm cell DNA is packaged by protamines while a small fraction remains associated with nucleosomes enriched at loci of developmental importance. But the distribution of other proteins, in addition to protamines and histones, in the different sperm chromatin fractions had not yet been explored. Therefore we initiated a detailed proteomic and genomic characterization of the sperm chromatin in order to increase the epigenetic knowledge of the male germ cell and determined whether quantitative alterations in chromatin proteins were present in infertile patients. Dissected sperm chromatin fractions were subjected to shotgun protein identification using mass spectrometry and deep genome sequencing of the DNA. Our results indicate that the sperm cell chromatin delivers to the offspring a rich combination of histone variants, transcription factors, chromatin-associated and chromatin-modifying proteins differing in chromatin affinity, which may be involved in the regulation of histone-bound paternal genes after fertilization. The differential proteomic results also suggest that alterations in the proteins involved in chromatin assembly and metabolism may originate epigenetic errors during spermatogenesis, resulting in inaccurate sperm epigenetic signatures, which could ultimately prevent embryonic development. Funded by a Marie Curie Initial Training Network (FP7-PEOPLE-2011-ITN-289880).

O-084 Is the promise of sperm transcriptomics being kept?

D. Miller[1]

[1]*University of Leeds General Infirmary, Institute of Genetics Health and Therapeutics, Leeds, United Kingdom*

Is the promise of sperm transcriptomics being kept?

Since the initial reports of transcription (RNA) in sperm appeared in the early 1960s, controversy over its purpose has existed ever since. A renewed interest in the field from the mid 1990s onward has led to many reports on the presence, characterisation and role of sperm RNA in a variety of species (including some plants). Because it can serve as a proxy for the testis, sperm transcriptomics promises to provide valuable, non-invasive insights into the health of the testis in general and individual fertility in particular. The key to wider acceptance is threefold. Firstly, there must be some clinical demonstration of the utility of sperm RNA in a truly diagnostic setting. Secondly, the diagnostic technology must be relatively inexpensive and thirdly, the technology (and its accompanying bioinformatics) must be more readily accessible (end-user friendly). To date, considerable progress with the first and second requirements has been made, but there is still some way to go before the third requirement is met. Recent publications of array-based approaches capable of identifying semen samples whose partners went on to achieve a pregnancy are very encouraging. Next generation sequencing is going to greatly improve our understanding of functional aspects of sperm RNA that will no doubt help inform the choice of a good panel of markers for a cheap diagnostic assay. Reprotrain is funding one such comparative transcriptomic study examining a range of animal species including human, bovine, porcine and ovine that seeks to define and hopefully refine the RNA profile of mammalian sperm in relation to its function, both in the spermatozoon and possibly the early embryo.

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SELECTED ORAL COMMUNICATION SESSION

SESSION 22: REPRODUCTIVE SURGERY

Monday 30 June 2014 **17:00 - 18:00**

O-085 A multicentre randomised study of pre-IVF outpatient hysteroscopy in women with recurrent IVF-et failure – the trophy trial

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[9]ESHRE, Reproductive Surgery SIG, Brussels, Belgium

Study question: Does outpatient hysteroscopy performed prior to starting IVF treatment improve treatment outcome in women with recurrent IVF-ET failure?
Summary answer: Outpatient hysteroscopy performed prior to starting IVF treatment appeared not to significantly improve IVF outcome in the study population.
What is known already: Current literature suggests that hysteroscopy performed before starting IVF treatment could increase the treatment success rate. Data from high quality randomised study is required.
Study design, size, duration: The TROPHY trial was a multicentre, allocation-concealed, single-blind randomised study completed across 8 European IVF centres between 2010 - 2013. In total, 760 women were recruited and 719 were randomised. Third party, internet-based independent randomisation method was used. "Minimisation" using a computer-based algorithm was used to avoid chance imbalances in important stratification variables.
Participants/materials, setting, methods: Patients recruited were younger than 38 years, had 2–4 failed IVF-ET cycles and planned a further IVF/ ICSI cycle. Exclusion criteria were BMI above 35, uterine fibroid(s) distorting the cavity or untreated hydrosalpinx. Patients in the intervention group had an outpatient hysteroscopy using a 3.7 mm continuous-flow hysteroscope (Trophyscope) without sedation before starting the IVF cycle. In the control group, patients started the IVF cycle without prior hysteroscopy.
Main results and the role of chance: In all, 367 were randomised to the outpatient hysteroscopy group, while 352 were randomised to the control group. At hysteroscopy, 12% of patients had an abnormality detected. There was no significant difference between the hysteroscopy and control groups with regard to mean age at recruitment (32.7 ± 3.1 vs 32.6 ± 3.5 years), duration of infertility (4.3 ± 3.5 vs 4.2 ± 2.8 years), number of previous failed IVF cycles (2.7 ± 0.9 vs 2.7 ± 1.0), BMI (23.4 ± 3.5 vs 23.3 ± 3.4 kg/m^2), basal FSH level (6.1 ± 2.4 vs 6.3 ± 2.5 IU/L) and antral follicle count (15 ± 7 vs 16 ± 8). The total gonadotrophin dose used (2416 ± 1359 vs 2254 ± 1261 IU), mean number of oocytes retrieved (11 ± 6 vs 11 ± 6) and oocytes normally fertilised (6 ± 4 vs 6 ± 4) and mean number of embryos transferred (1.8 ± 0.5 vs 1.8 ± 0.5) were comparable between the two groups, respectively. Per randomised patient, the pregnancy rate (37% vs 37%, P = 0.94) and clinical pregnancy rate (34% vs 32%, P = 0.63) were not different between the hysteroscopy and control groups. The implantation rate was also comparable between the 2 groups (28% vs 29%, P = 0.69).
Limitations, reason for caution: Results of 25 randomised patients are not available yet. All study results, including live birth data, will be presented in the conference. The study did not include patients who had less than 2 or more than 4 IVF-ET cycles and, therefore, our results may not apply to these patient populations.
Wider implications of the findings: Data from this study suggest that routine outpatient hysteroscopy prior to IVF treatment in women who have experienced 2–4 failed IVF-ET attempts may not significantly improve the subsequent IVF outcome. It is possible that endometrial scratching rather than routine outpatient hysteroscopy could be responsible for the previously reported improvement in IVF outcome. Further studies should establish the subgroup of patients who could benefit from such interventions.
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by national/international organization(s), Funding by commercial/corporate company(ies), All participating clinics contributed to the funding of the trial. The study was supported by the ESHRE and European Academy for Gynaecological Surgery for trial coordination and meetings and the hysteroscopy equipment were provided by Karl Stroz Company. The company had no influence in the design, conduct or reporting of the trial. The authors have no competing interests to declare.
Trial registration number: ISRCTN 35859078.

O-086 Perfusion of whole ovine ovaries: effect of perfusion speed and time elapsed after extraction

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[3]University Maternal Hospital, Department of Oncology, Cologne, Germany

Study question: The aim of this research was to study the effectiveness of perfusion of intact ovine ovaries with different rates of perfusion and time-period elapsed between extraction of these ovaries and the beginning of perfusion.
Summary answer: Effective perfusion of ovine intact ovaries with vascular pedicle by freezing medium at room temperature includes the rate of perfusion 25 ml/h or 50 ml/h. The ovaries must be perfused no later than 3 h after the death of animals.
What is known already: Ischemia is the serious problem accompanying the process of extraction, cryopreservation and re-transplantation of ovarian tissue. To date, no data about successful transplantation of human intact ovary with its vascular pedicle after cryopreservation. However, cryopreservation of whole ovaries with vascular anastomosis with post-thawing re-transplantation can be considered as a promising strategy for cancer patients.
Study design, size, duration: Ovaries were perfused for 60 min just after extraction with different rates of perfusion (first cycle of experiments) as well as after their storage by room temperature for the certain time-periods (second cycle of experiments).
Participants/materials, setting, methods: Ovaries (n = 96) were perfused for 60 min just after extraction with the rate of perfusion 150, 100, 75, 50, 25, 12.5, and 6.3 ml/h. Also ovaries (n = 26) were perfused with rate 25 ml/h for 60 min after extraction of ovaries and their storage by room temperature: 2 h, 3 h, 4 h, 5 h.
Main results and the role of chance: After successful perfusion, the approximately 100% of ovarian tissue and its vascular pedicle obtained blue colour. The ovarian tissue can show unstained areas that evidence about incomplete perfusion of freezing medium. The optimal perfusion rate was established for ovaries of Group 4 and Group 5 (50 ml/h and 25 ml/h, respectively). In the second cycle of experiments, good perfusion of the ovaries with the perfusion rate 25 ml/h was established for ovaries of Groups 1 only (3 h after extraction). The effectiveness of perfusion in Group 2 (4 h after extraction) was sharply decreased: the current of perfusion medium through capillaries of ovaries was blocked by coagulated blood cells in these capillaries.
Limitations, reason for caution: No limitation
Wider implications of the findings: Vascular leakage and tissue damage as a result of high pressure of perfusion (freezing) medium with the rates strong (+++), lack (++) and weak (+) was observed with a rate of perfusion 150 ml/h, 100 ml/h, and 75 ml/h (groups 1, 2 and 3, respectively).
Study funding/competing interest(s): Funding by University(ies), Cologne University.
Trial registration number: No trial registration number.

O-087 Feasibility of MRgFUS treatment in uterine adenomyosis: clinical results and technical approach

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[2]University of L'Aquila, Discab University Pathophysiology of Reproduction, L'Aquila, Italy

Study question: Can MRgFUS treatment allow a resolution of symptomatology and represent a valid alternative to hysterectomy, in women with adenomyosis?
Summary answer: This study demonstrates the efficacy of the technique in uterine adenomyosis treatment, shown by a complete resolution of symptomatology and uterine conservation, without applying hysterectomy.

What is known already: Actually MRgFUS is a well-known technique in medical literature, used to treat gynaecological disorders like uterine fibroids, so successfull in terms of symptoms resolution and possibility for fertile women to get pregnant. In the last years there was a growing interest in adenomyosis treatment, using MRgFUS.
Study design, size, duration: This is a prospective study. From October 2011 to March 2013, we treated 18 patients affected only by adenomyosis using MRgFUS. All patients were treated once and the longest treatment lasted about 120 min.
Participants/materials, setting, methods: Symptomatology was assessed through the symptoms severity score questionnaire before and after treatment. The technical plan was characterized by the use of a high-energy-grid-sonication. The mean energy delivered was of 3450 J. This allowed us to reach the therapeutic temperature also in more vascularized parts of the lesion.
Main results and the role of chance: We made a clinical and diagnostic evaluation. From a diagnostic point of view we evaluated "pre-treatment volume" measured in the T2-weighted sequences using an informatic method on single slice; "treated volume" obtained from the Exablate measurement system 2100; "Non Perfused Volume"(NPV), evaluated on the c.e. T1-weighted sequences made immediately after treatment. Results showed a "treated volume" mean value of 72.5% of the volume drawn by the operator. The NPV was meanly 14% greater than the "treated volume". Comparing the three different parameters we can demonstrate that we treated a mean of 86.5% of the lesion. From the clinical point of view, after 12 weeks, the symptomatic score showed a reduction of about 90% if compared to the pre-treatment one.
Limitations, reason for caution: This is a preliminary study performed in a single center and it was carried out in a small number of patients with a shorter follow-up time.
Wider implications of the findings: MRgFUS is a mini-invasive treatment for adenomyosis. It permits to maintain the integrity of the uterus, a good extension of NPV, a shorter hospitalization with significant reduction of the symptoms. In conclusion, it is a valid and conservative treatment in a pathology which had a limited therapeutic perspectives.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), DIAGNOSTIC RADIOLOGY DEPARTMENT OF SAN SALVATORE HOSPITAL-UNIVERSITY OF L'AQUILA.
Trial registration number: No trial registration number.

O-088 The effect of endometrial injury on ongoing pregnancy rate in unselected subfertile women undergoing in-vitro fertilization: A randomized controlled trial

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Study question: Does endometrial injury in the cycle preceding ovarian stimulation for IVF improve ongoing pregnancy rate in unselected subfertile women?
Summary answer: Endometrial injury induced by endometrial aspiration in the preceding cycle does not improve ongoing pregnancy rate in unselected subfertile women undergoing IVF
What is known already: Implantation failure remains one of the major limiting factors for IVF success. Mechanical endometrial injury in the cycle preceding ovarian stimulation of IVF treatment has been shown to improve implantation and pregnancy rates in women with repeated implantation failures. There is limited data on unselected subfertile women, especially those undergoing their first IVF treatment
Study design, size, duration: This randomized-controlled trial recruited 300 unselected women scheduled for IVF/ICSI treatment between March 2011 and August 2013. Subjects were randomized into endometrial aspirate (EA) (n = 150) and non-EA (n = 150) groups according to a computer-generated randomization list.
Participants/materials, setting, methods: Subjects were recruited and randomized in the assisted reproductive unit at the University of Hong Kong. In the preceding cycle, women in EA group underwent endometrial aspiration using Pipelle in mid luteal phase. 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 groups. 209 subjects (69.7%) underwent their first IVF cycles and 91 (30.3%) had repeated cycles. There was no significant difference in ongoing pregnancy rates [26.7% (40/150) vs 32% (48/150)] in EA and non-EA group (p = 0.370). Implantation rate [32.8% (67/204) vs 29.7% (68/229), p = 0.189], clinical pregnancy rate [34.0% (51/150) vs 38.0 (57/150), p = 0.548], miscarriage rate [28.6% (16/56) vs 18.6% (11/59), p = 0.272] and multiple pregnancy rate [28.6% (16/56) vs 18.6% (11/59), p = 0.272] were all comparable. Subgroup analysis in women having first embryo transfer (n = 209) also demonstrated no significant difference in ongoing pregnancy rate, while that in women undergoing repeated cycles (n = 91) were significantly lower in the EA group.
Limitations, reasons for caution: The study aimed at assessing an unselected population of subfertile women by recruiting consecutive women attending our fertility clinic. However, majority of the recruited women (69.7%) were having their first IVF treatments. The results may not be generalizable to all women undergoing IVF.
Wider implications of the findings: Previous RCTs had suggested improved pregnancy rates after pre-treatment endometrial injury in women with repeated implantation failure. A recent RCT also showed increased pregnancy rates in unselected subfertile women after endometrial injury, although that study was terminated early and thus underpowered. Our study showed with adequate power that no significant improvement in pregnancy rates has been observed after endometrial injury in unselected women undergoing IVF treatment, in particular treatment naïve patients.
Study funding/competing interest(s): The study is supported by the Committee on Research and Conference Grants, University of Hong Kong. The authors have nothing to disclose.
Trial registration number: HKCTR-1646 and NCT 01977976.
Keywords: Endometrial injury, endometrium, in-vitro fertilization, embryo transfer, pregnancy rate.

INVITED SESSION

SESSION 23: PROGRESS IN OVARIAN STIMULATION FOR IVF

Tuesday 1 July 2014 **8:30 - 9:30**

O-089 The genetics of ovarian capacity

F. Vialard[1], R. Boudjenah[1], F. Boitrelle[1], A. Torre[1], D. Molina Gomes[1], J. Selva[1]
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Assisted reproduction technology (ART) has solved many problems of infertility. The success of *in vitro* fertilization depends largely on the effectiveness of ovarian stimulation. Obtaining an adequate number of high-quality oocytes is a major challenge in controlled ovarian hyperstimulation (COH).

To date, a range of hormonal and clinical parameters have been used to optimize COH but none have significant predictive value. The search for new factors is then still valid.

Pharmacogenetics has shown the role of genetic factors in the prediction of ovarian response and patient variability could be due to the genetic predispositions of single-nucleotide polymorphisms (SNPs). Of these SNPs, the FSHR Asn^{680}Ser polymorphism has been largely studied, and even if results were sometimes contradictive, ^{680}Ser allele seems to be associated with lower ovarian response to COH than ^{680}Asn allele. But, explaining this variability, taking into account only one SNP seems to be limited and many SNPs have been further reported to have a potential impact.

Currently, few questions could be explored:

1. Why only consider one SNP. Can SNPs' associations permitted to better define group of patients?
2. Are SNPs the sole genetic variants that could be studied?
3. Is candidate gene approach the best way for pharmacogenetics studies?
4. Is ovarian capacity the sole IVF parameter that could be influenced by genetic variants?

First, a multiple gene polymorphisms multiplex PCR assay, using the candidate gene approach, has been designed to genotype women undergoing ICSI program. To minimize population bias, an analysis was done both for an overall study population and for a subgroup with homogeneous characteristics. Individual and combined impacts of SNPs have been reported to influence the outcome of *in vitro* fertilisation (IVF) on the ovarian response to rFSH stimulation for patients

undergoing intracytoplasmic sperm injection program (ICSI). For example, FSHR Asn^{680}Ser polymorphism impacts seems to be modulated by AMH-Ile^{49}Ser one, and permits to define different subgroups of patients. These results support an adjusted gonadotropin administration on the basis of the genetic component of each patient.

SNPs aren't the sole genetic variants that could influence ART results. Currently, copy number polymorphism (CNPs) or variants (CNVs) are supposed to predispose to mental retardation or autism or else. Could these variants have an impact on ART result, and how to design whole genome studies to identify variants or genes?

As it has also been postulated that genetic polymorphism, like TNF-308 polymorphism, could influence embryo implantation, it seems that IVF taking in charge needs to be individualized according to pharmacogenetics results. Further results are necessary and in particular large prospective study.

O-090 Diagnosis, treatment and significance of poor ovarian response

S.K. Sunkara[1]

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Poor ovarian response (POR) was first described in 1983 soon after the introduction of ovarian stimulation into IVF. It was recognised that poor responders have a worse prognosis and extensive research has been carried out since to improve the outcome of poor responders undergoing IVF. However, little has been translated to actual benefit mainly due to clinical heterogeneity among studies in their definition of POR. The recent (2011) ESHRE consensus on the definition of POR was aimed to overcome this hindrance and streamline research. Advances in the understanding and assessment of ovarian reserve have certainly contributed to the management of the challenging poor responder. To date, antral follicle count (AFC) and anti-Mullerian hormone (AMH) are the most sensitive predictors of ovarian response and use of either is valuable in planning personalized controlled ovarian stimulation (COS) regimens to maximise IVF outcomes.

Number of oocytes retrieved following COS is an important prognostic variable with an initial linear association between number of oocytes and live birth following IVF. Several therapeutic strategies at the various stages of COS have been advocated to improve oocyte yield and IVF outcome for poor responders. The long GnRH agonist and GnRH antagonist regimens are the more effective pituitary suppression regimens for poor responders over the short agonist regimen. Although there is a trend to using high gonadotrophin doses for ovarian stimulation in poor responders, there is doubtful benefit from high doses (gonadotrophin dose >300 IU/day) and COS regimens should aim to optimise costs. The role of natural cycle IVF, mild stimulation and double stimulation for POR need evaluation with robust studies.

Numerous adjuvant therapies with theoretical rationale have been proposed in the pursuit of an ideal regimen for poor responders. Adjuvant androgens and androgen modulating agents are advocated based on the biological plausibility of increased intra ovarian androgen levels in enhancing folliculogenesis. Growth hormone (GH) supplementation is centred on evidence of a stimulatory effect of GH on ovarian follicle growth and steroidogenesis thus anticipating a potential benefit from GH addition to COS regimens in poor responders. The underlying principle for use of luteal oestradiol (E_2) priming in COS regimens is to synchronise the stimulated cohort of follicles and thereby increase yield of mature oocytes. Current evidence from limited studies suggests a possible role for transdermal testosterone pre-treatment. There is inconsistent data on the role of DHEA, letrozole, r-LH or hCG supplementation generally compounded by heterogeneity among studies in their characterisation of POR. Although data on the use of GH supplementation and E_2 priming for poor responders seem favourable, the findings are restricted by the widely acknowledged limitations of lack of a concrete definition and small sample sizes involving existing studies of poor responders.

Poor responders have low live birth rates following IVF across all age groups compared to normal responders. POR is associated with a higher risk of miscarriage even among younger women suggesting a parallel decline in oocyte quality and quantity among poor responders. However, young poor responders have better prognosis compared to older poor responders. Live birth rates for poor responders range between ~1% to ~21% influenced mainly by age and number of oocytes. From an economic perspective, POR is associated with considerably increased costs per live birth.

POR is a continuum of events leading form ovarian senescence to ovarian failure and early menopause. Poor responders are therefore likely to have consequent potential long term health implications which may merit surveillance. Given the challenges, patient centred management should be prioritised in dealing with POR. Patients should be empowered with reliable information and adequately counselled in making decisions regarding their fertility treatments and future implications.

INVITED SESSION

SESSION 24: OOCYTE ACTIVATION

Tuesday 1 July 2014 **8:30 - 9:30**

O-091 Mechanisms of oocyte activation

J. Parrington[1]

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Changes in intracellular calcium (Ca^{2+}) play a central role in the activation of the egg or oocyte at fertilization. In echinoderms, amphibians, and fish, fertilization is accompanied by a single Ca^{2+} wave, whereas in mammals distinctive Ca^{2+} oscillations trigger oocyte activation. The mechanism by which the sperm induces Ca^{2+} release in the egg or oocyte has been the subject of much debate.

Studies of egg activation in echinoderms have identified a role for an egg PLCγ, which triggers Ca^{2+} release via the generation of IP_3. However, two other Ca^{2+} mobilizing messengers, cADPR and NAADP, also play an important role during egg activation in echinoderms. The sea urchin egg has proved to be very valuable for studying the mechanism of action of these two intracellular messengers. Recently we have used this system to gain important insights into the mechanisms whereby cADPR and NAADP are generated in response to physiological stimuli, and the molecular identity of the NAADP receptor.

Studies by my colleagues and I first showed that the mammalian oocyte activation factor appears to be a sperm-specific phospholipase C, PLCζ. We have also shown that PLCζ appears to play a similar role in the chicken, and we and others have identified PLCζ orthologues in fish, suggesting that PLCζ may play a universal role in vertebrates. Surprisingly, pufferfish PLCζ appears to be expressed in the egg, not the sperm, and may itself need to be activated by a stimulus from the sperm.

PLCζ is believed to be the physiological agent of oocyte activation in mammals because injection of recombinant PLCζ RNA or protein into the oocyte triggers a pattern of Ca^{2+} oscillations very similar to those seen at fertilization and depletion of endogenous PLCζ protein from sperm extracts removes their ability to induce Ca^{2+} oscillations in the mouse oocyte. Moreover, immunofluorescence studies performed by ourselves and others have shown that PLCζ is localized in the post-acrosomal or equatorial regions of the sperm head in rodent, pig and human sperm, the expected location for the oocyte activation factor.

Recently we provided the first evidence for a link between mutations in PLCζ and human infertility. We identified an infertile human male patient whose sperm were defective in the capacity to activate the oocyte and who had a mutation, H398P, in one of his PLCζ alleles passed down from the patient's fertile father, despite him carrying this mutant form of PLCζ, while a mutation on the other PLCζ allele, H223L, was inherited from the patient's mother. This, then, is a typical case of an autosomal recessive disorder, and the first recorded case, as far as we know, in which an autosomal point mutation resulting in a deficit of sperm function and male infertility, has been shown to have been passed from a mother to her son.

Treatment of oocyte activation defects is currently being performed in clinical IVF by inducing oocyte activation with a variety of stimuli such as Ca^{2+} ionophore, ethanol, strontium chloride, or electrical stimuli. We and others have recently shown that we can generate recombinant human PLCζ protein that triggers Ca^{2+} oscillations in mouse and human oocytes, opening up the possibility of using this approach therapeutically.

Despite the evidence in favour of PLCζ being the physiological agent of oocyte activation in mammals, an issue still to be fully resolved is whether PLCζ is both necessary and sufficient for activating the oocyte at fertilization. Studies of mouse models of infertility modelled on human patients with mutations in PLCζ will be important for resolving this issue and also provide a way to assess the efficacy and safety of clinically used artificial oocyte stimuli.

O-092 Induced oocyte activation in the lab – principles and practice

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Embryologists who face a particular problem in the laboratory for the first time are in a dilemma. Not only that a solution has to be found it also has to be an approach that is ethically acceptable and follows internal and external quality guidelines. In terms of fertilization failure or problems after ICSI in earlier years the only way to overcome oocyte- or sperm-derived problems was to modify the injection technique. These rather invasive procedures helped to deplete internal calcium storages and, thus, raised the chance to activate the female gametes. A mechanically less invasive mode of artificially activating oocytes is the usage of a wide range of various chemical agents. The most popular artificial activating substances for human oocytes are Ca^{2+}-ionophores, such as ionomycin or A23187. However, it must be considered that in-house mixing of ionophore solutions is a technical challenge and in times of the European Tissue Directive compounding of agents could be a potential source of contamination and/or controversy. This scenario led to the implementation of the first ready-to-use Ca^{2+}-ionophore (CultActive) into ART labs. Meanwhile studies have shown that apart from the original indications "fertilization failure after ICSI" and "severe male factor infertility", respectively, other indications are conceivable that might benefit from ionophore treatment (e.g., low fertilization rate after ICSI, developmental problems of embryos).

INVITED SESSION

SESSION 25: ASRM EXCHANGE SESSION - "OMICS OF INFERTILITY"

Tuesday 1 July 2014 **8:30 - 9:30**

O-093 Environment, epigenetics and reproduction

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There is increasing evidence that environmental chemicals and air pollution have adverse effects on one or more of the components leading to normal reproduction. These observations derive primarily from studies on wild-life, laboratory animals, and high dose exposures of women and their fetuses to specific, individual chemicals (e.g., diethylstilbestrol). Given that there are more than 85,000 synthetic chemicals registered for use, the question arises as to how these chemicals, individually or in combination, even at low levels, may compromise normal reproduction in humans. Exposure to some chemicals, e.g., bisphenol A and dioxin, during vulnerable developmental periods in utero and across the lifespan, demonstrate compromise of reproductive function and pregnancy outcomes, with epigenetic modifications (e.g., DNA methylation) occurring in gametes, endometrium, and placenta. This lecture will focus on representative examples of environmentally-based reproductive compromise and experimental evidence for epigenetic and trans-generational persistence of some modifications and phenotypes.

O-094 Omics of male infertility

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The molecular defects that underlie the vast majority of all male infertility are currently unknown. Clinicians primarily rely on the routine semen analysis to predict male fertility potential, although a semen analysis cannot usually distinguish a fertile from an infertile male, unless azoospermia is present. The development of new technologies has lead to innovative research aimed at defining novel male infertility biomarkers through the analysis of genes, RNA, proteins or metabolites. These molecular approaches are referred to as genomics (genes), transcriptomics (RNA), proteomics (RNA) and metabolomics (metabolites). A biomarker is a distinct biological or biologically derived indicator of a process, event or condition that can be objectively measured, evaluated and compared. The goal is to find unique markers associated with specific male reproductive phenotypes to improve diagnosis at an early stage of disease. A non-invasive, sensitive and specific biomarker might eliminate the need for invasive testing and expand the current, limited diagnostic categories that are largely descriptive to classify these patients. Despite the challenges involved with bringing the biomarkers from the "bench-to-the-bedside, biomarkers have the potential to reinvent the diagnosis and treatment of the infertile male.

INVITED SESSION

SESSION 26: PARAMEDICAL INVITED SESSION

Tuesday 1 July 2014 **8:30 - 9:30**

O-095 Qualitative nursing research in fertility and reproduction: Methods and findings

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The statistical association between individual and contextual characteristics of people living with infertility, and their related behaviour reveals little about what motivates individuals to consider, delay, or renounce pregnancy and parenthood. Information about individual attitudes, values, norms, identity, and expectations are difficult to obtain - and interpret - in quantitative form. Therefore, qualitative and or mixed methods studies are appropriate in the context of providing insight into how individuals perceive personal experience and relationships – and whether these change, or are maintained – and how they conduct themselves. Well executed qualitative studies capture individual perceptions of events, and in the process, yield rich biographical data in this context. Theoretical and practical aspects of in-depth interviewing in fertility and reproduction research; its application and findings will be discussed in the context of two qualitative studies; (1) *'Factors affecting decision making about fertility preservation after cancer diagnosis: A qualitative study'* and (2) *'A qualitative study of women's decision-making at the end of IVF treatment'*. Particular attention will be given to the interaction involved in the interview setting, the management of the gathered evidence, as well as data analysis and reporting of the qualitative material.

Finally, this session will examine the role of qualitative research, in revealing the components of women's personal, social and cultural existences that ultimately determine the evidence base and define clinical practice.

O-096 The young ones – extending endometrioses care to teenage girls

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Endometriosis associated with pain symptoms in adolescents has been reported, but the exact prevalence is unclear because pain symptoms may be atypical and endometriosis can only be diagnosed by laparoscopy. The laparoscopic appearance of adolescent endometriosis may vary between superficial active inflammatory highly vascularized peritoneal lesions to cystic ovarian endometriosis and associated adhesions, but deep endometriosis infiltrating 5 mm or more beneath the peritoneum is rare.

Dysmenorrhea at an early age is a risk factor for adult endometriosis (Treloar et al., 2010). Symptomatic adolescents with severe dysmenorrhea, nonmenstrual pelvic pain, and chronic pelvic pain (CPP) are usually prescribed nonsteroidal anti-inflammatory drugs (NSAIDs) and/or monophasic contraceptive pills. However, both incidence and duration of oral contraceptive use for severe primary dysmenorrhea during adolescence is higher in women who later develop deep endometriosis than in women without deep endometriosis (Chapron et al., 2010).

According to current evidence (Janssen et al., 2013), the overall prevalence of endometriosis in adolescents with pelvic pain is 62%, but varies between

75% in girls with chronic pelvic pain (CPP) resistant to treatment with oral contraceptive pills (OCPs), 70% in girls with dysmenorrhea and 49% in girls with CPP only. The overall prevalence of ASRM classified moderate-severe endometriosis is 32%, and varies between 16% in girls with CPP resistant to treatment with OCPs/NSAIDs, 29% in those with dysmenorrhea and 57% in the subgroup with CPP. These data suggest that about two third of adolescents with CPP or dysmenorrhea have laparoscopic evidence of endometriosis, and that about one third of adolescents with endometriosis have moderate-severe disease. Due to the lack of a noninvasive diagnostic test, the diagnosis of adolescent endometriosis is made with significant delay, sometimes after the disease has progressed to a stage with significant impact on future fertility and CPP.

During this lecture, we will further discuss the prevalence of menstrual cycle related pain problems in adolescents and the value of ultrasound and CA-125 in the diagnosis of endometriosis. We will also address the question how we can improve timely referral to endometriosis centers of expertise for adolescents with menstrual cycle related pain problems, resistant to medical management. Finally, we will discuss if early treatment of adolescent endometriosis can prevent the development and/or progression of adult endometriosis.

References:

Chapron C, Lafay-Pillet MC, Monceau E, Borghese B, Ngo C, Souza C, de Ziegler D. Questioning patients about their adolescent history can identify markers associated with deep infiltrating endometriosis. *Fertil Steril* 2011:95;877–881

Janssen EB, Rijkers ACM, Hoppenbrouwers K, Meuleman C, D'Hooghe T. Prevalence of Endometriosis diagnosed by Laparoscopy in Adolescents with dysmenorrhea or chronic pelvic pain: A Systematic Review. *Hum Reprod Update* 2013:19(5):570–582.

Treloar SA, Bell TA, Nagle CM, Purdie DM, Green AC. Early menstrual characteristics associated with subsequent diagnosis of endometriosis. *Am J Obstet Gynecol* 2010:202;534–536.

SELECTED ORAL COMMUNICATION SESSION

SESSION 27: EMBRYONIC BIOMARKERS - ARE WE GETTING NEARER?

Tuesday 1 July 2014 **10:00 - 11:30**

O-097 Cell-free DNA levels in human follicular fluid as a non-invasive biomarker of *in vitro* fertilization outcomes

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Study question: Is there any cell-free DNA in follicular fluid (FF) from preovulatory individual follicles and if so, is it correlated with *in vitro* fertilization (IVF) outcomes?

Summary answer: We detected cell-free DNA in human FF and observed that its level varies significantly according to the follicle size. Cell-free DNA levels in FF are significantly correlated with embryo quality in patients undergoing IVF process and could provide an innovative non-invasive IVF outcome biomarker.

What is known already: DNA fragments resulting from inflammatory, apoptotic or necrotic events, are present in blood circulation and their quantification are currently used for the detection of gynecological and pregnancy disorders. Furthermore, FF which constitutes oocyte micro-environment, contains both plasma components and secreted elements from granulosa cells. The presence of cell-free DNA in FF has never been investigated, nor its potential to be used as IVF outcome biomarker.

Study design, size, duration: A prospective collection of 100 individual FF from 44 patients undergoing conventional IVF (n = 26) or Intracytoplasmic Sperm Injection (ICSI) (n = 18) was conducted.

Participants/materials, setting, methods: The preovulatory follicles were aspired individually. Only blood-free FF samples were included. Follicle size has been calculated according to the volume of FF. Each corresponding cumulus oocyte-complex was isolated for IVF or ICSI. Cell-free DNA was quantified by ALU qPCR. Means ± SEM, correlations and linear regressions are presented.

Main results and the role of chance: FF samples from individual preovulatory follicles do contain measurable amounts of cell-free DNA (mean = 1.62 ng/µl). Cell-free DNA levels in small follicles (8–12 mm) were significantly higher than in large follicles (>18 mm) (2.54 ± 0.78 ng/µl vs 0.71 ± 0.44 ng/µl, respectively, p = 0.007). Likewise, cell-free DNA concentrations were significantly and negatively correlated with follicle size (r = –0.24; p = 0.017) and this correlation was strengthened in follicles containing an oocyte (r = –0.34; p = 0.003). Very interestingly, cell-free DNA level was significantly lower in FF related to low fragmentation rate embryo (≤25%) compared to those with high fragmentation rate (>25%) (p = 0.03). We also found a significant decrease of cell-free DNA from FF corresponding to top quality embryos comparing with no top (p = 0.022).

Limitations, reason for caution: A larger study will be conducted to investigate if cell-free DNA levels could be correlated with embryo implantation and pregnancy rates.

Wider implications of the findings: This study identified and quantified for the first time, cell-free DNA in human individual FF samples. The correlation between cell-free DNA levels and follicle size should reflect the functional state of each preovulatory follicle. High cell-free DNA amount in the follicle could have negative effects on oocyte and thus embryo health. Cell-free DNA measurement in follicles is easily performed and could be used as a supplemental non-invasive tool to predict embryo quality for IVF process.

Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Funding by commercial/corporate company(ies), This study was supported by the University-Hospital of Montpellier and Ferring pharmaceutical company. The authors of the study have no competing interests to report.

Trial registration number: Not applicable.

O-098 The metabolic profile of the human embryo as revealed by screening for >100 metabolites by HILIC-MS/MS and its association with development and chromosomal abnormalities

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[5]*University of Ioannina, Genetics and IVF Unit Department of Obstetrics and Gynaecology, Ioannina, Greece*
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Study question: Are there any differences in the levels of metabolites found in spent culture media of normal human embryos and embryos with chromosomal abnormalities?

Summary answer: Different metabolic profiles are observed between embryos with and those without chromosomal abnormalities.

What is known already: Metabolic profiling has been proposed as a tool to distinguish between human embryos with the potential to develop to the blastocyst stage and implant and those that arrest. Differential expression of metabolites has been observed in Down's syndrome embryos and Monosomy 21 embryos compared to normal embryos. Limited studies have however, investigated in detail the metabolic profiling of embryos with other aneuploidies in comparison to normal embryos.

Study design, size, duration: This an ongoing study, initiated in May 2012. 320 embryos were subjected to day 3 biopsy and processed for PGD/S by array-CGH or FISH. Culture media prior to biopsy (day 1–3) and following biopsy (day 3–5) were collected and analysed by hydrophilic interaction liquid chromatography tandem mass spectrometry (HILIC-MS/MS).

Participants/materials, setting, methods: PGD/S was conducted in an academic hospital with IVF/PGD laboratory and 2 IVF units. Metabolic analysis was conducted in a Forensic Toxicology Laboratory by HILIC-MS/MS which was developed to provide the quantitation of circa 100 metabolites in an UPLC system with a triple quadrupole spectrometer.

Main results and the role of chance: This is the first study to screen for >100 primary metabolites (aminoacids, sugars, amines etc) by a HILIC-MS/MS

method developed specifically for profiling and quantitation of metabolites in spent culture media. UPLC conditions were optimized in order to reach maximum peak capacity and retention for all hydrophilic metabolites in a single run of 40 min and the developed method was validated and proved to be reliable, robust and sensitive. Characteristic patient specific metabolic profiles were observed in day 3 and day 5 spent media which differed between aneuploid and chaotic embryos that arrested in culture and normal embryos that had developed to the blastocyst stage on day 5 and had resulted in a viable pregnancy.
Limitations, reason for caution: Although all abnormal embryos were meta-analysed on day 5 to confirm initial single cell diagnosis and their uniform or mosaic status was established, normal embryos transferred on the basis of single cell analysis but not resulting in a pregnancy could not obviously be tested for mosaicism.
Wider implications of the findings: This study provides unique biomarkers found in spent media from embryos identified as abnormal following PGS and reconfirmed as uniformy aneuploid, major mosaics or chaotics following meta-analysis of all the nuclei of the rejected embryos on day 5. Comparison of these metabolic profiles with those of chromosomally normal embryos by multivariate analysis (PCA, PLS-DA) showed distinct differences, which in the future could serve as non-invasive markers for the detection of aneuploidies before embryo transfer.
Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), This study was co-financed by the EU Social Fund (ESF) and the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Program: Thales.
Trial registration number: A9850.

O-099 Secreted miRNAs can be profiled with high accuracy and reproducibility from blastocyst spent culture media: a new potential biomarker for non-invasive embryo selection

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Study question: Do human embryos secrete miRNAs in the extracellular environment and if so, can these miRNAs be profiled from spent culture media of expanded human blastocyst?
Summary answer: miRNAs are secreted from human blastocysts in culture media in an appropriate concentration to be profiled with high accuracy and reproducibility.
What is known already: In the last decade it has been hypothesized that miRNAs could be extracellularly stable. The resulting evidence was that miRNAs secreted by donor cells can be taken up by recipient cells and exert a gene regulatory effect upon them. Accordingly, human embryos could secrete specific miRNAs as a part of the blastocyst – endometrial dialogue aiming at implantation success. Profiling the miRNA repertoire in easily collectable spent culture media holds the potential to identify non-invasive biomarkers of embryo quality.
Study design, size, duration: Ten good quality expanded human blastocysts donated for research between January and October 2013 underwent inner cell mass (ICM) isolation using a previously validated method. Trophectoderms (TE) samples (free of ICM cells) and their relative spent culture media (25 µl at 120 h post fertilization) were individually processed for miRNA analysis.
Participants/materials, setting, methods: MicroRNA expression was evaluated using TLDA MicroRNA Cards (Applied Biosystems) containing primer sets for 736 human miRNA sequences. *C*t values at a level ≥38 cycles and miRNAs expressed in less than 75% biological replicates were excluded from analysis. The mean expression level of expressed miRNAs was used for normalization.
Main results and the role of chance: 73 different miRNAs were consistently detected in blastocyst spent culture media. Comparative analysis with TE samples revealed that 95.8% (70/73) of those miRNAs were indeed expressed also in TE. The normalized Ct values of miRNAs detected in the media were also directly correlated with expression levels in the embryo (Spearman's correlation 0.68, $p < 0.001$). 11 miRNAs were found to be differentially expressed between TE and spent culture media. Interestingly, 4 of these miRNAs were significantly more abundant in the media suggesting an active secretion mechanism. Functional bioinformatics analysis revealed that 35,6% of the miRNAs found in the media have been already validated as circulating miRNAs, 13.7% were associated with endometrium receptivity, 12.9% with angiogenesis, 10.5% with placental function and 10.5% with stem cells regulation.
Limitations, reason for caution: To fully exploit the clinical translational relevance of these findings, further prospective studies are undergoing to identify key secreted miRNAs that can be used to predict developmental competence of preimplantation embryos based on quantification of secreted miRNAs from spent culture media.
Wider implications of the findings: In this study the population of miRNAs secreted by human blastocysts in spent culture media has been characterized for the first time in the IVF context. This knowledge will be used to develop a customized assay based on key secreted miRNAs for the non-invasive assessment of embryonic developmental potential. From a basic research perspective, this project is the first step towards outlining the role of microRNA dialogue at implantation.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), This project was funded by Merck Serono (Grant for Fertility Innovation 2013). The sponsor had no role in study design, data collection, data analysis, data interpretation. Authors declare no conflicts of interest.
Trial registration number: None.

O-100 Cell-free nucleic acids and IVF outcome: comparison between embryo on day 3 and blastocyst stage

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[3]*Institut De Recherche En Cancérologie De Montpellier, INSERM U896, Montpellier, France*

Study question: Is the detection of nucleic acids, including microRNAs (MiR) and cell free DNAs (cfDNA), released by human embryos into culture medium associated with embryo quality and IVF outcome?
Summary answer: Under *in vitro* conditions for IVF, changes in the nucleic acids levels in the embryo culture medium on day 3 and day 5/6 predict embryo quality and may be used as a new potential biomarker for selecting top quality embryos.
What is known already: The choice of the best embryos to transfer is based on subjective morphological parameters. The use of nucleic acids profile of culture medium as an objective approach to select the best embryo would clearly be more appropriate and reliable.
Study design, size, duration: Human fertilized oocytes were individually cultured from zygote to blastocyst satge. A total of 60 spent culture media were collected on day 3 (6–8 cells) and day 5/6 blastocyst stage.
Participants/materials, setting, methods: MicroRNAs were extracted from drops with the QIAamp kit and quantified by RT-qPCR using TaqMan technology. Cell-free DNA was quantified using Bio-Rad Supermix SYBR Green. Statistical analyses defined relationship between nucleic acid contents and embryo quality.
Main results and the role of chance: We demonstrate that the embryo culture medium samples, during *in vitro* embryo development, contained embryonic free nucleic acids, particularly *MiR-21* that acts as an anti-apoptotic factor. The concentration values of cell free DNA are lower in the culture medium in which emerge top quality embryo compared to no top ($p < 0.05$). The embryos that reached good blastocyst quality and leading to pregnancy, the variation in the cell free DNA concentration between day 3 and day 5/6 is significantly very decreased ($p < 0.05$). Conversely, this variation is very low from blastocyst which did not implant (5 ng/ml and 16.9 ng/ml respectively).
Limitations, reason for caution: It is necessary to establish the pregnancy prediction value of these nucleic acids in a large number of patients include *in vitro* fertilization program.
Wider implications of the findings: The analysis of cell-free nucleic acids released in the culture medium by embryos open the possibility to develop a new quick and low-cost test for the selection of the embryos viable with the highest implantation potential.

Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Funding by commercial/corporate company(ies), This work was supported by the University-Hospital of Montpellier and by a grant from the Ferring Pharmaceutical Company. The authors of the study have no competing interests to report.
Trial registration number: Not applicable.

O-101 Prokineticin 1 (PROK1) is a new non-invasive biomarker of embryo implantation in *in vitro* fertilization (IVF)

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Study question: To investigate the correlation of PROK1 concentrations in follicular fluid (FF) and fertilization culture media (FCM) with the corresponding reproductive outcome in patients who received conventional *in vitro* fertilization embryo transfer (cIVF-ET).
Summary answer: High concentrations of PROK1 in FF and FCM are both correlated with embryo implantation in cIVF-ET. PROK1 levels in FF and FCM are independent of transferred embryos morphokinetic parameters.
What is known already: Assessing the embryo implantation potential is crucial for increasing the success rates of cIVF. PROK1 is a well established actor in endometrial receptivity, trophoblast physiology and reproductive organs angiogenesis. In Assisted Reproductive Technology (ART), the rates of circulating PROK1 were recently reported as significantly linked to the rates of pregnancy. On the basis of these observations, it was intriguing to investigate the value of its measurement in FF and FCM in cIVF cycles.
Study design, size, duration: A prospective study was performed between January–November 2013 in the Assisted Reproduction Unit, Department of Obstetrics and Gynecology, University of Grenoble, France. A total of 67 infertile couples receiving first cIVF were recruited. Clinical pregnancy rate was use as endpoint with 15 cases/group for sample size calculation.
Participants/materials, setting, methods: Samples were collected through the CRB Germetheque. Signed informed consent was obtained from all patients who participated in the study. FF was collected immediately after oocyte retrieval and FCM after oocyte denudation steps. PROK1 concentrations in FF and FCM were measured by enzyme-linked immunosorbent assay (ELISA).
Main results and the role of chance: PROK1 was detected in all FF and FCM samples. The mean value of PROK1 concentrations in FF was 722 ± 306 pg/ml, ranging from 57 to 56,558 pg/ml. Follicular fluid PROK1 levels were significantly different between embryos which led to implantation (n = 15) versus no implantation (n = 15), with 19,279 pg/oocyte and 7,879 pg/oocyte respectively (p value = 0.0015, Mann Whitney test; RR = 5 for PROK1 ≥15,000 pg/oocyte). In FCM, the mean value of PROK1 concentrations was 114 ± 89 pg/ml, ranging from 3 to 274 pg/ml. PROK1 concentrations in FCM were significantly different between embryos which led to implantation versus no implantation, with 74 pg/oocyte and 40 pg/oocyte respectively (p value = 0.006, Mann Whitney test; RR = 6.8 for PROK1 ≥50 pg/oocyte).
Limitations, reason for caution: In non-pregnant women, PROK1 circulating levels were around 50 pg/ml. Extensive bleeding during transvaginal oocyte retrieval could reduce PROK1 concentrations in FF samples. PROK1 is up-regulated in ovarian hyperstimulation syndrome (OHSS) and recurrent miscarriage. These conditions constitute two non-inclusion/exclusion criteria in our study.
Wider implications of the findings: PROK1 concentrations assessment in FF and FCM by ELISA constitute a quick, non-invasive and non expensive test, which displays an appropriate sensitivity and specificity for cIVF cycles routine application. The long term objective is to evaluate the impact of including FF and/or FCM PROK1 quantification in the embryo transfer decisions. PROK1 levels determination in FF/FCM might improve the effectiveness of cIVF by reducing the time and cost required for obtaining a pregnancy.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Funding by national/international organization(s), Université Joseph Fourier (UJF), Centre Hospitalier Universitaire (CHU), Institut national de la santé et de la recherche médicale (INSERM).
Trial registration number: None.

O-102 Altered levels of mitochondrial DNA are associated with female age, aneuploidy, and reveal the implantation potential of chromosomally normal embryos

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Study question: Can differences in the quantity of mitochondrial DNA (mtDNA) or in the sequence of the mitochondrial genome affect embryo viability and aspects of embryo biology such as aneuploidy? Can measurement of mtDNA quantity provide information about the potential of an embryo to produce a viable pregnancy?
Summary answer: Elevated levels of mtDNA in human blastocysts were shown to be associated with advancing maternal age, aneuploidy and implantation failure. Not only does this have implications for our understanding of female reproductive aging and the origin of aneuploidy, but it also provides a clinically valuable new embryo viability biomarker.
What is known already: Mitochondria play a vital role in preimplantation development. They are the principal site of energy production and have various other critical cellular functions. The initial stages of preimplantation development are thought to be supported entirely by mitochondria derived from the oocyte. Despite the importance of this organelle, little is known about the extent of variation in mtDNA (quantity and sequence) between individual embryos or what the biological and clinical impacts of such variation might be.
Study design, size, duration: Quantification of mtDNA and detection of aneuploidy in 360 blastocysts and 21 cleavage stage embryos generated by 162 couples from seven clinics. Analysis of associations between mtDNA quantity and female age, embryo morphology, chromosome status and implantation success/failure.
Participants/materials, setting, methods: Embryos were biopsied at the cleavage or blastocyst stages. The samples were subjected to comprehensive chromosome analysis using a highly validated microarray method. These embryos were also tested using quantitative PCR and next generation sequencing (NGS), allowing highly accurate mtDNA quantification and detection of mutations of the mitochondrial genome.
Main results and the role of chance: A highly significant increase in embryo mtDNA quantity was observed with advancing female age ($P < 0.003$). Amounts of mtDNA were also significantly elevated in aneuploid embryos, independent of age ($P < 0.005$). The amount of mtDNA did not affect embryo morphology. Correlation with clinical outcome revealed that euploid embryos capable of implantation tended to contain lower mtDNA quantities ($P < 0.02$) than those failing to implant. This allowed establishment of a threshold mtDNA quantity above which implantation was never observed. 30% of chromosomally normal embryos that failed to implant had quantities of mtDNA above the threshold. 100% of euploid embryos that formed a viable pregnancy had mtDNA levels below the threshold. Sequencing of the entire mitochondrial genome using NGS revealed mutations in several blastocysts with abnormal levels of mtDNA.
Limitations, reason for caution: Further analyses of mitochondrial copy number and function should be undertaken in aneuploid and non-implanting euploid blastocysts in order to reveal whether organelle performance is altered in embryos with abnormal mtDNA levels and/or mtDNA mutations.
Wider implications of the findings: Our data suggests that elevated mtDNA amounts arise during oogenesis and may represent a compensatory mechanism, in response to defective organelles with mitochondrial genome mutations. The findings indicate that mitochondrial abnormalities may contribute to aneuploidy risk and female reproductive aging. Of clinical importance, an mtDNA content threshold was established, above which implantation never occurs. This threshold is independent of morphology, age and aneuploidy and implicates excessive mtDNA in approximately one-third of failed blastocyst implantations.
Study funding/competing interest(s): Funding by University(ies), University of Oxford.
Trial registration number: Not applicable.

INVITED SESSION

SESSION 28: LIVE SURGICAL TUTORIAL: UTERINE PATHOLOGY

Tuesday 1 July 2014 **10:00 - 13:00**

SELECTED ORAL COMMUNICATION SESSION

SESSION 29: NOVEL TECHNIQUES IN ANDROLOGY

Tuesday 1 July 2014 **10:00 - 11:30**

O-103 Can a patch fix it – electrophysiological studies of sperm and potential impact on clinical practice

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Study question: Can electrophysiology patch clamp interrogation of sperm cells from patients with total failed fertilisation diagnose underlying physiological defects?
Summary answer: Patch clamp studies of clearly defined subfertile men provide a fundamental insight into the functional role of CatSper and Slo1/3 in human spermatozoa.
What is known already: Patch clamping is revolutionising the understanding of human sperm function. CatSper and Slo1/3 are proposed to be involved in calcium entry and regulation of membrane potential respectively, but the synchrony of events and interplay between these channels is unclear (2; 3). Mouse knockout studies show they are both vital for fertilisation competence but there is no data on abnormalities in men and their effect on fertility (cf. 4).
Study design, size, duration: A series of functional assays on motility and $[Ca^{2+}]_i$ signalling on spermatozoa from subfertile men attending for ART over 12 month period (125 patients, 40 controls) identified those with phenotypic abnormalities (1). Patch clamping was instigated in patients with specified abnormalities and those with apparently unexplained total fertilisation failure following IVF/ICSI.
Participants/materials, setting, methods: Participants were men undergoing IVF/ICSI at Ninewells Assisted Conception Unit, Dundee specifically including those affected by failed fertilisation. Surplus semen samples at time of treatment, or produced specifically for research purposes, were subjected to analysis including patch clamping under quasiphysiological conditions (1; 3).
Main results and the role of chance: By studying a subpopulation with IVF/ICSI treatment complicated by total failed fertilisation we have identified 2 individuals affected by unique deficiencies. (1) Patient 1: Almost absent potassium conductance (current @68 mV ~3pApF^{-1} vs 42pApF^{-1} in fertile and ICSI controls; $p = 0.001$). This was not rescued by increasing internal Ca^{2+} demonstrating loss of function. Membrane potential was significantly different to controls (+5 mV vs. –22 mV $p = 0.001$) yet there was normal motility, CatSper (Cs+) current and expression of Slo3 (2) Patient 2: no effective CatSper current /rapidly inactivating cation conductance (I_{Tail}). Spermatozoa also showed repeated absent $[Ca^{2+}]_i$ response (FLUOStar) to progesterone (1/88 IVF patients). Abnormal CatSper, I_{Tail} was not a general phenomenon as a voltage activated potassium current was present and had similar magnitude compared to controls.
Limitations, reason for caution: The analysis is based on a limited numbers of patients but multiple samples from each one show robust defects. Proteomic and genetic data are not yet available (January 2014) but will be presented.
Wider implications of the findings: Identification of these individuals provides potential loss of function data to clearly determine the impact of these two key channels on human sperm function. Wider implications include the possibility to identify targets for therapeutic manipulation, either to fix the problem, or indeed, to aid development of non-hormonal male contraception, and without the need to generate knock-out mice models or to conduct animal studies.
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by national/international organization(s), Study funded by Chief Scientist Office [Scotland], Infertility Research Trust, NHS Tayside, Wellcome Trust and MRC.
References
1. Alasmari W, et al., (2013) Hum Reprod. 2013 28:866-76.
2. Mannowetz N, et al., (2013) Elife. 2013 Oct 8;2:e01009.
3. Mansell SA, et al., (2014) Mol Hum Reprod. Jan 16. [Epub ahead of print]
4. Smith JF, et al (2013) PNAS 110(17): 6823-8.
Trial registration number: Ethical approval granted by East of Scotland Research Ethics Service (EoSRES) REC 1: 12/ES/0091.

O-104 A modified protocol of sperm vitrification shows less cryodamage and enhanced sperm viability than classical slow freezing techniques

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Study question: Is a modified and improved vitrification technique of sperm able to reduce the cellular cryodamage produced by slow freezing? Is vitrification comparable to slow freezing in terms of sperm viability?
Summary answer: Using cellular damage parameters and redistribution of tubulin as biomarkers, we evaluated the cryodamage in two sperm cryopreservation techniques: classical slow freezing-thawing and modified vitrification-warming. The results obtained demonstrate that our vitrification technique is less harmful than conventional freezing in terms of cell viability and vitality survival.
What is known already: Previous studies have shown that slow freezing not only damages sperm membranes, but also cytoskeleton, mitochondrial function and DNA integrity suggesting sperm vitrification as an alternative. Results obtained in vitrification without cryoprotectant showed equivalence and two babies were born in 2012. Sucrose used as cryoprotectant in vitrification achieves better survival results. As other works have shown that the adding of HSA during the warming process may improve biological parameters, we performed preliminarily a dilution assay.
Study design, size, duration: Prospective randomized study, 20 normozoospermic samples divided into 3 aliquots: fresh (control), slow-freezing-thawing (group-A) and vitrification-warming (group-B). Protocols used: a) for freezing: Kit-Sperm-Cryoprotect-II (Nidacon), b) for vitrification: Sucrose 0.5 mol as cryoprotectant and HSA 0.5% as warming stabilisator. Preliminary tests performed with different Sucrose and HSA concentrations showed these as the best.
Participants/materials, setting, methods: Sperm samples collected after informed consent of 20 participants. After seminogram according to WHO-2010 and vitality testing, each one was tested for three different seminal parameters: spontaneous acrosome reaction (SAR) by *Pisum sativum*-FICT, DNA damage/fragmentation by SCD+TUNEL, and immunocytochemistry assay for cytoskeleton evaluation by α-tubulin antimouse and 488DYL anti-mouse antibodies.
Main results and the role of chance: We found no difference between study groups in classical spermiogram parameters. Vitality testing showed cell survival of 55.13 ± 20.33% in group-A vs 76.38 ± 6.50% in group-B, ($p < 0.01$).

DNA damage was 2.62 ± 5.11% (control) and 27.4 ± 8.37% vs 20.0 ± 6.1% in the study groups respectively ($p < 0.01$). Levels of SAR showed 26.6 ± 14.7% (control) and 58.2 ± 14.5% vs 44.3 ± 14.5% respectively, being differences statistical significant between study groups ($p < 0.001$). The tubulin assay distinguished three different patterns: continuous (P1), discontinuous (P2) and final pattern (P3). In the control and study groups the results showed equivalent patterns P2 and P3 only between fresh and vitrified.

P1: 58.18 ± 18.95%, 63.95 ± 15.98% and 59.96 ± 17.65%, respectively ($p > 0.5$); P2: 39.71 ± 19.07%^{a}, 3.55 ± 2.53%^{a,b} and 37.99 ± 17.89%^{b}, respectively ($p^{a,b} < 0.001$); P3: 2.07 ± 1.80%^{a}, 32.04 ± 13.57%^{a,b} and 1.65 ± 1.61%^{b}, respectively ($p^{a,b} < 0.001$).
Limitations, reason for caution: This assay has been undertaken only on normal sperm samples. It would be interesting to contrast these results in pathological samples and correlate results with clinical outcome parameters. A prospective study in this sense is ongoing.
Wider implications of the findings: Our sperm vitrification protocol shows improved recovery parameters and cellular viability after thawing than conventional freezing, as measured by TUNEL, SAR, tubuline assay and vitality testing. We observed that cellular damage after vitrification is even less, encouraging us to test results in clinical practice. The handling of the vitrification is

also easy and the procedure times shorter. Thus, vitrification has high potential to become a viable alternative as cryopreservation technique compared to current slow freezing.
Study funding/competing interest(s): Funding by hospital/clinic(s), IVFspain.
Trial registration number: A trial registration number is only required for clinical trials.

O-105 Sperm selection using MACS technology does not improve live-birth delivery rates when ICSI was performed in ovum donation. Prospective and randomized trial in unselected males

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Study question: does MACS increase live-birth delivery rates in ICSI procedures of ovum program?
Summary answer: MACS does not increase live-birth delivery rates in ICSI cycles of ovum donation 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 ART outcomes although it has been suggested as a potential way to improve results in male infertile couples.
Study design, size, duration: Two arms, unicentric, prospective, randomized and triple-blinded trial, a total of 237 infertile couples, October 2010 and December 2012.
Participants/materials, setting, methods: IVI-Valencia. Couples were divided into two groups: MACS (n = 125) and control (n = 114). Semen samples were both prepared by the swim-up method, apoptotic sperm in MACS-group were removed by annexin-V-MicroBeads and deposited into filtration columns under a magnetic field. The primary outcome was to determine if removing apoptotic sperm improves live-birth delivery rates.
Main results and the role of chance: Similar results were obtained in all the parameters studied compared between groups and the same occurs in live-birth delivery rates which slightly decreased 48.4% (CI95%39.6–57.1) vs. 56.4% (CI95% 47.3–65.5) in MACS group compared with control group with a relative risk reduction of 0.8 (0.6–1.2), but none of them reaching statistical significance.
Limitations, reason for caution: We did not include as a selection criteria an altered apoptotic profile on sperm samples, or increased DNA fragmentation. On the contrary we used unselected patients.
Wider implications of the findings: The absence of differences between two groups, indicates the need to design new strategies for MACS protocols in order to develop alternative sperm selection strategies, or clearly defined indications able to improve the outcome in ART.
Study funding/competing interest(s): Funding by hospital/clinic(s), Instituto Universitario IVI.
Trial registration number: Given that the main intervention was conducted on sperm samples and not human beings, Clinical Trial registration is not needed.

O-106 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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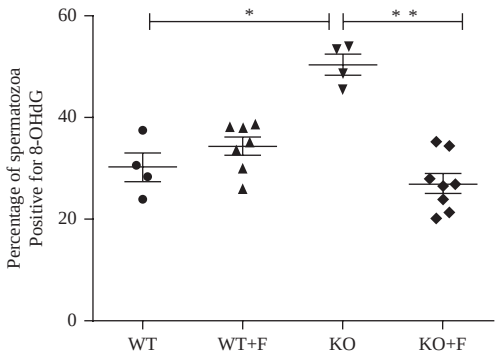
Study question: Does Fertilix™, a novel antioxidant formulation designed for the male reproductive tract, reduce Sperm DNA Damage (SDD) and increase pregnancy rates in mouse models of sperm oxidative stress?
Summary answer: Oral administration of Fertilix™ for a period of 2 weeks significantly reduces SDD in Glutathione Peroxidase 5 (GPX-5) knockout mice and restores normal pregnancy rates back almost to the normal levels in mice subjected to Scrotal Heat Shock (SHS).
What is known already: Moderate to severe damage to sperm DNA has been confirmed in over 60% of men visiting IVF centers and in 80% of couples diagnosed with idiopathic infertility. Numerous studies in animals and human document the adverse effect of SDD on fertilization rate, embryo quality, miscarriage rates and the transfer of higher rates of mutations in the offspring. Semen samples of infertile men are also known to be deficient in a number of 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: Two well-established mouse models of sperm oxidative stress from 2 independent laboratories were utilized to evaluate the efficacy of Fertilix. In both models 12 male mice were provided with Fertilix in their drinking water for 2 weeks (SHS) or 4 weeks (GPX-5) and compared with control groups for SDD and pregnancy rates.
Participants/materials, setting, methods: In the study performed on SHS mice, male fertility was tested by partnering three females with each male for 5 days, and the percentage of pregnant females, number of vaginal plugs, resorptions per litter, and litter size were recorded. For the GPX-5 model, sperm DNA oxidative damage was evaluated by the immunocytochemical detection of 8-OHdG residues.
Main results and the role of chance: The 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 4 week treatment of GPx5 KO mice diminishes DNA oxidative damage to WT levels.

In mouse models of SHS, only 35% (19/54) female mice got pregnant resulting in 169 fetuses. This is 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 make sure that every mouse took 100% of the product for the treatment period, despite careful monitoring.
Wider implications of the findings: SDD reduces fertilization rate, the maintenance of a healthy pregnancy and increases DNA mutational load passed onto the next generations. These results, if confirmed in humans, will impact the normal practice at ART centers as follow. Diagnosis of SDD in male partner of infertile couples followed by a simple course of antioxidant therapy will be considered routine prior to couples undertaking IUI or IVF treatments. This practice, if followed correctly, will lead to higher IVF success rates with lower sporadic mutational rates passed onto the children.
Study funding/competing interest(s): Funding by University(ies), University of Clermont-Ferrand. The corresponding 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.

O-107 *In vitro* fertilization versus *in vitro* fertilization-intracytoplasmic sperm injection in patients who previously failed intrauterine insemination

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Study question: In patients who have previously attempted and failed intra-uterine insemination (IUI), is in-vitro fertilization (IVF) or *in vitro* fertilization with intracytoplasmic sperm injection (ICSI) more effective in a subsequent ART cycle?
Summary answer: ICSI has a higher clinical pregnancy rate than IVF alone for patients who have previously failed IUI. This was statistically significant, and continued to be so when excluding male factor as the indication for treatment. When stratified by age, this was only significant in women aged 35 and under.
What is known already: IUI with or without ovarian stimulation is a common first-line fertility treatment for certain etiologies of infertility. Further treatments such as IVF and/or ICSI increase the pregnancy rate but are more costly and invasive. The indication for ICSI was initially severe male infertility but its indications have since been extended to the management of infertility in various other contexts.
Study design, size, duration: A single centre retrospective cohort study was performed on 449 patients who had their first IVF or ICSI treatment following IUI failure. The inclusion period was between January 2009 and May 2012.
Participants/materials, setting, methods: Patients who failed IUI and underwent their first IVF or ICSI after controlled ovarian stimulation were studied. Cycles using donor gametes and cycles canceled before the use of any method of fertilization were excluded. The main outcome was clinical pregnancy as defined by a gestational sac seen on ultrasound.
Main results and the role of chance: 180 patients had IVF and 269 had ICSI. 117 patients had male factor as the treatment indication. Fertilization rate was significantly lower in the IVF group (65.1%) than the ICSI group (74.3%, $p < 0.001$). The clinical pregnancy rate was 30.8% ($n = 55$) and 38.3% ($n = 103$) in the IVF and ICSI groups, respectively. This was statistically significant when adjusting for age and indication for treatment with an odds ratio of 1.96 (95% CI 1.20–3.20, $p = .008$). After removing patients with male factor as an indication for IVF, the clinical pregnancy rate remained significantly higher in the ICSI group ($p = 0.021$), with an adjusted odds ratio of 1.86 (95% CI 1.10–3.16).
Limitations, reason for caution: The study was limited to a single public centre, so caution should be used prior to extrapolating our findings to a wider population. The results were only significant in females under age 35 when stratified by age.
Wider implications of the findings: Clinical pregnancy rate in patients who pursue ART after IUI failure tends to be better with ICSI whether or not male factor is included in the analysis. It also agrees with the current state of the literature in that the fertilization rate is higher with ICSI. Although some studies have not found a higher clinical pregnancy rate with ICSI, our study clearly showed a benefit under 35 years of age.
Study funding/competing interest(s): Funding by hospital/clinic(s), McGill Reproductive Centre, Royal Victoria Hospital, McGill University Health Centre.
Trial registration number: Not an RCT.

O-108 A novel stepwise micro-TESE approach in non-obstructive azoospermia

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Study question: In the present prospective study we applied a conservative three-steps surgical approach with attention to organ preservation. We refer to this as to step-wise micro-TESE (Microsurgical Testicular Sperm Extraction). We report our results of stepwise micro-TESE on azoospermic NOA patients in terms of sperm retrieval rate (SRR).
Summary answer: Our proposed stepwise micro-TESE approach in NOA patients can optimize sperm retrieval reducing the invasiveness of the surgery procedure. Patients who benefit from stepwise micro-TESE include previous unsuccessful TESE, unfavorable diagnosis (maturation arrest, MA; Sertoli-cell-only syndrome, SCOS), and Klinefelter syndrome.
What is known already: Conventional TESE consists in a few superficial testicular incisions. This procedure can cause irreversible damage to the subtunical vessels and scar formation. On the other hand, micro-TESE consists in an deep equatorial incision of the whole testicle for selectively pick up single seminiferous tubules. Micro-TESE was introduced with the aim of improving sperm recovery and reducing invasivity of conventional TESE. However, some concern has recently been raised on the risk of hormonal impairment after micro-TESE.
Study design, size, duration: We performed testicular sperm retrieval on 64 NOA patients (March 2007–April 2013). The presence of spermatozoa was examined with a three-steps biopsy: i) single superficial TESE in the first testis; ii) micro-TESE on the same testis; iii) conventional TESE on the contralateral testis; SRR from the three steps were compared.
Participants/materials, setting, methods: At histological evaluation of the 64 patients, 37 had SCOS, 15 MA, 6 Leydig-cells hyperplasia and 6 sclera-hyalinosis; 37/64 patients with azoospermia had previously undergone testicular surgery: 12 for cryptorchidism, 2 for seminoma and 23 for previous fertility treatment with negative sperm retrieval. No pre-operative hormonal treatment was planned.
Main results and the role of chance: In all 64 procedures the outcome of the first single TESE and that of micro-TESE were overlapping, with a SRR of 28.1%. The contralateral biopsy was always negative for sperm retrieval. Levels of FSH, LH, inhibin B and testicular volume were not predictive of sperm retrieval. The mean age of 18/64 men with positive sperm retrieval was 34.2 ± 7.16; levels of FSH was 25.9 ± 15.10 mIU/ml, LH was 12.5 ± 9.40 mIU/ml and inhibin B was 14.9 ± 5 pg/ml. The histological diagnosis of these patients were: 10 MA, 1 sclera-hyalinosis and 7 SCOS. In 46/64 patients with negative sperm retrieval, male mean age was 35.9 ± 3.94; levels of FSH, LH and inhibin B were 25.7 ± 11.60 mIU/ml, 9.7 ± 4.88 mIU/ml and 20.0 ± 18.86 pg/ml, respectively. The histological diagnosis were: 10 MA, 5 sclera-hyalinosis, 6 Leydig-cell hyperplasia and 30 SCOS.
Limitations, reason for caution: During surgery, a prompt response from the laboratory on sperm recovery is needed to decide whether proceeding to the next step or not. If no sperm is immediately found (very severe cases), it is possible that the surgeon proceeds and only later sperm are found in the initial sample.
Wider implications of the findings: Data obtained suggest that in the majority of poor prognosis NOA patients ($N = 46$) even micro-TESE does not allow a successful sperm retrieval; in 18 patients with successful sperm recovery, the initial, less invasive single biopsy would have been enough to obtain sperm. Due to the priority given to organ preservation, stepwise micro-TESE could be an ideal approach in all azoospermic men. Micro-TESE can be offered only when no sperm is found in the initial incision.
Study funding/competing interest(s): Funding by hospital/clinic(s), No external funding was obtained for the present study; no conflict of interest has to be declared with any financial organization regarding the material discussed in the manuscript.
Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATION SESSION

SESSION 30: BASIC SCIENCE ENDOMETRIOSIS

Tuesday 1 July 2014 **10:00 - 11:30**

O-109 microRNA miR-142-3p regulates endometriotic cell invasiveness by targeting integrin-related molecular pathways

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Study question: Could miR-142-3p, a microRNA dysregulated in endometriosis, be mechanistically involved in the pathogenetic process?
Summary answer: miR-142-3p affects endometriotic cell motility and invasiveness via targeting of several target mRNAs implicated in integrin-mediated processes.
What is known already: microRNAs are potent posttranscriptional regulators of gene expression which are dysregulated in endometriotic tissue and sera of endometriosis patients compared to controls. miR-145 and miR-10b were previously show to affect endometriotic cell invasiveness (Adammek et al. Fertil Steril 2013, Schneider et al. Fertil Steril 2013), but the role of miR-142-3p is unclear.

Study design, size, duration: In this cross-sectional *in vitro* study, the endometriotic cell line 12Z (Zeitvogel et al. 2001) was transiently transfected with control reagents or miR-142-3p precursors. 72 h after transfection, changes in cell motility and invasiveness, as well as changes in target gene expression were monitored. All measurements were performed in triplicates on three independent biological replicates.
Participants/materials, setting, methods: miRNA-dependent cell motility was monitored by video microscopic analysis. Cellular invasiveness was investigated by matrigel invasion chamber assays. Alterations in cytoskeletal structure were investigated by confocal laser microscopy using fluorescently labelled phalloidin and antibodies against vinculin and integrin alpha V. Changes in predicted target gene expression were monitored by qPCR, Western blotting and 3′-UTR luciferase reporter assays.
Main results and the role of chance: Upregulation of miR-142-3p results in increased 12Z cell motility and invasiveness ($p < 0.05$, $n = 3$). Altered motility correlates with a more rounded cell shape of miR-142-3p-transfected cells on laminin, but not on fibronectin substrates. Integrin alpha V, the integrin-associated small GTPase Rac1, the kinase ROCK2 and additional cytoskeletal elements are downregulated upon miR-142-3p upregulation as determined by qPCR ($p < 0.05$, $n = 3$). Integrin downregulation was confirmed at the protein level by Western blotting, and by 3′UTR luciferase assays ($p < 0.01$, $n = 3$).
Limitations, reason for caution: The use of an immortalized cell line is a limitation of this study. Furthermore, transient transfection of miRNA precursors may not reflect physiological miRNA levels. As miRNAs have several predicted targets, changes in other mRNAs may additionally contribute to the observed changes in cell behaviour.
Wider implications of the findings: Our *in vitro* data indicate a novel role for miR-142-3p in regulating endometriotic cell invasiveness. An extracellular matrix-dependent regulation of integrins and integrin-associated cytoskeletal modulation by miR-142-3p are mechanistic elements of the phenotype. A targeting of miR-142-3p, e.g. by locked nucleic acid antimirs, may be a future experimental approach for reducing endometriotic cell invasiveness. However, more details on the molecular mechanisms, targets and potential side effects need to be elucidated in the near future.
Study funding/competing interest(s): Funding by University(ies), Funded by a grant of the Medical Faculty of the University of Münster to M.G.
Trial registration number: Not applicable.

O-110 Aberrant activation of the Akt–S6K1 signal pathway in a fibrotic microenvironment in deep endometriosis

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[2]*CHU Clermont-Ferrand, Department of Pathology, Clermont-Ferrand, France*

Study question: Why deep endometriotic stromal cells can proliferate and persist in a fibrotic environment where normal fibroblasts display the tightly regulated proliferation essential for physiological repair?
Summary answer: Aberrant activation of the Akt–S6K1 signal pathway in a fibrotic microenvironment might support growth of deep endometriotic lesions by enhancing endometriotic stromal cells persistence and survival.
What is known already: Histologically, endometriosis is characterized by dense fibrous tissue surrounding the endometrial glands and stroma. The formation of endometriosis lesions shares characteristics with pathological wound healing.
Study design, size, duration: A laboratory study. Seventy patients (40 with and 30 without endometriosis) of reproductive age with normal menstrual cycles were recruited. A total of 25 nude mice received a single injection of 10 proliferative endometrial fragments.
Participants/materials, setting, methods: *In vitro* studies using a three-dimensional type I collagen gel culture system were performed to evaluate cell proliferation, apoptosis, the activation status of the Akt signaling pathway in endometrial and endometriotic stromal cells. Immunohistochemistry for phospho-Akt expression was performed in endometriosis and endometriotic implants in a mouse model of endometriosis.
Main results and the role of chance: Polymerized collagen significantly inhibited proliferation of endometrial stromal cells compared with endometriotic stromal cells ($p < 0.0004$ and 0.0004 at days 3 and 6, respectively). Furthermore, polymerized collagen significantly increased caspase 3/7 activity in endometrial stromal cells compared with endometriotic stromal cells ($p < 0.004$). The levels of phosphorylated Akt, S6K1 and PTEN were suppressed on polymerized collagen in endometrial stromal cells. In contrast, the levels of phosphorylated Akt, S6K1 and PTEN were maintained in endometriotic stromal cells. Few endometrial stromal cells were positive for phospho-Akt expression compared to endometriotic tissues. However, phospho-Akt positive stromal cells were already present on day 3 after endometrial tissue implantation in a nude mouse model of endometriosis.
Limitations, reason for caution: The precise mechanisms of aberrant activation of the Akt–S6K1 signal pathway in a fibrotic microenvironment need to be elucidated. In addition, the present study investigated only stromal cells. Further studies are necessary to investigate extracellular matrix- epithelial cell-stromal cell interactions in a three-dimensional multicellular culture model.
Wider implications of the findings: Aberrant activation of Akt appears to play a critical role in drug resistance. Deep endometriosis usually does not respond well to hormonal suppressive therapy, although endometriosis is an estrogen-dependent disease. Aberrant activation of the Akt–S6K1 signal pathway may cause drug resistance in deep endometriosis and targeting this mechanism may be exploited for the treatment of deep endometriosis.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Karl Storz Endoscopy & GmbH (Tuttlingen, Germany).
Trial registration number: Not applicable.

O-111 Endometrial telomerase activity is limited to human epithelial cells, plays a fundamental role in glandular formation and might have therapeutic implications in endometrial proliferative diseases

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Study question: Does endometrial telomerase activity (TA) and telomere lengths (TL) play a role in human endometrial glandular epithelial cell proliferation and gland-formation?
Summary answer: Endometrial TLs and TA change according to the menstrual cycle. TA is higher in the endometrial epithelial population, proliferating epithelial cells in short term culture and inhibition of TA appears to attenuate glandular formation in 3D culture, therefore epithelial cell proliferation is likely to be regulated by telomerase.
What is known already: The Human Endometrium expresses the enzyme telomerase and TA changes according to the menstrual cycle. Endometriosis is associated with high endometrial TA and longer TLs. The effect of menstrual cycle on endometrial TLs and if TLs are different in discrete endometrial-cellular compartments is not known. We hypothesised that endometrial epithelial cell proliferation may be regulated by the telomere maintenance function of telomerase and that inhibition of TA may have a function in endometrial gland formation.
Study design, size, duration: A prospective-observational study included 125 healthy-women without endometrial pathology. Matched endometrial and blood samples collected ($n = 60$). Sorted primary endometrial-epithelial and stromal cells in short-term culture (3 days, $n = 5$/group). Epithelial cells, freshly-sorted from healthy-women in proliferative, secretory phases of the cycle, women treated with synthetic-progestogens or combined-contraceptive pill (COCP, $n = 3$/group, total 12).
Participants/materials, setting, methods: TL (by qPCR) and TA (by TRAP-assay). Mean relative TLs of paraffin-embedded full-thickness endometrial biopsies (proliferative $n = 3$ and secretory $n = 8$, postmenopausal (PM) = 5) were also examined *in vivo* by Quantitative fluorescence-in-situ-hybridization (qFISH). The effect of telomerase-inhibition on endometrial gland-formation was assessed in 3D-culture using a spheroid-forming assay of primary human endometrial-epithelial cells ($n = 6$).

Main results and the role of chance: Blood and endometrial TLs did not correlate suggesting a local-regulation. Endometrial TLs changed with cycle phase shortest TL in mid-secretory phase ($p = 0.04$). TA was higher in cultured primary-epithelial cells than the stromal population (0.42 vs. 1.59, $p = 0.004$). Freshly-sorted epithelial-cells from progestogen-treated group had low TA when compared to proliferative-phase or COCP samples (0.3vs.1.7, $p = 0.007$). In-vivo examination of relative TL with qFISH of full-thickness pre-menopausal endometrium showed that epithelial TLs in the functionalis was significantly longer than functionalis stroma ($p = 0.0006$), but this difference was not apparent in the basalis compartment. In proliferative basalis and postmenopausal samples stroma had significantly longer TL than epithelium ($p = 0.01$). Treatment of epithelial cells with telomerase inhibitor for 5 days in 3D-culture disrupted the primary human endometrial epithelial cells forming spheroids in 3D culture ($p < 0.01$).
Limitations, reason for caution: The *in vitro* telomerase inhibition data is tested in a mono-cellular system for a short-term. Further confirmation of the results in an *in vivo* model will be necessary.
Wider implications of the findings: Telomerase activity has shown to regulate cell proliferation in many tissue types. Progestogens may exert their inhibitory effect on endometrial proliferation via inhibiting telomerase. Direct inhibition of telomerase may have a potential therapeutic effect on many endometrial proliferative diseases including endometriosis.
Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), The study was funded by Wellbeing of Women project grant RG1073 and University of Liverpool Department of Women's and Children's Health.
Trial registration number: Collection of human samples was approved by Liverpool Adult Ethics committee (REC references; 09/H1005/55 and 11/H1005/4).

O-112 PEDF based therapy for angiogenesis-related reproductive disorders

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Study question: Can administration of a physiologic intra-ovarian anti-angiogenic factor as Pigment epithelium-derived factor (PEDF) treat angiogenesis-related reproductive disorders?
Summary answer: Reco recombinant PEDF (rPEDF) exerts its potent therapeutic properties on several angiogenesis-related reproductive disorders including endometriosis and ovarian hyperstimulation syndrome (OHSS), without adversely affecting ovulation, implantation and pregnancy rates
What is known already: PEDF is a non-inhibitory member of the serine protease inhibitors (serpin) superfamily, possessing potent physiologic anti-angiogenic and anti-inflammatory activities; we have extensively investigated PEDF/VEGF counter regulation in the reproductive system demonstrated PEDF's anti-angiogenic activity. Evidence targets VEGF as the main effector in the pathogenesis of angiogenesis-related gynecological disorders as endometriosis and OHSS. Utilizing our comprehensive knowledge of PEDF's role in the reproductive system makes it a promising therapeutic agent for angiogenesis-related reproductive pathologies.
Study design, size, duration: We established mouse models for both endometriosis and OHSS.
Endometriosis: We plotted treatment concentration-curve evaluating rPEDF regimen given on 4 or 2 administration times, and tested for endometrial lesions recurrence following treatment cessation.
OHSS: We plotted treatment concentration-curve evaluating rPEDF regimen comparing between preventive and acute treatment.
Participants/materials, setting, methods: Endometriosis: Uterine pieces were transplanted onto the abdominal wall of estrogen-treated ICR mice. Treatment with rPEDF started 10 days later. OHSS: ICR mice were treated with PMSG (3*20 IU/day)/hCG (7 IU). Control mice administered with PMSG (1*5 IU)/hCG (7 IU). Changes in body weight, ovarian weight and vascular permeability were recorded.
Main results and the role of chance: PEDF and VEGF are counter regulated both in-vivo and in-vitro in reproductive model systems. Treating edometriosis-induced mice with rPEDF reduced the weight of the endometrial transplants significantly ($p < 0.001$). A concentration of 2 mg/kg rPEDF was chosen as the preferable treatment dose. After cessation of treatment there was no recurrence of endometrial lesions under a continuous 2-weeks estrogenic treatment. Treating OHSS-induced mice with rPEDF alleviated OHSS symptoms, including edema ($p < 0.001$) and vascular leakage ($p < 0.001$). We found that the preferable treatment dose of PEDF needed for alleviating OHSS symptoms either as a preventive or therapeutic treatment was also 2 mg/kg. Administration of rPEDF had no effect on ovulation and pregnancy rates.
Limitations, reason for caution: The experiments were performed in a mice model.
Wider implications of the findings: The therapeutic properties of PEDF and the fact that it does not affect reproductive parameters, offer a rationale for using PEDF as a treatment for endometriosis with a potential of treating other angiogenic-related reproductive pathologies.
Study funding/competing interest(s): Funding by University(ies), Tel-Aviv University.
Trial registration number: N/A.

O-113 Comparison of the anti-inflammatory effect of resveratrol and leuprolide acetate in an experimental endometriosis model

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Study question: Can resveratrol inhibit the growth of endometriotic-like lesions in an experimental model of endometriosis, and is this effect comparable with leuprolide acetate?
Summary answer: Resveratrol is comparably effective on reduction of endometriotic lesions and suppression of inflammatory response.
What is known already: resveratrol is a novel treatment agent that has an anti-inflammatory effect by inhibition of cytokines. It is known that endometriosis is a chronic inflammatory disease and related with increase of inflammatory markers.
Study design, size, duration: This was a randomized study in a rat model of endometriosis. Twenty one female Sprague-Dawley rats with surgically induced endometriosis were treated with resveratrol (10 mg/kg/day intramuscular for 14 days, $n = 7$), leuprolide acetate (1 mg/kg subcutaneous depot, $n = 7$) or vehicle ($n = 7$).
Participants/materials, setting, methods: Endometriosis was surgically induced in 21 female rats during estrus by implantation of an autologous fragment of the endometrium to the anterior wall of the abdominal cavity. After 4 weeks, repeat laparotomies were performed to check the implants and the animals were randomized into three groups: Group I, resveratrol group; Group II, leuprolide acetate group and group III, no medication. The resveratrol administration was continued for 2 weeks after the surgery. Two weeks later rats were sacrified and the implants were evaluated morphologically and histopathologically, and plasma and peritoneal fluid interleukin (IL)-6, IL-8, TNF-α levels were assayed.
Main results and the role of chance: In this experimental rat model, both treatment modalities significantly reduced the volume of the endometriotic lesions ($P < 0.05$ versus control). IL-6 levels in plasma and peritoneal fluid, and TNF-α plasma levels significantly decreased in group I and II ($P < 0.05$ versus control). No statistical significance was observed in IL-8 plasma and peritoneal fluid levels, and TNF-α peritoneal fluid levels ($P > 0.05$).
Limitations, reason for caution: Experimental models were induced by implantation of autologous rat endometrial tissue. Confirmation of the results with the human tissues would be required in the future.
Wider implications of the findings: Our results are proved that resveratrol is a potential agent for treatment of endometriosis and an alternative of leuprolide acetate.
Study funding/competing interest(s): Funding by University(ies), Recep Tayyip Erdogan University, Scientific Research Project Unit.
Trial registration number: Project number: 2012.106.02.3.

O-114 Influence of different oocyte maturation triggering and luteal phase support protocols on dendritic cell and metalloproteinase endometrial protein expression

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Study question: Do immunological endometrial factors, like MMP expression, NK and dendritic cell number, differ between different modes of oocyte maturation triggering and luteal support?
Summary answer: MMP-14 protein and dendritic cells (CD1a protein) show a trend towards a differential endometrial expression in different oocyte maturation triggering and luteal support protocols and may be further explored as potential immunological markers in IVF. CD56 cell counts are in line with known high inter-patient and -cycle variability.
What is known already: Natural killer (uNK) cells count for the majority of the immune cells in luteal phase endometrium. However, several mice studies also pointed out an orchestrating role for uterine dendritic cells (uDCs) by contributing to the decidualization of the endometrium and to tolerance induction towards the implanting embryo. In human endometria however, uDCs have only been studied a few times. Metalloproteinases play a role in endometrial receptivity. Earlier studies showed variable gene expression according to the IVF protocol.
Study design, size, duration: A prospective randomized study was performed in four oocyte donors recruited from an oocyte donation program during the period 2010–2011. The endometrial biopsies from these donors (n = 16) were used for gene expression analysis and further protein analysis by immunohistochemistry.
Participants/materials, setting, methods: Four oocyte donors each prospectively underwent four consecutive stimulation protocols, with different modes of triggering final oocyte maturation and a different luteal phase support (**A**) 10.000 IU hCG and standard luteal phase support, (**B**) Triptorelin 0.2 mg (GnRH agonist trigger) and 1500 IU hCG with standard luteal phase support, (**C**) Triptorelin 0.2 mg and standard luteal phase support and (**D**) Triptorelin 0.2 mg – without luteal phase support), followed by endometrial biopsy on Day 5 after oocyte retrieval. The endometrial biopsies were analyzed for gene expression with microarrays and histologically dated according to Noyes. IHC was performed, based on differentially expressed gene data, for MMP proteins (MMP1 and MMP14), and dendritic cell and NK cell surface markers (CD1a and CD56).
Main results and the role of chance: All 16 biopsies were dated to be in the secretory phase, except in protocol D where the endometrium had a consistent premenstrual appearance. Absolute CD1a+ DC counts showed a large inter-patient variation with the highest amount in the luminal epithelium and the lowest in the stroma. A trend was observed towards lower luminal CD1a+ DC numbers in protocols B, C and D, the lowest being in protocol D, associated with a histological premenstrual appearance. CD56+ counts for NK cells showed no differential expression between the different stimulation protocols. Low MMP1 protein expression was found, mainly in the glands, without differential expression between the protocols. MMP14 proteins were strongly expressed in all endometrial biopsies. In the biopsies with a premenstrual histology, MMP14 showed a trend towards a high expression in the stroma and a lower expression in the glands.
Limitations, reason for caution: The study was performed in four stimulated oocyte donors only; however, it is a strength of the study that the same donor underwent four consecutive stimulation protocols within 1 year to avoid inter-individual variations. A non-quantitative descriptive approach was used to analyze different proteins.
Wider implications of the findings: The present findings will be useful not only in the study of recurrent implantation failure but also in the study of endometrial receptivity and implantation and the influence of different stimulation protocols hereon.
Study funding/competing interest(s): Funding by national/international organization(s), Funding by commercial/corporate company(ies), This study was supported by a research grant by MSD Belgium. Shari Mackens is supported by FWO as a doctoral fellow.
Trial registration number: This study was performed on samples from an RCT with EudraCT number 2009-009429-26, protocol number 997 (P06034).

SELECTED ORAL COMMUNICATION SESSION

SESSION 31: CLINICAL TRIALS IN OVARIAN STIMULATION

Tuesday 1 July 2014 **10:00 - 11:30**

O-115 Does extending the time interval between human chorionic gonadotrophin administration and oocyte pick-up by two hours affect oocyte retrieval rate – a randomized clinical trial

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Study question: Does extending the time interval between human chorionic gonadotrophin (hCG) administration and oocyte pick-up (OPU) by 2 h affect oocyte retrieval rate (ORR) in women undergoing in-vitro fertilization (IVF)/ intracytoplasmic sperm injection (ICSI) with gonadotrophins and gonadotrophin releasing hormone (GnRH) antagonists?
Summary answer: Extending the time interval between hCG administration and oocyte pick-up by 2 h does not seem to have an impact on the oocyte retrieval rate.
What is known already: The time interval between hCG administration and OPU varies among IVF centres, although an interval of 32–36 h is the most widely adopted. However, no data have been published to date regarding the optimal time interval in cycles in which GnRH antagonists are used.
Study design, size, duration: The study was carried out between 11/2009 and 12/2013. Patients underwent oocyte retrieval either 36 h or 38 h following hCG administration according to a computer-generated randomization list. The primary outcome measure, oocyte retrieval rate, was defined as cumulus-oocyte complexes (COCs) retrieved/ follicles >11 mm on the day of hCG administration.
Participants/materials, setting, methods: According to sample size estimation (aiming to detect a difference of 10% in ORR), 156 normo-ovulatory women <45 years of age, without polycystic ovarian syndrome, endometriosis stage III–IV or history of previous ovarian surgery were included in this study. Stimulation was performed using recombinant gonadotrophins and GnRH antagonists.
Main results and the role of chance: No significant differences were observed regarding the baseline characteristics of patients in the two groups compared. Moreover, no statistically significant differences were observed between the 36 h (n = 78) and the 38 h (n = 78) groups regarding the oocyte retrieval rate (63.3 ± 24.2% vs. 59.1 ± 26.1%, p = 0.29), the number of COCs retrieved (6.12 ± 3.8 vs. 6.19 ± 4.0, p = 0.90), the number of mature oocytes (4.85 ± 2.8 vs. 4.77 ± 3.1, p = 0.87) and fertilization rates (54.4 ± 29.6% vs. 49.7 ± 28.7%, p = 0.33), respectively. The proportion of patients with embryo transfer was similar between the 36 h and the 38 h groups [87.2% vs. 82.1%, rate difference (RD): +5.1%, 95% confidence interval (CI): –6.3 to +16.5, respectively] as were clinical pregnancy rates per randomized patient (29.5% vs. 21.8%, RD: +7.7%, 95% CI: –6.2 to +21.5, respectively).
Limitations, reason for caution: Sample size estimation was based on a difference of 10% in the oocyte retrieval rate between the two groups compared and thus smaller differences cannot be excluded. Moreover, due to sample size restrictions, no conclusions can be drawn regarding the probability of pregnancy.
Wider implications of the findings: Based on the results of the current study, prolongation of the time interval between hCG administration and oocyte pick-up by 2 h does not seem to be associated with a decreased oocyte retrieval rate and thus clinicians may be more flexible regarding the timing of oocyte pick-up. On the other hand, no benefit in terms of oocyte maturity should be expected by following this approach.
Study funding/competing interest(s): Funding by University(ies), Aristotle University of Thessaloniki, Greece.
Trial registration number: ClinicalTrials.govID**:** NCT02044445.

O-116 Endocrine effects of rhCG or rLH supplementation to rFSH stimulation in a GnRHa long down-regulation protocol – a randomized controlled study

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Study question: Do significant differences exist in follicular steroidogenesis depending on the type of LH-activity used during ovarian stimulation for IVF.
Summary answer: RLH and rhCG are equally effective for controlled ovarian stimulation when tested in a 1:1 bioactivity ratio in a GnRHa long down regulation protocol.
What is known already: During follicular growth and steroidogenesis both LH and FSH are required for normal function. FSH is the principal regulator, and the precise requirements of LH are unclear. LH receptors in the theca cell stimulate the synthesis of androgens, which act as a substrate for the oestrogen (E2) synthesis. Therefore, circulating levels of E2 reflect the combined effects of FSH and LH on follicular steroidogenesis.
Study design, size, duration: A randomized controlled study enrolling a total of 100 normogonadotropic IVF/ICSI patients in a public IVF setting. The primary endpoint of the study was the E2 level on the day of triggering final oocyte maturation.
Participants/materials, setting, methods: IVF patients down-regulated with GnRHa using a fixed dose of 150 IU rFSH combined with either 25 IU rhCG (Group A) or 150 IU rLH (Group B) daily from day 1 of stimulation. The gonadotropin dose was modified according to ovarian response, keeping the rFSH/LH bio-activity level constantly at 1:1.
Main results and the role of chance: There were no significant differences regarding the demographic characteristics between the two groups and the majority of participants were Caucasians. No differences in rFSH and total LH-activity consumption, the number of follicles at the time of ovulation induction, and the distribution in follicular sizes were seen. The endocrine levels on day of hCG trigger were similar between groups, in particular the E2 level was 11482 (±8210 pmol/l) in group A versus 12262 (±8116 pmol/l) in group B (NS).

The study was not powered to detect differences in reproductive outcome; however, similar clinical pregnancy rates were seen between groups.
Limitations, reason for caution: The present study is the first to explore possible endocrinological and clinical differences between use of hCG or LH in a 1:1 bio-activity ratio for ovarian stimulation from day 1. To draw firm conclusions, a larger trial is needed.
Wider implications of the findings: When used in a 1:1 bio-activity ratio the actions of rLH and rhCG are similar, and both compounds secure the development of good quality oocytes.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), The study was supported by an unrestricted research grant from Serono, Denmark.
Trial registration number: EUdraCT: 2009-009375-35; number: 2612-3960.

O-117 Kisspeptin – a novel physiological trigger for oocyte maturation in IVF treatment

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Study question: Kisspeptin is a recently identified naturally occurring hormone, which potently increases endogenous luteinising hormone (LH) secretion in women without adverse effects. Kisspeptin has been shown to trigger the LH surge and ovulation in rodents and sheep. It is not known whether administration of kisspeptin can induce oocyte maturation in women.
Summary answer: We demonstrate for the first time that a subcutaneous bolus injection of kisspeptin can be used therapeutically to induce oocyte maturation in women following a standard FSH/GnRH antagonist superovulation protocol. This study provides proof of concept that kisspeptin can effectively induce oocyte maturation in IVF treatment.
What is known already: IVF is an effective treatment for infertility. However, it can cause life-threatening complications such as ovarian hyperstimulation syndrome (OHSS) due to the pharmacological use of hCG to stimulate oocyte maturation during IVF. Developing a more physiological stimulus for oocyte maturation would prevent OHSS. We have shown that subcutaneous injection of kisspeptin to healthy women increases endogenous LH secretion potently during the pre-ovulatory phase of menstrual cycle.
Study design, size, duration: This was a single-centre prospective clinical trial. Fifty three women underwent an FSH/GnRH antagonist protocol using kisspeptin (1.6–3.2 nmol/kg, $n = 5$; 6.4 nmol/kg, $n = 24$; 12.8 nmol/kg, $n = 24$) to trigger oocyte maturation (instead of hCG). Oocyte retrieval was performed 36 h after kisspeptin injection. Following intracytoplasmic sperm injection (ICSI) one or two embryos were transferred to the patient.
Participants/materials, setting, methods: All IVF cycles were performed at Hammersmith Hospital, London. Inclusion criteria were as follows: age <35 years; body mass index <30 kg/m^2; serum anti-Mullerian hormone >10 pmol/L or good response during previous IVF cycle. The primary outcome was number of mature oocytes (defined as MII oocytes) following egg collection.
Main results and the role of chance: No adverse events were noted at any time during the study. Serum LH was increased 9.0-fold at 12 h following kisspeptin injection, when compared with serum LH immediately prior to kisspeptin injection. Oocyte maturation (defined as at least one mature oocyte) was observed at all doses of kisspeptin: 96% (51/53) of patients, with a mean of 7.9 ± 3.9 MII oocytes/cycle. Fertilisation occurred in 92% (49/53) of cases, with a mean of 5.6 ± 3.5 zygotes/cycle. Biochemical and clinical pregnancy rates were 40% (21/53) and 23% (12/53), respectively. Eight women have already given birth to healthy babies including two sets of twins.
Limitations, reason for caution: This study suggests that injection of kisspeptin is sufficient to stimulate oocyte maturation in women following superovulation. Further work is required to directly compare the efficacy of kisspeptin with other currently used triggers of oocyte maturation such as hCG and GnRH agonists.
Wider implications of the findings: We suggest for the first time that kisspeptin can effectively induce oocyte maturation in women undergoing IVF treatment. Kisspeptin may therefore offer an entirely novel trigger for oocyte maturation during IVF treatment. Further studies are warranted to investigate whether kisspeptin-induced oocyte maturation is associated with lower rates of OHSS when compared with hCG.
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.

O-118 Mild versus standard ovarian stimulation in poor responder women undergoing ivf and icsi (prima) – multicenter randomised controlled study

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Study question: How does – in couples with poor response or women with advanced age undergoing IVF or ICSI- one cycle of mild ovarian stimulation IVF followed by cryo-cycles compare with one cycle of standard ovarian hyperstimulation IVF- plus subsequent cryo-cyclesin terms of ongoing pregnancy rate?

Summary answer: Mild ovarian stimulation is comparable to the standard ovarian stimulation in women with poor ovarian response or advanced age undergoing IVF/ICSI treatment cycles.
What is known already: Poor responders are estimated to comprise approximately 9–24% of IVF/ICSI patients. Various stimulation protocols have been tried to improve pregnancy outcomes in poor responder women. Since most studies included small numbers of patients and used different definitions of poor response, the best stimulation protocol is still unknown. Most treatment comparisons include high doses of gonadotropins and only vary in their means of ovarian suppression. High doses of gonadotropins did not result in higher pregnancy rates. The mild stimulation using a GnRH antagonist and a low dose of gonadotrophins may present a good alternative
Study design, size, duration: This open-label, multicentre randomized controlled trial was conducted between March 2011 and January 2014. A web-based program was used for randomization and 394 IVF/ICSI patients were included.
Participants/materials, setting, methods: The study group (mild stimulation group, *n* = 197) was pretreated with oral contraceptive pills (OCP) started on cycle day 2–3 of the preceding cycle, then a fixed dose of 150 IU/day HP/rec FSH, s.c was initiated on day 5 after the last OCP. GnRH antagonist was commenced on stimulation day 6 (Fixed protocol). The control group (standard stimulation, *n* = 197) was treated with the GnRH agonist triptoreline starting 1 week before the expected menses. After down-regulation is achieved, ovarian stimulation was commenced with a fixed daily dose of 450 IU/day HMG. After establishing ovarian and uterine quiescence using vaginal ultrasound.
Main results and the role of chance: There were no significant differences in ongoing pregnancy rate between the two groups (OPR: 16.0% (18/111) versus 22.6% (26/115), *P* = 0.12).
Limitations, reason for caution: None.
Wider implications of the findings: The present study shows that the mild ovarian stimulation with GnRH antagonist protocol could be an optimal alternative to standard ovarian stimulation with mid luteal long GnRH agonist protocol.
Study funding/competing interest(s): Funding by national/international organization(s), This study was partially supported by a grant from NUFFIC, STDF.
Trial registration number: NTR2788.

O-119 Corifollitropin-alfa compared to daily rFSH in poor responders undergoing ICSI: a randomized clinical trial

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Study question: Is corifollitropin-alfa treatment equally effective compared to daily recombinant follicle stimulating hormone (rFSH) in terms of cumulus-oocyte complexes retrieved (COCs) in poor responders undergoing intracytoplasmic sperm injection (ICSI) using gonadotrophin releasing hormone (GnRH) antagonists?
Summary answer: Corifollitropin-alfa appears to be at least as effective as daily rFSH for ovarian stimulation regarding the number of COCs retrieved in poor responders treated by ICSI.
What is known already: The effectiveness of substituting the first seven daily injections of rFSH with a single injection of corifollitropin-alfa in women undergoing ovarian stimulation for ICSI has been demonstrated in previous studies, which showed similar ongoing pregnancy rates. However, no data exist regarding the effectiveness of corifollitropin-alfa treatment compared to daily rFSH in poor responders.
Study design, size, duration: This was a non-inferiority trial performed from 1/2011 to 12/2013 on poor responders (<5 COCs in a previous attempt with a starting dose of at least 450 IU/d). Following sample size estimation, patients were randomized to receive either corifollitropin-alfa (*n* = 40) or rFSH (*n* = 39), using a computer-generated randomization list.
Participants/materials, setting, methods: On day 2 of the cycle patients received either a fixed dose of 450 IU/d of rFSH or a single dose of 150 mg corifollitropin-alfa. In the corifollitropin-alfa group 450 IU of rFSH were used from day 8 of stimulation until the day of human chorionic gonadotrophin (hCG), if necessary.
Main results and the role of chance: No differences in the patient baseline characteristics were observed between the corifollitropin-alfa and the rFSH group. Duration of stimulation was comparable between the two groups (10.6 ± 2.6 vs. 10.2 ± 2.3 days, $p = 0.43$) as were estradiol levels on the day of hCG administration (1105 ± 490 vs. 1163 ± 597 pg/ml, $p = 0.64$), the number of COCs retrieved (3.10 ± 2.6 vs. 2.79 ± 2.4, $p = 0.59$), the number of mature oocytes (2.65 ± 2.4 vs. 2.46 ± 2.3,$p = 0.72$), the number of 2-pronuclei oocytes (1.98 ± 2.0 vs. 1.77 ± 1.9, $p = 0.64$), fertilization rates (59.7 ± 32.9% vs. 57.4 ± 30.2%,$p = 0.77$) and the number of embryos transferred (2.17 ± 0.9 vs. 1.96 ± 1.2, $p = 0.45$), respectively. Pregnancy rates were not statistically different between the corifollitropin-alfa and the rFSH groups (17.2% vs. 7.1%, Rate Difference: +10.1%, 95% CI: −7.4 to +27.6) as were the proportions of patients with embryo transfer (72.5% vs. 71.8%, $p = 0.94$), respectively.
Limitations, reason for caution: Although the pregnancy rates in the corifollitropin-alfa group are higher than the rFSH group, this study was not powered to detect a difference in pregnancy rates, and thus no conclusions can be drawn regarding this finding.
Wider implications of the findings: The results of this randomized clinical trial indicate that corifollitropin-alfa is at least as effective as daily rFSH for the ovarian stimulation of poor responders. Considering that the use of corifollitropin-alfa simplifies treatment by reducing the burden of multiple daily injections, it appears to be an advantageous alternative to daily rFSH for the treatment of poor responders undergoing ICSI.
Study funding/competing interest(s): Funding by University(ies), Aristotle University of Thessaloniki.
Trial registration number: ClinicalTrials.govID: NCT02046655.

O-120 Fully human glycooptimized recombinant FSH: a randomized, assessor-blind, multi-center, multi-national phase II trial to investigate the efficacy and safety of FSH-GEX in women undergoing ART

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[10]Glycotope GmbH, Clinical Development, Berlin, Germany
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Study question: The aim of the current study was the determination of the recommended standard treatment dose of FSH-GEX, a fully human glycooptimized recombinant FSH molecule, in women undergoing ICSI treatment in a long GnRH agonist protocol as assessed by follicle growth.
Summary answer: Compared with follitropin-alfa 150 IU, the lower dose of 112.5 IU FSH-GEX exhibits a statistically higher activity with respect to number of COCs and MII oocytes retrieved, whereas 75 IU FSH-GEX is at least as active. FSH-GEX shows a good safety profile with a low incidence of ovarian hyperstimulation syndrome.
What is known already: Recombinant FSH products on the market comprise a non-human glycosylation pattern expressed in Chinese Hamster Ovary (CHO) cells. FSH-GEX is a fully human molecule with optimized glycosylation produced in human glycoengineered GlycoExpress™ cells. Single dose and multiple dose studies in healthy volunteers demonstrated a dose dependent increase in follicle size and numbers which were explicitly higher as compared with equivalent doses of follitropin alfa (Gonal-f) or urofollitropin (Bravelle).
Study design, size, duration: Phase II, randomised, assessor-blind, comparator-controlled study in women undergoing ICSI treatment. Patients were randomized to 5 treatment groups receiving fixed doses of FSH-GEX (52.5, 75, 112.5, 150 IU daily; 150 IU every other day) compared with a group receiving 150 IU Gonal-f daily. Study period: January to September 2013.

Participants/materials, setting, methods: A total of 247 female patients aged 18–37 underwent controlled ovarian stimulation by using the long agonist protocol. After down-regulation with triptorelin (100 µg/day) patients were stimulated with fixed doses of FSH until at least one follicle reached a diameter of ≥20 mm. Number of follicles >12 mm were assessed.
Main results and the role of chance: FSH-GEX displayed a dose dependent effect on follicle growth and oocyte numbers. In comparison with a standard dose of Gonal-f (150 IU) the pharmacodynamic effects of 112.5 IU FSH-GEX were stronger resulting in higher numbers of follicles >12 mm [FSH-GEX: least square mean = 13.7 (95% CI = 12.3–15.0); Gonal-f: 12.2 (10.8–13.6), n.s.]; COCs [FSH-GEX: 14.3 (12.6–15.9), Gonal-f :10.9 (9.2–12.6), $p < 0.01$]; MII oocytes [FSH-GEX: 10.4 (9.2–11.7), Gonal-f: 8.4 (7.1–9.7), $p < 0.05$]; and 2PN oocytes [FSH-GEX: 7.4 (6.2–8.7), Gonal-f: 6.1 (4.9–7.3), n.s.]. Pharmacodynamic effects of 150 IU Gonal-f ranged between the dosages of 52.5 and 75 IU FSH-GEX. Every other day administration of 150 IU FSH-GEX™ resulted in pharmacodynamic parameters comparable to 75 IU every day. OHSS occurred after FSH-GEX treatment in 2.9% and after Gonal-f treatment in 7.7% of patients.
Limitations, reason for caution: The study was powered to detect differences of 3 follicles between treatment groups ($\beta = 0.80$; $\alpha = 0.05$). Secondary outcome parameters were analyzed by descriptive statistics. Additional analyses of subgroups were hampered by small sample sizes.
Wider implications of the findings: FSH-GEX displays strong activity on follicle growth and, subsequently, the development of oocytes for fertilization. In comparison with a standard dose of recombinant CHO derived FSH the activity ratio is estimated to be at least 2:1. The equipotent dose of FSH-GEX in comparison with 150 IU of CHO derived FSH is estimated at between 52.5 and 75 IU. Even a low dose of 52.5 IU FSH-GEX leads to sufficient numbers of oocytes in most cases.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Glycotope GmbH, Germany.
Trial registration number: ClinicalTrials.gov identifier: NCT01794208.

SELECTED ORAL COMMUNICATION SESSION

SESSION 32: PATIENTS' NEEDS AND WISHES: WHERE TO DRAW THE LINE?

Tuesday 1 July 2014 **10:00 - 11:30**

O-121 *In vitro* screening and selection of embryos by whole-genome sequencing and analysis – assessment of its usefulness and ethical implications

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[5]*Katholieke Universiteit Leuven, Department of Electrical Engineering, Brussel, Belgium*

Study question: What is the analytical and clinical validity and the clinical utility of *in vitro* screening of embryos by whole-genome sequencing? What are the associated ethical questions?
Summary answer: At present there are still many limitations in terms of analytical and clinical validity and utility. In addition many ethical questions remain. Therefore, using whole genome sequencing and analysis for embryo selection is not advised.
What is known already: With the advent of single-cell whole genome sequencing and analysis, whole genome sequencing of IVF/ICSI f embryos for selection purposes is technically possible. However, many loss of function mutations exist in the general population without serious effects on the phenotype of the individual. Moreover, annotations of genes and the reference genome are still not 100% correct.
Study design, size, duration: We used publicly available samples from the 1000 Genomes project and Complete Genomics, together with 42 samples from in-house research samples of parents from trios to investigate the presence of loss of function mutations in healthy individuals.
Participants/materials, setting, methods: To assess for clinical and analytical validity of WGS/WG for embryo selection, we looked for mutations in genes that are associated with a selection of severe Mendelian disorders with a known molecular basis. We looked for mutations predicted to be damaging by PolyPhen and SIFT and for mutations annotated as disease causing in HGMD.
Main results and the role of chance: More than 40% of individuals who can be considered healthy have mutations that are predicted to be damaging in genes associated with severe Mendelian disorders or are annotated as disease causing. This has an impact on the clinical and analytical validity of WGS/WGA for embryo selection, and therefore on its clinical utility for embryo selection, as it would lead to discarding embryos that may well develop into healthy children. At present, the drawbacks of WGS-based embryo screening appear to outweigh the possible benefits for prospective parents, making the introduction of such screening in clinical practice unwarranted and at best premature.
Limitations, reason for caution: The analysis relies on current knowledge and databases are continuously updated to reflect our increasing knowledge about the genome. In the process of our analysis several updates were already made.
Wider implications of the findings: At this moment it is not advisable to use whole-genome sequencing as a tool to set up health profiles to select embryos for transfer. We also raise some ethical questions that have to be addressed before this technology can be used for embryo selection.
Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), Raf Winand is supported by Research Council KU Leuven: GOA/10/09 MaNet, KUL PFV/10/016 SymBioSys; IWT O&O ExaScience Life; Hercules Stichting: Hercules III PacBio RS; iMinds; Art&D Instance; VSC Tier 1: exome sequencing; COST: Action BM1006: NGS Data analysis network. Kristien Hens was supported by the Dutch Centre for Society and Life Sciences (CSG, non-profit organization) (project number: 70.1.074).
Trial registration number: N/A.

O-122 Maybe we shouldn't give infertile patients what they want, but what they need

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Study question: The ever-expanding options in medically assisted reproduction illustrate that we are willing to go to great lengths to establish genetic parenthood. To what extent should we defend an alternative, paternalistic approach, in which a greater emphasis is put on helping patients to cope with infertility, rather than remedying it?
Summary answer: As reproductive autonomy is highly valued in our society, assisting people in their reproductive endeavors is a duty of the medical community. However, sometimes restricting patients' options, rather than enlarging them, will benefit them, despite going against their reproductive autonomy.
What is known already: An infertility diagnosis often leads to psychological problems such as depression. However, follow-up shows that long-term quality of life is as high for involuntarily childless couples as for parents. Thus, although accepting childlessness is psychologically very difficult, the perception that having children will bring about a degree of happiness that cannot be achieved otherwise is at least partially flawed.
Study design, size, duration: In this study the prevailing arguments in theoretical normative ethics on 'real interests' versus 'perceived interests' are applied to the clinical ART-setting. A literature search was performed on short-term and long-term effects of parenthood and childlessness on wellbeing.
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 fact that many people find it difficult to cope with an infertility diagnosis while research shows that having children does not significantly contribute to overall wellbeing leads to a discrepancy between perceived interests and real interests. Whereas going to great lengths to enable an infertile couple, same-sex couple or single to conceive a child appears to be in their best interest, helping them develop coping mechanisms and convincing them that *not* having children will not preclude future wellbeing may serve their interest even more. Just as offering a tenth or fifteenth cycle after multiple failures is not always in the patients' best interest, we may also do them a disservice by constantly expanding their options and thus hindering the grieving process.
Limitations, reason for caution: The social stigmatization that accompanies infertility in certain developing countries has not been taken into consideration. Our findings mainly apply to the Western world.
Wider implications of the findings: Fertility clinics may want to rethink their measure of success. In light of our analysis, patient drop-out can oftentimes be considered as a positive outcome, specifically when patients choose to discontinue treatment because they have accepted the prospect of remaining childless.
Study funding/competing interest(s): Funding by national/international organization(s), Research Foundation Flanders.
Trial registration number: N/A.

O-123 Donor conception disclosure: directive or non-directive counselling

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Study question: Which counselling approach is most in accordance with the counsellor's general deontology with regard to the topic of donor conception disclosure: a directive or a non-directive approach?
Summary answer: A directive counselling approach in favour of both disclosure and secrecy cannot be justified on the basis of probability and severity of harm to a third person.
What is known already: It is widely agreed among health professionals and governmental organizations that couples using Assisted Reproductive Techniques (ART) should be offered counselling (before, during and after treatment). It differs from country and legislation whether this is mandatory or not. Specifically with regard to the topic of DC disclosure: for a long time, a directive approach pro secrecy was used. Nowadays, both a directive or a non-directive approach are defended by counselors organisations, but the directive approach now favours disclosure.
Study design, size, duration: This analysis is based on a literature research focusing on genetic counselling and counselling of donor conception disclosure.
Participants/materials, setting, methods: We made an analysis of the arguments used in favour of both counselling approaches (directive and non-directive). A comparison between the context of donor conception disclosure and the genetic context was made.
Main results and the role of chance: In the genetic context, counselling is generally non-directive. However, directive counselling is accepted in some cases, for instance, where sharing information with relatives is important. We compared donor conception disclosure to such an 'exception to the rule': the case of BRCA. The basis of our comparison is the probability and severity of harm to a third person. The analysis shows that the risks for the child due to non-disclosure of donor conception are much less severe and much less likely than the risks for the at-risk relatives in the case of non-disclosure of BRCA. It is therefore not possible to present directive counselling in the case of donor conception as an exception to the rule.
Limitations, reason for caution: We hold a strict dichotomy between non-directive and directive counselling for the sake of the argument. In practice, this distinction is often not so clear.
Wider implications of the findings: Counsellors should aim to counsel 'as neutrally as possible', paying equal attention to the possible (psychological and medical) harms of secrecy as well as of disclosure. Also, the broader social environment should be taken into account during counselling sessions as this is an important factor in deciding whether or not it is best to disclose.
Study funding/competing interest(s): Funding by University(ies), University Ghent.
Trial registration number: None.

O-124 Low ovarian stimulation using tamoxifen/fsh compared to conventional IVF: a cohort comparative study from Kazakhsztan

M. Nitzschke[1], S. Baikoshkarova[2], A. Ibragimov[2], B. Abdilmanova[2], G. Murzagaliyeva[3]
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[2]*Ecomed, Private IVF Clinic, Astana, Kazakhstan*
[3]*Ecomed, Private IVF Clinic, Almaty, Kazakhstan*

Study question: Is it possible that a low stimulation protocol based on Tamoxifen and FSH may be as efficient as a conventional IVF program in a routine IVF clinic in Kazakhstan?
Summary answer: The clinical pregnancy rate was found to be NOT significantly different between the Tamoxifen treated group and the Conventional IVF group (24.7% *vs.* 21.2%), however we found lower numbers of visits, lower costs for medication, less side effects and better acceptance for treatment in the Tamoxifen group
What is known already: Old types of medicine such as Tamoxifen and Clomiphene have been introduced in the market again for several reasons, such as lower costs, safety, less stress for the patient and convenience. The wide spread use of single embryo transfer makes is unnecessary to harvest more than a few eggs in an IVF cycle. It has been demonstrated that the health of offsprings from mild stimulation is comparable to conventional IVF.
Study design, size, duration: A total of 85 regular short antagonist IVF cycles were retrospectively compared to 97 Tamoxifen low stimulation IVF cycles. All patients were recruited in the same time period and allocated to the different treatments on their own request. The study took place in our clinic 'Ecomed' (Astana) from 06/2011–04/2013.
Participants/materials, setting, methods: In a cohort study including all patients admitted to IVF for unexplained infertility, male factor or tubal factor, the patients decided on their own to go for regular IVF using a short antagonist protocol with daily 225 IU HMG or low stimulation using daily Tamoxifen 40 mg +150 IU HMG.
Main results and the role of chance: No differences in age and number of previous cycles were found in between the groups. The pregnancy rate per transfer and started cycle, as well as the implantation rate was equal between the protocols. The mean number of HMG units used in conventional IVF was significantly higher than for low stimulation. The mean number of visits during the conventional stimulation was 3 before oocyte collection versus 1 visit for the Tamoxifen protocol. The costs for medication and the time spent in the clinic were found to be significantly lower in the low stimulation group as well as patients' distress during treatment. The clinical pregnancy rate was found to be NOT significantly different between the Tamoxifen treated group and the conventional stimulation group (24.7% *vs.* 21.2%).
Limitations, reason for caution: The relatively small size of the study groups may not have the statistical power to show significant differences between the groups.
Wider implications of the findings: Low stimulation using Tamoxifen, without any other agonist or antagonist, is an efficient treatment regime and fully comparable to conventional IVF. These data are among the first to present the benefits of Tamoxifen in IVF stimulation. Therefore we are convinced that the low stimulation will be the preferred treatment by patients in the future, due to excellent pregnancy rates following oocyte collection, less stress, fewer drop-outs and a reduced economic burden.
Study funding/competing interest(s): Funding by hospital/clinic(s), Ecomed – Clinic For Human Reproduction, Almaty, Katakhstan.
Trial registration number: None.

O-125 Amazing low cost IVF/ICSI induction protocol in developing countries

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Study question: No questionair given. Only clinical study of follicular monitoring by transvaginal ultrasonography & serum estradiol levels.

total 140 patients were randomly selected under 37 year of age. group 1 was stimulated with standard GnRh analogues, group 2 was stimulated with aromatase inhibitors, indomethacine and gonadotrophins.

Summary answer: Dose of gonadotrophins was adjusted according to results of follicular size & serum estradiol level.
What is known already: Since avoidance of premature LH surge remained the main issue of IVF/ICSI. It is proven fact that estrogen peak comes before LH surge. A concept behind this regime was that if we suppress this estrogen peak, we can also control this LH surge. For poor patients, we used low dose antiestrogen along with flexible dose of gonadotropins. Since ovulation is basically inflammatory event, we used anti-inflammatory agents to stop ovulation before ovum pick up.
Study design, size, duration: RCT study conducted on 140 patients under 37 years of age (2011 to 2012). Group 1monitored according to standard protocol. Second group observed estradiol levels by transvaginal ultrasonography on day second & eighth, then serial monitoring until LH surge was given & oocyte retrival done after 36 h of LH surge.
Participants/materials, setting, methods: Total 140 patients below 37 years of age were studied. They were divided into two equal groups. The agonist group underwent long GnRH analogue protocol. Other group received aromatase inhibitors with gonadotropins & anti-inflammatory agent indomethacin (indomethacinate) added 8 hourly intra muscular till the day of ovum pick up.
Main results and the role of chance: In group 1

In 20 patients (10–14) M2 oocytes retrieved with 4–6 embryos at morula stage.6 patients conceived. 5 ended in full term.

In 40 patients (6–9) M2 oocytes retrieved with 3–5 embryos at morula stage, 8 patients conceived, 6 ended in full term.

In 10 patients (2–6) M2 oocytes retrieved with 0–3 embryos at morula stage, 2 patients conceived & ended in full term.

In group 2

In 30 patients (6–8) M2 oocytes retrieved with 2–4 embryos at morula stage, 7 patients conceived, 6 ended in full term.

In 40 patients (3–6) M2 oocytes retrieved with 1–4 embryos at morula stage, 9 patients conceived, 7 ended in full term.

In group 1 pregnancy rate 22.8% & take home baby 18.5%.

In group 2 pregnancy rate 22.8% & take home pregnancy 18.5%.

Limitations, reason for caution: Significantly difference in gonadotropins usage in both groups, leading to marked economical saving for poor patients. According to results although mean number of oocytes retrieved were higher in group 1, but clinical pregnancy rate remained similar. Limitations are there as regarding sample size & random selection of patients.
Wider implications of the findings: Since people of Pakistan are suffering from population explosion. Government is only giving attention to restrict population but the poor 20%population who is facing infertility is not having funds & resources. Our center 'Pakistan institute of andrology' is offering low cost ICSI & even free of cost IVF in some cases. This forum will give us opportunity to explore the genius minds of expertise to find some solution for ART in developing countries.
Study funding/competing interest(s): Funding by national/international organization(s), Women friendly association.
Trial registration number: Applied for trial registration number.

O-126 A prospective randomized controlled study (RCT) depicting favourable IVF-ICSI outcomes following anti-tubercular treatment in cases with positive PCR test for endometrial non tuberculous mycobateria

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[1]*Bhopal Test Tube Baby Centre, Gynaecology and Obstetrics, Bhopal, India*
[2]*Bhopal Test Tube Baby Centre, Infertility, Bhopal, India*

Study question: Does anti-tubercular treatment (ATT) for positive endometrial TB-PCR test for non tuberculous mycobacteria (NTMB) yield better IVF-ICSI outcome in developing countries like India with very high prevalence of Tuberculosis disease?
Summary answer: Infertile women prior given anti-tubercular treatment (ATT) for non tuberculous mycobacterial infection had improved IVF-ICSI outcome.
What is known already: Genital Tuberculosis is a major cause of infertility in developing countries like India. Prompt ATT can dramatically improve female reproductive function. Non-tuberculous mycobacteria (NTMB) are environmental organisms capable of causing *chronic disease in humans. The prevalence of* NTMB disease is steadily increasing and has emerged in previously unrecognized populations. The diagnosis is often difficult or unconvincing.
Study design, size, duration: 612 infertile patients below 42 years of age were evaluated by laparoscopy and hysteroscopy between January 2006 and December 2013. Each patient underwent endometrial aspiration biopsy on day-1 of menstrual cycle for detection of MTB by PCR. Out of these, 126 patients having a positive NTMB PCR and abnormal endoscopic 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 126 NTMB PCR positive women who were randomized into two groups, one receiving standard anti-tubercular treatment and the other receiving no treatment before IVF/ICSI cycles. Out of 126 patients, 18 were excluded from the study either because of failure to comply to drug therapy, or other reasons. The primary outcome measured was the clinical 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 clinical pregnancy rate being higher following IVF-ICSI in the group who took prior anti tubercular treatment (34.9% versus 21.2%).

Although the abortion rate was higher in the non-treatment group, it was not statistically significant (4.2% versus 3.1%).
Limitations, reason for caution: NTMB diseases are seen worldwide. However, surveillance data are limited and NTMB infections are noncommunicable and therefore not reportable. As such, the true prevalence is not fully appreciated. Regardless, NTMB infections have increased by 8% to 10% per year. There are more than 120 identified mycobacteria species known to cause disease in humans. By far, *Mycobacterium avium* complex (MAC) and *M. kansasii* are the most common NTMB species causing disease in humans. NTMB is commonly isolated in both natural and indoor water sources, which are the primary reservoir for most human infections. As such, not all NTMB isolates represent true infections and may be colonization or contamination.

Genitourinary involvement account for less than 10% of all NTMB infections, therefore accounting for the paucity of literature.
Wider implications of the findings: Indication for the treatment of tuberculous 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 positive NTMB cases improves the IVF-ICSI results.
Study funding/competing interest(s): Funding by hospital/clinic(s), BTTB CENTRE.
Trial registration number: BTTB /2006/12.

INVITED SESSION

SESSION 33: EUROPEAN AND GLOBAL ART MONITORING

Tuesday 1 July 2014 **11:45 - 12:45**

ICMART World Report 2010

D. Adamson[1]
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How the Latin American ART Registry transitioned from summary to individualized data

F. Zegers[1]
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O-127 Assisted reproductive technology in Europe, 2011: results generated from European registers by ESHRE. Preliminary results

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[9]ESHRE, Central Office, Grimbergen, Belgium

Introduction: This is the fifteenth 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 2011.
Methods: Data were collected from existing national registries in 29 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, 997 IVF clinics participated (80.0% of registered clinics in the participating countries. Next to these also 825 IUI units reported their data. The IVF clinics reported 582 423 treatment cycles: IVF (132 610), ICSI (288 563), frozen embryo replacement (FER, 118 933), egg donation (ED, 29 631), preimplantation genetic diagnosis/screening (PGD/PGS, 6 926), in-vitro maturation (IVM, 511) and frozen oocytes replacements (FOR, 5 249). This preliminary data setting shows that the number of cycles compared to 2010 is somewhat stable. However since a number of countries still need to send in their data, the total number of ART cycles will still increase. Data on intrauterine insemination using husband/partner's (IUI-H) and donor (IUI-D) semen were reported from 20 countries (data sent in at the time of abstract deadline). A total of 171 934 IUI-H and 40 337 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 28.8% and 33.0%, respectively and comparable to 2010. For IVF the delivery rate (DR) per aspiration was at 21.5%. For ICSI, the corresponding rates were 26.8%, 31.6%, and 19.0%. For frozen embryos replacements, PR was 21.2% per thawing and 23.4% per transfer. The corresponding delivery rates were 14.2% and 15.7%. In oocyte donation cycles, PR and DR were 45.7% and 28.6% per transfer, respectively in the fresh cycles and 33.6% and 19.5% in the frozen cycles. For the ED cycles with FOR, the numbers are as follows: PR was 42.7% and DR was 25.1% per transfer. For PGD/PGS, in the fresh cycles the PR was 35.2% per transfer. In the frozen cycles this was 37.9%. For *in vitro* maturation, PR and DR were 29.1% and 19.9% per transfer respectively. Finally, 4377 replacements after oocyte freezing were reported, mainly from Italy and Spain. They resulted in 27.0% PR and 16.2% DR per thawing, respectively. Following IUI-H the pregnancy rate and delivery rate was 11.7% and 8.2%, while it was 16.9% and 12.1% respectively in IUI-D. The transfer of 1, 2, 3 and 4 or more embryos following IVF or ICSI occurred in 27.8%, 56.6%, 14.3% and 1.3% 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 2010, at 82.9%, 19.0%, and 0.7% respectively. The proportion of very preterm deliveries (≤33 weeks) increased from 4.8% for singleton pregnancies to 14.4% for twin pregnancies and 41.3% 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 9.0% twin and 0.7% triplet pregnancies, thus not higher than in IVF-ICSI.
Conclusions: In comparison with previous years, 2011 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.

INVITED SESSION

SESSION 34: LONG-TERM HEALTH IN TURNER SYNDROME WOMEN

Tuesday 1 July 2014 **11:45 - 12:45**

O-128 Pregnancy outcome after oocyte donation in women with Turner syndrome – what are the risks?

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Turner syndrome affects 1 in 2500 live born females and is characterized by complete or partial loss of one X chromosome causing reduced adult height, ovarian insufficiency, and several endocrine disorders. Cardiac malformations, such as bicuspid aortic valves and aortic coarctation, are common and the risk of aortic dissection is high. Due to cardiovascular complications and other health problems women with Turner syndrome have reduced life expectancy and increased mortality compared to the general population.

Most women with Turner syndrome are infertile and they report infertility to be one of the greatest challenges they have to face affecting quality of life. To date, for the majority of patients with Turner syndrome, oocyte donation is the only way to achieve a viable pregnancy.

Clinical pregnancy rate in oocyte recipients with Turner syndrome has reported to be similar to other recipients of donated oocytes. Increased miscarriage rate noted in early studies may be due to insufficient hormonal support before and in the treatment cycle.

In 1997, the first reports on serious cardiac complications and deaths in pregnant women with Turner syndrome were published. Later there have been at least six more reports on aortic dissections related to pregnancy. Most, but not all of these women had had some cardiac abnormality in the background. In a study from 2003 the estimated risk of maternal death during pregnancy was 2%. However, only 49% of these women had any cardiac evaluation before embryo transfer.

A high risk of several obstetric and perinatal complications has also been noted in OD pregnancies in patients with Turner syndrome. In published studies, the incidence of hypertensive complications and pre-eclampsia has been 36%–63%, the risk of preterm birth 20–50% and Cesarean section rate 82–100%. The largest of these reports is a Nordic cohort study including 106 women with Turner syndrome giving birth after OD treatment in 1992–2011. These women had altogether 122 deliveries and the multiple delivery rate was 7.4%. In this cohort, the karyotype was 45X in 44% of the Turner recipients. Ten women had a known cardiac defect before pregnancy, 64% had a pre-pregnancy cardiac examination and only 29% had had an echocardiogram or MRI during pregnancy. In total, 35% of the pregnancies were associated with a hypertensive disorder including pre-eclampsia in 20.5%. Potentially life-threatening complications occurred in four patients (3.3%). No maternal deaths were reported. The mean birth weight of the singletons was 3150 g and among the twins 2200 g. In singletons the incidence of low birth weight was 12% and of prematurity 9%.

In a Swedish registry study published in 2013 maternal mortality was zero during pregnancy and during an average of a 10 year's follow-up after childbirth in 124 women with Turner karyotype, but a higher rate of circulatory and endocrine diseases and a high risk of aortic aneurysm was confirmed.
Conclusion: Pregnancies among women with Turner syndrome carry a substantial risk, especially for hypertensive disorders. General health and cardiac pre-pregnancy evaluation is of utmost importance in these women. A history of aortic surgery or aortic dissection, coarctation of the aorta, aortic size index >2.5 cm/m^2 and uncontrolled hypertension are contraindications for oocyte donation in women with Turner syndrome. However, the presence of normal cardiac anatomy and normal aortic dimensions does not exclude the risk of a sudden aortic dissection. Elective single embryo transfer is mandatory, as multiple pregnancies increase the obstetric and perinatal risks many times over. Close collaboration with the cardiologist is necessary during the whole pregnancy and the postpartum period.

O-129 Optimizing health care for adult women with Turner syndrome

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Treatment with growth hormone (GH) during childhood and adolescence allows a considerable gain in adult height. SHOX deficiency explains some of the phenotypic characteristics in TS, principally short stature. Puberty has to be induced in most cases, and female sex hormone replacement therapy should continue during adult years. These issues are normally dealt with by the paediatrician, but once a TS female enters adulthood it is less clear who should be the primary care giver. Morbidity and mortality is increased, especially due to the risk of dissection of the aorta and other cardiovascular diseases, as well as the risk of type 2 diabetes, hypertension, osteoporosis, thyroid disease and other diseases.

The proper dose of hormone replacement therapy (HRT) with female sex steroids has not been established, and, likewise, benefits and/or drawbacks from HRT have not been thoroughly evaluated. In most countries it seems that the transition period from paediatric to adult care is especially vulnerable and the proper framework for transition has not been established. Likewise, no framework is

in place for continuous follow-up during adult years in many countries. Today, most treatment recommendations are based on expert opinion and are unfortunately not evidence based, although more areas, such as GH treatment for increasing height, are well founded.

Osteoporosis, diabetes, both type 1 and 2, hypothyroidism, obesity and a host of other endocrinological diseases and conditions are seen more frequently in Turner syndrome. Prevention, intervention and proper treatment is only just being recognized. Hypertension is frequent and can be a forerunner of cardiovascular disease.

The description of adult life with Turner syndrome has been broadened and medical, social and psychological aspects are being added at a compelling pace.

Proper care during adulthood should be studied and a framework for care should be in place, since most morbidity potentially is amenable to intervention.

Many women turn up and present a wish for fertility and oocyte donation is now an option in many countries in Europe. However, before embarking on an oocyte donation program, it is vital to perform a thorough examination of every woman, including MR of the heart and great vessels, 24 h blood pressure measurement and a comprehensive biochemical profile. During pregnancy, we follow every woman with repeat echocardiography and MR if indicated. Pregnancy in females with Turner syndrome is to be viewed as a high-risk endeavour. We treat even slightly elevated blood pressure vigorously. More research is needed in order to provide the most optimal condition during oocyte donation and ensuing pregnancy.

In summary, Turner syndrome is a condition associated with a number of diseases and conditions which need the attention of a multi-disciplinary team during adulthood.

SELECTED ORAL COMMUNICATION SESSION

SESSION 35: THE VOICE OF DONOR SPERM RECIPIENTS

Tuesday 1 July 2014 **11:45 - 12:45**

O-130 Solo mothers and women with a partner seeking donor insemination – attitudes towards motherhood and socio-demographic characteristics – a national study from public Danish fertility clinics

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Study question: To compare intentions and attitudes towards motherhood and family background and socio-demographic characteristics among single women seeking treatment with donor sperm versus cohabitating women.
Summary answer: Solo mothers were similar in intentions and attitudes towards motherhood as well as in socio-demographic background when compared with cohabitating women. Both groups had in their early 20s identical intentions of motherhood and sharing their life with a partner. Solo mothers generally hoped to have a partner in the future.
What is known already: In Denmark 1.3% of all births are children born by solo mothers or lesbians after fertility-treatment using donor sperm. There is a lack of Danish studies, but previous international studies found the majority of solo mothers to be 35–40 years old and from mid-high social classes. Solo mothers seeking treatment due to concern that their reproductive period would otherwise run out and the majority would have preferred having a child within a relationship.
Study design, size, duration: Prospective cohort multi centre study involving all nine public fertility clinics in Denmark. All single women and all cohabitating women (heterosexual or lesbian) initiating donor insemination during February 2012 and July 2013. In all 360 were invited, 339 accepted, and $n = 315$ (93%) returned the questionnaire.
Participants/materials, setting, methods: A total of $n = 182$ single women were compared to a group of $n = 133$ women cohabitating with a partner (67 heterosexual; 66 lesbian). The response rate was 93% (315/339). Study participants received a questionnaire at initiation of their first donor insemination treatment cycle.
Main results and the role of chance: Solo mothers were significantly older than cohabitating women (mean age 36.3 years versus 33.0 years, $p < 0.001$). There were no differences between solo mothers and cohabitating women in intension of and expectations to motherhood or in family background. Solo mothers' educational level was distributed similarly to cohabitating women. Overall 70.6% of solo mothers preferred to have a child compared to having a partner if they had to choose, but 85.2% desired a relationship with a man in the future. Among these, 55.8% preferred a future partner to have parental responsibilities for the child. Solo mothers considered it in general more important to, have a sufficient network and a career that could be combined with raising a child, compared to women in heterosexual couples.
Limitations, reason for caution: The study included only women treated at public fertility clinics, where treatment is free of charge for the patients and limited to women below 40 years of age. This could influence the results, as women treated at private clinics and women above 40 could differ in their responses.
Wider implications of the findings: This study adds to our limited knowledge about the growing number of solo mothers using semen donation. Data may be used by health professionals and social workers in their efforts to improve the well-being of this new type of families. The study also provides up-to-date information to pass on to the next generations. Due to the multicentre design the results are generalisable to public fertility treatment in Denmark
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by commercial/corporate company(ies), This study received financial support from Ferring, MSD and Nordic Cryo Bank who had no influence on data collection or analysis. The authors have no conflict of interest to declare.
Trial registration number: No trial registration number.

O-131 Family decision making: a qualitative study in lesbian couples after donor conception

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Study question: We describe what decisions lesbian couples made while building their family via donor insemination [DI] 8–10 years ago and how they organise(d) family life post treatment.
Summary answer: Family decision making was clearly influenced by the fact that one mother was genetically related to the child while the other was not. Decisions were also made to protect the social mother both within and outside the family.
What is known already: It is known that lesbian couples commit to equality in their relationships. While preparing for parenthood, they are confronted with unique decisions in addition to the ones that all parents encounter. Previous research has shed light on the decisions about the conception, family narratives, the division of tasks and roles, social support, and legal arrangements. However, most studies mainly focused on the time of the transition to parenthood.
Study design, size, duration: We included 18 lesbian parents (9 biological & 9 social mothers). Data from the in-depth semi-structured couple interviews 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. The study was approved by the appropriate Ethics Committee.
Participants/materials, setting, methods: Lesbian couples with at least one child (7–10 years old) conceived through anonymous DI were recruited at the Department of Reproductive Medicine of the Ghent University Hospital. The interviews were performed at their homes (8) or at the department (1) between October and December 2012.
Main results and the role of chance: For most couples it seemed as self-evident that Mother's day was a celebration of the biological mother. Social mothers who were celebrated on Father's day saw this as a favour, not a right. The choice for anonymous sperm donation was made to protect the social

mother: the parents feared that a known donor could get involved and pull rank on the social mother because of his genetic link to the child.

Most couples complained about the lack of societal recognition of social mothers. They stated that, without adoption, Belgian non-biological mothers cannot sign legal documents and have no rights but still have to perform their duties. For them, this emphasized that for society, the social mother was not equal to the biological one.

Limitations, reason for caution: A consequence of the semi-structured interview guide was that there were differences between the couples in the decisions they mentioned. Advantages of this interview structure were that participants could talk about topics that they considered important and that it allowed for decisions new to the literature to be raised.

Wider implications of the findings: These findings offer important insights for professionals involved in counselling lesbian couples. The study also shows the importance of social acceptance of same sex couples and of legal arrangements that safeguard the status of the social parent.

Study funding/competing interest(s): Funding by University(ies), The project is funded by the Special Research Fund of Ghent University.

Trial registration number: N/A.

O-132 How do lesbian parents and their children experience family communication about the donor conception – a multi-perspective qualitative study

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Study question: How do lesbian parents and their school-age children describe and experience family communication about the donor conception? The child's conception narrative is explored in detail and related to parents' accounts of how they discuss the donor conception with their children.

Summary answer: Analysis revealed that both parents and children situated the initiative for talking about the donor conception mostly with the child. Family members can have different needs/wishes with regard to the disclosure: in some families children expressed a need for dialogical space and further reflection whereas the parents leaned towards 'closure'.

What is known already: Literature considers disclosure of donor conception in lesbian families as obvious and straightforward. Studies focus mainly on the meaning of donor anonymity and what information children want about the donor. The ways in which this donor conception is discussed and family members' individual experiences of the disclosure process do not receive much research attention. Even more lacking are studies including both children's and parents' voices offering a comprehensible perspective on family communication within families.

Study design, size, duration: As part of a larger qualitative research project on family members' perspectives on social and genetic parenthood, lesbian couples and their children were interviewed between October 2012 and May 2013 using semi-structured interviews. Approval by the Medical Ethics Committee of University Hospital Ghent has been obtained.

Participants/materials, setting, methods: Lesbian couples treated at the Ghent University Hospital were contacted by their counselor seven to 10 years post treatment. Six couples and seven of their children were interviewed separately at their homes. 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: Analysis revealed that both parents and children situated the initiative for talking about the donor conception mostly with the child. Children were sensitive in picking the moment when such a conversation was possible and welcomed. In some families both parents and children alike expressed no need for further reflection on or conversation about the donor conception. In other families preferences and expectations differed among family members: parents wanted to disclose essential information to the child without too much elaboration, while for children this seemed to be only the starting point of further reflection about the donor conception.

Limitations, reason for caution: This qualitative explorative study does not have the intention to generate generalizable findings. Our study mainly elaborates on parents' and children's views on family conversations about the donor conception. Our findings serve as a rich exploration of this dialogical process between parents and children.

Wider implications of the findings: Both parents and professionals can benefit from the insights offered by this study. Our study illustrates the complexity of this dialogical process and the various meanings that lesbian parents and their children can attribute to disclosure. Moreover, it shows that parents and children can have different needs or wishes with regard to the disclosure.

Study funding/competing interest(s): Funding by University(ies), Special Research Fund of Ghent University.

Trial registration number: None.

O-133 Handing over control: lesbian couples' views on and experiences with limited input in sperm donor selection

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Study question: In Belgium, gamete donor screening and selection follows a 'law-dominated' policy that grants the recipients very little input or personal choice. This qualitative study explores how Belgian lesbian (candidate) recipients view and experience the selection and screening of their donor.

Summary answer: The participants felt they did not have (or need) a right to further control over the donor selection. Important decisions, initiatives and responsibilities were left to the hospital. Most of the participants described their experiences from a compromising, accommodating attitude and were foremost relieved they could obtain treatment.

What is known already: Various studies have shown that recipients of donor gametes want greater control and input in the donor selection (Heinemann-Kuschinksy et al., 1995; Adair & Purdie, 1996). The main reasons for this appears to be the need for reassurance about the quality of the donated gametes and an interest in the donor as a person.

Study design, size, duration: As part of a larger qualitative research project, 20 lesbian couples were recruited via the Department of Reproductive Medicine of the Ghent University Hospital. Couple interviews were performed between October and December 2012. These were semi-structured and lasted 90–120 min. Approval of the Ghent University Hospital EC was obtained.

Participants/materials, setting, methods: Of the 20 couples recruited, 10 already had a child conceived after successful donor conception treatment and 10 were in treatment at the time of data collection. Inductive thematic analysis was performed on data where couples referred to the selection of their donor and their expectations towards the hospital.

Main results and the role of chance: Many respondents did not express a desire for more input in the donor selection process. Those who did, were very nuanced and careful about their motivations: they focused on benefits for the child or for family coherence. They acknowledged their lack of control on the selection outcome and negotiated this as part of an anonymous donation policy which provides an opportunity to have a child. They actively and passively downplayed (initial) concerns about the donor selection procedure. They appeared to take on the view that the chance of having a child is a gift that should not be scrutinized. In adopting this 'subordinate' position, they felt they must trust the hospital and hoped that the hospital fulfilled (rather high) screening standards.

Limitations, reason for caution: This was a qualitative explorative study and did not intend to produce generalizable findings. An inherent risk is that the respondents at times produced socially desirable responses. However, the interviewers emphasized their neutrality and the fact that they were not affiliated to the clinic.

Wider implications of the findings: Given their high reliance on the hospital, it is important that candidate recipients are well informed about how donors are screened. They should also feel welcome to ask questions and not be fearful of being too critical. These findings bring to light an opportunity for enhancing informed consent.

Study funding/competing interest(s): Funding by University(ies), Ghent University.

Trial registration number: N/A.

INVITED SESSION

SESSION 36: ADVANCES IN GENETIC TESTING

Tuesday 1 July 2014 **14:00 - 15:00**

O-134 Non invasive prenatal diagnosis by genome sequencing

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The presence of cell-free fetal DNA in the plasma of pregnant women was first reported in 1997. After just over a decade of research, tests based on massively parallel sequencing of maternal plasma DNA achieve fetal chromosomal aneuploidy detection at 99% accuracy. Since 2011, such non-invasive tests have been introduced clinically as part of prenatal screening programmes in many parts of the world. Chromosomal aneuploidies covered by these tests include trisomy 21, 18 and 13 as well as the sex chromosome aneuploidies. Methods that assess the fetal molecular karyotype have subsequently been developed. In fact, sequencing analysis of maternal plasma DNA allow the entire fetal genome to be decoded in the prenatal period non-invasively. Thus, non-invasive scanning of the fetal genome for the prenatal detection of genetic variants associated with single gene diseases was possible. In addition, methylation profile of the placenta could be deduced using whole genome bisulfite sequencing of maternal plasma DNA. This development offers a non-invasive means to monitor placental function. Non-invasive prenatal diagnosis has therefore become a clinical reality and resulted in shifts in prenatal practices.

Acknowledgement: Supported by the University Grants Committee of the Government of the Hong Kong Special Administrative Region, China, under the Areas of Excellence Scheme (AoE/M-04/06) and Innovation and Technology Fund (ITS/095/13FP).

O-135 Direct to consumer genetic testing

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In recent years various companies have started to advertise and sell genetic tests through the internet. Many different types of tests are offered and include, among others, testing for monogenetic disorders, (preconceptional) carrier testing, susceptibility testing for common complex disorders, nutrigenomic testing, paternity testing, ancestry testing and, pharmacogenomic testing. Supporters of DTC genetic testing claim that the benefits of such services include, among others, increased access for consumers, increased genetics and genomics education, added support for consumer autonomy and individual empowerment. With respect to health-related genetic tests, some companies also suggest that knowing their disease risk (based on genetic and genomic information) will encourage individuals to modify their behaviour in order to achieve better health. Concerns about DTC genetic testing, include, among other issues, the lack of clinical validity and clinical utility of tests, especially for complex traits. Concerns have also been raised regarding the lack of pre and post-test counselling as well as the lack of individualized medical supervision, the inappropriate testing of minors, and the potential burden on public health resources. The advent of DTC genetic testing companies is challenging frameworks previously set up to address issues of single gene or multi gene testing in a clinical context and questions which regulatory frameworks should be in place to deal with this development. Next to health-related issues, genetic and genomic services for genealogical or ancestry purposes is new development that can undermine the privacy and confidentiality of the participating customers and their relatives. Anecdotes report individuals being able to identify close relatives, including gamete donors. This development could have an impact on (i) the disclosure of donor conception to donor-conceived offspring (ii) the anonymity debate in donor conception, and (iii) the information available to donor-conceived offspring.

INVITED SESSION

SESSION 37: ASSISTED REPRODUCTION AND HUMAN RIGHTS (JOINT SESSION ESHRE SIG ETHICS AND LAW AND FIGO'S ETHICS COMMITTEE)

Tuesday 1 July 2014 **14:00 - 15:00**

O-136 Access to assisted reproduction as a human right

B. Dickens[1]
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The European Court of Human Rights has issued judgments that the comparable Inter-American Court of Human Rights drew upon, in November, 2012, to identify human rights that support access to assisted reproduction. Costa Rica had banned IVF by claiming that religious values in embryonic human life prohibit the wastage of human embryos that IVF risks. The Inter-American Court applied the equivalent of Article 9(2) of the European Convention on Human Rights, which states that "Freedom to manifest one's religion or beliefs shall be subject only to such limitations as are…necessary… for the protection of the rights and freedoms of others", and ruled that the ban on IVF violated the human rights of infertile individuals and couples.

The Court ruled that human embryos are not "human beings" in law, and do not possess legally recognized human rights. The Court further ruled that no human right can prevail absolutely over other human rights, but that each right must be balanced against others. The principal human rights that the Court ruled protect access to assisted reproduction are:

1. Rights to private and family life;
2. Rights to found a family; and
3. Rights to equality with others and to non-discrimination.

Private and Family Life: Article 8 of the European Convention on Human Rights provides that "Everyone has the right to respect for his private and family life…" Public authorities are not able to interfere with exercise of this right, except in the interests of national security, public safety, or the economic well-being of the country, or for prevention of disorder or crime, or for protection of health or morals, or of the rights and freedoms of others. The Court ruled that access to IVF, and by implication to other forms of medically assisted reproduction, do not endanger any of these interests, and that accordingly the state cannot interfere with people's private decisions for development of their families.

Founding Families: Article 12 of the European Convention provides that "Men and women of marriageable age have the right to marry and to found a family…" This leaves unanswered the question whether marriage is a necessary prior legal condition of founding a family, because the claimants before the Inter-American Court were all married couples. Laws may prohibit for instance incestuous marriage and bigamy, but not prevent otherwise capable individuals from resort to medically assisted reproduction.

Equality and Non-Discrimination: Article 14 of the European Convention provides that the rights it establishes "shall be secured without discrimination on any ground such as sex, race, colour, language, religion, political or other opinion, national or social origin, association with a national minority, property, birth or other status". The Court found that the ban on IVF constituted unlawful discrimination:

i) Against those who are infertile, because fertile people are not generally banned from reproduction;
ii) Against women, because women are generally more prejudiced in their families and communities by childlessness than men; and
iii) Against those who lack financial or other means to travel to other countries where assisted reproduction is not legally banned.

The European Convention on Human Rights may be applied to address additional issues of assisted reproduction, such as by partners in same-sex relationships, those with sexually transmissible infections or with transmissible adverse genetic conditions, and long-term prisoners. Decisions of the European and Inter-American Courts of Human Rights on access to medically assisted reproduction provide guidance on which acceptable future practices may be built.

O-137 Cross-border reproductive care and global justice

G. Pennings[1]
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The issue of global justice in health care is clearly separated from the issue of global justice in medically assisted reproduction. Infertility treatment in developed countries does not generate much enthusiasm and is only partially reimbursed. In developing countries, it encounters even fierce opposition. There are several reasons for the negative reception but the status of infertility as a disease is certainly part of the explanation.

We will focus on cross-border reproductive care (CBRC) and global justice. Two broad categories of concerns can be distinguished in this context: the exploitation of poor women in both rich and poor countries; and the effects on access to ART in general due to the inflow of patients.

Four theories on the just distribution of scarce resources can be distinguished: utilitarianism, egalitarianism, prioritarianism and sufficientarianism. In the discussion on general medical tourism, the main concern is a prioritarian one: medical travelling should not make the poor worse off than they are now. This rule defends the most minimalistic condition. We will scrutinize whether the same question applies to CBRC. To consider this question, we will separate developing from developed countries. In the developing countries, we will look more closely at two examples: 1) the introduction of fertility clinics in developing countries and the effect on access for the local population, and 2) the brain drain of qualified personnel. In developed countries, we will look at the effects of CBRC on the situation of the local patients in terms of costs and waiting time. Still, also positive effects of CBRC on justice should be mentioned such as the possibility of some groups to access treatment denied to them at home.

No definite conclusions can be drawn at the moment regarding the effects of CBRC on global justice for infertility care due to lack of data.

O-138 Reproductive ethics: human rights or religious authority

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WHO defined bioethics as a field of ethical enquiry that examines ethical issues and dilemmas arising from health, health care and research involving humans. Bioethics is closely interrelated with Human Rights. It is therefore not surprising that UNESCO combined Bioethics and Human Rights in its recently published document "Universal Declaration of Bioethics and Human Rights". This included 15 ethical and human rights principles including autonomy, justice, beneficence and non malficence, pleural diversity and solidarity.

Women have the right to the highest attainable standard of health care for all aspects of their reproductive health. The principle of autonomy implies that women themselves should be able to choose when to reproduce, how often, how many and what method they would use and protection of those incapable of autonomy.

Human reproduction is an essential component of human rights. They should never be transferred, renounced or denied for any reason based on race, age, language, religion, national origin, political opinion or economic conditions. However disparities in Reproductive and Sexual Health are so huge between North and South and in country between different sectors of the community. In this regards Assisted Reproductive Technology (ART) is not an exception.

The principle of Justice implies that all be treated with equal standard irrespective of their socioeconomic status and have equal access to quality reproductive health services and information without discrimination or coercion.

Religions play a major role in shaping cultural diversity, norms, regulations and laws in many societies. Respect for cultural diversity and pluralism has to be given due course without infringing on basic human rights. Many reproductive practices as ART were not mentioned in the original texts of practically all religions. The birth of Luis Brown, the first IVF baby in 1978 initiated intense ethical and religious debate all over the world. Various religious bodies, authorities and leaders issued their recommendations on IVF and continued to do so on the emerging new practices in ART as gamete donation, cryopreservation, PGD, and surrogacy. Then guidelines varied between too restrictive and very permissive.

In Judaism the Mishnah emphasizes that only prohibitive, strict decisions require juridical substantiation while permissibility or leniency needs no supportive precedent.

Rome, as it puts absolute value on an unbreakable nexus between coitus and conception, forbids to its members all practices which bypass the sexual union of man and woman. In the Protestant Churches, moral reasoning is the perennial task: to establish the ethical permissions and prohibitions attaching to new developments in science and technology, including ART. The Church of Alexandria, in the Middle East and Africa, encourages the use of ART for infertile married couples. It forbids sperm donation, egg donation, embryo donation and surrogacy. The basic guidelines for ART in the Sunni sector in Islam: if ART is indicated in a couple (husband & wife) with no third party participation as a necessary line of treatment it is permitted. Shi'aa Guidelines has allowed egg donation, sperm donation and surrogacy.

However, when religious guidelines were restrictive patients crossed borders to fulfill their reproductive choices. This implied discrimination against and exploitation of the needy and disadvantaged women. Furthermore it created socioeconomic and health problems, burdening health systems of countries of origin with the complications of unregulated practices in ART.

In conclusion, not uncommonly there are conflicts between Human Rights principles and religious authorities on contemporary issues in Human Reproduction. Health care providers should ensure women's access to ethically acceptable quality health services in Human Reproduction in a patient's friendly facility which ensures patient's confidentiality, satisfaction and non stigmatization based on Human Rights. If the physician has conscientious objections to provide various ART practices to his/her patient, it is an ethical obligation to refer the patient to where such treatment is available.

O-139 Limits of reproductive autonomy

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Respect for autonomy in general and reproductive autonomy in particular is a core ethical principle. It is, however, not absolute. This lecture regards some possible (moral) limits of reproductive autonomy in the context of medically assisted reproduction.

Professionals involved have the responsibility to take into account the interests of both applicants (prospective parents) and possible future children thus conceived. There seems to be a strong consensus that if there is a 'high risk of serious harm' to the child, professionals should not assist in reproduction. Obviously, this implies limits to applicants' reproductive autonomy – and raises further questions about how to make this professional responsibility operational, as will be illustrated by means of two cases.

The first case regards an infertile couple at high risk of having a child affected with a serious genetic disease, like beta thalassemia. The couple applies for IVF, but states that it is willing to accept the genetic risk – 'the child is welcome, whatever its condition'. In the case of serious *non*-genetic risks for the welfare of the future child, the question is usually: 'will we give access to fertility treatment: yes or no?'. In the case of genetic risk, however, there is a third option: to give access on the condition that the couple consents in preimplantation genetic diagnosis (PGD), in order to transfer 'unaffected' embryos only. This strategy is in fact a so-called 'coerve offer'. Would this be morally acceptable? In traditional genetic counseling, especially in the context of prenatal diagnosis, non-directive counseling, aimed at facilitating reproductive choice, is the professional standard. But in view of the specific responsibility of professionals involved in assisted reproduction, this normative framework cannot be simply extrapolated to the current context. Offering PGD as a condition for access to IVF (or any other type of medically assisted reproduction) may, then, be morally justified – if not morally obliged.

The second case regards the request of PGD in order to conceive an affected child. Prospective parents affected with a particular handicap may prefer to have a child with the same handicap. Think of e.g. (hereditary, non-syndromic) deafness. Applicants may argue that their 'handicap' is just a variant on the spectrum of normalcy, and that both they themselves and any future children will benefit if they share the same phenotype. How, then, to handle such requests? Assuming that (outside the Deaf subculture) deafness is

a real disability, and that prospective parents and professionals involved have the responsibility to ensure that future children will have more rather then less health and well-being, one should, if one engages in PGD, opt for the selective transfer of hearing embryos.

That said, it might, paradoxically, be justified to transfer a deaf embryo if an infertile, deaf couple, preferring a hearing child, would engage in PGD, but, unfortunately no hearing embryo is available for transfer. Conditions include that the couple will also be happy with a child with the handicap that they first intended to avoid, and that such transfer is not at odds with the responsibility to avoid *serious* suffering for future children.

Even if we agree that professionals should not assist in reproduction in case of a 'high risk of serious harm', and that they should ensure, as far as is reasonably possible, that future children will have more rather then less health and well-being, reasonable people may well disagree about how to make this operational in individual cases. In view of the importance of respect for reproductive autonomy and in order to avoid arbitrary decisions, procedural safeguards and regular clinical ethics deliberations are of utmost importance.

INVITED SESSION

SESSION 38: INVITED PATIENT SESSION - SOCIAL INFERTILITY (E.G. GAMETE FREEZING FOR SOCIAL REASONS; DELAYED PREGNANCY; AGE FACTOR

Tuesday 1 July 2014 **14:00 - 15:00**

O-140 Social egg freezing- the clinician point of view: information, indications and expectations

R. Vassena[1]
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O-141 The conflict between psychology and medicine - Social freezing motivation and consequences

V. Savova[1]
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The world is not the same as it was back in 1978, when the first test-tube baby was born; and IVF today does treat many more cases than tubal factor infertility. IVF has come to be one of the ways for humans to reproduce. Besides patients with physiological infertility problems, today's IVF clinics welcome also people with no medical pathology. These come in not for fertility treatment proper, but rather for a reproductive 'service' – for a multitude of social reasons. One particularly interesting case is the social freezing patient, who eventually might reveal deeper social problems. The clinical psychologist will treat this person as normal, with seemingly no deviations. When, however, the time comes for them to return for IVF service, they tend to reveal certain psychological issues of social origin.

Apart from the oncofertility group, more and more women are tempted to cryopreserve oocytes and delay pregnancy for years with different motives: these could be severed partners; people in search of the right partner; people abstaining from sexual intercourse; such that are pursuing a career; or ones that desperately want to save an otherwise malfunctioning relationship. Too often a hypertrophic social individualism is opposing biological fertility.

Two pivotal questions arise for the clinical counsellor: to what extent IVF as a medical procedure is a therapeutic one in such cases, and to what extent medical-supporting practices are therapeutic, in view of the definition of health as a unity of physiological, psychological and social wellbeing, and not just as the absence of disease.

While the social freezing patient, often pressed by ageing anxiety, insists on medical treatment, medical ethics and psychological ethics might not always support this. Such cases include patients with clinical psychological symptoms, and with family issues. The very motivation of the social freezing patient might lead to psychopathology symptoms during the IVF procedure; and this is not because their wish for a child itself is pathological, but because the social etiology of this wish as a background, and the IVF procedure as a consequence, are affecting it.

The essence of the conflict between the points of view of psychology and IVF in such cases is actually a conflict between the social and medical aspects. If the IVF treatment is clear and well defined, the underlying social issues remain untouched, and ultimately deepen any psychological suffering the patient may already be in. As a result, we are facing psychopathology, and such which is supported by medicalization.

The baby is no longer deemed a value per se, but is downgraded to an instrument for satisfying needs. The paradox is seen when during the course of the IVF procedure, the desired pregnancy becomes a goal to be achieved by all means, whatever it takes. In our Clinic we call this The "I Want" Syndrome. Sadly, that syndrome further deepens during and after pregnancy and is demonstrated through various symtomps, including anxiety peak, and, later, attachment disorders. Last, but not least, attachment disorders accompany post-social freezing parenting.

The main goal of psychological counseling is to relieve suffering; the road to relief, however, requires work (transformation) on patient motivation. And how do we provide psychological support to somebody who needs it but does not ask for it? The answer lies in introducing psychological counselling as routine practice - an integral part of an IVF procedure. Only in a setting which allows a close, day-to-day collaboration between the IVF team and the psychologists, it will be possible to achieve a perfect orchestration of their efforts, and their different points of view will not clash but together contribute to the ultimate wellbeing of the patient.

O-142 Social freezing, unlimited access and open identity - A rainmaker?

H. Konecna[1], C. Menzies[2], M. Zeman[1]
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Hope is the most important thing in life. In certain situations it is more important and of greater value than the reality, the truth. Technological progress in medicine brings hope and is often capable of fulfilling it. The current emphasis on democratisation, human rights, especially equality and autonomy regardless of sex, race, education, ethnicity, gender, and so forth have all led to a situation where society seeks to treat all those who wish treatment. This is also true of reproductive medicine – medically assisted reproduction brings enormous hope not only to those who long for a child but have been unable to conceive, but also to those who have delayed parenthood. This brings with it new ethical, psychological, social, medical, political and legal challenges. The challenges currently attracting most attention are parenthood at a 'physically' post-reproductive age, freezing gametes for social reasons, reproduction by single individuals and gay and lesbian couples, surrogacy, pre-implantation genetical screening for social reasons, and so forth.

This talk will look at the role hope plays in reproductive medicine. Taking as our metaphor a film about hope, *Rainmaker* (1956) directed by Joseph Anthony, we will analyse how hope plays out in social freezing, unlimited access and open identity. What is it that gives people hope and what is it that takes away hope? What is the relationship between truth and hope? Should we pay more attention to hope in reproductive medicine? What are the ethics of hope?

O-143 Medicalizing and socializing infertility in the 21st century

F. Oliveira[1]
[1]*Coimbra, Portugal*

INVITED SESSION

SESSION 39: PARAMEDICAL INVITED SESSION

Tuesday 1 July 2014 **14:00 - 15:00**

O-144 "The de jure stateless children": The dilemma of cross-border surrogacy

H. Sol Olafsdottir[1]
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Surrogacy is an age-old method of dealing with infertility. What is new today is that society and the health care system is involved in the process through assisted reproduction treatments (ART). Surrogacy's legal situation in Europe is far from unanimous and there are known cases were children conceived by surrogacy are

in fact *de jure* stateless, that is have no citizenship and are formally orphans. The legislation and courts are lacking behind, and the need of the child and media discussion may force countries to act quickly and from case to case.

In September 2013 the Directorate general for internal policies policy department *C: Citizens' rights and constitutional affairs – Legal affairs* presented a rapport regarding surrogacy in EU states, *A Comparative Study on the Regime of Surrogacy in EU Member States*. It presented the current legislative situation in the EU states and court cases.

Irrespective of whether the countries allow surrogacy, are thinking of allowing it or surrogacy is forbidden, their main concern is the wellbeing the actors; surrogate mother/family, intended parent/s and the child. Another concern is the control and surveillance of domestic surrogacy contra cross-border surrogacy. Even in UK, were it has been possible to have surrogacy for many years, residents have nevertheless sought help abroad where they may pay less for the procedures, meaning the domestic family court system still has to deal with cases where evidence can be hard to find.

The families, surrogacy mothers/families and especially the children are in a vulnerable situation when the legislation is unclear or not being used as intended. Nevertheless, a surrogacy agreement is much more than a legal contract, there has to be a consensus built on trust from all actors to make surrogacy work. Counselling is a crucial part of helping people to understand the consequences of their actions in the long run.

O-145 The pivotal role of the nurse in a fertility clinic

H. Kendrew[1]
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Nurses and midwives working in assisted conception centres undertake tasks and procedures which vary widely from country to country. Decisions about who performs certain procedures have historically always been made by doctors. Where it is seen to be beneficial to allow other personnel in a clinic to be able take on some procedures, this allocation, to nurses, again is made by the medical team and evolved from what is allowed legally within a country and culturally within a clinic.

I believe that nurses occupy a central position within the multidisciplinary fertility team and have a clear overview of the clinic day to day activities. They are the individuals with possibly the most patient contact and are aware of the practical issues that patients have to manage whist going through treatment, juggling work and hospital attendances.

Patients attending clinic appointments throughout their IVF treatment cycle, for example for follicular tracking of the ovaries, value a decision at the time of the scan and a clear plan of what to do next. It is extremely helpful if nurses can make these clinical decisions rather than wait for a doctor to review the scan findings and for the patient to be contacted by phone at a later time.

Providing access to appropriate training and promoting the development of nurses into experts and specialists improves their decision making abilities. Coupled with a clear understanding of how the specific clinic "ticks" the patient experience of the clinic improves.

Nurses in the UK have long experience of working in this way and I hope to demonstrate in my talk that embracing educational development and practical training of nurses, leads to a more efficient clinic and provides a highly satisfactory service for patients.

SELECTED ORAL COMMUNICATION SESSION

SESSION 40: TIME-LAPSE IMAGING - WHICH EMBRYO GETS THE OSCAR?

Tuesday 1 July 2014 **15:15 - 16:30**

O-146 External validation of meseguer's prediction model according to embryo transfer policy (cleavage stage or blastocyst)

N. Le Fleuter[1], T. Freour[1], J. Lammers[1], C. Splingart[1], P. Barriere[1]
[1]*Chu Nantes, Service De Médecine Et Biologie De La Reproduction, Nantes, France*

Study question: In 2011, Meseguer et al proposed a hierarchical predictive model of implantation based on morphokinetics. The purpose of this study was to compare the accuracy of this model in predicting implantation according to embryo transfer strategy, i.e. cleavage stage or blastocyst, in an external setting.

Summary answer: We did not confirm the predictive interest for implantation of Meseguer's model in our database, neither in blastocyst nor in cleavage stage transfer strategy. Each ART centre should learn from Meseguer's model and build a centre-specific predictive model fitted to its patients and practice.

What is known already: Several recent studies have addressed the relevance of time-lapse technology in the selection of embryos with good developmental competence. Meseguer's model, published in 2011, is the first and only published hierarchical model using early morphokinetic parameters. However, no external validation has been undertaken to our knowledge up to now in order to confirm its accuracy in a different setting and in various embryo transfer policies.

Study design, size, duration: This retrospective observational study was conducted in a public IVF centre, on all ICSI cycles performed with the Embryoscope between February 2011 and December 2013.

Participants/materials, setting, methods: Our population consisted in 450 couples undergoing ICSI cycle, with 2240 fertilized embryos, and 528 transferred embryos, among which 169 were blastocysts. We used Meseguer's hierarchical model and calculated the implantation rates (IR) corresponding to each category. We then performed the same analysis in subgroups according to embryo transfer strategy.

Main results and the role of chance: Overall implantation rate was 22.7%. IR in subgroups defined by Meseguer's model were: A+ 33.9%, A– 28.8%, B+ 31.4%, B– 15.8%, C+ 32.4%, C– 22.2%, D+ 37.4%, D– 10.5%, E 8.2%. When only cleavage stage transfers were considered, IR were: A+ 26.5%, A– 19.4%, B+ 44.4%, B– 7.1%, C+ 36.6%, C– 14.3%, D+ 28.6%, D– 3.8%, E 8.8%. When blastocyst transfers were considered, IR were: A+ 45.5%, A– 40%, B+ 17.7%, B– 40%, C+ 26.7%, C– 39.1%, D+ 46.2%, D– 25%, E 5%. Although A category had higher IR than D and E categories, we did not find a continuous trend towards lower IR from A to E category. The model's accuracy seems to be different according to embryo transfer policy.

Limitations, reason for caution: This study was retrospective and monocentric, preventing from generalizing our results to other ART centres. However, our aim was exactly to evaluate the performance of the prediction model in a local setting.

Wider implications of the findings: Hierarchical prediction models based on morphokinetics should apparently not be universally used in any IVF unit. Each team using time-lapse technology should take inspiration from Meseguer's model in order to build a centre-specific prediction model fitted to its data. Specific prediction models could be built for each embryo transfer strategy in order to have improved accuracy.

Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), University hospital of Nantes.

Trial registration number: None.

O-147 A prospective randomised controlled trial of the efficacy of using a closed time-lapse system for embryo culture

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Study question: To evaluate the effect of culture in a closed system in terms of number of high quality embryos and implantation rate compared to culture in a conventional incubator system.

Summary answer: Incubation during 2 days in a closed time-lapse system resulted in a similar number of good quality embryos per patient compared with incubation in a conventional incubator system.

What is known already: The EmbryoScope is both an incubator and a system for monitoring morphological events taking place during culture. The EmbryoScope does not have to be opened during embryo assessment, thereby ensuring more stable culture conditions. There have been a few studies conducted which have compared the outcome of closed time-lapse incubators with culture in a standard incubator, however no large prospective controlled randomized trials.

Study design, size, duration: The study was performed between May 2010 and January 2014. Randomization was carried out after oocyte retrieval and the patients' oocytes were allocated to culture in either a conventional incubator or the EmbryoScope in proportion 1:2. The primary endpoint was the number of good quality embryos in the two groups.

Participants/materials, setting, methods: Patients (n = 357) undergoing their first IVF cycle using ICSI, where at least one oocyte was retrieved, were included. All observations were made at the same time points for the embryos cultured in the EmbryoScope and those cultured in standard incubators. Morphological assessment was based on the exact same criteria in the two groups.

Main results and the role of chance: The mean number of aspirated oocytes were 9.6 for the study group vs. 9.4 for the control group. The mean number of good quality embryos per patient were 2.6 ± 2.2 vs. 2.2 ± 1.8 (p = 0.20). Embryo transfer was performed on day 2, and a mean of 1.06 vs. 1.04 embryos were transferred. The pregnancy rate was 29.5% vs. 30.4% per OPU and 32.7 vs. 33.3 per ET. The clinical implantation rate (ultrasound) was 26.2% for the study group and 28.6% for the control (p = 0.12)
Limitations, reason for caution: This study has focused on the concept of culturing in two different systems. Culture media, temperature etc. have been kept similar, but different culture dishes were used in the different systems, which could influence the culture conditions and possibly embryo quality.
Wider implications of the findings: The EmbryoScope has been introduced into a large number of clinics without showing improvement in embryo development or pregnancy rates in well designed controlled trials. Further studies need to be performed to investigate if this system has other advantages i.e. for selection of embryos for transfer.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Funding by commercial/corporate company(ies), Sahlgrenska Academy and Reproductive Medicine, Sahlgrenska University Hospital, Ferring Research Infertility and Gynecology Grant, Unisense, Fertilitech.
Trial registration number: ISRCTN 13118173.

O-148 An examination of the ability of six published, time-lapse imaging embryo selection algorithms to predict implantation when applied to the same cohort of implanted embryos

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Study question: The literature currently provides several differing sets of morphokinetic embryo selection algorithms (ESAs). This investigation seeks to determine the efficiency of six different ESAs at predicting implantation when applied to an exclusive set of embryos known to implant, derived within the same laboratory system.
Summary answer: The data suggest that previously published ESAs are inefficient and vary in their ability to predict embryo implantation when applied to a cohort of exclusive implanted embryos derived within the same laboratory system. Therefore, published ESAs may not be readily transferrable to centres other than those in which the criteria were defined.
What is known already: Since the introduction of time-lapse imaging into clinical practice, many have sought to utilise the information these systems can offer by developing ESAs and identifying embryo selection criteria. The usefulness of these criteria can be determined by examining their positive predictive value (PPV); the ESAs capability to predict an embryo's ability to either form a good quality blastocyst, to implant or produce a live birth, specificity; true negative rate, and sensitivity; true positive rate.
Study design, size, duration: 97 known implantation embryos derived between December 2012 and December 2013 were included. Six published ESAs were retrospectively applied to the embryos' morphokinetic data to derive the PPV, sensitivity and specificity of each ESA in terms of its ability to predict implantation.
Participants/materials, setting, methods: Embryos from patients undergoing treatment by ICSI were included. Embryos were cultured using Vitrolife™ G5 series medium in an atmosphere of 5% O_2, 6% CO_2 at 37°C in EmbryoScope® instruments throughout. Morphokinetic data were derived retrospectively by manually annotating the captured images.
Main results and the role of chance: Each published ESA specified time ranges into which embryos must fall to be identified as having the highest potential for the stated end point. The ESAs included a differing variety of observable events including time to 2, 3, 4, 5, 6, 7 and 8 cell, cell cycle durations and time to blastulation. Although some of the ESAs use blastocyst formation rate and live birth rate as an end-point, this investigation used implantation rate as the end-point of interest. When applied to the exclusive cohort of embryos, the PPV for the six ESAs was 50.8%, 50.0%, 43.8%, 43.3%, 54.5%, and 50.8%, respectively. The specificity was 3%, 95.5%, 35.7%, 10.5%, 0% and 40%, respectively. Finally, the sensitivity was 91.7%, 12.5%, 87.5%, 72.2%, 85.7% and 66%, respectively.
Limitations, reason for caution: This preliminary investigation was performed on a relatively small cohort of embryos of known implantation status and further investigation including more embryos is prudent. Morphokinetic annotations are, by their nature, subjective, and culture conditions and patient management varied for each ESA's development.
Wider implications of the findings: When applied to the same cohort of known implantation embryos derived within the same laboratory system, all six of the published embryo selection algorithms examined appeared to be relatively inefficient at predicting implantation with some being less efficient than others. These data suggest that embryology laboratories should proceed with caution when implementing embryo selection criteria derived from published sources and consider thorough in-house derivation and validation of such criteria before clinical use.
Study funding/competing interest(s): Funding by hospital/clinic(s), The Hewitt Fertility Centre.
Trial registration number: Not applicable.

O-149 Computer-automated time-lapse analysis test results correlate to clinical pregnancy and embryo implantation: a prospective, blinded, multi-center study

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Study question: Do computer-automated time-lapse analysis results correlate with pregnancy outcome and/or embryo implantation?
Summary answer: Quantitative time-lapse analysis results generated by state-of-the-art computer vision software programs correlated well with clinical pregnancy rates and embryo implantation in a prospective, blinded, multi-center study. Automated time-lapse analysis provides objective, quantitative information that could be used by embryologists to improve embryo selection.
What is known already: Previous studies (Conaghan et al., 2013) have shown that computer-automated time-lapse analysis together with traditional morphology could help embryologists identify Day 3 embryos with higher potential of becoming a usable blastocyst. As computer-automated time-lapse analysis may help increase embryologists' efficiency in embryo assessment, it is valuable to examine the correlation between computer-derived test results and patient pregnancy/embryo implantation in a large sample-sized, multi-center study.
Study design, size, duration: Prospective, blinded, multi-center study (Jun.2011-Oct.2013). 177 patients from 6 clinics consented to have embryos imaged using *Eeva*™, a platform that integrates time-lapse imaging and automated analysis of P2 (time between first and second mitosis) and P3 (time between second and third mitosis) to categorize embryos into three groups (High/Medium/Low) based on developmental potential.
Participants/materials, setting, methods: Time-lapse analysis results (High: $9.33 \leq P2 \leq 11.45$ h and $0 \leq P3 \leq 1.73$ h; Medium: if not High and $9.33 \leq P2 \leq 12.65$ h and $0 \leq P3 \leq 4$ h; Low: if not High or Medium) were blinded to embryologists. Embryos were selected for transfer using morphology assessment. Pregnancy was confirmed by ultrasound at 6–7 weeks. χ^2-test was used for statistical analysis; data analyzed using SAS9.3.
Main results and the role of chance: Patients with at least one computer-rated High embryo transferred had significantly higher clinical pregnancy rates than those with only Low embryos transferred (54%, 48/89, vs. 34%, 14/41, p = 0.02). The clinical characteristics of these two populations were comparable. Further analysis of 286 transferred embryos with known implantation data revealed that computer-generated time-lapse results also correlated to embryo implantation: High embryos had statistically significantly higher implantation rates than Low embryos (38%, 35/91, vs.15%, 18/121, $p < 0.0001$). A trend was observed that showed implantation rates decreased as time-lapse grading transitioned from High to Medium to Low (38%, 34%, 15%). This correlation between computer-generated time-lapse results and implantation was observed for Day 3 and Day 5 transferred embryos, and in different IVF centers each using their own laboratory protocols.

Limitations, reason for caution: Only embryos with a known implantation outcome were included in this analysis, and therefore, non-implanted embryos were overrepresented in the dataset.
Wider implications of the findings: In this study we have demonstrated that computer-automated time-lapse analyses show strong correlation to clinical pregnancy and embryo implantation. These data, together with previous research and clinical studies (Wong et al., 2010; Meseguer et al., 2011; Chavez et al., 2012), confirm that Eeva provides valuable information regarding embryo developmental potential. Therefore, selecting an Eeva High embryo to transfer may improve the clinical outcome of IVF procedures and ultimately provide an improvement in single embryo selection.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), This work was supported by Auxogyn, Inc., M.D. VerMilyea has provided technical consultation to Auxogyn, Inc.
Trial registration number: ClinicalTrials.gov # NCT01369446.

O-150 The relationship between early cleavage morphokinetic time points is more predictive of implantation and live birth than single time points alone

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Study question: Can the way in which morphokinetic variables at the cleavage stage relate to each other impact on implantation and live birth and can this approach be used to develop models to recognise cleavage patterns that can maximise implantation potential when selecting embryos from a cohort?
Summary answer: The classification power of a selection model was improved by comparing two variables CC2 (time to 3 cell minus time to 2 cell) and CC3 (time to 5 cell minus time to 3 cell) as proportions relative to each other, in a new variable, RELCC2 (t3–t2)/(t5–t3)*100.
What is known already: We have previously shown using Known Implantation Data (KID) that time to reach 2 cell (t2), time to reach 4 cell (t4), CC2 (t3–t2) and CC3 (t5–t3) are morphokinetic variables which can be used to aid embryo selection. A new variable, relCC2, considered the proportion of time that CC2 occupies from the duration of CC2+CC3 and therefore describes the regularity with which cleavage is occurring.
Study design, size, duration: Morphokinetic variables were assessed for 600 ICSI embryos with known implantation outcome (KID), using EmbryoScope™ (Fertilitech, Denmark). A new algorithm was derived using recursive partitioning which ranked embryos by implantation potential calculated using the KID outcome.
Participants/materials, setting, methods: Morphokinetic data from 4 clinics was collected (May 2011–August 2013). CC2, CC3 and relCC2 differed significantly between KID positive (implanted) and negative (not implanted) embryos. The new algorithm was compared to previous in house derived algorithms and compared using AUC of the ROC curve to establish their comparative classification power.
Main results and the role of chance: The first in house algorithms showed deselecting embryos by CC2 (<5 h) significantly increased the implantation rate ($p = 0.04$). The second model gave an area under the ROC curve of 0.77 for live birth when using t2 <30 h, CC2 >2 h and t4 <38.4 h. The new model has improved the area under the ROC curve to 0.8 for live birth, when t2 < 27.1, CC2 > 2 and CC3 > 5 and relCC2 is 0.44–0.47.
Limitations, reason for caution: KID embryo data will continue to be collected and analysed, to increase the live birth data set. Models may not be transferrable between clinics and prospective studies are required to confirm the efficacy of such morphokinetic selection algorithms.
Wider implications of the findings: This work is identifying early cleavage patterns that are more likely to give rise to live births, the ultimate outcome measure. It highlights the potential importance of the regularity of early cleavage divisions in relation to each other, aiding the selection of embryos from a cohort with the best chance of live birth.
Study funding/competing interest(s): Funding by hospital/clinic(s), Clinic – CARE Fertility.
Trial registration number: None.

SELECTED ORAL COMMUNICATION SESSION

SESSION 41: IMPROVING ART OUTCOME

Tuesday 1 July 2014 **15:15 - 16:30**

O-151 Predictive value of serum and follicular fluid levels of 25-OH vitamin D, osteopontin and sclerostin on the outcome of assisted reproductive treatment

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Study question: Do 25-OH vitamin D (25(OH)D), osteopontin (OPN) and sclerostin (SOST) serve as predictive markers for outcome of IVF/ICSI?
Summary answer: Only 25(OH)D-levels in serum the day of oocyte retrieval and 25(OH)D-levels in follicular fluid showed a significant difference between pregnant and non-pregnant women undergoing IVF/ICSI.
What is known already: There are several studies indicating that levels of G-CSF, vitamin D, glycodelin, leptin and anti-Mullerian hormone in follicular fluid might be predictive for oocyte maturation and possible outcome of IVF/ICSI. Furthermore, there is ongoing evidence the ART patients do have low levels of 25(OH)D in peripheral blood.
Study design, size, duration: This retrospective case control study was performed between February 2011 and August 2013 with a total of 58 women undergoing ART for *in vitro* fertilization (IVF) or intracytoplasmatic sperm injection (ICSI) and 17 healthy controls without hormonal stimulation during the follicular and luteal phase.
Participants/materials, setting, methods: Blood samples were taken at oocyte pick up [+ follicular fluid (FF)] and 14 days later. 25(OH)D, OPN and SOST were analyzed by chemiluminescence immunoassay and ELISA in patients and controls and compared to outcome of ART. Statistical analysis was performed using SPSS for windows (22.0), $p < 0.05$ significant.
Main results and the role of chance: The clinical pregnancy rate was 50% ($n = 29$), miscarriage rate 31% ($n = 9$). So far 18 patients delivered and 2 pregnancies are ongoing. Levels of 25(OH)D, OPN and SOST did not differ significantly during menstrual cycle nor between patients and controls. However, there were significant differences in levels of 25(OH)D at oocyte pick up in peripheral blood as well as follicular fluid between pregnant and non-pregnant patients ($p = 0.003$ peripheral blood, $p = 0.018$ follicular fluid).
Limitations, reason for caution: The study population is small, but the study is ongoing.
Wider implications of the findings: The measurement of 25(OH)D) in peripheral blood and follicular fluid might serve as predictive marker in ART.
Study funding/competing interest(s): Funding by hospital/clinic(s), Womens' University hospital Heidelberg.
Trial registration number: A trial registration number was not required due to the retrospective study design.

O-152 Circulating microRNAs and cell-free DNA in infertile patient bloodstream as innovative non-invasive diagnostic biomarkers for assisted reproductive technology

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Study question: We identified the microRNAs present in oocytes and cumulus cells. Some of them are detected in the bloodstream, as well as small amounts of cell-free DNA. Could circulating nucleic acids be related to ovarian reserve and thus, be used as non-invasive biomarkers for infertile patients included in IVF/ICSI program?
Summary answer: Several circulating microRNA expression was significantly up-regulated or down-regulated in serum samples from infertile patients either with low ovarian reserve or with hormonal or metabolic disorders, respectively. Furthermore, cell-free DNA was significantly related to patient age.

What is known already: MicroRNAs are small non-coding RNA molecules that regulate specifically messenger RNAs. They play important physiological roles and their deregulations lead to pathologies. Cell-free DNA results from apoptotic/necrotic cells. Both are intensively studied and used as diagnostic/prognostic biomarkers of many diseases. Several microRNAs are present in the oocyte-cumulus complex and constitute crucial regulators of folliculogenesis process. The potential use of circulating nucleic acids as potential non-invasive biomarkers of infertility and IVF/ICSI outcomes has never been investigated.
Study design, size, duration: This study includes 60 serum samples from natural cycle day 3 of 60 patients, included in IVF/ICSI program. Ovarian reserve biomarkers such as FSH, LH, AMH and Estradiol in serum were measured and compared with microRNAs levels and cell-free DNA amounts.
Participants/materials, setting, methods: MicroRNAs were extracted from serum samples with the QIAamp kit from QIAGEN and quantified by RT-qPCR, using TaqMan technology. Serum cell-free DNA was prepared in Proteinase K buffer and quantified by ALU sequence qPCR. Means ± SEM are presented. The *p*-values were calculated by using the unpaired *t*-test, on GraphPad software.
Main results and the role of chance: Circulating Let-7b was significantly increased ($p = 0.05$) in serum samples from patients with a low ovarian reserve (AMH <2 ng/ml). Moreover, miR-30d and miR-320a were drastically reduced ($p = 0.02$; $p = 0.01$, respectively) in serum samples from patients with high LH rates (>5 UI/l), suggesting a role of these both microRNAs in folliculogenesis. Furthermore, miR-125a and miR-191 were both also significantly decreased in serum samples from patients with a BMI >25 compared to those with a BMI ≤25 ($p = 0.001$) and serum cell-free DNA is significantly higher in aged patients, >37-years-old ($p = 0.02$). This is interesting since obesity or aging are bad prognosis for ART success. All together, our results suggest that the profiling of circulating nucleic acids opens new perspectives for the diagnosis/prognosis of ovarian disorders and maybe for the prediction of ART outcomes.
Limitations, reason for caution: Further investigations with large number of patients and their attempt outcomes are will be done to confirm these results and to highlight new circulating nucleic acids related to ART outcomes.
Wider implications of the findings: Using this approach, we identified new biomarkers of ovarian disorders and ART outcome and we will establish diagnostic tests based on the quantification of these nucleic acids in blood. Such results could give new clinical biomarkers for a rapid and easy diagnosis of infertile patients to individualized IVF/ICSI cycles.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Funding by commercial/corporate company(ies), This work was supported by the University-Hospital of Montpellier and by a grant from the Ferring Pharmaceutical Company. The authors of the study have no competing interests to report.
Trial registration number: Not applicable.

O-153 Preventive effect of oral mucosal epithelial cell-sheets on the endometrial adhesion

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Study question: Can regenerative-medicine technique with cell sheets become a new treatment method for intrauterine adhesion causing female infertility?
Summary answer: Oral mucosal epithelial cell sheet (OMECS) transplantation was confirmed to be effective to prevent intrauterine adhesions from endometrial damage in rats.
What is known already: Uterine disorder such as endometrial adhesion, Asherman's syndrome, is one of the infertility factors. Therapy for this disease is the prevention of re-adhesion by surgical synechiotomy, hormonal administration after operation, and the use of intrauterine device. 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: OMECS was prepared from rat oral mucosal tissues. An endometrial adhesion model was made in rat uteri, and OMECS were transplanted to the model. Uteri transplanted with OMECS were compared to the non-transplanted control uteri by histological analysis at 1, 2, and 8 days after surgery ($n = 3$).
Participants/materials, setting, methods: Oral mucosal tissues resected from neonatal rats, and oral mucosal epithelial cells were collected with enzymatic treatment. Isolated cell suspension was seeded on a temperature-responsive-cell-culture-insert and incubated. After being detached from the insert, a cell sheet was transplanted into the defect of endometrium. After surgery, uteri were resected and examined.
Main results and the role of chance: Histological examination of the non-treated specimens at 1, 2, and 8 days after surgery showed the absence of uterine cavity caused by endometrial adhesion. In contrast, the histology of uterus transplanted with OMECS immediately after endometrial damage showed the presence of uterine cavity and, furthermore, the exist of stratified squamous epithelial cells on the luminal surface ($n = 3$).
Limitations, reason for caution: Because the structure and function of rat uteri are different from those of human, the results of this study were unable to be extrapolated to the human.
Wider implications of the findings: Transplantation OMECS can have a high possibility to prevent not only intrauterine re-adhesion after synechiotomy for endometrial adhesions (Asherman's syndrome) or uterine lumen adhesion, and degeneration in female infertility, but also adhesion after other intrauterine surgeries in clinical case.
Study funding/competing interest(s): Funding by national/international organization(s), 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: No applicable.

O-154 Urine hormones in relation to ultrasound observed ovulation and serum hormones in cycles from women with reportedly normal menstrual cycles

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Study question: To examine the precise relationships and inter-individual variation, in urinary hormone levels in relation to ultrasound observed ovulation and serum hormone levels in menstrual cycles of apparently normal women.
Summary answer: There was surprising variety in cycle characteristics within normal women, including anovulatory cycles. Surge in urinary luteinising hormone (LH) was an excellent predictor of ovulation and rise of urinary pregnanediol-3-glucuronide (P3G) above baseline was a consistent maker of luteinization.
What is known already: Several recent studies examining urinary hormone profiles, have demonstrated that normal women can exhibit considerable inter-individual variation in their menstrual cycle characteristics. This can make it difficult to predict the fertile period using calendar methods alone and therefore objective methods for fertile phase identification, such as urinary hormone monitoring can be useful both for conception and contraception purposes. However, these studies had no independent reference method for ovulation. Older studies have compared urine hormones to ultrasound, but ultrasound methods have since improved, and population demographics altered, and therefore a current study on this topic is important.
Study design, size, duration: Prospective study of 42 women who collected daily urine samples for one complete menstrual cycle and recorded menses in a daily diary. Serum samples and trans-vaginal ultrasonography to examine ovaries/endometrium were taken every 2 days (daily from follicle size >16 mm until post ovulation). Study design was approved by University of Cologna ethics committee.
Participants/materials, setting, methods: Women were aged 18–40 with a minimum of 2 natural cycles prior to study start, and no known infertility. Serum LH, Progesterone, estradiol were measured by ADVIA Centaur XP. Urine LH, P3G, E3G (estrone-3-glucuronide) were measured by Auto DELFIA using validated, in-house assays. Ultrasound was conducted by two clinicians and all images stored for central review.
Main results and the role of chance: Ultrasound revealed 2 women had an anovulatory cycle (cycle lengths 26 and 28 days). For one, follicle growth, but

not rupture was seen and there was luteinisation of the unruptured follicle. Serum and urine hormones matched ultrasound findings with no LH surge, but elevated luteal progesterone.

Cycle length varied from 22 to 37 days (median 27). Ovulation by ultrasound ranged from days 8 to 26 (median 15). Correlation between urinary hormones and their serum equivalents was good, and for LH was excellent.

Urinary LH surge preceded ovulation for most women (mean time from surge to ovulation 0.81 days, SD 0.89), but LH peak could be post-ovulatory (mean time from LH peak to ovulation 0.42 days, SD 0.87). Urinary P3G rise from baseline occurred after ovulation for all volunteers. Assays conducted in duplicate, and image review was done to reduce error.

Limitations, reason for caution: Daily ultrasound prior to ovulation was not possible for all volunteers and therefore for some volunteers, day of ovulation was estimated, introducing an error ±1 day. Volunteer recruitment targeted women who reported normal menstrual cycles, so study could under-represent the extent of population variability and frequency of anovulation.

Wider implications of the findings: Having a normal length cycle, does not guarantee the cycle is ovular, and, very short or long cycles can be fertile. In addition, we found considerable inter-individual variation in day of ovulation, therefore a prospective method should be used for identification of the fertile period, for women wishing to time intercourse either to avoid or achieve pregnancy. Our study has found that urinary hormone monitoring can be used to predict and confirm ovulation, with P3G rise providing an excellent marker of the end of the fertile period. As urinary LH peak did not always precede ovulation, LH surge appears a more effective prospective marker. Most importantly, urinary hormone monitoring provides an accurate, and individual, reflection of the fertile phase for a woman.

Study funding/competing interest(s): Funding by commercial/corporate company(ies), SPD Development Company Ltd., a fully owned subsidiary of SPD Swiss Precision Diagnostics GmbH, the manufacturers of Clearblue™ products.

Trial registration number: NCT01802060.

O-155 Arcuate uterus: is a normal or septate uterus

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Study question: What is now the clinical approach to arcuate uterus after the introduction of the new ESHRE/ESGE classification system of female genital anomalies in 2013?

Summary answer: The arcuate uteri previously diagnosed with ultrasound Salim classification (2003) on 3D coronal section are now, according the new ESHRE/ESGE classification, normal uteri (U0) or septate uteri (U2) with important consequences in the prognosis for the patients.

What is known already: Congenital uterine anomalies are associated with adverse reproductive outcomes such as infertility, recurrent miscarriages and early preterm labor. A 3D transvaginal ultrasound scan is performed when a uterine anomaly is suspected but the classification systems proposed for their categorization are associated with some limitations.

Study design, size, duration: We assessed a retrospective study in which we re-evaluated and reclassified the stored 3D ultrasound uterine volume of the all women who underwent in our Unit between January 2012 and January 2014 a 3D transvaginal ultrasound scan with diagnosis of arcuate uterus according to Salim classification.

Participants/materials, setting, methods: 327 women were included in the study. The assessment of uterine morphology was performed in a coronal plane with the interstitial portions of the Fallopian tubes used as reference points. Three measurements were taken: uterine cavity width (W), fundal distortion or septal length (F) and length of unaffected myometrium (M).

Main results and the role of chance: According to ESHRE/ESHE classification of the 327 uteri classified previously as arcuate, 152 (46.5%) uteri were septati because the length of F exceeded the 50% of the uterine wall thickness (F + M) while 174 (53.2%) were considered normal. The mean length of F and M were in the now normal uteri significantly different compared to the now septate uteri (3.9 ± 0.7 vs. 7.1 ± 1.5 mm and 11.0 ± 2.1 mm vs. 8.5 ± 1.5 mm, respectively) while the mean width of W was not significantly different. In the reclassified patients who had according to ESHRE/ESGE septate uterus, 52% (79/152) had infertility and 8% (12/152) recurrent miscarriage, whereas the now 174 patients with normal uterus showed 36% (62) of infertility and 18% (31) of recurrent miscarriage.

Limitations, reason for caution: Minor uterine anomalies, essentially the arcuate uterus, with the 3D TVS diagnosis and the new ESHRE/ESGE classification, arise several diagnostic and clinical implications and may result in difficulties in counselling and in the treatment options in women who experience miscarriage and subfertility.

Wider implications of the findings: The diagnosis of arcuate or septate uterus on the basis of relative ESHRE/ESGE criteria is not supported by retrospective results and prospective studies of corrective surgery. The only way to overcome these limitations is to perform prospective randomized studies with 3D TVS diagnosis and ESHRE/ESGE classification followed by metroplasty and fertility outcomes. This could help to refine selection criteria for surgery, resulting in improved long-term outcomes in women with uterine anomalies.

Study funding/competing interest(s): Funding by University(ies), Department of Obstetrics and Gynecology, University of Rome Tor Vergata, Rome, Italy, Department of Obstetrics and Gynecology, University of Bari, Bari, Italy, Department of Obstetrics and Gynecology, University of Siena, Siena, Italy.

Trial registration number: None.

SELECTED ORAL COMMUNICATION SESSION

SESSION 42: SPERM DNA INTEGRITY

Tuesday 1 July 2014 **15:15 - 16:30**

O-156 Revisiting DNA integrity in function of sperm motility

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Study question: Sperm chromatin fragmentation does not always correlate with ICSI outcome. Therefore, we revisited the role of DNA fragmentation index (DFI) by measuring the DFI of exclusively motile spermatozoa in relation to clinical outcome.

Summary answer: Increasing DFI had a positive correlation with paternal age and lengthening of abstinence period. There was an inverse correlation between sperm DFI and motility. DFI above and below thresholds did not affect ICSI fertilization and pregnancies. Once DFI was corrected for motile spermatozoa, men above threshold yielded higher embryo implantation.

What is known already: During the later stages of spermiogenesis DNA breakages are physiologically induced to allow tight chromatin compaction and only those spermatozoa with repaired chromatin reach the ejaculate. Throughout the male genital tract, oxygen-free radicals mostly from decaying spermatozoa and other cells are the main cause of DNA damage and responsible for the compromised ART outcome. Therefore, tests for sperm DNA integrity are gaining popularity to assess gamete competence as a supplemental assay to traditional semen analysis.

Study design, size, duration: Couples with previous ART failure were screened for sperm DNA fragmentation and inseminated by ICSI. DFI was plotted against abstinence period and semen parameters. We then correlated DFI and normalized DFI (mDFI) according to clinical outcome.

Participants/materials, setting, methods: A total of 253 couples with 568 ICSI cycles were analyzed and compared. DFI was assessed by TUNEL and SCSA tests. An mDFI for TUNEL and SCSA was calculated by taking the initial DFI × a constant × motility and was correlated with clinical outcome.

Main results and the role of chance: A total 253 couples were included with a male age of 40.2 ± 9 years (range 21–69 years) and female of 36.4 ± 7 years. The abstinence period ranged from 1 to 18 days, with a concentration of 51.1 ± 14 million/ml, motility of 49.5 ± 11%, and morphology of 3.2 ± 2%. When DFI was plotted against sperm concentration and morphology no difference was observed. DFI positively correlated with paternal age and abstinence period ($p < 0.001$). Higher sperm motility had the lowest incidence of DNA fragmentation ($p < 0.001$). No correlation was observed between the mDFI below/above threshold with fertilization 73.7/73.6%, clinical pregnancies 32.9/29.7% or losses 3.9/5.4%. However, when we looked at the implantation ability of embryos generated through ICSI, we saw that the abnormal mDFI had a compromised implantation (15.9 vs. 10.6%; $p = 0.02$).

Limitations, reason for caution: While it is clear that fragmented sperm DNA may limit embryo developmental competence, this should take into account only the motile portion of spermatozoa particularly with ICSI.
Wider implications of the findings: This finding theorizes that DNA fragmentation originates from aggressors in the male genital tract. In men with ART failure or recurrent pregnancy loss the proportion of high sperm DNA fragmentation in the ejaculate may provide guidance towards the utilization of testicular sperm. A relative DFI as a function of the proportion of motile spermatozoa more appropriately depicts the ability of a sperm genome to generate a healthier embryo.
Study funding/competing interest(s): Funding by University(ies), Reproductive Medicine, Weill Cornell Medical College.
Trial registration number: N/A.

O-157 Age of the sperm donor: does it really matter - an analysis of 1,048,576 assisted reproduction treatment cycles

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Study question: Does the age of sperm donor affect the chances of success in women undergoing assisted reproduction for the first time?
Summary answer: Age of the sperm donor does not significantly alter the live birth rate in females undergoing assisted reproduction. Older females (>37 years) might have a slightly better chance of success with sperm donors of increasing age.
What is known already: Although it is well known that advanced maternal age has a negative effect on fertility and pregnancy outcome, the influence of paternal age or sperm donor age on these outcomes is less well researched. In UK, Human Fertilization and Embryology Authority (HFEA) recommends that sperm donors should be aged between 18 and 41 years while in Australia, Human Reproduction Act quotes 21–45 years but substantial evidence of reproductive outcome based on male donor age is lacking.
Study design, size, duration: Retrospective analysis of anonymised HFEA long term data registry from year 1991 to 2012 correlating live birth rate with sperm donor age was performed for first sperm donor assisted reproduction treatment cycles.
Participants/materials, setting, methods: Out of 1,048,576 cycles, 237,853 were in-vitro fertilization (IVF) and Donor Insemination (DI) cycles using donor sperm. Binary logistic regression analysis was performed on first treatment cycles for women aged 18–34 or >37 years based on impact of sperm donor age on live birth rate.
Main results and the role of chance: Of the 237,853 donor sperm cycles, first treatment cycles with recorded sperm donor age (n = 39,282) were analysed in two female age groups (18–34 and >37 years). Of the 30,097 cycles in 18–34 years female group, 25,925 were DI and 4172 IVF/ICSI cycles. In >37 years female group, 6490 and 2695 were DI and IVF cycles respectively. The sperm donor age was collated in 6 different subgroups (<20, 21–25, 26–30, 31–35, 36–40 and 41–45 years).

In this study impact of sperm donor age on live birth failed to reach statistical significance in any of the groups. However the study showed a trend of increased likelihood of live birth with rising sperm donor age with lowest chance in <20 years sperm donor subgroup.
Limitations, reason for caution: As the study is based on available data registry, potential confounders such as smoking, body mass index were not accounted. Grouped donor age rather than continuous age data can impact on the accuracy of the prediction model. Transcription errors were likely for information returned to the HFEA on paper forms.
Wider implications of the findings: This is probably the first study to conduct an analysis of impact of sperm donor age on live birth using a large national IVF database. Although female age is a key predictor of outcome in assisted reproduction and sperm donor age has lesser impact on outcome especially in optimum reproductive age group females (18–34 year), donors between 26 and 30 years might be considered to give older female age group (>37 years) maximum chances of success.
Study funding/competing interest(s): Funding by hospital/clinic(s), Authors have no funding or support to report. The authors have declared that no competing interests exist.
Trial registration number: Not applicable.

O-158 Seminal plasma round cell as an indicator of spermatogenic integrity

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Study question: To characterize the origin and meaning of round cells (RC) in human ejaculates and its relationship to spermatogenesis. The role of RC on clinical outcome was assessed in specimens used for ART.
Summary answer: The large majority germ cells are not exclusively WBC in the ejaculate. These RCs are immature germ cells (IGC) confirmed by their ploidy status, chromatin fragmentation, protamine, and their encasement in sertoli-cell cytoplasm. Seminal round cells indicate suboptimal gamete production as confirmed by ART outcome.
What is known already: The source of round cells in the ejaculate has always been puzzling and enigmatic. White blood cells may represent infection raising concerns on their impact of *in vitro* culture contamination and the possible effects of peroxidase on the sperm cell genome. These round cells can also be immature germ cells therefore raising doubts on the spermatogenic conditions of the individual.
Study design, size, duration: In a prospective fashion, a total of 3152 men undergoing male infertility screening in a period of 24 months were included in the study. Semen parameters were assessed according to the WHO criteria 2010. Cases displaying ≥2 million RC were screened for bacterial presence and LeucoScreen® to determine WBC proportion.
Participants/materials, setting, methods: Men with ≥2 million RC represented the study group. After identification of immature germ cells, specific stains were utilized to identify sertoli-cell cytoplasmic remnants as well as protamine to assess their spermiogenic stage. Sperm DNA fragmentation and aneuploidy (9 chromosome FISH) was also performed. Maternal age, fertilization, and pregnancy outcomes were recorded.
Main results and the role of chance: Male age was 44.1 ± 10 years and female 36.6 ± 4 years. Prevalence of RC was 1.1% (n = 35) with a concentration of $29.9 \pm 27 \times 10^6$/ml, motility of 34.9 ± 20%, and morphology of 1.7 ± 1%. The actual proportion of WBC was 29.7% with the remainder being IGC (range 0.79–17.2 million). All specimens screened were negative for uropathogens. The IGC were characterized by single/multiple nuclei as proven by their ploidy content. Ejaculates with IGC had higher incidence of aneuploidy in their spermatozoa (p = 0.0001). There was a concordant presence of DNA fragmentation between IGC and spermatozoa. Interestingly, IGC stained for vimentin indicating their encasement in sertoli-cell. A subgroup of men (n = 13) underwent 37 cycles with 61.5% fertilization, 27.0% clinical pregnancy and a pregnancy loss of 16.2%. Presence of RC clearly impaired fertilization (p = 0.0001), clinical pregnancies, and accompanied by higher pregnancy losses (p = 0.001).
Limitations, reason for caution: These exciting findings related to the seminal round cells on the integrity of spermatogenesis and ART outcome need to be validated in a larger number of cases.
Wider implications of the findings: The traditional perception of the seminal round cell has ranged from an infection marker to a "good Samaritan" cell. However, genetic and epigenetic assessments of these cells carried out in this study evidenced that they are largely abnormal spermiogenic products engulfed in sloughed sertoli-cell cytoplasm. This suggests that the presence of round cells in the ejaculate may serve as an indicator of an acute spermatogenic insult and may yield knowledge on compromised gamete competence.
Study funding/competing interest(s): Funding by University(ies), Reproductive Medicine, Weill Cornell Medical College.
Trial registration number: N/A.

O-159 Topographic mapping of sperm DNA fragmentation within the male genital tract

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Study question: Site of sperm DNA damage within the male genital tract remains puzzling. A recent tendency to suggest testicular biopsy to men with high DNA fragmentation in their ejaculate has resulted in an enhanced clinical outcome. This finding has called for DFI testing in spermatozoa retrieved at different surgical exploration sites.

Summary answer: It is clear that DNA fragmentation has its source within the seminiferous tubules, however, progression through the genital tract towards the ejaculate dynamically increases DFI. This finding may justify offering testicular biopsy to men with very high sperm DNA fragmentation in their ejaculate aiming at better embryo development and implantation.
What is known already: During the later stages of spermiogenesis DNA breakages are physiologically induced to allow tight chromatin compaction and only those spermatozoa with intact chromatin reach the ejaculate. Throughout the male genital tract, oxygen-free radicals mostly from decaying spermatozoa and other cells are the main cause of DNA damage and responsible for the compromised ART outcome. Therefore, testing for sperm DNA integrity is gaining popularity to assess gamete competence.
Study design, size, duration: Men with extremely high DFI in their ejaculates (n = 20) were counseled to undergo surgical sampling. DFI analysis was carried out on ejaculate, vasal fluid, epididymis, and testis. To determine whether a testicular biopsy would yield spermatozoa with healthier chromatin and superior embryo developmental competence, men underwent ICSI with these specimens.
Participants/materials, setting, methods: We identified infertile men (n = 20) with high DNA fragmentation in their ejaculates that agreed to undergo surgical sampling for this indication. Chromatin fragmentation index was assessed by SCSA and/or TUNEL on specimens isolated from all sites. ICSI outcomes with ejaculated and testicular spermatozoa were analyzed and compared.
Main results and the role of chance: In ejaculated spermatozoa the average DFI was 43.0 ± 16% (range 26–96) assessed in 25 occasions. In some of these men, aspiration of the vas deferens (n = 2) yielded a DFI of 16.5 ± 1% (range 15.7–17.3) while spermatozoa from the epididymis (n = 8) had a DFI of 15.8 ± 5 (range 11.7–25.9) and testicular spermatozoa (n = 15) 11.4 ± 7.9 (range 2–26.2). This topographic representation of the DFI in favor of testicular spermatozoa encouraged us to utilize these gametes to inseminate oocytes. These couples (n = 8) obtained 50% fertilization and an embryo cleavage of 100% that resulted in a clinical pregnancy of 25.0%. This finding appears superior to their respective ICSI cycles carried out with ejaculated spermatozoa that resulted in fertilization of 55.9%, embryo cleavage of 63.6%, and pregnancy rate of 12.5%.
Limitations, reason for caution: Patients need to be informed of risks regarding surgery, anesthesia, and the possibility that even with TESE a pregnancy may not occur. Thus, engaging counseling should be conducted since these men have spermatozoa in their ejaculate. These data are still preliminary and a clinical concensus have not been reached.
Wider implications of the findings: The topographic assessment of sperm chromatin integrity throughout the male genital tract indicates a disruption of DNA packing during spermiogenesis that does not allow sperm chromatin to withstand oxidative stressors, possibly compounded by a compromised total antioxidant capacity in the seminal fluid of these men. When couples have recurrent pregnancy failures, related to high DNA fragmentation of their ejaculated spermatozoa, they may benefit from undergoing testicular biopsy for diagnostic and therapeutic purposes.
Study funding/competing interest(s): Funding by University(ies), Reproductive Medicine, Weill Cornell Medical College.
Trial registration number: N/A.

O-160 The quest for the less than ideal spermatozoon - does it generate good quality embryos

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Study question: Pseudo-/azoospermic men requires extensive search for the spermatozoa to be injected. The increasing search time is inversely related to sperm availability, morphology, kinetic characteristics and genomic integrity. In this study, we assessed the clinical outcome of less than ideal spermatozoa identified after an exhaustive search in ejaculated and testicular specimens.
Summary answer: An exhaustive sperm quest in specimens from men with severely compromised spermatogenesis yielded morphologically and genomically impaired spermatozoa that retained the ability to activate oocytes and generate developing embryos and healthy offspring. This can only be explained by the ability of the oocyte to overcome eventual male genomic errors.
What is known already: Paternal factors ordain fertilization and trigger early embryo development, induce genomic activation, which ultimately yield a conceptus. Cryptozoospermic and azoospermic men can often provide extremely scarce and dysmorphic spermatozoa plagued by poor motility. The genomic integrity and competence of these spermatozoa are of concern. The ability of the ootid to repair sperm epigenetic damage has been postulated. We tried to assess to what extent the oocyte DNA polymerase repair mechanism can overcome male genomic dysfunction.
Study design, size, duration: In a retrospective cohort analysis, ICSI outcomes were reviewed as a function of the length of microscopic sperm search carried out in ejaculated (n = 2197) and testicular specimens (n = 1180). Fertilization, pregnancies, deliveries, and child health in relation to increasing search time were compared to controls with a large selection of spermatozoa.
Participants/materials, setting, methods: An extended sperm quest of ejaculated and TESE samples was performed with an inverted microscope in droplets under oil for up to >6 h and carried out by several embryologists that identified the spermatozoa utilized in this study. Sperm genome were screened by TUNEL, FISH, and protamine assay.
Main results and the role of chance: The maternal and paternal ages were comparable for ejaculated and TESE couples as well as the number of oocytes retrieved. The fertilization rates for both ejaculated and TESE progressively decreased with abating sperm quality ($p < 0.0001$). The ability of testicular spermatozoa to activate the oocyte remain clearly inferior to ejaculated counterparts independent of the quality of the spermatozoa injected ($p < 0.01$). Clinical pregnancy rates for both ejaculated and testicular spermatozoa were independent of the diminishing quality of spermatozoa used in comparison to their respective control groups (Ejac 39 vs. 43%; TESE 36 vs. 41%). While implantation decreased with the worsening of the ejaculated sperm cell injected ($p = 0.028$) this was not true for the testicular sperm. Offspring health was unaffected from sperm origin and quality.
Limitations, reason for caution: It remains difficult to standardize the consistency of the quality of spermatozoa that are retrieved following extensive microscopic search carried out by several embryologists. In fact, the quest for these spermatozoa are dictated by the number of oocytes retrieved or by exhaustion of the sperm specimen.
Wider implications of the findings: From this study, we learned that through ICSI the ability to achieve a pregnancy is virtually unaffected by the abating quality and scarcity of spermatozoa retrieved, particularly when they are retrieved directly from the seminiferous tubule. The ability of dysmorphic, kinetically impaired spermatozoa – with possibly compromised chromatin – to establish a pregnancy and live offspring remain puzzling. It may only be explained by a well functioning oocyte DNA polymerase repair mechanism.
Study funding/competing interest(s): Funding by University(ies), Reproductive Medicine, Weill Cornell Medical College.
Trial registration number: N/A.

SELECTED ORAL COMMUNICATION SESSION

SESSION 43: SAFETY ISSUES FOR MOTHER AND CHILD

Tuesday 1 July 2014 **15:15 - 16:30**

O-161 Cervical conisation and risk of preterm delivery in assisted reproductive technology (ART) singleton and twin pregnancies - Danish national cohort study

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Study question: Does cervical conisation add an additional risk of preterm birth in singleton and twin pregnancies conceived by ART?
Which embryo transfer strategy should be recommended to women with previous cervical conisation?
Summary answer: The prevalence of conisation is higher in ART vs. spontaneously conceived (SC) singleton pregnancies but similar in ART vs. SC twin

pregnancies. After adjustment for maternal age, parity and year of birth conisation adds an additional risk of PTB in ART singletons of 1.55 and in ART twin deliveries conisation doubles the risk of PTB.

What is known already: ART conception and cervical conisation both increases the risk of preterm birth. One study has shown that conisation adds an additional risk to the risk of preterm birth in ART pregnancies. This additional risk, however, was mostly related to higher maternal age and more nullipara among women pregnant after ART.

Study design, size, duration: Danish national cohort study from 1995 to 2009 including ART singleton (n = 16,923) and twin (n = 4829) deliveries identified through the Danish IVF and Medical Birth register (MBR). A random sample of spontaneously conceived (SC) singletons, two-fold the size of the ART singleton group matched by date and year of birth (n = 33,835) and all SC twin deliveries born 1997–2009 were extracted via the MBR (n = 15,112).

Participants/materials, setting, methods: Information on all women who had a cervical conisation prior to delivery was retrieved from the Danish Registry of Pathology (DRP). Risk of preterm birth was assessed in multiple logistic regression analyses with adjustment for maternal age, parity and year of birth. Risks were presented as adjusted odds ratio (aOR).

Main results and the role of chance: The prevalence of conisation was higher in women with ART singleton deliveries 3.4% compared to the SC singleton group (2.4%) ($p < 0.001$). In ART and SC twin deliveries the prevalence of mothers with cervical conisation did not differ.

In ART singleton deliveries the prevalence of PTB was 13.1% if conisation was performed prior to delivery vs. 8.2% in women without conisation and the adjusted risk of PTB in ART mothers with conisation was aOR 1.55 (95% CI: 1.21–2.06). In ART twin deliveries the prevalence of PTB was 58.0 vs. 41.3% in women with and without conisation and the adjusted risk was aOR 1.93 (95% CI: 1.35–2.76).

The risk of PTB in ART vs. SC singletons with previous conisation was aOR 1.58 (1.05–2.36) and in ART vs. SC twins with previous conisation the aOR of preterm birth was aOR 1.34 (95% CI: 0.88–2.02). These added risks of ART were similar to those observed in women without conisation.

Limitations, reason for caution: We were not able to adjust for the depth of the cervical cones, which has recently been shown to the associated with the risk of preterm birth. Only pregnancies with gestational age 22 + 0 at delivery were included as deliveries with lower gestational age are defined as miscarriages and not included in MBR.

Wider implications of the findings: With nearly 60% of the ART twins being born preterm in case of conisation and with a doubled risk of PTB (in case of conisation), single embryo transfer should be performed in all women with conisation prior to ART independent of age and number of ART cycles performed. With more than ten percent being born preterm and with an added risk of 1.55 of PTB in ART singletons with previous conisation, the antenatal care should be intensified in ART singleton pregnancies with previous conisation.

Study funding/competing interest(s): Funding by hospital/clinic(s), The study was funded by the fertility clinics at Skejby Hospital, Aarhus University and Rigshospitalet, Copenhagen University.

Trial registration number: N/A.

O-162 Predictive factors of poor perinatal outcomes in 6338 singletons born after intrauterine insemination in Denmark 2007 to 2012 - the influence of ovarian stimulation

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Study question: We aimed to evaluate obstetric and perinatal outcomes in singletons born after intrauterine insemination (IUI) compared with children born after IVF, ICSI and spontaneous conception (SC). Furthermore we wished to assess predictors of poor perinatal outcome in singletons born after IUI, exploring the effect of ovarian stimulation.

Summary answer: Children born after IUI have increased perinatal risks compared with SC-children. Outcomes are favourable compared with IVF, but similar to ICSI. Stimulation with clomiphene citrate (CC) is associated with increased risk of small for gestational age compared to natural-cycle IUI. Stimulation with follicle-stimulating hormone (FSH) did not increase perinatal risks.

What is known already: A limited number of previous studies have investigated children born after IUI, finding poorer perinatal outcomes compared to SC children. Studies on IUI vs. IVF are few and limited by small sample sizes. A negative effect of ovarian stimulation on birth weight, in medical assisted reproductive procedures other than IVF, has been described by Källén et al. in 2002, but without specification of type of medication used.

Study design, size, duration: We conducted a controlled national cohort study from 2007 to 2012. Cases were 4208 singletons born after IUI with husband semen (IUI-H) and 1881 singletons born after IUI with donor semen (IUI-D). Controls were 4135 singletons born after IVF, 3635 singletons born after ICSI and 229,749 SC singletons.

Participants/materials, setting, methods: Data originated from the Danish IVF register, medical birth register and hospital discharge register. Stratified analysis was performed for IUI-singletons born after ovarian stimulation vs. natural-cycle. Multivariate logistic regression analysis adjusted results for maternal age, parity, year of birth, child sex, BMI, smoking, elective caesarean section and induction of labour.

Main results and the role of chance: Among children born after IUI-H, risks of preterm birth (PTB), low birth weight (LBW) and small for gestational age (SGA) were increased vs. SC-singletons, adjusted odds ratios (aOR) 1.3 [1.1–1.5], 1.4 [1.2–1.7] and 1.4 [1.2–1.6], respectively. Compared with IVF, risk of SGA were similar for IUI-H, but risks of PTB and LBW were decreased, aOR: 0.6 [0.5–0.8] and 0.8 [0.6–0.9]. Compared with ICSI, no differences were found. For children born after IUI-D, results were similar to the IUI-H group. In the stratified analyses, we found increased risk of SGA and LBW in IUI singletons born after ovarian stimulation with CC, compared with natural-cycle IUI, aOR: 1.7 [1.1–2.5] and 1.5 [1.1–2.1] respectively. Treatment with FSH alone vs. natural-cycle IUI did not seem to affect perinatal outcomes.

Limitations, reason for caution: Odds-ratios could not be adjusted for cause and duration of infertility; this is of concern since couples offered ovarian stimulation may be more reproductively challenged compared with couples that receive IUI in natural cycles. However, children born after stimulation with FSH alone did not have adverse outcomes vs. natural-cycle IUI.

Wider implications of the findings: Adverse obstetric and perinatal outcome after IUI vs. SC children have previously been reported by Ombelet et al. (2006). Nakashima et al. (2013) compared children born after ovarian stimulation/IVF with natural-cycle IVF, and found, similar to our results, increased risk of LBW after treatment with CC, but not after treatment with FSH alone. The increased risk of SGA after CC treatment may be related to the anti-estrogenic effect off CC, affecting implantation and early placentation.

Study funding/competing interest(s): Funding by hospital/clinic(s), Fertility Clinic, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen.

Trial registration number: None.

O-163 Pregnancies issued from egg donation are associated to a higher risk of hypertensive pathologies then control ART pregnancies. Results of a large comparative cohort study

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Study question: The purpose of this study was to find out whether pregnancies issued from egg donation (ED) had a higher risk of pathologies and, in particular, of hypertensive disorders, than those issued from ART using the patients own oocytes, in term of hypertension alone, eclampsia and pre-eclampsia.

Summary answer: The percentage of hypertensive disorders was multiplied by 3 in the ED group compared to the control group (17.7 vs. 5.3%, $p < 0.001$), and this was observed for pre-eclampsia (11.2 vs. 2.8%, $p < 0.001$) and eclampsia (1.8 vs. 0.0%, $p < 0.05$, Fisher test) more than for isolated hypertension (4.7 vs. 2.5%, $p = 0.19$).

What is known already: Several papers have shown results in favour of an increased risk of pregnancy induced hypertension (PIH). However, most of them rely on small samples, without an adequate control group or do not analyse the role of some important confounders (women's age, multiple pregnancies, previous fertility). This has a growing importance since the number of egg donation is increasing, reaching more than 22,000 cycles (6600 deliveries) in the last EIM-ESHRE report (year 2010).
Study design, size, duration: This anonymous multicentre (7 centres) comparative cohort study included all singleton ED pregnancies diagnosed at 7–8 weeks, January 2005–December 2011. For each, two controls were selected among ART pregnancies. In total, 580 pregnancies were included (217 ED, 363 controls). This allowed to detect a doubling PIH incidence.
Participants/materials, setting, methods: Controls were matched on pregnancy date, parity, and women's age. Each centre coordinator collected information on infertility, pregnancy pathologies and outcome, and on neonate data. Comparison were made with chi-square and variance analysis, and a multivariate logistic model was conducted to take into account the main confounders (age, parity, ….)
Main results and the role of chance: ED indication was premature ovarian deficiency, failed ART or both in 68.1, 17.1 and 14.8%. There was no difference in women's age (34.5 ± 8.6 vs. 34.5 ± 4.5, $p = 0.95$), BMI, previous pregnancies and deliveries. Transfer was more frequently the first in ED (69.2 vs. 50.1%, $p < 0.001$); 3 embryos transfers were less frequent ($p < 0.01$). The percentages of frozen embryos transfers (FET) were similar, as was pregnancy outcome (78.6 vs. 78.7% deliveries). PIH was increased in ED, in total and for preeclampsia (see above). In the logistic model, PIH risk increased with ED (OR = 3.84, 95% CI = 1.89–7.77), and women's age OR = 1.08; 1.00–1.16). There was no significant effect of previous pregnancies (neither from current nor other partner), cycle rank, FET. For preeclampsia, the risk was still higher (OR = 4.60; 1.81–11.67).
Limitations, reason for caution: This study had power to detect a doubling in PIH incidence, which was demonstrated, and confirmed by multivariate analysis. However, no conclusion can be drawn for eclampsia itself (only 2 cases). The donor effect could not be analysed. However, in France, donors are anonymous and aged <37 years.
Wider implications of the findings: This study confirms several findings in the literature, on a large sample, with a matched control ICSI group. Egg donation itself is a risk factor for PIH and pre-eclampsia. The origin of this phenomenon remains uncertain, even if immunological hypothesis has currently most favours. The foetus, fully allogenic to its mother may be less tolerated. This higher risk must be acknowledged by clinicians, to inform the couples and to provide a careful pregnancy monitoring.
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by national/international organization(s), Institut Mutualiste Montsouris, Paris; CHRU Lille; CMCO, Strasbourg; Hôpital Jean Verdier, Bondy; Hôpital Tenon, Paris; CHU Amiens; CHI 4 villes, Sèvres; INSERM, Paris.
Trial registration number: N/A.

O-164 Cervical mucus removal prior to embryo transfer in women undergoing IVF/ICSI: a systematic review and meta-analysis of randomised controlled trials

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Study question: Is cervical mucus removal prior to embryo transfer efficient in terms of increasing pregnancy, implantation and live birth rates for women undergoing IVF/ICSI?
Summary answer: A meta-analysis from the eight available moderate to low quality trials provides very little evidence of an overall benefit of cervical mucus removal prior to embryo transfer for women undergoing IVF/ICSI.
What is known already: Cervical mucus has been suggested to interfere with adequate embryo transfer in different ways: blocking the passage of embryos through the tip of the catheter, dragging the embryos back from the releasing site, contaminating the intra-uterine environment with micro-organisms. It has been recommended that cervical mucus should be removed prior to embryo transfer in order to increase the rates of pregnancy and live births.
Study design, size, duration: Two of the present authors independently performed a literature search based on the PICO Method using the standard medical databases in order to find all the randomised controlled trials reporting on the use of cervical mucus removal prior to embrio transfer in women undergoing IVF/ICSI published prior to October 2013.
Participants/materials, setting, methods: We used the software package RevMan 5.2.7, provided by the Cochrane Collaboration, for statistical analysis. The risk ratio (RR) with a 95% confidence interval (CI) was calculated using the Mantel-Haenszel method for binary data variables. We displayed the results from the meta-analysis as forest plots.
Main results and the role of chance: Eight RCTs evaluating 1715 women allocated to experimental group (851 women) or control group (864 women) for reporting the effect of cervical mucus removal prior to embryo transfer were included in the systematic review.

The clinical pregnancy rate was similar (RR, 1.25; 95% CI, 0.96–1.63; $z = 1.63$; $p = 0.10$) in the experimental group compared to the control group. The implantation rate was similar in the experimental group (RR, 1.44; 95% CI, 0.93–2.21; $z = 1.64$; $p = 0.10$) compared to the control group. The live birth rate was similar in the experimental group compared to the control group (RR, 1.59; 95% CI, 0.99–2.55; $z = 1.92$; $p = 0.05$).
Limitations, reason for caution: The trials with relatively small number of women included in this review may not have been sufficient to recognise small differences between groups. The quality of the included trials was moderate to poor because of limitations in the allocation concealment, blinding and possible incomplete reporting.
Wider implications of the findings: Due to problems of clinical diversity, statistical heterogeneity, and risk of bias, additional pragmatic multicenter RCTs are needed to evaluate the possible small benefit of cervical mucus removal prior to embryo transfer.
Study funding/competing interest(s): Funding by hospital/clinic(s), We received no funding for this study.
Trial registration number: N/A.

O-165 Risk of cancer in children born after maternal use of fertility drugs: results from a nationwide Danish population-based cohort study

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Study question: To examine the effect of maternal use of fertility drugs on the risk of cancer in children, while taking into account the effect of the underlying infertility.
Summary answer: We found no associations between maternal use of fertility drugs and risk of overall cancer in childhood and young adulthood. However, maternal use of progesterone increased the risk of acute lymphocytic leukemia and sympathetic nervous system tumors in childhood markedly.
What is known already: A number of studies have examined short-term health consequences, and found that children born after fertility treatment are at an increased risk of adverse perinatal factors. In contrast, few large-scale epidemiological studies have assessed potential long-term health consequences, such as cancer, and the results are inconsistent. Furthermore, even if negative health effects of fertility treatment are found they could be related to the underlying infertility rather than the treatment itself.
Study design, size, duration: A nationwide population-based cohort of 123,322 children born in Denmark between 1964 and 2006, to 68,255 women evaluated for infertility, was established. Of these, 90,888 were born after maternal infertility evaluation and were followed for first cancer occurrence until date of emigration, date of death, or December 2006.
Participants/materials, setting, methods: We used case-cohort techniques to calculate hazard ratios (HRs) of cancer in childhood (0–19 years) and in adulthood (>20 years), associated with maternal use of six groups of fertility drugs [clomiphene, gonadotropins (i.e., human menopausal gonadotropins and follicle-stimulating hormone), gonadotropin releasing hormone analogues, human chorionic gonadotropins, progesterone and other fertility drugs].
Main results and the role of chance: We found no statistical significant associations between maternal use of fertility drugs and risk of overall cancer in childhood and young adulthood. However, concerning specific cancers in childhood our results showed that maternal use of progesterone prior birth increased the risk of acute lymphocytic leukemia [ever use (HR, 4.95; 95% CI, 1.69–14.54) and >3 cycles of use (HR, 9.96; 95% CI, 2.63–7.77)] and sympathetic nervous

system tumors [ever use (HR, 5.79; 95% CI, 1.23–27.24) and >3 cycles of use (HR, 8.51; 95% CI, 1.72–42.19)] markedly.
Limitations, reason for caution: Although we tried to minimize the effects of the underlying infertility, the severity of infertility can have affected our risk estimates as women with more severe fertility problems may receive more treatment. Furthermore, as we conducted multiple statistical tests some results may have been due to chance findings.
Wider implications of the findings: Additional large epidemiological studies are pressingly needed to confirm our finding. Although potential adverse effects of fertility treatment must be put into perspective and balanced against the benefits of childbirth, it may be considered if specialists should consider the increased risk when performing fertility treatment and if couples seeking fertility treatment should be informed.
Study funding/competing interest(s): Funding by national/international organization(s), This work was supported by a grant from Børnecancerfonden (Childhood Cancer Foundation). The funding organization had no role in the design and conduct of the study; the collection, management, analyses, and interpretation of the data; or the preparation or approval of the manuscript.
Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATION SESSION

SESSION 44: UNDERLYING MECHANISMS OF EARLY PREGNANCY PATHOLOGY

Tuesday 1 July 2014 **15:15 - 16:30**

O-166 Inflammatory cytokines in the placenta and maternal circulation of chromosomally abnormal first trimester miscarriages

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Study question: The impact of abnormal placental karyotype on the inflammatory response within the villous tissue and peripheral circulation of women with miscarriage was evaluated.
Summary answer: In miscarriage with abnormal karyotype, there is an exacerbated placental inflammatory response, in contrast to miscarriage of normal karyotype where maternal systemic response is increased.
What is known already: Cytokines at the feto-maternal interface plays an important role in determining the outcome of a particular pregnancy.
Study design, size, duration: Consent was obtained prospectively from 106 women presenting with a missed miscarriage, undergoing ERPC at UCLH over a period of 10 months. Karyotyping of the miscarried chorionic villous samples resulted in 29 cases with normal male karyotype and 25 with abnormal male karyotype.
Participants/materials, setting, methods: Villous and venous blood samples were obtained from women with missed miscarriage. T Concentration of tumour necrosis factor alpha (TNFα), TNF-R1 and TNF-R2, and interleukin (IL)-10 were measured using flowcytometric bead array in fresh villous homogenate, cultured villous extracts, culture medium, maternal whole blood, and plasma.
Main results and the role of chance: Plasma TNFα/IL-10 ratios were significantly ($p < 0.05$) lower in miscarriages with abnormal karyotype. In the abnormal karyotype group, there were significantly higher levels of TNFα ($p < 0.01$), IL-10 ($p < 0.01$), TNF-R1 ($p < 0.001$), and TNF-R2 ($p < 0.001$) in the villous extracts and culture-conditioned medium compared to normal karyotype group.
Limitations, reason for caution: Only miscarried pregnancies with a male karyotype fetus were included, to avoid the risk of maternal contamination during interpretation of the results.
Wider implications of the findings: This study gives a better insight into the mechanisms involved during miscarriage, and may pave the way for predicting the risk of miscarriage and other placental-related adverse pregnancy outcomes, such as pre-eclampsia, intra-uterine growth restriction and preterm delivery.
Study funding/competing interest(s): Funding by hospital/clinic(s), UCLH charities for early pregnancy research.
Trial registration number: Not applicable.

O-167 Standardisation of uterine natural killer (uNK) cell measurements in endometrium of women with recurrent miscarriage

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Study question: Can assessment of density of uterine natural killer (uNK) cells be standardised across different research laboratories?
Summary answer: Analysis of the same fifteen sections in three different centres showed variation which could be attributed to tissue processing, image capture, where in the tissue sample assessment images were taken and what was counted as an immunopositive cell. A standardised protocol has been agreed and is currently being tested.
What is known already: Several studies have demonstrated that uNK cells are increased in mid-luteal phase endometrium in a subset of women with recurrent miscarriage (RM) and recurrent implantation failure (RIF). However, what is classified as "high" density varies between research groups. In addition, whether this increase in uNK cell numbers contributes to the aetiology of the condition or is a marker of widespread endometrial dysfunction is unclear.
Study design, size, duration: Three centres participated in the study. Each centre exchanged 5 × 3 μm formalin fixed paraffin embedded sections of endometrium from women with recurrent miscarriage or after hysterectomy for non-malignant conditions from 5 different cases.
Participants/materials, setting, methods: Sections were immunostained for CD56 in routine pathology or research laboratories. Images were taken of 10, 40× random fields of view that contained the luminal epithelium, total stromal and immunopositive uNK cells were counted manually in Image J and results expressed as % positive uNK cells/stromal cells.
Main results and the role of chance: Each sample was immunostained 5 times across 3 different centres with different methods (Newcastle - 2× research laboratory and 1× pathology laboratory; Sheffield – research laboratory; Warwick – pathology laboratory). The level of variation in % immunopositive uNK cells differed for each of the 15 different samples assessed in the 5 different settings (variance: mean ± SEM 17.4 ± 5.9, range 0.16–71.3; coefficient of variation: mean ± SEM 0.4 ± 0.05, range 0.18–0.88; range: mean ± SEM 7.7 ± 1.7, range 1.0–19.5). The variation could be attributed to length of tissue fixation, potential accumulation of dust in water baths and processing stations, selection of areas for assessment, quality of image analysis, definition of immunopositive cells, and inclusion or exclusion of notable blood vessels.
Limitations, reason for caution: This study confirms the variation in uNK cell measurement in different centres even on the same cases. A standardised protocol has been agreed and is currently being tested to assess its ability to reduce the variation.
Wider implications of the findings: From time to time women with RM or RIF request uNK cell density testing. It is not clear whether the association between "high" uNK cell density and these conditions is causal, nor whether "high" uNK cell density predicts poor outcomes or need for treatment. However, standardisation of testing is urgently needed to foster collaboration and progress in this field.
Study funding/competing interest(s): Funding by University(ies), Newcastle University; Sheffield Hallam University; University of Warwick.
Trial registration number: Not applicable.

O-168 Relation between cytokine gene polymorphisms and concentration of autoantibodies and CD markers in Iranian recurrent miscarriage patients

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Study question: Which cytokine gene polymorphisms (IL-6, IL-1 β, IL-10, IL-13 and IL-17) are related with autoantibodies (anti-nuclear Ab, anti-ds DNA, anti-cardiolopin Ab, anti-thyroid peroxidose, anti-thyroglobulin) and CD markers (CD3, CD4, CD8, CD4/8 CD5, CD19, CD5/19, CD16, CD56, CD16/56) to increase the risk of recurrent miscarriage (RM)?
Summary answer: It seems 2 polymorphisms of IL-10 (–819 and –592) are related with CD5, CD5/19, CD56 and CD16/56 that maybe increased the risk of RM.
What is known already: This is the first study that evaluated the relation of 10 polymorphisms of 6 cytokine genes with concentration of autoantibodies and CD markers in recurrent miscarriage**.**
Study design, size, duration: In this case – control study, 104 women with history of at least three miscarriages were recruited between April 2010 and March 2011 as the case group. Seventy healthy women with a history of two successful deliveries, without any pregnancy complications, were also selected as the control group.
Participants/materials, setting, methods: IL-1β (–31, –511 and +3954), IL-1RN (+9589 and +11100), IL-6 (–174), IL-10 (–819, –591 and –1082), IL-13 (+2044) and IL-17 (–197) polymorphisms were compared in patients and healthy controls using a PCR-RFLP. The data about autoantibody concentrations and CD markers were collected from patient records.
Main results and the role of chance: The data showed significant differences in IL-10 promoter gene polymorphism (–592 and –819) frequencies between RM patients and healthy controls ($p < 0.01$). However no significant differences in the frequencies of interleukin IL-1β, IL-1RN, IL-6, IL-10 (-1082), IL-13 and IL-17 polymorphisms were detected between RM patients and healthy controls. Significant differences in the frequencies of CD5 ($p < 0.001$, $p = 0.035$), CD5/19 ($p < 0.001$), CD56 ($p = 0.002$, $p = 0.016$) and CD16/56 ($p = 0.002$, $p = 0.001$) positive cells were found between RM patients carrying different genotypes of IL-10 –819 (normal, heterozygous and homozygous) and IL-10 –592, respectively, but not other cytokine gene polymorphisms.
Limitations, reason for caution: Because the sample size was relatively small in the study, we could not confirm polymorphisms of IL-10 (–819 and –592) as a predictive marker for RM. Therefore a larger study is needed to warrant this information.
Wider implications of the findings: Among the studied cytokine polymorphisms, that are related to Th1, Th2 and Treg activities, two polymorphisms of IL-10 (–819 and –591) were significantly different between RM patients and the control group. Furthermore, the frequencies of CD5, CD5/19, CD56 and CD16/56 positive cells in homozygous genotype of IL-10 (–819 and –591) were significantly higher than those in heterozygous and normal genotypes of IL-10. In addition, no significant relation between autoantibody concentrations and frequency of other CD markers were found with IL-1β, IL-1RN, IL-6, IL-10 (–1082), IL-13 and IL-17 cytokine polymorphisms. It may be suggested that the two polymorphism of IL-10 might play a role in increasing the autoreactive B cell (CD5 and CD 5/19) or NK cell (CD56, CD16/59) frequencies that can influence on pregnancy outcome. To verify these findings we suggest evaluation of cytokine levels in RM patients and healthy controls.
Study funding/competing interest(s): Funding by University(ies), This work was supported by a grant from Avicenna Research Institute.
Trial registration number: No trial registration number.

O-169 Unusual inverse relationship of DR and IL-2R expression on blood T-lymphocytes and decreased in-vitro responsiveness of T-cells in recurrent miscarriage

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Study question: Is it possible to identify deregulated immunological parameters in patients with recurrent miscarriage (RM) that might serve as targets for therapy?
Summary answer: The inverse relationship of increased DR but decreased IL-2 receptor (CD25) expression on blood CD4+ T-cells, the increased IL-10 plasma levels and the decreased in-vitro response of T-lymphocytes characterize an immunological abnormality that might contribute to the pathogenesis of RM.
What is known already: Immunological aspects are becoming increasingly important in the context of diagnosis and treatment of patients with recurrent miscarriage. Some studies showed that regulatory T-cells as well as natural killer and dendritic cells are involved in the pathophysiology of recurrent miscarriage. However, studies are limited by small study size.
Study design, size, duration: Within this retrospective case control study a total of 220 RM patients underwent a standardized diagnostic procedure including haemostatic, anatomic, genetic and immunologic risk factors between November 2011 and November 2013. Finally 97 idiopathic RM patients with 3 and more miscarriage were analyzed and compared to 31 healthy women aged 20–40 years.
Participants/materials, setting, methods: 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: RM patients had significantly higher proportions of activated CD3+DR+ ($p = 0.007$) and CD4+DR+ ($p = 0.006$) lymphocytes in the blood and a lower in-vitro stimulation of lymphocytes with phytohaemagglutinin (PHA, $p = 0.007$) than healthy controls. Patients with higher proportions of CD3+DR+ blood lymphocytes showed higher absolute counts of CD3+DR+ T-cells ($p < 0.001$), higher relative and absolute numbers of CD4+DR+ and CD8+DR+ T-lymphocytes (all $p < 0.001$), higher proportions of CD8+ T-lymphocytes ($p = 0.005$), lower relative and absolute numbers of CD16+CD56+ NK cells ($p = 0.026$, $p = 0.002$), lower absolute numbers of CD45+ total lymphocytes ($p = 0.009$), CD19+ B-cells ($p = 0.014$), and CD4+CD25+ T-cells ($p = 0.004$) and lower in-vitro lymphocyte proliferation with pokeweed mitogen (PWM, $p < 0.001$), PHA ($p = 0.012$), concanavalin A (ConA, $p = 0.021$), anti-CD3 monoclonal antibody (anti-CD3 mab, $p = 0.002$) and pooled allogeneic stimulator cells ($p = 0.005$).
Limitations, reason for caution: Although a large study population was included immunological testing was performed only once during the menstrual cycle and therefore cycle-specific differences can not be excluded.
Wider implications of the findings: By identifying immunologic disorders in patients with recurrent miscarriage targeted therapies might be developed.
Study funding/competing interest(s): Funding by University(ies), Ruprecht-Karls University.
Trial registration number: A trial registration number was not required due to the retrospective study design.

O-170 An early pregnancy assessment unit improves quality of care and reduces health care costs

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Study question: What is the impact of an early pregnancy assessment unit (EPAU) on clinical care in women with early pregnancy complications?
Summary answer: In a University Hospital setting, the establishment of an EPAU improved the quality of care for women with early pregnancy complications and health care costs were halved for the diagnosis and treatment in women with miscarriage and a history of recurrent miscarriage.
What is known already: An EPAU helps streamlining the service for women with suspected miscarriage or ectopic pregnancy, and for women with history of recurrent miscarriage. Whether EPAUs improve early pregnancy care and save costs is largely unknown as stated in the recently published NICE guideline.
Study design, size, duration: Quality of early pregnancy care was measured after the establishment of an EPAU in June 2008. Measurable derivatives of quality of care were percentage of inpatient admissions, surgical management for miscarriage, number of repeat consultations, karyotypes for recurrent miscarriage, and associated costs.
Participants/materials, setting, methods: The care for women with early pregnancy complications was registered during a three-month-period in 2006 (baseline), 2009 and 2012. Mean total costs per woman were calculated on derivatives mentioned per time period. Actual cost differences with 95% CI were calculated. In addition, a scenario analyses were done using non-parametric bootstrapping.
Main results and the role of chance: In 2006, 14% of women with a miscarriage were admitted, whereas in 2009 and 2012 none. The surgical management rate for miscarriage decreased from 79% (2006) to 6% (2009) and 5%

(2012). Repeat consultations by phone for women with recurrent miscarriage decreased from two in 2006 to less than one (2009 and 2012). Karyotyping in these women decreased from 100% in 2006 to 17% in 2009 and 33% in 2012. The mean total costs per woman treated in 2006 were €535 (95% CI 412–655), €302 (95% CI 226–377) (2009) and €454 (95% CI 370–537) (2012). If in women (2009, 2012) diagnosis and treatment would have been done based on the standards in 2006 (baseline), then calculated costs are doubled.
Limitations, reason for caution: A disadvantage of this study is the relatively low number of women and partially retrospective data collection.
Wider implications of the findings: An EPAU has a positive impact on the quality of care provided to women with early pregnancy complications. It reduces health care costs by 50%. This study may motivate other clinicians in OB/GYN to initiate the establishment of an EPAU in their hospital.
Study funding/competing interest(s): Funding by national/international organization(s), Funding by commercial/corporate company(ies). This study was supported by a grant of the Netherlands Organisation for Health Research and Development (ZonMw Clinical fellow grant 907-00-154 and a fund of the AGIS Health Insurance Company.
Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATION SESSION

SESSION 45: EPIGENETICS IN GAMETE COMPETENCE AND IMPLANTATION

Tuesday 1 July 2014 **15:15 - 16:30**

O-171 Imprinting landscape of human placenta as discovered by whole transcriptome RNA-sequencing and exome variant data analysis

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Study question: The purpose of our study was to get more information about the imprinted genes in human placenta by applying whole transcriptome RNA-sequencing and exome variant data analysis.
Summary answer: We combined whole transcriptome sequencing (WT RNA-seq) and exome SNP microarray genotyping to map allelic imbalances characteristic to imprinting and to detect the parental specificity for the expressed alleles. We generated a list of 7 novel imprinted genes and confirmed imprinting of 5 genes in healthy full term placenta.
What is known already: The prenatal and postnatal health is determined by genetic factors with epigenetic regulation. Genomic imprinting is an epigenetic phenomenon common to placenta and refers to monoallelic expression of a gene in parental-specific manner. So far the conclusive list of placental imprinted genes is missing. Although being very promising, the use of RNA-seq to characterize imprinting has been limited by the inability to determine the allelic origin of expressed genes from the transcriptome-wide data.
Study design, size, duration: WT RNA-seq was employed to gather information for the placental transcriptome, while exome SNP microarray genotyping provided the tool to estimate the allelic abundance of parental transcripts. Ten family trios (mother, father and newborn) with healthy spontaneous single term pregnancy were recruited.
Participants/materials, setting, methods: Parental peripheral and umbilical cord blood, and placental tissue samples were collected to extract DNA and RNA, respectively. The combination of RNA-seq with exome SNP-microarray analysis enabled to detect deviations in expression of maternal and paternal alleles, whereby the genes were reported as having allele-specific expression and being potentially imprinted.
Main results and the role of chance: Our data revealed the list of 12 imprinted genes, out of which 7 (*ABP1*, *BCLAF1*, *IFI30*, *LGALS8*, *LGALS14*, *PAPPA2*, and *SPTLC3*) were newly detected as imprinted and 5 (AIM1, PEG10, RHOBTB3, ZFAT, and ZFAT-AS1) were confirmed to be imprinted. Five genes showed preferential maternal expression and seven genes were expressed paternally, demonstrating similar proportion of maternally and paternally expressed genes. The main function of the proteins encoded by these genes is associated with tissue development, metabolic regulation or control of immune mechanisms in promoting fetal immune tolerance and providing protection against infections to safeguard the fetal and maternal wellbeing.
Limitations, reason for caution: The discovery of the novel imprinted genes was limited by the restricted list of the genes included into the SNP-microarrays. Thus in future studies, whole exome sequencing should be used instead to obtain the entire list of imprinted genes.
Wider implications of the findings: Imprinted genes are highly expressed in the placenta and contribute to the fetal and maternal wellbeing. Therefore it is important to identify all of the genes regulated by genomic imprinting during embryonal and fetal development in order to understand the risks the baby might meet during pregnancy and how these developmental stages might impact a person's health later in life. Our study extended substantially the list of genes being imprinted in placental tissue.
Study funding/competing interest(s): Funding by University(ies), This study was supported by grants from Centre of Excellence in Genomics (grant EXCEGEN), Estonian Ministry of Education and Research (grants SF0180044s09, SF0180008s12 and SF0180035s08), Enterprise Estonia (grant EU30020), EU-FP7 Eurostars program (grant EU41564), EU-FP7 IAPP project (grant EU324509), Estonian Centre of Excellence in Computer Science (grant EXCS) and by the Tiger University Program of the Information Technology Foundation for Education.
Trial registration number: None.

O-172 Global transcriptome analysis of oocytes and granulosa cells from all stages of human folliculogenesis via next generation sequencing

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Study question: From the primordial- to the pre-ovulatory follicle; which genes are activated and transcribed in the oocytes, and granulosa cells, respectively, throughout human folliculogenesis?
Summary answer: The expression profile throughout the different stages of human folliculogenesis is dynamic and cell specific. As an example, several genes encoding isoforms of the phosphatidylinositol 3-kinase (PI3K) signalling pathway show significant variance in expression levels when comparing different follicle stages.
What is known already: From previously conducted transcriptome studies, we know the transcriptional activity of the oocyte differs when comparing primordial oocytes with the later antral stages of folliculogenesis. Follicle loss, maintenance and growth are strictly controlled processes governed by complex molecular interactions including the phosphatidylinositol 3-kinase and protein kinase (Akt) signalling pathway, where PI3K promotes Akt phosphorylation causing follicle survival and activation of growth. These actions are suppressed by actions of phosphatase and tensin homolog (PTEN).
Study design, size, duration: The mRNA of oocytes, and granulosa cells, respectively, were extracted from all stages of human folliculogenesis via Laser Capture Microdissection (LCM), and subsequently sequenced using Next Generation Sequencing (NGS). Expression patterns were compared the various stages between to map transcriptional dynamics.
Participants/materials, setting, methods: Ovarian tissue was procured from the Danish Ovarian Cryopreservation Programme with permission from the three donating women. Oocytes and granulosa cells were identified based on morphology, and isolated using LCM. mRNA was extracted, purified, and submitted to NGS. Expression levels of selected genes were verified using qPCR and immunohistochemistry.

Main results and the role of chance: During the cause of human folliculogenesis, we observe, that several genes encoding isoforms of the PI3K complex are expressed both cell specific, i.e., when comparing oocytes with granulosa cells, and stage specific. Moreover, the expression profiles of the Akt 1–3 isoforms add another level of regulations to the PI3K signalling pathway actions, by the highly stage-specific expression of this gene. PTEN appears to be present throughout the evolving stages, suggesting that PTEN function is essential throughout folliculogenesis.

Knowing the gene expression of specific genes linked to follicle activation and growth, we can elucidate the molecular requirements for these processes.

The analyses presently being undertaken also cover areas such as: FSH/LH/androgen receptor expression levels, and expression levels of genes involved in steroid synthesis.

Limitations, reason for caution: Inter-individual variance in transcriptomes was limited using mean expression levels from three women. This is a small population base. The time connected with preparing and morphologically identifying the different stages in the spares human ovarian tissue set limitations in the number of patients included.

Wider implications of the findings: To our knowledge, the study presents the most comprehensive dataset on the molecular pathways at play throughout human folliculogenesis. The findings include novel genes not previously associated with human folliculogenesis in dynamic expression patterns identified for the first time. Our data suggests that there is a stage-specific function of several genes in human oocytes that controls follicular activation. These expression dynamics are potentially interesting for treatment purposes via Assisted Reproductive Techniques (ART).

Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), Aarhus University, the Aarhus University Resarch Fundation, Fonden for Lægevidenskabens Fremme, Kong Christian den Tiendes Fond.

Trial registration number: Basic Science.

O-173 DNA methylation analysis of *in vitro* matured human oocytes retrieved from small antral follicles

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Study question: ART coincides with genome-wide epigenetic reprogramming during late oocyte and early embryo development. Due to the increased vulnerability of the oocyte to adverse environmental factors during *in vitro* culture and maturation, it is important to evaluate whether human *in vitro* maturation (IVM) interferes with epigenetic reprogramming of maternal methylation patterns.

Summary answer: The human IVM technique by itself does not significantly alter the epigenetic status of oocytes.

What is known already: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. When undergoing standard assisted reproductive technologies (ARTs) for infertility treatment, PCOS patients have a high risk of developing ovarian hyperstimulation syndrome (OHSS), which is due to an increased response to ovarian stimulation. *In vitro* culture and maturation of human oocytes can be used to avoid conventional hormonal stimulation and OHSS.

Study design, size, duration: Immature oocyte-cumulus complexes were retrieved from 2 to 9 mm follicles without an ovulatory dose of hCG and matured *in vitro*. To investigate the influence of IVM on the epigenetic status of human oocytes, we compared the DNA methylation patterns of 71 *in vitro* and 38 *in vivo* matured oocytes from PCOS patients.

Participants/materials, setting, methods: Altogether, we analysed four imprinted genes (maternally methylated *LIT1*, *PEG3*, *SNRPN* and paternally methylated *GTL2*) as well as three developmentally important non-imprinted genes (*DNMT3Lo*, *OCT4* and *NANOG*), using limiting dilution bisulfite pyrosequencing. This technique allows one to determine the methylation patterns of individual alleles of several genes in single cells.

Main results and the role of chance: Both *in vitro* and *in vivo* matured oocytes showed only few abnormal alleles, consistent with epimutations. Although the number of abnormally (de)methylated imprinted alleles was slightly higher in the *in vitro* group, this difference was not statistically significant. The observed abnormalities may be due to the fact that the *in vitro* cultured oocytes were not yet completely matured. We also found slightly increased numbers of abnormal alleles in immature, compared to mature *in vivo* grown oocytes.

Limitations, reason for caution: Single cell methylation analysis was restricted to a limited number of well selected candidate genes. Genome-wide methylation analysis of single human oocytes is currently not possible.

Wider implications of the findings: Our study shows for the first time that optimized human IVM procedures have no significant effects on the establishment of maternal DNA methylation patterns. Follicle culture and oocyte IVM are emerging ARTs with potentially important future applications in the fertility clinic.

Study funding/competing interest(s): Funding by University(ies), Universitaet Wuerzburg and Vrije Universiteit Brussel.

Trial registration number: Does not apply.

O-174 Conventional IVF versus ICSI; differences in placental gene expression

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Study question: Are there differences in placental gene expression when women who have conceived naturally and women who have conceived through different assisted reproductive techniques (ART) are compared?

Summary answer: Placental gene expression differs when women who have conceived naturally and women who have conceived through assisted reproduction are compared and there seems to be a major difference related to the techniques used.

What is known already: Infants conceived by ART are at an increased risk of birth defects and congenital malformations and there are also increased risks for a number of obstetric and neonatal complications related to ART.

Study design, size, duration: This is a case control study. Seven women were included in each group for the initial microarray analysis. Women who conceived through IVF were compared with women who conceived naturally and were matched for age, BMI and parity. For validation, the study groups were extended to 22 women in each group.

Participants/materials, setting, methods: Total RNA was prepared from frozen placenta collected at partus. Whole genome expression analysis was performed with GeneChip® Human Gene 1.0 ST Array. Hierarchical clustering was investigated and to determine significantly deregulated genes and pathways, ingenuity pathway analysis (IPA) was used. For validation quantitative real-time PCR (qRT-PCR) was performed.

Main results and the role of chance: There are differences in global placental gene expression when women conceiving naturally and women conceiving through ART are compared. Placental gene expression differs to a higher extent in women treated with ICSI compared with conventional IVF or women conceiving naturally. The gene expression profile differed in 387 genes between the ART group and naturally conceiving women. Cluster and pathway analysis indicated that mechanisms vital for cellular function are of main relevance. In the validation a difference in expression of Noggin was confirmed in placentas from ART pregnancies as compared to naturally conceiving women ($p < 0.05$). In a subgroup analysis, the main difference in expression of Noggin was found when placentas from women treated with ICSI were compared with controls ($p = 0.001$).

Limitations, reason for caution: A limitation with the study is the fairly low number of women included, meaning that the results, even if they are significant, need to be analyzed and used with care and that they also need to be confirmed in larger studies in the future.

Wider implications of the findings: The findings are of main relevance to, and have possible implications for, how and when ICSI should be used in assisted reproduction. Based on the results, ICSI should be recommended preferably on indication and not only as a technique available to every infertile couple undergoing IVF treatment.

Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by national/international organization(s), Funding was granted by Stiftelsen

Olle Engkvist Byggmästare, Stockholm; the Family Planning Foundation, Uppsala; and the Swedish Medical Society, Sweden.
Trial registration number: Not applicable.

O-175 Variable expression of the major POI/POF candidate gene FMR1 in human granulosa cells under epigenetic control: genetic marker for human ovarian reserve?

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Study question: Identification of epigenetic control elements within the *FMR1 (Fragile X Mental Retardation 1)* core promoter region which can be used to diagnose the age-dependent ovarian reserve in females entering the infertility clinic.
Summary answer: FMR1-protein (FMRP) is expressed in granulosa cells during folliculogenesis. Distinct blocks of CpG-methylation in one allele and complete CpG-demethylation in the second allele of *FMR1* core promoter could be distinguished in COV434 granulosa cells. This suggests a distinct epigenetic control on both *FMR1* gene alleles when expressed in granulosa cells.
What is known already: The *FMR1* gene contains within its core promoter a mutational hotspot due to tandem repetitive CGG-triplets (n = 26–34 in general population). Expansion or reduction of this CGG-triplet block seems to influence follicular recruitment and ovarian reserve most likely due to reduced expression of its protein. Triplet block extension over 53–56 (Fragile-X premutation) is even associated with POI/POF (premature ovarian insufficiency/failure) in 20–30%. It can therefore be assumed that *FMR1* is a major regulator of ovarian reserve.
Study design, size, duration: Establishment of gene-specific bisulfite assays to score the allele specific CpG methylation pattern in *FMR1* core promoter region including CGG-triplet block. Functional analysis of variable CpG methylation densities to *FMR1* gene expression in human granulosa cells.
Participants/materials, setting, methods: Collection of follicular tissue samples for extraction of granulosa and cumulus cells from women experiencing different ovarian response after controlled ovarian stimulation for IVF/ICSI. Human granulosa cell line COV434 as model system to investigate functional *FMR1*/FMRP expression after specific epigenetic modification within the *FMR1* core promoter region.
Main results and the role of chance: The *FMR1* core promoter region seems to contain distinct epigenetic control elements far beyond the tandem repetitive CGG-triplet block. In the COV434 model system for human granulosa cells we identified different CGG triplet numbers in each gene allele. Most interesting, while the larger allele (n = 42) was completely CpG-demethylated, the shorter allele (n = 23) displayed distinct blocks of CpG-methylation and demethylation upstream and downstream of the CGG-triplet block. This suggests that the *FMR1* gene in granulosa cells escapes the X-chromosomal inactivation process normally expressed in somatic cells. Both alleles of this gene on the X-chromosome are thus functional during folliculogenesis. Analysis of specific *FMR1*/FMRP-expression profiles in granulosa cells of women with variable ovarian stimulation response will thus help to elucidate the function of this gene during folliculogenesis.
Limitations, reason for caution: Results got with the COV 434 cell line need to be verified with fresh cultures of granulosa cells extracted from mature follicles of a large number of women with different ovarian response in order to reach statistical significance.
Wider implications of the findings: Molecular expression analysis of *FMR1* transcripts and protein in granulosa cells will eventually also help to understand the molecular mechanism behind the risk of women for suffering from ovarian insufficiency/failure (POI/POF) when their FMR1 gene alleles contain a CGG-triplet premutation (n > 53).
Study funding/competing interest(s): Funding by University(ies), Short time grant for female medical scientists - Medical Faculty of Heidelberg, Germany.
Trial registration number: Vote S-602/2013, Ethical Review Committee - Medical Faculty of Heidelberg, Germany.

SELECTED ORAL COMMUNICATION SESSION

SESSION 46: CLINICAL ENDOCRINOLOGY (1)

Tuesday 1 July 2014 **17:00 - 18:00**

O-176 Polycystic ovary syndrome and its symptoms increase the risk of pregnancy complications: population-based cohort study

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[4]*University of Helsinki and Helsinki University Central Hospital, Department of Obstetrics and Gynaecology, Helsinki, Finland*

Study question: Do self-reported PCOS or PCOS-related symptoms (presence of both oligo-/amenorrhea and excessive hair growth) increase the risks of gestational diabetes (GDM), pre-eclampsia (PE) or pregnancy induced hypertension (PIH) during reproductive life independently from body mass index (BMI)?
Summary answer: PCOS-related symptoms were risk factors for PE and PIH, but not for GDM. Self-reported PCOS diagnosis was a risk factor for GDM and PE, but not for PIH, and after adjustment for BMI and change in BMI between age 31 and 46 the significances decreased for GDM and disappeared for PE.
What is known already: The risk for pregnancy complications such as GDM, PE and PIH has been shown to be increased in women with PCOS. In most population-based studies, however, adequate adjustment for BMI is missing, and the respective roles of obesity and PCOS *per se* are still under debate.
Study design, size, duration: The study population comprised all women in the population-based Northern Finland Birth Cohort 1966 (NFBC 1986, n = 5889) that includes all expected births from year 1966 in the two northernmost provinces of Finland. Collection of the database (postal questionnaires and clinical examinations) was performed at age 31 and 46.
Participants/materials, setting, methods: The questionnaires at age 31 (N = 5608, 81% answered) included questions on PCOS-related symptoms (both oligo/amenorrhea and excessive hair growth N = 153, no symptoms N = 3340), and PCOS diagnosis and lifelong obstetric outcomes at age 46 (n = 5123, 70% answered, PCOS N = 154, no symptoms N = 2933). 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: PCOS-related symptoms at age 31 were associated with an increased risk of PE [OR 2.67, 95% confidence interval (CI) 1.47–4.84] and PIH (OR 2.02, 95% CI 1.16–3.51), but the risk for GDM was not significantly increased. After adjusting for BMI at age 31 and change in BMI between age 31 and 46, the significances decreased for PE (OR 1.94, 95% CI 1.03–3.66) and disappeared for PIH (OR 1.35, 95% CI 0.74–2.48).

Self-reported PCOS diagnosis was a risk factor for GDM (OR 1.94, 95% CI 1.24–3.04) and PE (OR 1.80, 95% CI 1.14–2.83). After adjusting for BMI at age 31 and change in BMI the significances decreased for GDM (OR 1.79, 95% CI 1.13–2.82) and disappeared for PE (OR 1.56, 95% CI 0.98–2.50). Smoking was not a confounding factor in the analyses.
Limitations, reason for caution: The diagnosis of PCOS, the obstetric outcomes and the BMI at age 46 were based on self-reporting, which may have led to information bias. Ovarian ultrasonography was not available to aid the diagnosis of PCOS.
Wider implications of the findings: PCOS-related symptoms are risk factors for PE and PIH, and PCOS diagnosis is a risk factor for PE and GDM, but obesity still seems to play a crucial role as regards these pregnancy complications. Our results highlight the importance of early diagnosis of PCOS and intervention and guidance of women with PCOS or PCOS-related symptoms

in preventing the occurrence of these pregnancy complications, which are the most common causes of preterm birth and postnatal morbidity.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Funding by national/international organization(s), Finnish Medical Society Duodecim, the North Ostrobothnia Regional Fund, the Academy of Finland, Biocenter, Sigrid Juselius Foundation, the European Commission and Medical Research Council, the National Institute for Health Research.
Trial registration number: None.

O-177 Hyperhomocysteinemia rather than hyperinsulinemia - the major determinant for long-term thrombophilic manifestations of polycystic ovary syndrome

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Study question: Does hyperhomocysteinemia (HHcy) play a dual role, thereby, resulting in pregnancy wastage during early reproductive life along with metabolic dysregulation in later years, in polycystic ovarian syndrome (PCOS) patients?
Summary answer: HHcy impairs reproductive outcome, and furthermore causes a metabolic insult during later stages of life in PCOS subjects.
What is known already: HHcy, a close associate of metabolic syndrome and an increasingly frequent finding among PCOS women in India, plays a significant role in recurrent pregnancy loss (RPL).Type 2 diabetes and cardiovascular disease are some of the most important emerging issues regarding long-term sequel in PCOS. Moreover, insulin inhibits transsulfuration thereby resulting in an increment of serum homocysteine. However, current reports have not documented the proper influence of the molecule in broader aspects of life.
Study design, size, duration: Five-year (January 2008–December 2013) prospective cohort analysis of lab parameters and reproductive outcome in 2355 subjects was analyzed.
Participants/materials, setting, methods: One thousand five hundred and thirty-nine women selected as study cohort were divided into hyperhomocysteinemic, insulin resistance (IR) and obese groups based on individual cut-offs and were analyzed for outcome parameters. Communication was maintained with the patients, even after successful delivery, to assess their long-term sequel.
Main results and the role of chance: Rates of miscarriage (52.23 vs. 24.84%) in PCOS were significantly higher ($p < 0.006$) in patients diagnosed with HHcy ($n = 469$) when compared to the non-PCOS ($n = 157$) cohort. After evaluation of HHcy, IR and obesity between both the groups, repressor operating characteristic curve – area under curve (ROC-AUC) values suggest increased tendency of HHcy-mediated RPL when compared to other factors (HHcy: 0.778; IR: 0.601; BMI: 0.548). A probabilistic causal model evaluated the time-series data points before, during and after pregnancy by dynamic Bayesian network to assess the chances of metabolic disturbances by HHcy which revealed a possibility of 32.24% ($n = 79$) of PCOS cohort developing hypertension and 26.94% ($n = 66$) onset of diabetes following 2 years of pregnancy.
Limitations, reason for caution: Prospective study; Maternal health information was incomplete in the years following the delivery of the child. Methylene tetra hydro folate reductase gene polymorphisms were not elucidated in either the mother or the child.
Wider implications of the findings: HHcy controls insulin axis, thereby acting as a fulcrum to divide the long-term sequences of the syndrome into either thrombotic (RPL) or metabolic avenues. HHcy poses as a possible threat towards the development of a "thrombophilic syndrome" with its possible culmination in coronary artery disease later in life. Until more detailed data are available, the observation provided herein may be merely viewed as a prelude to what the future holds.
Study funding/competing interest(s): Funding by hospital/clinic(s), This study was supported by Institute of Reproductive Medicine. No competing interests have been declared.
Trial registration number: Not applicable.

O-178 Testosterone levels and FAI in adolescent girls are related to the rate of childhood growth but not to intrauterine growth

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Study question: Does birth weight and length as a markers of intrauterine growth and child hood rate of growth in height and weight influence testosterone levels and free androgen index in adolescent girls?
Summary answer: Intrauterine growth has no impact on testosterone levels or FAI in adolescence. However, the rate of growth at different stages of childhood impacts, subject to regional variations, on both testosterone and FAI.
What is known already: Life course studies have shown that intrauterine growth and early childhood growth influence health in adulthood. Several studies have shown a relationship between childhood growth and obesity and risk of PCOS, onset of puberty and cancer risk. However most of the studies have been in small cohorts.
Study design, size, duration: This is a retrospective cohort epidemiological study of two European birth cohorts, Avon Longitudinal Study of Parents and Children (ALSPAC) and North Finland Birth Cohort_86 (FBC-86). 1526 girls in NFBC-86 and 742 in ALSPAC were included in the study.
Participants/materials, setting, methods: The testosterone levels and free androgen index of adolescent girls (16 years of age) of ALSPAC and NFBC-86 were correlated with birth weight and weight and height gain at different chronological points in their childhood. Adjustments were made for age of menarche, birth order and maternal smoking and social class.
Main results and the role of chance: No significant correlation between birth weight or length and testosterone or FAI in either cohort. In ALSPAC: Testosterone correlated significantly with weight gain between 1 and 3 years of age ($p = 0.005$) and with height gain between 3 and 7 ($p < 0.02$) and 7–10 ($p = 0.044$) years. However after adjusting for previous weight gain the height gain at 7–10 became insignificant. FAI correlated significantly with weight gain between ages 1–3 ($p < 0.0001$), 3–7 ($p = < 0.0001$) and 7–10 ($p < 0.0001$) years (adjusted for previous growth). FAI also correlated significantly with height gain between ages 1–3 ($p < 0.0001$), 3–7 ($p < 0.0001$) and 7–10 ($p = 0.016$). In NFBC-86: there was no significant correlation between weight or height gain and testosterone however, there was a significant correlation between weight gain between ages 3–7 years ($p = 0.005$) and height gain between 1 and 3 years ($p = 0.015$).
Limitations, reason for caution: The numbers included in the analysis varied according to the availability of early growth data which were obtained from the health visitors records in childhood.
Wider implications of the findings: Data from both cohorts indicate that weight and height gain between 1 and 3 years impact on FAI and not testosterone suggesting that growth in that period can influence sex hormone binding globulin in adolescence. This may have implications for pubertal development and possibly PCOS. There was a variation in the relationship between growth rates at different stages of childhood and testosterone and FAI in the two cohorts may be due to regional variations.
Study funding/competing interest(s): Funding by national/international organization(s), Wellcome Trust (089549Z/09/Z).
Trial registration number: None.

O-179 The effectiveness of a structured lifestyle program in overweight and obese subfertile women. Preliminary data from a randomised controlled trial (LIFEstyle study)

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[11]Univerity Medical Center Utrecht Utrecht University, Obstetrics and Gynaecology, Utrecht, The Netherlands
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Study question: Is a structured lifestyle program for overweight and obese subfertile women aiming at weight reduction prior to fertility treatment more effective (in terms of ongoing pregnancy rates) as compared to conventional fertility treatment?
Summary answer: Although lifestyle intervention in overweight and obese subfertile women did not result in higher overall ongoing pregnancy rates, lifestyle intervention reduced the need for fertility treatment as more natural conceptions occurred.
What is known already: In Europe, 30% of women of reproductive age are overweight or obese and, at present, there is no evidence-based agreement on weight reduction prior to fertility treatment for overweight and obese subfertile women. Data from observational and small intervention studies suggest that modest weight loss (5–10%) increases the chance of conception, but the issue has not yet been addressed in large randomised controlled trials (RCT).
Study design, size, duration: Between 2009 and 2012 a multicenter RCT was performed in 23 hospitals in the Netherlands. Subfertile women (18–39 years) with BMI ≥29 kg/m^2 were allocated to a 6-months structured lifestyle intervention program followed by fertility treatment (intervention group) vs. fertility treatment only (control group). Follow-up period was 24 months.
Participants/materials, setting, methods: The intervention program consisted of a diet, physical activity and behavioural modification. Ongoing pregnancy rates of women for whom follow-up is complete, are reported. Data on primary outcome (vaginal birth of a healthy singleton ≥37 weeks of gestation) are not available yet. Analyses were by intention-to-treat.
Main results and the role of chance: We randomly allocated 582 women, of whom the data of 447 women (77%) were available for the current analysis (intervention group: $N = 223$, control group: $N = 224$). Median BMI at randomisation was 36.0 kg/m^2 (IQR 33.5–38.4) and 36.0 kg/m^2 (IQR 33.4–38.1) respectively. Adherence to the lifestyle intervention was 69.2% of all consultations. Median weight change was −4 kg (IQR −7 to −1) in the intervention group vs. −1 kg (IQR −3 to +1) in the control group ($p < 0.001$). Ongoing pregnancy rates were 51.1% ($N = 114$) in the intervention group vs. 58.9% ($N = 132$) in the control group (RR 0.87, 95% CI 0.73–1.03). In the intervention group 44.7% of the ongoing pregnancies were achieved spontaneously vs. 28.0% in the control group (RR 1.60, 95% CI 1.13–2.24).
Limitations, reason for caution: These preliminary results are based on ongoing pregnancy rates of 77% of the included women. All data of participants on the primary endpoint, i.e., vaginal birth of a healthy singleton after ≥37 weeks of gestation, will be available in June 2014 and presented at the meeting.

[M1]Dit is het stukje van BW, hij wil/verwacht dus kennelijk dat wij wel data over het primaire eindpunt kunnen presenteren. Lijkt mi eigenlijk ook wel haalbaar (misschien niet compleet, maar een groot deel wel).

Wider implications of the findings: These preliminary data suggest that lifestyle intervention in overweight and obese subfertile women preceding fertility treatment is effective in increasing spontaneous pregnancy chances and hence reduce the number of fertility treatment cycles needed. If this strategy proves to be effective in terms of maternal and neonatal outcome lifestyle intervention should be implemented in regular fertility care for overweight and obese subfertile women.
Study funding/competing interest(s): Funding by national/international organization(s), ZonMW, the Dutch Organization for Health Research and Development.
Trial registration number: NTR 1530.

SELECTED ORAL COMMUNICATION SESSION

SESSION 47: BASIC PHYSIOLOGY IN EMBRYOLOGY

Tuesday 1 July 2014 **17:00 - 18:00**

O-180 A modified human growth differentiation factor 9 is potently active in a porcine ivm model with inherent low oocyte developmental competence

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Study question: To investigate the amino acid residues of human growth differentiation factor 9 (GDF9) which when modified can activate the latent form of human GDF9.
Summary answer: Our results suggest that both Gly^{391} and Pro^{412} of human GDF9 are important residues for the stimulation of oocyte development competence in a porcine IVM low competence model.
What is known already: GDF9 belongs to the TGFβ superfamily and plays a critical role in the regulation of granulosa and cumulus functions, folliculogenesis and oocyte developmental competence. Recombinant human GDF9 is secreted from mammalian host cells in a latent form with the pro-region noncovalently attached to the mature region.
Study design, size, duration: We designed and produced four forms of mutant human GDF9 (M1–M4) pro-mature proteins. These proteins were tested for their effects on mouse murine granulosa cell proliferation, porcine cumulus cell expansion and expression of expansion related genes, as well as porcine oocyte developmental competence, in comparison with human wild type GDF9.
Participants/materials, setting, methods: Proteins were produced with a poly-His tag and purified by affinity chromatography. The mice granulosa cell bioassay was performed by a ^{3}H-thymidine incorporation assay at concentrations of 12.5–200 ng/ml. Cumulus expansion, expression of related genes and oocyte developmental competence were examined in a porcine model utilizing 200 ng/ml GDF9 proteins.
Main results and the role of chance: All four modified human GDF9 forms could stimulate murine granulosa cell proliferation whereas wild type human GDF9, being latent, lacked bioactivity. Of the modified proteins, M2–M4 stimulated cumulus expansion and the expression of the *TNF*αIP6 gene in porcine cumulus cells after a 22 h period of *in vitro* maturation (IVM). GDF9-M3 in particular, when added to IVM, notably improved the subsequent blastocyst rate on day 7 (17.7 vs. 41.1%, $p < 0.05$).
Limitations, reason for caution: We tested the bioactivity of GDF9 M1–M4 in mouse and pig models, but not human.
Wider implications of the findings: This is the first time that a purified form of human GDF9 has been shown to stimulate oocyte developmental competence and subsequent embryogenesis. GDF9-M3 is a promising GDF9 protein for the development of human IVM.
Study funding/competing interest(s): Funding by national/international organization(s), Funding by commercial/corporate company(ies), This study was supported by research grants and fellowships from the National Health and Medical Research Council of Australia(1017484 and APP1023210), and by grants from Cook Medical.

The University of Adelaide owns a patent family on the application of BMP15/GDF9 in oocyte *in vitro* maturation. Gilchrist RB is an inventor.
Trial registration number: None.

O-181 Effect of dehydroepiandrosterone on ovine *in vitro* oocyte maturation

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[2]Royal Derby Hospital, Reproductive Medicine and Fertility Unit, Derby, United Kingdom

Study question: What are the effects of exposure of cumulus-oocyte complex (COC), from the antral follicle, to dehydroepiandrosterone (DHEA) and/or oestradiol in ovine oocyte maturation media *in vitro*?
Summary answer: Neither DHEA nor oestradiol (E2) had an effect on nuclear maturation. Cumulus expansion is significantly lower in COCs exposed to E2 (DHEA−E2+; DHEA+E2+) and even less if exposed to a 100 times higher concentration of DHEA (200 ng/ml). DHEA may diminish Hyaluronan synthase expression but significantly stimulates expression of androgen receptor.
What is known already: DHEA has been used worldwide to improve ovarian response after stimulation in women with diminished ovarian reserve undergoing IVF treatment. Recent evidence suggests that it stimulates primordial follicles initiation and preantral/early antral follicular growth in the gonadotrophin-responsive stage via granulosa cell proliferation and modulation of local growth factors such as AMH. Nonetheless, little is known regarding benefit/adverse effect of DHEA and hyperandrogenism during the final maturation step of oocyte development.
Study design, size, duration: Two ovine *in vitro* experiments were conducted in parallel; comparing effects of: (i) presence/absence of DHEA and/or oestradiol (DHEA+E2+; DHEA+E2−; DHEA−E2+; None), and (ii) various doses of DHEA (0; 0.2; 2; 20; 200 ng/ml with oestradiol) in oocyte maturation media. Each experiment was conducted in at least 6 replicates.
Participants/materials, setting, methods: Cumulus-oocyte complexes aspirated from antral follicles of abattoir derived ovaries were matured for 24 h in TCM199-based media containing various concentrations of DHEA and E2, as mention above. The dependent outcomes were nuclear maturation rate, cumulus expansion, and mRNA expression (androgen, FSH and LH receptors, BMP receptor and Hyaluronan synthase-2).
Main results and the role of chance: There was no difference in nuclear maturation rates among all groups (73–85%; $p = 0.28$).
DHEA/E2 experiment: Means cumulus expansion was significantly lower in media containing E2 (DHEA+E+ 52.68 ± 3.38%; DHEA−E+ 59.05 ± 4.09%) compared to control (DHEA+E2− 80.41 ± 5.71%; DHEA−E2− 96.38 ± 6.20%, $p < 0.05$). No significant difference in cumulus gene expression was observed.

Varying DHEA concentrations

Cumulus expansion was significantly reduced in media containing DHEA 200 ng/ml (37.92 ± 3.09%) compared to the others (60.57 + 4.06%; $p = 0.001$). Using granulosa cells as a calibrator, COCs from any DHEA concentrations have androgen receptor expression relative quotients more than 1 and higher than control (DHEA groups average 1.73 ± 0.43 vs. control 0.82 ± 0.14; $p = 0.017$). There was a trend of reducing HAS-2 expression with increasing DHEA concentration but not statistically significance. Neither FSH nor BMP receptor expression was difference.
Limitations, reason for caution: Differences between *in vitro* and *in vivo* oocyte maturation is the major limitation of this study. In addition, cumulus gene expression may not result in gain or loss of the cell function. Further studies are needed to elucidate whether the findings have a negative impact on embryo quality and implantation.
Wider implications of the findings: The results indicate that DHEA does not affect oocyte nuclear maturation when supplied *in vitro*. However, higher dose of DHEA (with presence of E2) have a detrimental effect upon cumulus expansion potentially mediated through reduction of Hyarolunan synthase type 2 production. DHEA at least acts through androgen receptor. Because degree of cumulus expansion is associated with oocyte quality, this warrants concern over exaggerate use of DHEA supplementation especially during the final stage of oocyte maturation.
Study funding/competing interest(s): Funding by University(ies), The University of Nottingham.
Trial registration number: None.

O-182 The role of WNT signaling in human preimplantation development

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Study question: This study was aimed to describe new molecular players in early human embryogenesis. In particular, we investigated the possibility of WNT pathway to regulate human preimplantation development via beta-catenin. We wanted to define specific players of this signaling cascade and some of the gene targets critical for normal human embryogenesis.
Summary answer: The WNT/beta-catenin pathway is necessary for proper trophectoderm (TE) specifications rather than for inner cell mass (ICM) formation in the human preimplantation embryo.
What is known already: WNTs constitute to a gene family encoding secreted signaling proteins. WNTs participate in oncogenesis and developmental processes balancing between pluripotency and differentiation. Based on the downstream players, WNT pathway can be beta-catenin-dependent or -independent. In the classical view, WNT signals stabilize beta-catenin and cause its translocation to the nucleus followed by specific target gene activation. Recently, more attention has been paid to membrane-associated beta-catenin contributing to intracellular contacts and its regulation by WNT cascade.
Study design, size, duration: Day 3 8-cell stage human embryos were thawed after cryopreservation (slow cooling/thawing DMSO protocol) and cultured until the blastocyst stage in the presence of drugs inhibiting (Cardamonin (CAR) or stabilizing (1-Azakenpaullone (AZA) beta-catenin.
Participants/materials, setting, methods: The study was approved by Local and Federal Ethical Committees for research on human embryos. Human embryos ($n = 199$) were obtained from patients treated for infertility at our IVF Centre after the legally determined period of cryopreservation and with informed consent. Samples were analysed by qRT-PCR and immunocytochemistry.
Main results and the role of chance: Stabilizing beta-catenin had a positive effect on compaction, while any imbalance in protein levels negatively affected blastocyst formation. Beta-catenin was critical for *CDX2* and sufficient for *SOX2* expression, both normally present in TE of expanded blastocysts. TCF1, a well-known WNT-target, was analyzed as control. Pluripotency/ICM markers *NANOG* and *SALL4* were unaffected. We suggest WNT signaling to define TE via intracellular junctions, since beta-catenin was only present in the nucleus at the 8-cell stage and ubiquitously dominated on the membrane from compaction onwards. Indeed, CAR inhibited most of the genes critical for TE integrity like *CX43*, *CDH1*, *EOMES*, *GATA3* and *KRT18*. We propose WNT3 as potential candidate for TE specification due to its exclusive expression in outer cells of compacted embryos and in TE of blastocysts.
Limitations, reason for caution: There are limited numbers of good quality human embryos donated for research.
Wider implications of the findings: The better knowledge about factors influencing human preimplantation development will contribute to the refinement of artificial reproductive techniques and consequently increase live birth rates. Extracellular proteins critical for TE or ICM formation could be used as medium supplements to correct developmental defects in human preimplantation embryos. Recent accent on the importance of TE quality for the implantation rates especially emphasizes significance to increase available information on critical factors for TE specification.
Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), Fund for Scientific Research - Flanders (FWO-Vlaanderen) and the Methusalem (METH) of the VUB.
Trial registration number: None.

O-183 Differences in gene expression in human preimplantation embryos cultured in two different IVF culture media

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[4]Swammerdam Institute for Life Sciences-University of Amsterdam, MicroArray Department and Integrative Bioinformatics Unit, Amsterdam, The Netherlands

Study question: Is gene expression in human preimplantation embryos affected by the culture medium used?
Summary answer: Culture of human preimplantation embryos in either HTF (Lonza, Verviers, Belgium) or G5 (Vitrolife, Göteborg, Sweden) medium resulted in differential expression of genes involved in apoptosis, protein degradation and cell cycle regulation.
What is known already: Several studies showed effects of culture medium on embryonic development, pregnancy outcome and birthweight. The underlying mechanisms are not known; however, culture of preimplantation embryos affects gene expression in animals. The effects of culture medium on gene expression in non-cryopreserved human preimplantation embryos are not known.
Study design, size, duration: In a multicenter trial, patients were randomized to two culture medium groups. Data on embryonic development were collected for all embryos. In one center, embryos (originating from 2PN zygotes), which were not used for transfer or cryopreservation, were cultured until day 6 and collected for this study if patients consented.
Participants/materials, setting, methods: Ten blastocysts from each culture medium group, matched for fertilization method (IVF or ICSI), maternal age and embryo quality, were selected and their mRNA was amplified. Embryos were individually assessed for genome-wide gene expression using Agilent microarrays and PathVisio was used to identify pathways that were affected by culture medium.
Main results and the role of chance: Amplification was successful for all samples and all 20 arrays passed the quality control for sample quality, hybridization quality and signal comparability. Expression of 951 genes differed significantly ($p < 0.01$) between human preimplantation embryos that had been cultured in HTF or G5 medium. Affected pathways included apoptosis, protein degradation and cell cycle regulation. Findings were in line with the observations from the multicenter trial that for this center more fragmentation was seen in the embryos on day 2 in the HTF group (1.93 ± 0.9 vs. $1.75 \pm 0.8\%$, $p < 0.05$) and that embryos in the G5 group consisted of more cells on day 3 (8.03 ± 2.1 vs. $6.72 \pm 2.1\%$, $p < 0.05$).
Limitations, reason for caution: Despite careful matching of the embryos, it cannot be ruled out that differences between the groups could be affected by factors not investigated, for example the hormonal stimulation.
Wider implications of the findings: This study shows that gene expression in human preimplantation embryos is affected by the culture medium used during an IVF treatment. Furthermore, it provides insights in the mechanisms involved in human embryonic development during *in vitro* culture in different media, as our findings confirm clinical results. This knowledge can be used for improving the outcome of IVF treatments.
Study funding/competing interest(s): Funding by hospital/clinic(s), Maastricht University Medical Centre.
Trial registration number: N/A.

SELECTED ORAL COMMUNICATION SESSION

SESSION 48: IMPLANTATION FAILURE AND THE ENDOMETRIUM

Tuesday 1 July 2014 **17:00 - 18:00**

O-184 Increased previous spontaneous abortions rate in endometriotic women

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Study question: To evaluate previous history of spontaneous abortions in women with endometriosis as compared to unaffected women.
Summary answer: Endometriotic women display significantly higher previous spontaneous abortion rate than disease free controls independently of the existence of previous history of infertility or disease severity.
What is known already: The association between endometriosis and spontaneous abortion rates has long been debated without reaching a consensus.
Study design, size, duration: We conducted a cross sectional study in a tertiary-care university hospital between January 2004 and March 2013. This study enrolled a cohort of 1851 patients: 870 with histologically proven endometriosis and 981 unaffected women. A thorough surgical examination of the abdominopelvic cavity was performed in all study participants.
Participants/materials, setting, methods: Data were collected preoperatively using a structured questionnaire. Among women who conceived before the surgery, the type and number of the different previous first trimester pregnancies outcomes were studied. Previous history of spontaneous abortions rate was studied according to the existence of previous infertility history and the disease severity (rAFS and surgical classification).
Main results and the role of chance: Endometriotic women were more likely to be nulligravida as compared to controls [585/870 (67.3%) vs. 513/981 (52.4), respectively; $p < 0.001$]. Among women who conceived (284 endometriotic women and 466 controls) previous spontaneous abortion rate is significantly increased in women with endometriosis as compared to controls [139/478 (29%) vs. 187/964 (19%), respectively; $p < 0.001$]. After subgroup analysis PSA rates of women with endometriosis and controls are respectively: 20 vs. 12% ($p = 0.003$) among women without previous history of infertility, 53 vs. 30% ($p < 0.001$) in case of previous history of infertility, and finally 58 vs. 33% ($p < 0.001$) among women with previous infertility and assisted reproductive treatment (ART). No effect of the disease severity (using rAFS stages or the surgical classification) was observed on spontaneous abortion rate.
Limitations, reason for caution: There was a possible selection bias due to inclusion of only surgical patients. Therefore our control group consisted of women who underwent surgery for benign gynaecological conditions. This may lead to biases stemming from the fact that certain of these conditions, such as tubal infertility or ovarian cysts, might be associated with higher spontaneous abortion rates.
Wider implications of the findings: We demonstrate in a large cross sectional study that endometriosis is associated with higher spontaneous abortion rate as compared to disease free women. However, even if an association does not constitute proof of cause and effect, investigating the mechanisms that underlie spontaneous abortion rates in endometriosis is a step towards understanding this enigmatic disease. This study opens the doors to future, more mechanistic studies to establish the exact link between endometriosis and spontaneous abortion rates.
Study funding/competing interest(s): Funding by University(ies), no funding.
Trial registration number: None.

O-185 Network meta-analysis of interventions used to improve clinical pregnancy rate in women with implantation failure

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Study question: To investigate the effectiveness of various interventions (hysteroscopy, endometrial scratch, low molecular weight heparin (LMWH), hysteroscopy and biopsy and no treatment) in improving clinical pregnancy rates (CPR) for women with implantation failures. In addition, investigate if one intervention ranks better than the other in improving pregnancy outcome.
Summary answer: Network-meta-analysis (NMA) using Bayesian framework for direct and indirect comparisons between interventions showed hysteroscopy and biopsy ranked the best (OR = 4.06, 95% Crl 1.48, 11.13) and scratch ranked second (OR = 3.01, 95% Crl 1.52, 5.31) in improving CPR. However, individual patient data (IPD) highlighted issues of heterogeneity and uncertainty around effect estimates.
What is known already: Recurrent implantation failure (RIF) is a challenging and distressful condition, and various interventions have been reported to improve clinical pregnancy rates for these patients undergoing assisted reproduction. Five interventions (hysteroscopy, endometrial scratch, low molecular weight heparin, hysteroscopy and biopsy and no treatment) have been investigated in randomized (RCT) and non-randomized trials (NRCT). Thus far, meta-analyses on individual intervention have reported variable results.
Study design, size, duration: Evidence synthesis was performed doing systematic review of the literature; pooled effect estimates were obtained from standard pairwise meta-analysis; then NMA performed within Bayesian

framework for direct and indirect intervention comparisons and analysis will performed using both aggregate and individual patient data.
Participants/materials, setting, methods: Online database searches performed (January 1980–September 2013) and RCT, quasi-randomized and prospective NRCT that compared use of interventions of endometrial scratch, hysteroscopy and LMWH with placebo/no adjuvant treatment in women with RIF undergoing IVF/ICSI, were included. Primary outcome measure was CPR/woman. Final inclusions made after full manuscript examinations.
Main results and the role of chance: Eight-eighty citations were identified and 13 studies (8 RCTs, 1 quasi-RCT and 4 NRCTs) with total of 2820 participants included for evidence synthesis. Three studies compared hysteroscopy with no treatment; 5 compared endometrial scratch with no treatment; 3 compared LMWH with no treatment or placebo; 2 compared hysteroscopy and biopsy with hysteroscopy only. NMA of aggregate data using Bayesian framework random effects model showed hysteroscopy and biopsy and scratch as interventions have estimates of OR = 4.06, 95% Crl 1.48, 11.13 and OR = 3.01, 95% Crl 1.52, 5.31, respectively indicating increased odds of clinical pregnancy. On ranking, hysteroscopy and biopsy ranked the best, followed by scratch and no treatment or placebo arm ranked the worst. IPD highlighted issues of heterogeneity and uncertainty around effect estimates.
Limitations, reason for caution: The number of studies in each arm of the intervention was small, which impacts the inferences made from the effect estimates. IPD was obtained from only three studies, therefore further limiting evidence synthesis.
Wider implications of the findings: This study highlights that of the various interventions used to improve pregnancy outcomes in women with implantation failure, hysteroscopy and biopsy and scratch are most effective. However, individual patient data emphasizes the issues of publication bias, heterogeneity and uncertainty around effect estimates. Adequately powered randomized controlled trials are required to robustly ascertain efficacy of these interventions and researchers should be able to share individual patient data to support evidence-based medicine.
Study funding/competing interest(s): Funding by University(ies), University of Leicester.
Trial registration number: N/A.

O-186 Intra-uterine administration of human chorionic gonadotrophin (hcg) before embryo transfer in recurrent implantation failure (RIF) patients improves implantation and pregnancy rates in IVF-ICSI cycles

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Study question: Does intrauterine administration of human chorionic gonadotropin (hcg) before embryo transfer in recurrent implantation failure (RIF) patients improve pregnancy rates in IVF-ICSI cycles?
Summary answer: Intrauterine injection of hCG before embryo transfer in IVF/ICSI cycles may increase endometrial regulatory T cells and improve the implantation and pregnancy rates.
What is known already: Human chorionic gonadotrophin (hCG) was found to secreted immediately after fertilization by the embryo. It plays an important role in implantation and in attracting regulatory T cells to the endometrium**.**
Study design, size, duration: 216 Infertile recurrent implantation failure (RIF) patients younger than 42 years from 2006 to 2013 were included in this study. Patients were randomly divided into two groups using computer generated list. The study group received intrauterine administration of 500 IU of HCG and control group received nothing before ET.
Primary Outcome Measures: Implantation and pregnancy rates.
Secondary Outcome Measures: Miscarriage and delivery rates.
Participants/materials, setting, methods: The study group (n = 108) received intrauterine injection of 500 IU of hCG, and the control group (n = 108) underwent ET without hCG.
Main results and the role of chance: The IR and PR were statistically significantly higher in the group received intrauterine injection of 500 hCG (23.2 and 36.8%, respectively) as compared with the control group (12.5 and 23.6%, respectively).
Limitations, reason for caution: A relatively new concept in recurrent implantation failure, requiring more multicentric trials worldwide.
Wider implications of the findings: Implantation and pregnancy rates are very low in RIF patients.
This study shows improved Implantation and pregnancy rates by intra-uterine hCG administration before embryo transfer.
Study funding/competing interest(s): Funding by hospital/clinic(s), BTTB CENTRE.
Trial registration number: BTTB/2006/19.

O-187 Serum VEGF level on day of embryo transfer is indicative of a vascularized receptive endometrium and is a non-invasive implantation marker in ongoing IVF cycles

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Study question: To determine if serum VEGF level on day of embryo transfer and days 7 and 14 post embryo transfer reflects endometrial vascularization and whether serum VEGF can be construed as a non-invasive marker for clinical pregnancy and embryo implantation in ongoing IVF cycles.
Summary answer: Serum VEGF level on day of embryo transfer correlates with clinical pregnancy and embryo implantation rates with a threshold value of >486.7 pg/ml as a determinant for clinical pregnancy and hence may be considered as a robust non-invasive marker for embryo implantation in ongoing IVF cycles.
What is known already: Endometrial vascularization at the feto-maternal interface is essential for successful embryo implantation. Vascular endothelial growth factor (VEGF) mediates vascular permeability during the peri-implantation phases and higher VEGF expression in endometrium has been associated with pregnancy. However endometrial biopsy for VEGF estimation constitutes an invasive method for implantation studies and cannot be applied to an ongoing cycle. Exploring new biochemical markers for endometrial receptivity and embryo implantation is required for increasing pregnancy rates in IVF cycles.
Study design, size, duration: This prospective cohort study involving 227 non-PCOS, normal responder infertile females undergoing fresh IVF treatment cycles using standard conventional protocol was carried out at our academically affiliated private infertility clinic from July 2012 to December 2013. 7 cycles were cancelled due to fertilization failure (n = 3) or no embryo transfer (n = 4).
Participants/materials, setting, methods: Serum VEGF levels were measured by ELISA method on day of embryo transfer (dET) and days 7 (d7ET), 14 (d14ET) post ET. Cycles were divided into pregnant and non-pregnant and into high and low dET serum VEGF groups. Clinical pregnancy rate (CPR) and Implantation rate (IR) were main outcome measures.
Main results and the role of chance: Overall Clinical Pregnancy Rate (CPR) 30% and Implantation rate (IR) 27.60% was achieved. Serum VEGF level on dET (658.1 ± 19.90 vs. 495.7 ± 15.98 pg/ml; $p < 0.0001$) was significantly higher in pregnant (n = 66) than in non-pregnant (n = 154) group. High (>495 pg/ml) dET VEGF group (n = 106) depicted significantly much higher CPR (55.66 vs. 6.14%; $p < 0.0001$) and IR (51.92 vs. 5.29%; $p < 0.0001$) compared to low (≤495 pg/ml) dET VEGF group (n = 114). dET VEGF level correlated strongly with clinical pregnancy rate (Spearman r = 0.4109, 95% CI: 0.2910–0.5181; $p < 0.0001$) and implantation rate (Spearman r = 0.4215, 95% CI: 0.3027–0.5274; $p < 0.0001$). Threshold value of serum dET VEGF to achieve clinical pregnancy was found to be >486.7 pg/ml (Sensitivity 93.94%, Specificity 75.32%, Likelihood ratio 3.81, ROC_{AUC} 86.50%, 95% CI 0.82–0.91, $p < 0.0001$).
Limitations, reason for caution: This study is limited by a small sample size and is not a randomized controlled trial. Also, we have not evaluated VEGF levels in endometrial tissue.
Wider implications of the findings: This novel study offers dET serum VEGF level as a robust 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 may be a decisive factor for either transferring embryos in same cycle or cryopreserving them and postponing ET to subsequent natural cycle.
Study funding/competing interest(s): Funding by hospital/clinic(s), The study was funded by our own Private Infertility Centre: Vaunshdhara Clinic and Assisted Conception Centre.
Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATION SESSION

SESSION 49: NURSES' PERSPECTIVE IN PATIENT CARE AND QUALITY OF LIFE

Tuesday 1 July 2014 **17:00 - 18:00**

O-188 Clinical research in reproductive medicine: the contribution of the research nurse

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Study question: Can a research nurse improve clinical research, i.e., patient recruitment and participation in clinical studies in reproductive medicine?
Summary answer: A research nurse was instrumental in recruitment of patients for an increasing number of studies and played a key role in facilitating and coordinating clinical studies in reproductive medicine.
What is known already: Tasks and responsibilities of research nurses have been extensively discussed and include coordinating study visits, participant recruitment and obtaining informed consents. Yet very limited data is available if involvement of a research nurse actually improves patient recruitment and facilitates participation of a centre in multiple studies.
Study design, size, duration: The Centre for Reproductive Medicine of the Academic Medical Centre in Amsterdam is involved in clinical research and has a prominent role within the Dutch consortium for Obstetrics, Gynaecology and Fertility. From 2009 until 2014, study participation and patient recruitment was registered and linked to activities of a research nurse.
Participants/materials, setting, methods: Descriptive study. Since January 2010, a research nurse was actively involved, and had a coordinating role in the implementation of study protocols at the clinical site(s). To evaluate the role of a research nurse we here evaluate patient recruitment and study participation in the years 2009 and 2013.
Main results and the role of chance: From 2009 until 2013 there was an average increase of 55 recruitments per year. In 2009, 47 couples participated in 3 clinical trials and in 2013, 266 couples participated in 14 clinical trials. There were 3.9 inclusions per month in 2009 and 22 in 2013, showing a five-fold increase. The research nurse had a coordinating role in preparing and implementing study protocols at the clinical site(s). The main tasks were: presenting new studies, identifying, counselling and randomizing eligible patients, performing study visits and collecting data. The research nurse worked 3 days a week.
Limitations, reason for caution: The experience and number of hours per research nurse might differ. The information about recruitment and randomizations are from a single centre, performing clinical research within the Dutch consortium for Obstetrics, Gynaecology and Fertility.
Wider implications of the findings: A research nurse plays a vital role in the identification of eligible patients, counselling and data collection, keeping an overview of all studies and recruitment. If given a key role in clinical research, our results show that the number of inclusions and the number of studies can be significantly improved. A research nurse should be trained in good clinical practice and research ethics, and potentially follows the progress of all studies.
Study funding/competing interest(s): Funding by hospital/clinic(s), Amsterdam University Medical Centre.
Trial registration number: Not applicable.

O-189 Spanish patient's desires and attitudes about single or multiple embryo transfer

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Study question: The aim of this study is to assess the patients preferences about having a multiple or single gestation, their preferences regarding the number of embryos transferred (single or multiple embryo transfer (SET/DET)) and their knowledge about the risk of multiple pregnancies.
Summary answer: A significant proportion of fertility patients consider multiple birth an ideal treatment outcome.

Patient education may play an important role in order to improve the patient's knowledge about the risks of multiple gestation and their preference by SET and to improve their confidence in the chance of pregnancy with SET.
What is known already: We already know this data with other european population but not with spanish patients.
Study design, size, duration: It's a prospective study. It was performed from February 2012 to June 2013, and there were 292 subjects involved from Seville, Valencia and Barcelona.
Participants/materials, setting, methods: The patients were invited to fill a questionnaire about different issues concerning the single or multiple embryo gestation and transfer and their perception of the risks associated with these pregnancies. Data were analyzed by Chi square test or Anova, according to the variables.
Main results and the role of chance: There are not statistical differences between the number of patients who desire single gestation or twin gestation as an ideal treatment outcome (average 1.46 vs. 1.59).

The 3rd most desirable option is a triple pregnancy and 50% of the patients prefer a quadruplet gestation better than no gestation.

Single gestation is more desirable as first option for the women than for the men (63 vs. 45%).

A 50% of the patients who desire single gestation as first option, prefer double embryo transfer against 32% who prefer SET. The rest, have no preferences.

When carrying out an ANOVA analysis, our data shows that the larger the knowledge of the risks of multiple pregnancy, the higher the preference by single gestation.
Limitations, reason for caution: Because of the type of data, we estimate there is no reasons for caution.
Wider implications of the findings: Our data show that the patients still have few confidence in the chance of pregnancy with SET. It would be interesting to know the reasons for this lack of security in the technique and work to get that those patients who prefer a single pregnancy really want to do SET.
Study funding/competing interest(s): Funding by national/international organization(s), IVI SEVILLA, IVI VALENCIA, IVI BARCELONA.
Trial registration number: This study is not an RCT.

O-190 Is the quality of life in poor ovarian responders (POR) worse than normal responders? a prospective study using fertiqol

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Study question: Is the previous diagnosis of POR an important factor to impair the quality of life of the patients during the ovarian stimulation for IVF?
Summary answer: In a center with a specific unit for poor responders, the quality of life of this patients is not different from the normal responders (NR).
What is known already: Many studies demonstrate that infertile patients commonly experience feelings of depression, isolation, anxiety…. As a result, quality of life assessment is becoming increasingly important in reproductive medicine.

Patients with low response are a group of women in a critical situation facing an uncertain prognosis treatment. Many of these patients have already considered the possibility of abandoning the treatments or move on to egg donation. This could affect even more their quality of life.

Study design, size, duration: Cohort prospective study. 59 patients under ovarian stimulation for ivf completed the fertiqol questionnaire. All the POR patients were evaluated in the POR unit with previous counselling and personalized follow during the ovarian stimulation. The study was started in september 2013 and finished in January 2014.

Participants/materials, setting, methods: From the 59 patients, 24 fulfilled the Bolonia Criteria to be considered POR and 35 were NR. Fertiqol was use to determine the quality of life in patients. *t*-student test and linear regression were applied for statistical analysis, *p* values <0.05 were considered statistically different.

Main results and the role of chance: The total FertiQol score was 75.4 in POR and 77.1 in NR. The difference was not significant.

The scores on the core of the survey were 75.2 for POR and 77.7 for NR. For the different subscales the results were for POR and NR respectively: Emotional subscale: 68.75 and 76.6; Mind-Body subscale: 71.0 and 73.6; Relational subscale: 82.6 and 82.8; Social subscale: 79.0 and 77.8. None of the differences found was statistically significant.

In the treatment domain the results were 74.4 for POR and 73.9 for NR. For the different subscales the results were for POR and NR: Environment subscale: 75.2 and 78.5; Tolerability subscale: 73.3 and 69.7. None of the differences found was statistically significant.

Limitations, reason for caution: Size of sample. Accuracy of tools to evaluate quality of life.

Wider implications of the findings: The evaluation in a specific multidisciplinary unit for PORs and the special follow of this patients could improve the quality of life of this women.

Study funding/competing interest(s): Funding by hospital/clinic(s), Instituto Bernabeu.

Trial registration number: None.

O-191 Quality of care in an IVF programme: differences and similarities between genders

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Study question: Do men and women assess quality of care differently when measured with the validated instrument Quality of *In Vitro* Fertilisation treatment from the Patient's Perspective (QPP-IVF)?

Summary answer: Men and women rated the subjective importance higher than the perceived reality in almost all aspects. Both gender stressed the need for more participation in decisions, continuity in caregivers, more information about treatment steps and better availability. Men and women had similar experience of perceived quality of care during IVF.

What is known already: A few validated tools concerning IVF measuring quality of care have been developed. Earlier instruments have either focused on women or have been tailored to couples rather than men and women separately.

Study design, size, duration: A two-centre study ran between September 2011 and May 2012. In all, 363 women and 292 men evaluated quality of care by answering the QPP-IVF questionnaire.

Participants/materials, setting, methods: The measurements consisted of two kinds of evaluations: the rating of perceived reality and rating of subjective importance of various aspects of treatment. Comparison between gender on subjective importance and perceived reality ratings were performed on scale and single item level. An Index of measures took both measurements into account.

Main results and the role of chance: In general the ratings of subjective importance were significantly higher than the ratings of perceived reality for both men and women. Women rated subjective importance significantly higher than men in almost all factors while the rating of perceived reality was similar. At item level women rated subjective importance of "information about examinations and drugs" and "staffs respect, commitment and empathy" significantly higher than men. Men rated perceived reality of "having the same doctor," and "same responsible midwife/nurse" and "understanding doctor" significantly higher than women. The Index of measures indicated that the most deficient aspects were: "meeting the same doctor during treatment," "participation" and "responsibility/continuity." The aspects receiving the best evaluation were "respectful doctors and midwifes/nurses" and "privacy."

Limitations, reason for caution: The lower response rate of men compared to women (60.6 vs. 74.2%) might have influenced the results through selection bias. Only patients who had adequate fluency in the Swedish language participated.

Wider implications of the findings: This study is an important contribution for equating men and women in fertility treatments and illuminate men's needs and experiences in quality of care. The QPP-IVF instrument is a suitable instrument for revealing deficiencies in quality of fertility care experienced by both men and women and stimulate quality improvements within and between clinics.

Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), The study was supported by the LUA/ALF agreement at Sahlgrenska University Hospital, Gothenburg, Sweden, and by Hjalmar Svensson's Research Foundation.

Trial registration number: None.

SELECTED ORAL COMMUNICATION SESSION

SESSION 50: THE PSYCHOLOGY OF FERTILITY PRESERVATION AND GAMETE DONATION

Tuesday 1 July 2014 **17:00 - 18:00**

O-192 Oocyte freezing for social indications: an internet based survey of knowledge, attitudes and intentions among women in Denmark and the United Kingdom

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Study question: To assess knowledge and intentions of women in Denmark and the United Kingdom regarding oocyte freezing for reproductive life planning, and describe the general and personal circumstances which would make women more likely to undergo the procedure.

Summary answer: 83% were aware of social freezing, 89.1% thought it acceptable, but only 20% were actively considering it. Greater uptake was dependent on chance of conceiving rather than reassurance on safety. Other factors encouraging use were fear of impact of early pregnancy on career, and no partner by age 30.

What is known already: Until recently, no options for preserving fertility in order to delay childbearing existed. Oocyte freezing is now possible. It remains unclear to what extent women are aware of its possibilities and limitations, their attitudes to use or the circumstances under which they might consider this technique.

Study design, size, duration: A cross-sectional survey was designed to investigate knowledge and attitudes of women in Denmark and the 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) and completed anonymously.

Participants/materials, setting, methods: From October 2012 to September 2013, 973 females completed the questionnaire (60% Denmark and 40% UK). The acceptability of the procedure was calculated by analysing categorical variables. Univariate analyses were used to identify the characteristics of women more likely to intend oocyte freezing for reproductive life planning.

Main results and the role of chance: The median age of respondents was 31 years (range 18–68). 83% reported having heard of oocyte freezing. 99.4%

considered it an acceptable procedure for medical indications, and 89.1% considered "social" freezing to be acceptable. 19% reported considering undergoing the procedure.

The key factor shown to encourage uptake was a more than 50% chance of having a baby ($p = 0.001$). Characteristics significantly associated with intention to freeze were career impact concerns, no partner by age 30, 35 or 40, age under 25 years, being single, divorced or childless, intending to have children and having a history of infertility. Interestingly, the effect of the procedure on future fertility, its safety for the future children and the cost of the procedure weren't significant factors.

Limitations, reason for caution: The generalizability of the results is limited due to the internet-based data collection. Respondents may be a selected group of the population with higher education.

Wider implications of the findings: This study indicates that 89.1% of women consider reproductive planning to be an acceptable indication for oocyte freezing. Nearly 20% of women would actively consider oocyte freezing. Interestingly, reassurance regarding efficacy is more important than the safety and cost of the procedure. In terms of personal circumstances, career aspirations and not having found a partner by age 30 remain significant.

Study funding/competing interest(s): Funding by commercial/corporate company(ies), This study is partly funded by an unrestricted grant from Merck Serono which had no influence on data collection or data analyses. The authors have no conflicts of interest.

Trial registration number: Not applicable as not an RCT. Local ethics ID number: 1783 (Southampton University).

O-193 Fertility preservation in young cancer patients – how to facilitate decision-making best?

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Study question: The objectives were (1) to assess attitudes toward fertility preservation (FP) in young female cancer survivors, (2) to measure decisional conflict associated with consideration to opt for FP and (3) to assess specific needs of patients when making their decision and the helpfulness they attributed to various sources of support.

Summary answer: The online survey showed that young cancer patients' attitude towards FP is positive, but decisional conflict is considerable. The complementary focus groups revealed additional ethical considerations and confirmed the need for comprehensive support and standardized decision-aid tools.

What is known already: Impaired fertility is a frequent consequence of successful treatment and a major concern in young cancer patients. Thus, options to preserve fertility are welcome to these patients, even if having to make decisions on fertility preservation (FP) in the short time period after cancer diagnosis and before onset of treatment causes additional distress to them.

Study design, size, duration: A cross-sectional online survey (phase 1) was followed by focus groups (phase 2), combining a quantitative with a qualitative approach. Sample size in phase 1 was 155 women and its duration 11 months in total. In phase 2 four focus groups were held with a total of 12 participants.

Participants/materials, setting, methods: Cancer patients aged 18–45 years had access via 18 websites to a 136-items questionnaire including the validated Decisional Conflict Scale (DSC). In focus groups cancer survivor discussed attitudes towards FP, difficulties with decision-making and identification of supportive strategies. Besides quantitative analysis, the program MAXQDA served for management of qualitative data.

Main results and the role of chance: Positive attitudes towards FP significantly outweighed negative attitudes, but 47.4% of online survey participants considered decisions on preserving fertility as difficult. While the survey revealed few ethical considerations, focus groups identified additional aspects, such as concerns about the fate of unused cryopreserved embryos. Ninety-four (62.3%) survey participants had a high decisional conflict represented by a score over 37.5 of 100. Mean DSC score was 49.59 (SD = 2.90). For the subscales "informed," "support," "uncertainty" and "value clarity" the means were 55.1 (SD 2.69), 49.17 (SD2.45), 48.06 (SD 2.67) and 45.93 (SD 2.69) respectively. Mean score was significantly lower in women who had undergone a FP procedure (30.88 vs. 57.57; $p = 0.0001$). Besides a well-informed physician, checklists and decision-guides were identified as especially helpful.

Limitations, reason for caution: The composition of an online sample is arbitrary and prone to bias, such as higher education, and thus not representative for young cancer patients in general. Focus groups were performed to provide more specific and in-depth information and to counter-balance and amplify online data.

Wider implications of the findings: Decisional conflict with regard to FP is considerable and a standardized instrument that facilitates decision-making would be desirable. The authors believe that an online tool would best meet the needs of the patients concerned and a study with the aim to develope and evaluate such a tool is in preparation.

Study funding/competing interest(s): Funding by national/international organization(s), Swiss cancer league (KLS – 02577-02-2010).

Trial registration number: Not applicable.

O-194 How to improve clinical pathways for fertility preservation in a pediatric population: results of a survey on decisional process in the patients and parents

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Study question: How to improve the decision-making process for fertility preservation (FP) in adolescents and prepubertal boys based on analysis of: (1) patient and parent attitudes depending on whether FP options have been proposed; (2) influence of the emotional state of patients and parents; (3) availability of medical staff and family.

Summary answer: The survey analysis revealed that patient and parent expectations were useful to improve FP pathways.

What is known already: Reproductive capacity is a major quality-of-life issue. Lack of awareness of patient and parent expectations leads to poor application of guidelines on FP by specialists. However, patients have a strong desire to be informed of available FP options and their illness strengthens the value they place on parenthood and family. Less than 10% would choose to adopt or use donated gametes to become parents. Consequently, FP measures must assume a higher priority.

Study design, size, duration: Questionnaire survey via regular mail to an eligible population between January 2005 and May 2013.

Participants/materials, setting, methods: 348 prepubertal boys and adolescents aged 0–18 years, diagnosed with cancer in a university setting, were eligible. Three different questionnaires, for children (<12 and >12) and parents respectively, were established based on information from focus groups. Questions were subsequently reviewed by the institutional ethics board before being sent.

Main results and the role of chance: Of the 348 eligible patients, 44 died and 14 were lost to follow-up. 290 patients (77 > 12 and 213 < 12) were sent a questionnaire. 128 questionnaires were recovered with 45.45% from adolescents and 39.9% from children.

50/85 boys <12 years and 28/35 > 12 were informed about FP approaches. Only 1/6 boys <12 years and 3/6 > 12 years considered they were fully informed. 31/50 boys <12 years and 22/28 > 12 years felt they were able to digest this information and most considered the volume of information too much all at once. 27/50 boys <12 years and 13/28 > 12 years felt anxious mainly because of the experimental nature of the procedure, their physical condition, or the need for an additional procedure. Family support did not appear to be of benefit.

Limitations, reason for caution: This single center survey does not allow extrapolation of gathered information to other settings. Results after adaptation of the usual FP pathway should be taken into account.

Wider implications of the findings: Acknowledging the issues faced and familiarizing oneself with the care of patients undergoing fertility-threatening therapies supplies primary care providers with appropriate quality management tools in the field of FP in centers for reproductive medicine. Expectations reported in the survey allow appropriate support to be included within the FP clinical pathway design.

Study funding/competing interest(s): Funding by hospital/clinic(s), Cliniques Universitaires Saint Luc, Brussels.

Trial registration number: Not applicable.

O-195 Preferences and needs regarding future contact with donation offspring among identity-release gamete donors. Results from the Swedish study on gamete donation

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Study question: What are the preferences and needs regarding future contact with donation offspring among identity-release gamete donors?
Summary answer: More than half of identity-release donors want to be notified about offspring requesting identifying information about them, and one in four reports a need for counselling.
What is known already: Globally, there is an increase of identity-release donor programmes in which offspring at mature age can obtain identifying information about the donor. Research on donors' attitudes towards contact with offspring is predominantly based on sperm donors in anonymous programmes who actively have made themselves identifiable to offspring and there is limited knowledge about the preferences of female and male identity-release donors regarding potential future contact with offspring.
Study design, size, duration: The Swedish Study on Gamete Donation is a longitudinal cohort study including all clinics performing gamete donation treatment in Sweden. During 2005–2008 donors were recruited consecutively and 181 oocyte donors (83% response) and 119 sperm donors (79% response) were included prior to donation. Long-term follow-up was performed 5–8 years post-donation.
Participants/materials, setting, methods: The present study includes questionnaire data from 126 women and 84 men provided 5–8 years after donating to couples that were unknown to them (74–83% of participants at baseline). Data collection was performed with nine study-specific items on preferences and attitudes (Likert scales) developed by experts and pilot-tested.
Main results and the role of chance: While more than half of donors (59%) wanted to be notified by the clinic when an offspring requests information about them, about a third was negative to receiving such information. A majority of donors were positive or neutral towards offspring contacting them and towards offspring meeting the donor's family, e.g., their own children. Few wanted a potential meeting with offspring to take place at the fertility clinic or in the donor's home, but preferred a meeting at a neutral place, e.g., a café. One in four donors reported a need for counselling regarding future contact with offspring.
Limitations, reason for caution: No information is available about the donors who chose not to participate in the study and it is possible that they have different views on the studied variables.
Wider implications of the findings: Oocyte and sperm donors in identity-release programmes have positive or neutral attitudes towards potential future contact with offspring, which is reassuring. However, donors appear to have different preferences regarding information and support from the clinic. Fertility clinics should offer counselling regarding contact with offspring to donors who express a need for this.
Study funding/competing interest(s): Funding by national/international organization(s), The Swedish Research Council.
Trial registration number: N/A.

INVITED SESSION

SESSION 51: IMPROVING MEDICAL DECISION MAKING

Wednesday 2 July 2014 **08:30 - 09:30**

O-196 Improving decision making in the clinic and laboratory. The importance of Non-Technical Skills

R. Flin[1]
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Many safety-critical tasks are characterised by teams of workers dealing with significant risks, time pressure and increasingly complex technology. In these domains, practitioners need both technical and non-technical skills. The term non-technical skills comes from European aviation and they can be defined as "the cognitive, social and personal resource skills that complement technical skills, and contribute to safe and efficient task performance." They are not new or mysterious skills but are essentially what the best practitioners do in order to achieve consistently high performance: the skills include situation awareness, decision making, team work and leadership. There are now methods for training and rating the non-technical skills of surgeons (NOTSS) and anaesthetists (ANTS), with applications being developed for other clinical specialists, such as histopathologists and radiologists. In this presentation, I will briefly outline the non-technical skills approach and will explain how this could be applied for staff working in clinical and laboratory settings for Assisted Reproduction Technologies.

O-197 Use of modern business intelligence systems in medicine

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My presentation will be based upon inspiration from three important books: Groopman (2007), *How Doctors Think*, Reiser (2009), *Technological Medicine, The Changing World of Doctors and Patients* and Topol (2012), *The Creative Destruction of Medicine. How the Digital Revolution Will Create Better Health Care*. Insights from these sources and my own research about decision making and expertise system ("Business Intelligence," "Big Data") leads to following questions and answers during my presentation:
How do we make decisions in general, and specifically in a medical practice?: It is not uncommon that we make biased decisions. One reason for that is that our decisions often are more based upon intuition and earlier own experience, than actual (latest) facts/evidence. Often our knowledge is based upon insights from populations and by that average. At the same time, we know that we have much complexity and variations at the individual level. With the support of new technology, we can wider and deepening our individually and collective perception about granularity in data. We can increase our knowledge about the individual case.
How can we improve our capability to make decisions?: By using different forms of digital technology in the best way, in the right mix between human and the machine, we can increase our productivity at the same time we continuously develop or own and our common organisational knowledge. With the support from modern technology, we can move from a System 1 – thinking (rapid and intuitive) to a System 2 – thinking (analytical and reflective). By that we can improve our decision-making.
From insights to actions: Even if it is good with an increased insight, knowledge, it is not enough. We still have to overcome some barriers to action. From our interpretation of a defined situation we need to find a suitable, not always perfect solution. We also understand it is not enough to have knowledge you must also motivation and possibilities to take action. Here we discuss some of the problems/hindrance we can see working with modern digital technology at a medical workplace.

From my presentation, it is possible to learn more about the impact modern digital technology can have for contemporary medical practice.

INVITED SESSION

SESSION 52: UPDATE ON ULTRASOUND IN REPRODUCTIVE HEALTH CARE

Wednesday 2 July 2014 **08:30 - 09:30**

O-198 Automated ultrasound in reproductive medicine

N. Raine-Fenning[1]
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Ultrasound has become an essential tool for the assessment of women with subfertility and in their subsequent management. It is commonly used to assess the pelvis to exclude pathology that may be related to the couples' inability

to conceive or to identify abnormalities that may impair their treatment and reduce the chance of conception or increase the risk of aberrant pregnancy outcome. Ultrasound is also used to assess ovarian reserve through estimation of ovarian volume and the total number of antral follicles. Such assessments are important as antral follicle counts have a good predictive value for the response to controlled ovarian stimulation and may perform as well as serum levels of Anti-Müllerian Hormone (AMH). Once treatment is initiated ultrasound remains an integral part of the patient's management as it is used to track follicles regardless of whether a unifollicular or multifollicular response is desired.

All of these ultrasound assessments involve subjective interpretation of the image display and are operator dependent therefore. Objective assessment, including counting the number of antral follicles or measuring the mean diameter of a follicle, are time consuming and will vary according to the skill and diligence of the observer. The validity and reliability of measurements of follicular diameter are particularly likely to reduce as the number of follicles increases and the time required for these measurements also increases, which may also adversely affect measurement reliability. Despite this there are no standards for these measures or audit process to monitor their application. Automated measurement of follicular number and size could potentially address these issues and is a now viable option.

Sono-AVC (Automatic Volume Calculation: GE Healthcare) is a software programme that identifies and quantifies hypoechoic ("fluid-filled") regions within a three-dimensional dataset and provides automatic estimation of their absolute dimensions, mean diameter, and volume. As each and every volume, regardless of its absolute size, is given a specific colour and Sono-AVC is an ideal tool to study follicles within the ovary. Sono-AVC also provides automated measurements of the follicles mean diameter (relaxed sphere diameter) and its volume. It can be used to perform antral follicle counts and or track follicle growth during natural/stimulated cycles.

The talk will demonstrate the technical and practical use of automated ultrasound within reproductive medicine and clarify its current clinical role.

O-199 Introducing virtual ultrasound training as a new teaching tool in early pregnancy

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Ultrasonography has been adopted in many clinical specialties during the last decade including in obstetrics/gynaecology. Though ultrasonography, when used correctly, can be an effective diagnostic tool, it is at the same time highly operator-dependent. Insufficient training may lead to diagnostic errors that, in turn, may endanger patient safety. Increased awareness of the importance of proper training, as well as of the potential patient safety threats associated with the lack thereof, has intensified the search for optimised training methods. During this talk, current evidence on challenges to ultrasound training and assessment of trainee competence is reviewed. Discrepancies between trainees' confidence and their expected levels of performances have raised concerns about the adequacy of current ultrasound training programmes. Trainees and educators have long called for more supervised hands-on time but this remains time consuming and may lead to increased patient discomfort if the examination is prolonged. Simulation-based ultrasound training has therefore been suggested to improve basic training without the risk of any patient discomfort. However, to examine the effectiveness of simulation-based ultrasound training, reliable and valid assessment instruments are needed. To assess trainee competence, the Objective Structured Assessment of Ultrasound Skills (OSAUS) scale has been shown to discriminate between differences in ultrasound competence and defensible pass/fail standards have been established for transvaginal ultrasound. Using this scale, a recent study has explored the effectiveness of simulation-based transvaginal ultrasound training and found that training leads to large immediate effects on performances with patients. Another study has shown that these effects persist even after 2 months of clinical practice, suggesting that ultrasound simulation does in fact lead to long-term effects on diagnostic performance for novice trainees. Finally, the preliminary results of a large multi-centre randomized trial on the effect of simulation-based training on patient satisfaction, discomfort, the use of time during the examination, and finally need for supervision by a senior colleague is reviewed.

INVITED SESSION

SESSION 53: PARAMEDICAL INVITED DEBATE SESSION - LABORATORY (DEBATE) - IS THERE TIME FOR TIME-LAPSE IN THE LABORATORY?

Wednesday 2 July 2014 **08:30 - 09:30**

O-200 Pro

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After more than 30 years of *in vitro* fertilization the challenge now is to improve results by increasing healthy take-home baby rates and diminishing multiple pregnancy rates. By optimizing embryo culture and making a more precise embryo evaluation we can improve the difficult task of identifying the best embryo for elective single-embryo transfer.

In recent years time lapse methodology has rushed into many ART laboratories and straight away it became widely accepted. Time-lapse systems give us the opportunity to stabilize embryo culture, minimizing the negative effects of traditional incubator door openings and embryo observations under the microscope. Moreover, this non-invasive system offers large numbers of images that allow us to know constantly what is happening to the embryos. By checking the embryos at only 4 or 5 precise timings we are not aware of the continuity of embryo development. We miss valuable information that could be crucial for selecting the best embryo for transfer. It is well known now that during the intervals between traditional check points phenomena such as abnormal pronuclear behaviour or abnormal cleavage occur.

By introducing time-lapse methodology in the ART laboratory we have improved our knowledge about first stages of human *in vitro* embryo development, and relevant morphokinetic parameters that could contribute to a better embryo scoring have been identified. Synchrony, timing of cleavage, duration of cell cycles, pronuclear fading, initiation of compaction, and blastulation have been identified as relevant parameters for predicting blastocyst formation and/or implantation potential. Although differences have been reported between groups in identifying which parameters offer the best prediction, the clinical value of these parameters for embryo selection is widely accepted and increased pregnancy rates have been reported. As a consequence, we now know that some morphokinetic parameters could be crucial in excluding or selecting embryos, especially in young patients with many good quality embryos. It is obvious, however, that time-lapse methodology is a rather time-consuming process that needs to be adapted to the IVF laboratory routine.

In order to identify predictive parameters, confounding factors such as maternal age or day of transfer have to be taken into account. Intrinsic characteristics of patients, stimulation protocols and laboratory conditions lead to different scenarios that could vary between centres. For this reason it is necessary for each ART laboratory to analyse its own data, identifying which morphokinetic parameters are relevant and developing an embryo selection model incorporating these internal parameters.

In order to optimize daily work it is essential to have standardized operating procedures highlighting the time-lapse annotations to be made and the embryo selection model to be applied. Only in strict compliance with the defined protocols can we avoid drowning in data.

O-201 Contra

S. Mastenbroek[1]
[1]*Center for Reproductive Medicine, Academic Medical Center University of Amsterdam, Amsterdam, The Netherlands*

In vitro fertilization (IVF) has revolutionized reproductive medicine and it has established itself firmly in modern day society. In the past decades great progress has been made and success rates after IVF have improved. In the majority of current day IVF cycles multiple embryos are created after ovarian hyperstimulation. The viability of these embryos, and as a consequence the chance for an embryo to successfully implant, is subject to biological variation. To achieve the best possible live birth rates after IVF while minimizing the risk for multiple pregnancy, one or two embryos that are considered to have the best chance

of implanting are selected for transfer. Subsequently, supernumerary embryos with a good chance of implanting are selected for cryopreservation and possible transfer in the future while remaining embryos are discarded.

Embryos are most often selected for transfer based on multiple morphological characteristics at one or several stages of preimplantation development. However, implantation rates in general do not exceed 35 percent with embryo selection based on such morphological evaluation. This has resulted in a strong drive for finding alternative selection methods. One of the promising methods of recent years is the more detailed evaluation of morphology and developmental kinetics of the available embryos using time-lapse imaging.

On itself, time-lapse imaging of embryos is not new, but the technology gained traction due to the introduction, and active promotion by the companies involved, of new time-lapse devices that allow easy clinical adoption. Continuous monitoring of embryo morphokinetics within the stable environment of an incubator is possible with these devices, where evaluation in a regular setting outside the incubator exposes embryos to possibly undesirable changes in temperature, humidity, gas concentrations, and pH values. Time-lapse systems increase the precision and sensitivity of regular morphological evaluation and introduce new dynamic parameters, such as timing and synchrony of cleavage stages. They could allow for standardization, objectivity, and facilitate laboratory logistics. It has been suggested that time-lapse systems can be used to predict blastocyst formation, to distinguish aneuploid from euploid embryos, and that they improve the success rate after IVF.

But do these time-lapse systems actually deliver on all these promises? In this debate, a review of current available evidence will be presented to discriminate between hype and truth. This will answer whether it is time for time-lapse in the laboratory.

SELECTED ORAL COMMUNICATION SESSION

SESSION 54: CLINICAL ENDOCRINOLOGY (2)

Wednesday 2 July 2014 **10:00 - 11:45**

O-202 AMH predicts menopause; results of an ongoing follow up study in normo-ovulatory women

M. Depmann[1], M.J.C. Eijkemans[2], G.J. Scheffer[1], I.A.J. van Rooij[1], A. de Vet[1], B.W.J. Mol[3], F.J.M. Broekmans[1]
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[3]*Academic Medical Center, Obstetrics and Gynaecology, Amsterdam, The Netherlands*

Study question: This study aims to determine whether endocrine markers (Anti-Müllerian hormone (AMH) and Follicle Stimulation Hormone (FSH) or ultrasound markers (Antral Follicle Count (AFC)) are capable of predicting the occurrence of menopause in a group of normo-ovulatory female volunteers.
Summary answer: Even after adjusting for age at the time of inclusion, AMH remains significantly accurate in predicting time to menopause.
What is known already: The current study is an extension of a previously published study (Broer SL, 2011). By extending the follow up time from 11 to 15 years and thus analyzing a larger group of postmenopausal women, a more accurate analysis for predicting time to menopause could be performed.
Study design, size, duration: A long-term follow up study was conducted. 302 normo-ovulatory women aged 21–46 were included between 1983 and 2001. Follow up was performed between 2012 and 2013. 218 women remained eligible for analysis.
Participants/materials, setting, methods: At time of inclusion AMH, FSH, AFC were measured. At follow up, women were asked to fill out a questionnaire designed to determine cycle state (regular, menopausal transition, menopausal). Cox regression analysis was performed to measure predictive power of age and ovarian reserve tests (ORT) for time to menopause.
Main results and the role of chance: 88 women (40.37%) had reached menopause during the follow up. Univariate Cox regression analysis for predicting time to menopause using age and ORTs (AMH, FSH, AFC) showed that all variables significantly correlated with time to menopause. Multivariate Cox regression analysis, adjusting for age at the time of inclusion, proved AMH alone to remain significantly correlated with time to menopause.
Limitations, reason for caution: The database was composed by pooling 3 cohorts. The inclusion criteria for these cohorts were highly comparable. AMH was measured using 2 different assays performed in the same laboratory and both assay systems were later converted to the Gen II assay level. This conversion factor has been previously published.
Wider implications of the findings: Forecasting of menopause timing may allow for individual counseling on reproductive lifespan and individual adjustments in family planning to avoid age related infertility.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Unrestricted grant from Merck-Serono.
Trial registration number: Observational study with IRB approval in volunteers.

O-203 Ovarian reserve assessment in users of oral contraception seeking fertility advice on their reproductive time span

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Study question: To what extend does oral contraception (OC) impair ovarian reserve parameters in women who seek fertility assessment and counselling to get advice on their remaining reproductive time span?
Summary answer: Ovarian reserve parameters were found to be significantly reduced among users of OC compared to non-users. Anti Müllerian Hormone (AMH) and Antral Follicle Count (AFC) were decreased by 28% and 31%, respectively, whereas the reduction in ovarian volume ranged from 29–53%, being most pronounced in the group 35–39.9 years.
What is known already: AMH and AFC have proven to be reliable predictors of ovarian ageing and onset of menopause. In women, AMH declines with age and data suggests a clear relationship with remaining reproductive life span and age at menopause. OC may alter parameters related to ovarian reserve assessment, but the reduction in the various parameters is more uncertain.
Study design, size, duration: A prospective, population-based, cross sectional cohort study of the first 500 women aged 20–45 years seeking fertility counselling at the Fertility Assessment and Counselling Clinic (FACC) at Copenhagen University Hospital from 2011 to 2013. The study compared AMH, AFC and ovarian volume in users and non-users of OC.
Participants/materials, setting, methods: The FACC was initiated in order to provide individual fertility assessment and counselling. The first 500 consecutive women were included in the present study. All women were examined by a fertility specialist, who performed a transvaginal ultrasound (AFC, ovarian volume, pathology) uptake of a full reproductive history and AMH measurement.
Main results and the role of chance: Among the 500 women, the proportion of OC-users was 133 (26.6 %). The ovarian volume was markedly reduced in all OC-users ranging from 29% to 53% with the most pronounced reduction in the age group 35–39.9 years ($p < 0.0005$). In linear regression analyses with adjustment for age, AMH was 28% (95% CI 10–50%) lower and AFC was 31% (95% CI 17–46%) lower in OC-users compared to non OC-users. Furthermore, we found a significant decrease in antral follicles sized 5–7 mm ($p < 0.001$) and antral follicles sized 8–10 mm ($p < 0.0001$) among OC-users, but no decrease in antral follicles sized 2–4 mm ($p < 0.247$). The two groups (OC-users vs. non-OC-users) were comparable regarding age, BMI, smoking and maternal age at menopause.
Limitations, reason for caution: The study population consisted of women attending the FACC with a concern about their ovarian reserve and reproductive time span, which could imply a potential selection bias. Both AMH and AFC can be assessed independently of the cycle, but documentation of the accuracy in predicting residual reproductive time span is still needed.
Wider implications of the findings: Oral contraception has a major impact on the ovarian volume, and a moderate impact on AFC and AMH with a shift towards the small size in antral follicle subclasses. The most evident reduction occurs in the AMH producing follicles (5–7 mm and 8–10 mm follicles), which have the highest number of AMH secreting granulosa cells. Knowledge on these changes in ovarian morphology is important when using ovarian reserve parameters for counselling on reproductive time span.
Study funding/competing interest(s): Funding by national/international organization(s), the FACC was established in 2011 as part of the Reprosund

collaboration and is 50% co-financed by EU-regional funding. This study is also funded by the Capital Region Research Fund. There are no competing interests.
Trial registration number: The establishment of the Bio Bank is approved by the Scientific Ethical Committee (nr: H-1-2011-081).

O-204 Anti-Müllerian hormone (AMH) serum levels are correlated with the number of primary follicles in ovaries of female-to-male transgender persons

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[4]*University Hospital Ghent, Endocrinology, Gent, Belgium*

Study question: Are serum levels of anti-Müllerian hormone correlated with the number of follicles in ovaries of female-to-male transgender persons?
Summary answer: AMH serum levels are significantly positively correlated with the number of primary follicles found in ovarian tissue cortex of prelevated ovarian tissue.
What is known already: Anti-Müllerian hormone, expressed in granulosa cells, is a very interesting marker for ovarian reserve. AMH is currently used to evaluate the reproductive lifespan or to predict the ovarian response stimulation in IVF/ICSI cycli (Grynnerup et al., 2012).
Study design, size, duration: From April 2013 to January 2014, ovaries from female-to-male transgender persons were included after informed consent. At the moment of hysterectomy with bilateral oöphorectomy, hormone serum levels were determined. One piece of ovarian cortex (5 × 5 mm) was obtained per person, fixed in 4% buffered formalin and embedded in paraffin.
Participants/materials, setting, methods: Fifteen persons were included with a mean age of 25.8 ± 7.1 years. The cortical piece was serially sectioned at 5 µm and stained with haematoxylin/eosin. Follicles were classified according to Fortune (2003), and Pedersen and Peters (1986). Statistical analysis was performed using Spearman correlation tests.
Main results and the role of chance: AMH serum levels are significantly positively correlated ($p = 0.022$) with the number of primary follicles ($n = 230$, mean per person = 15.3). There is no correlation with the total number of follicles ($n = 1170$, mean per person = 78), the number of primordial ($n = 641$, mean per person = 42.7) or secondary follicles ($n = 20$, mean per person = 1.3) (respectively $p = 0.087$; $p = 0.349$; $p = 0.955$). The correlation with the number of antral follicles could not be studied as no antral follicles were found in the randomly selected ovarian tissue strips. A significantly negative correlation with age is confirmed ($p = 0.03$).
Limitations, reason for caution: Although we did not find a significant correlation with serum levels of total testosterone, free testosterone nor with sex hormone binding globulin, the influence of hormonal treatment in transgender persons on AMH serum levels or ovarian follicle reserve is unknown.
Wider implications of the findings: Although AMH is believed to play a role in keeping primordial follicles in a dormant state, in our study AMH serum levels do not mirror the size of the resting primordial follicle pool, which is in contradiction with previous findings in mouse and monkey ovaries (Kevenaar et al., 2006, Appt et al., 2009). Therefore, low serum AMH-levels do not exclude the possibility of ovarian preservation on oncological patients.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Ghent University Hospital - Ghent University.
Trial registration number: Not applicable.

O-205 The relationship between follicle-stimulating hormone receptor polymorphism status and ongoing pregnancy in IVF; a large retrospective observational study

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Study question: Is there an association between follicle-stimulating hormone receptor (FSHR) gene polymorphism at position 680 and ongoing pregnancies in women undergoing IVF/ICSI?
Summary answer: In this largest study so far the homozygous Ser/Ser genotype of FSHR polymorphism at position 680 is associated with a reduced ovarian response to ovarian stimulation in IVF/ICSI, while Asn/Asn genotypes showed a significantly lower pregnancy rate.
What is known already: FSH is essential for normal reproductive function and is essential for oocyte maturation. Its action is mediated by the follicle-stimulating hormone receptor. In women undergoing ART, the response to FSH is highly variable. There is a need for identification of predictive markers of ovarian response due to this individual variability. Predicting the ovarian response is the main step towards developing individualized treatments with better effectiveness and improved safety. Several studies investigated the relationship between FSHR genotypes and ovarian stimulation albeit only in small groups, but results are conflicting.
Study design, size, duration: In this retrospective observational study data was collected from the Electronic Patient Database of the VU University Medical Centre, Amsterdam, The Netherlands. This database contained all treatment cycles from January 2008 till January 2012. The study population consisted of 1173 women.
Participants/materials, setting, methods: Women starting their first standard IVF/ICSI ovarian stimulation cycle, of whom the FSHR genotype was determined, were included. Three groups of patients were classified according to the three different FSHR 680 genotypes of asparagine (Asn) and/or serine (Ser); Asn/Asn, Asn/Ser and Ser/Ser polymorphisms. The primary outcome was ongoing pregnancy rate. Secondary outcomes were total number of follicles, oocytes, embryos and top quality embryos.
Main results and the role of chance: The FSHR genotype distribution was as follows; 331 women in the Asn/Asn group (28.1%), 610 in the Asn/Ser group (52.0%) and 232 in the Ser/Ser group (29.8%). Patient characteristics did not differ significantly, except for the higher basal FSH in the Ser/Ser group ($p = 0.005$). The number of oocytes ($p = 0.01$) and number of embryos ($p = 0.03$) were significantly lower in the Ser/Ser group. The Asn/Asn group showed a significantly lower clinical and ongoing pregnancy rate. Ongoing pregnancies were 21.1% versus 30.2% and 25.9% ($p = 0.01$), for respectively Asn/Asn, Asn/Ser and Ser/Ser. Logistic regression analysis showed a significant lower chance on ongoing pregnancy in the Asn/Asn group compared to patients in the Asn/Ser and Ser/Ser group ($p = 0.03$).
Limitations, reason for caution: The strengths of this study is the large sample size and the use of treatment naive women (only first cycle IVF/ICSI were included). A limitation of this study was the retrospective aspect.
Wider implications of the findings: This large retrospective study reveals that FSHR gene polymorphism at position 680 is associated with a different ovarian response to controlled ovarian stimulation. These data may implicate that a natural modest response with a lower sensitive receptor may have advantages over a higher sensitive receptor. Negative effects of high ovarian stimulation may result in increased number of poorer oocytes or adverse effects on the endometrium development with a low pregnancy rate as a result.
Study funding/competing interest(s): Funding by University(ies), SWOG: Stichting Wetenschappelijk Onderzoek Gynaecologie (Gynaecological Research Foundation)
Trial registration number: N/A.

O-206 Ovarian response in BRCA1 carriers: a case-control study

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Study question: To assess ovarian response to conventional ovarian stimulation (COS) as part of a pre-implantation genetic diagnostic (PGD) treatment in BRCA1 mutation carriers.
Summary answer: BRCA1 mutation is not associated with a reduced response to ovarian stimulation.
What is known already: Loss-of-function mutations in the tumour suppressor genes BRCA1/2 are associated with an increased lifetime risk of developing breast- and/or ovarian cancer. In more recent years it has been hypothesized that germline mutations in BRCA-genes may be associated with in fertility-related problems. This is because BRCA genes participate in repair and maintenance of chromosome telomeres and the integrity of those is central in reproductive wellbeing.

Study design, size, duration: A retrospective, case-control, non-randomised and mono-centre study. In the study group and both control groups 39 first treatment cycles were included (13:13:13). They were matched (1:1) for ovarian stimulation protocol, starting dose of gonadotrophins, for age ±3 years and pick-up date ±1 year. The study period covered 2006–2013.
Participants/materials, setting, methods: Academic PGD reference centre. 13 BRCA1 cases (group A) stimulated in an antagonistic scheme with starting dose of 150 units of gonadotrophins, matched to two control groups, group B, patients for PGD for monogenic disorders not associated with infertility, and group C patients for ICSI and blastocyst transfer.
Main results and the role of chance: Statistical analysis was performed using SPSS software (version 22.0). Numerical variables were compared using Mann-Whitney U-test, continuous variables were analysed by Fisher's exact test. Compared with the control groups, B1 and B2, BRCA patients do not produce a significant lower amount of mature oocytes (A: 10.077 (SD:5.6); B: 11.62 (SD:5.1); C:9.308 (SD:5.9)) and no significant difference was found in the total dose of gonadotrophins used in the three groups to achieve this result (A: 1431 (SD:257) U; B:1450 (SD:303) U; C:1315(SD:264) U). No difference in pregnancy rate was found; the pregnancy rate in all groups was 23.1%. Overall there are no significant differences in outcome parameters between the groups compared.
Limitations, reason for caution: Although this series is larger than those previously reported, the numbers remain small and more extensive studies are required. The retrospective character of the study reflects clinical practice, however does not exclude selection bias.
Wider implications of the findings: Contrary to recent studies that have suggested that BRCA1 mutations are associated with an earlier menopause and occult primary ovarian insufficiency, our study did not confirm reduced ovarian response. This implies that BRCA patients presenting for IVF and/or PGD can use conventional stimulation schedules.
Study funding/competing interest(s): Funding by hospital/clinic(s), Universitair Ziekenhuis Brussel.
Trial registration number: N/A.

O-207 Transfer of frozen embryos in an artificial cycle after a failed long Gn-Rh agonist protocol: hormonal profiles of 2 separate transfers in one month

C. Avril[1], A. Finet[1], V. Grzegorczyk-Martin[1], J. Roset[1]
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Study question: Is there persistent pituitary suppression after failed fresh embryo transfer with a long acting Gn-Rh agonist protocol which could allow cryo-preserved embryo transfer (FET) in an artificial cycle with adequate endometrium and embryo implantation rates (IR)?
Summary answer: Pituitary suppression was observed for all the patients and an appropriate endometrium was obtained for 57/60 patients. This protocol led to 28% IR, not statistically different from fresh embryo transfer IRs.

2 separate transfers over a one-month period permit a high mono foetal pregnancy rate in a record time.
What is known already: For couples, the key issue is the time to obtain pregnancy which is the real obstacle to a single embryo transfer.

An artificial cycle is an effective preparation of the endometrium for FET. When fresh embryo transfer cycles fail to yield pregnancies, another injection of long acting agonists is usually administered before FET.
Study design, size, duration: Prospective cohort study: 128 women with cryopreserved embryos after a long agonist protocol From October 2012 to June 2013.
Participants/materials, setting, methods: Hormone levels were measured before and during the cycle for FET, following failed long agonist protocols (Triptoreline LP 3 mg on cycle day 1, followed by gonadotrophin day 15–25, luteal support by progesterone 400 mg intra-vaginally). FET artificial cycles began 7 days after stopped progesterone, by administration of 6 mg oestradiol valerate.
Main results and the role of chance: 170 fresh embryos were transferred (1.29 ± 0.61 per cycle) leading to 54 singleton pregnancies, and one twin (IR 0.32).Of the 74 not pregnant, FET was delayed for 6 due to closing of the center and 4 due to patient's choice. On starting day of oestradiol, desensitisation was persistant for all: E_2:33.87 ng/ml ± 10.94; LH:1.18 mUI/ml ± 0.9; Progesterone: 0.365 pg/ml ± 0.20; endometrial thickness: 1.655 mm ± 0.755. On starting day of progesterone, no premature progesterone elevation was observed: E_2 352.43 ng/ml ± 235.19; LH 3.28 mUI/lm ± 2.61; progesterone 0.45 pg/ml ± 0.38; endometrial thickness: 8.26 mm ± 1.57.60 FET were performed. 78 cryopreserved embryos were transferred (1.3 ± 0.65 per transfer) resulting in 20 singleton pregnancies, 2 twin pregnancies (IR: 0.28). The cumulative pregnancy rate was 57.8% with a twin rate of 3.9%.
Limitations, reason for caution: - Is the Gn-Rh-persistent desensitization responsible for the hormonal profiles and the embryo implantation rates observed, or can other protocols give similar results? - FET after a long-agonist protocol may delay closing of IVF centers during closing periods.
Wider implications of the findings: Couples easily agree to a limited number of embryos transfered when they know they have the opportunity to undergo FET in the near future. This transfer strategy reduces twin rates without losing time. The long-agonist protocol was studied here because it is widely used; it was therefore logical to study its desensitization role in FET artificial cycles. Future studies are ongoing to understand how to manage artificial cycles immediately after failed fresh embryo transfers using antagonists.
Study funding/competing interest(s): Funding by hospital/clinic(s), Clinique Mathilde, centre d'assistance médicale à la procréation, 7 boulevard de l'Europe, 76100 Rouen, France
Trial registration number: Comité d'éthique, Clinique Mathilde.

O-208 ESHRE guideline: Management of women with Premature Ovarian Insufficiency

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Introduction: Premature/Primary ovarian insufficiency was first described in 1942 and has, since then, been described with different names and definition. Premature ovarian insufficiency is a clinical syndrome characterised by menstrual disturbances, raised gonadotrophins and low estradiol in women below the age of 40. There are multiple possible causes of POI, including genetic abnormalities, autoimmune disorders and iatrogenic causes.
Methodology: In 2011, a multidisciplinary group of European experts, including gynaecologists, endocrinologists, a paediatrician, psychologist, bone specialist and a patient representative started the development of a guideline on the management of POI. Following the 12-step process described in the ESHRE manual for guideline development, the group started by scoping the guideline and drafting 29 key questions on the management of women with POI to be answered in the guideline. A thorough literature search was performed, evidence tables were drafted and quality assessment was taken into account in a process of gathering all the relevant information from the literature to answer the different key question. Finally, recommendations were written based on the collected evidence, benefits and harms, the preferences of the patients and the expertise of the guideline group members.
Results: The guideline on the management of women with Premature Ovarian Insufficiency consists of clinical evidence and recommendations on diagnosis and initial assessment of POI. The guideline also explores the consequences of POI for fertility, bone health, cardiovascular health, quality of life, sexuality, and neurological health and described the management options for each of these consequences. All the information on the management of symptoms

and consequences of POI, is summarized in a chapter on treatment options, including hormone replacement therapy, and alternative treatments. Finally, life expectancy of women with POI and implications their relatives are described.
Conclusion: The final draft of the guideline on the management of women with Premature Ovarian Insufficiency will be presented. The next step in the process of guideline development is an extensive review by the stakeholders and future users of the guideline.

SELECTED ORAL COMMUNICATION SESSION

SESSION 55: GENES AND EMBRYO DEVELOPMENT

Wednesday 2 July 2014 **10:00 - 11:45**

O-209 RNA sequencing provides new insights into the timing of embryonic genome

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Study question: During maternal-to-embryonic transition control of embryonic development gradually switches from maternal RNAs and proteins stored in the oocyte to gene products generated after embryonic genome activation (EGA). Detailed insight into the onset of embryonic transcription is obscured by the presence of maternal transcripts. Using the bovine model system, we established by RNA sequencing a comprehensive catalogue of transcripts in germinal vesicle and metaphase II oocytes, and in embryos at the 4-cell, 8-cell, 16-cell and blastocyst stages.
Summary answer: By combining a dedicated cross breeding design of *Bos taurus taurus* × *Bos taurus indicus* with the sensitivity and single nucleotide resolution of RNA-Seq, we created a basis for detailed analyses of oocyte maturation, early embryo development and expression of parental alleles. We established novel strategies for identification of *de novo* transcribed RNAs, providing detailed insight into the timing of activation of almost 8,000 genes during early bovine embryo development.
What is known already: EGA occurs in several waves, and the timing of major EGA is species dependent: it occurs at the 2-cell stage in mouse embryos, at the 4- to 8-cell stage in human and pig embryos, and at the 8- to 16-cell stage in bovine embryos. At the time of EGA, both maternal and embryonic transcripts are present in the embryo, thus hampering a precise mapping of the onset of embryonic expression of specific genes. First insight into the timing of global EGA came from incorporation studies of radiolabeled uridine triphosphate (UTP). However these studies did not provide the resolution of activation of individual genes.
Study design, size, duration: We used high throughput sequencing to generate comprehensive transcriptome profiles of oocytes and early embryos.
Participants/materials, setting, methods: These were produced by *in vitro* fertilization of *Bos t. taurus* oocytes with sperm from a *Bos t. indicus* bull to facilitate parent-specific transcriptome analysis.
Main results and the role of chance: Transcripts from 12.4 to 13.7×10^3 different genes were detected in the various developmental stages. EGA was analyzed by i) detection of embryonic transcripts which are not present in oocytes; ii) detection of transcripts from the paternal allele; and iii) detection of primary transcripts with intronic sequences. These strategies revealed (i) 220, (ii) 937, and (iii) 6,848 genes to be activated from the 4-cell to the blastocyst stage. The largest proportion of gene activation, i.e. (i) 59%, (ii) 42%, and (iii) 58%, was found in 8-cell embryos, indicating major EGA at this stage. Gene ontology analysis of genes activated at the 4-cell stage identified categories related to translation, RNA processing and transport, consistent with preparation for major EGA.
Limitations, reason for caution: Notably, the results of the three methods to detect the onset of gene expression were consistent with respect to the timing of minor EGA at the 4-cell stage and major EGA at the 8-cell stage; however the absolute numbers of activated genes detected were rather different. This is due to the facts that i) method 1 covered only genes which are not transcribed in oocytes; and ii) method 2 relied on SNPs distinguishing the parental alleles, which were - in our experiment - found only in about 20% of the known bovine genes. Thus, method 3 based on the presence of primary transcripts identified the largest proportion of activated genes. Nevertheless, the results of method 2 and 3 were remarkably concordant. The limitations of our study in detecting all activated genes might be overcome by labeling and enriching nascent RNA and by increasing the sequencing depth.
Wider implications of the findings: Gene expression profiling is widely used to get insight into mechanisms of early embryonic development and to characterize embryos generated by various techniques or exposed to different culture conditions. RNA sequencing in bovine oocytes and embryos facilitated for the first time mapping of the onset of embryonic expression for almost 8,000 genes. The timing of embryonic gen(om)e activation offers an additional level of information for embryo biosystems research and for detecting disturbances of early development due to genetic, epigenetic, and environmental factors.
Study funding/competing interest(s): Funding by national/international organization(s), Deutsche Forschungsgemeinschaft, EU (PLURISYS, FECUND).
Trial registration number: FOR 1041.

O-210 PGS results in cycles producing day 5 and day 6 blastocysts

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Study question: To determine the chromosome status as well as implantation potential of embryos reaching blastocyst either on day-5 or day-6, in PGS cycles producing both types of embryos and resulting in SET.
Summary answer: Chromosome abnormalities were significantly higher in day-6 than day-5 blastocysts, but 42% of day-6 were still euploid. Implantation rates of day-6 blastocyst were similar to day-5.
What is known already: Some centers produce more day-6 blastocysts than others depending on their culture conditions and stimulation. Time-lapse studies suggest that delayed morula formation is linked to aneuploid and lower implantation potential. These observations could be center-dependent, and have not been focused on centers producing a sizable number of day 6 blastocysts.
Study design, size, duration: Cohort study. Between 1/1/2013 and 6/1/2013, 373 cycles underwent PGS at three fertility centers, and of those we selected for this study 71 cycles (17.8%) that produced both day-5 and day-6 blastocysts.
Participants/materials, setting, methods: Embryos were biopsied at blastocyst stage either on day-5 or -6, vitrified, and the biopsies analyzed by array CGH. SET of warmed euploid embryos was performed and pregnancies followed up. Euploid day-5 embryos were prioritized for replacement if both euploid day-5 and -6 were available.
Main results and the role of chance: Of 311 day-5 embryos 56.6% were euploid, 33.4% aneuploid (one or two aneuploidies), 7.4% complex abnormal (>2 aneuploidies), and 2.6% not analyzable, compared to 42%, 44.6%, 11.5%, and 1.9%, for day 6 embryos ($n = 157$), respectively. The euploidy rate between day-5 and day-6 embryos ($p < 0.025$) was significantly different.

Of the 71 cycles, 30 had only day-5 euploid blastocysts, 27 had euploid day-5 and day-6 blastocysts, 4 had only day-6 euploid blastocysts, and 10 had no euploid blastocysts.

There were 11 cycles with no normal embryos, 15 cycles that banked the embryos and have not yet been replaced, 39 with one day-5 blastocysts replaced resulting in 20 pregnancies (51%) and 6 with a day-6 embryos replaced, resulting in 3 pregnancies (50%).
Limitations, reason for caution: Some biases were eliminated by including similar numbers of cycles from each center ($n = 24, 23, 23$), and by only including from SET cycles producing day-5 and day-6 blastocysts. The major limitation was the low number of cycles with a day-6 embryo replaced.
Wider implications of the findings: Day-6 embryos are worth biopsy since 42% of them are still euploid, and when replaced in a warmed cycle they implant at similar rates as day-5 embryos. Time-lapse selection of embryos based on late morula formation may discard embryos with potential to implant.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Reprogenetics.
Trial registration number: Not applicable.

O-211 Analysis of clinical outcome performing fresh or vitrified-warmed blastocyst transfer after trophectoderm biopsy in 307 Preimplantation Genetic Screening with array comparative genomic hybridization cycles

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Study question: The aim of this study is to retrospectively compare clinical outcomes of fresh versus frozen/thawed Embryo Transfer (ET) in 307 cycles with Preimplantation Genetic Screening (PGS) cycles with array comparative genomic hybridization (aCGH) performing fresh or frozen-thawed blastocyst transfer.
Summary answer: In PGS-aCGH cycles, fresh or frozen/thawed biopsied blastocysts give similar clinical pregnancy and implantation rates. Cryopreservation of blastocyst after trophectoderm biopsy does not interfere with clinical outcomes.
What is known already: PGS with trophectoderm biopsy is becoming a frequent strategy in infertility centers. When applying aCGH, a time of approximately 24 h is needed for the genetic results. In some occasions it is not possible to perform a fresh transfer and blastocyst cryopreservation is necessary. Blastocyst vitrification/warming procedure give very high survival and implantation rates. Therefore, the combined use of PGS-aCGH and cryopreservation could be used for achieving good clinical results.
Study design, size, duration: The outcome of 307 Preimplantation Genetic Screening cycles performed from September 2012 to October 2013 with trophectoderm biopsy and aCGH were retrospectively analyzed. Mean female age was 36.6 ± 4.20 years. One-hundred-twelve fresh and 129 vitrified-warmed euploid blastocysts were transferred in 103 and 111 cycles respectively.
Participants/materials, setting, methods: When blastocyst stage was achieved on day-5, the biopsy was performed and fresh ET was possible on day-6 after receiving the genetic results. When blastocysts were obtained later (day-6 or 7), ET was delayed to a new natural cycle and blastocysts were individually vitrified after the biopsy.
Main results and the role of chance: A total of 3153 oocytes were collected, 2500 MII were injected and 1946 fertilized (77.8%). On day-3, 1946 embryos were obtained, 1671 (85.9%) were of excellent-good quality. On day-5-6-7, a total of 1035 blastocysts were obtained (53.2%) and all of them were biopsied. The genetic result was available for 1017 (98.3%) of them and 341 (32.9%) resulted euploid. In 103 cycles it was possible to perform a fresh transfer of 112 blastocysts. The clinical pregnancy and implantation rates were 54.4% and 52.7% respectively ($N = 56$ and $N = 59$). In 111 cycles, ET were performed with 129 vitrified-warmed blastocysts. Clinical pregnancy and implantation rates were 56.8% and 53.5% respectively ($N = 63$ and $N = 69$). All warmed blastocysts survived. No statistical differences were observed between the two groups.
Limitations, reason for caution: It has been reported that a late transfer on day-6 of an expanded blastocyst can lead to a reduced success rate due to an asynchrony with endometrial receptivity. In addition, some country have limitations in blastocyst cryopreservation for legal reasons. Finally, some blastocysts could not survive after warming.
Wider implications of the findings: Several studies comparing fresh versus vitrified-warmed blastocysts show controversial results. The advantage of cryopreserved transfer is that there is a better synchronization with endometrium during a natural cycles, where the estradiol levels are physiological. Anyway, the fresh embryo transfer is less expensive than embryo replacement after cryopreservation and have not legal issue but, when the blastulation start later than day-5, cryopreservation become indispensable. The data of the present study demonstrate that both procedures are efficient and can be applicable.
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 declared.
Trial registration number: Not applicable.

O-212 Morphology dynamics of multinucleation affects embryo chromosome content

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Study question: Does multinucleated embryos in 2 cells stage present higher aneuploidy rate than those multinucleated in 4 cells?
Summary answer: Embryos with at least one blastomere multinucleated (more than two nuclei per cell) in two cells stage are more frequent and present lower aneuploidy rate than embryos where multinucleation is present in the four cells stage. Multinucleation in 2 cells stage seems to be a transitory event in IVF embryos.
What is known already: Multinucleation is a relatively frequent event in those embryos generated in IVF laboratories, which has been related with abnormal embryo development and increased aneuploidy rate, although there are live births from multinucleated embryos. It has been widely analyzed in 4 cells embryos. Time-lapse incubation allows identifying it during early development without compromising embryo viability. Recent studies point that it is a transitory event.
Study design, size, duration: Retrospective study of 564 embryos from 96 PGD patients incubated in a time lapse incubator 37°C 6% CO2 atmosphere and analyzed by arrays CGH (24 chromosomes) between September 2011 and January 2014 in IVI VIGO.
Participants/materials, setting, methods: Embryos over 6 cells on day 3 were biopsied for CHG-arrays analysis. They were classified as normal or abnormal according to the genetic results. Multinucleation was annotated in 2 cells and 4 cells stage as NM = no multinucleation, and M = multinucleated. χ^2-t and t-test were performed.
Main results and the role of chance: 127 embryos were M in 2 cells stage (21.8%), whereas 28 were normal (22.0%). 437 embryos were NM in 2 cells, being 123 normal (28.1%) ($p = 0.172$). Considering multinucleation in 4 cells stage, 71 out of 564 were M (12.5%), whereas 9 were normal (12.6%), 493 embryos showed no multinucleation on 4 cells, being 142 of them normal after array CGH (28.8%) ($p = 0.004$).
Limitations, reason for caution: Data analysis is based in a retrospective analysis, no description of the complexity of the chromosomal abnormality is reported. The number of multinucleated embryos is reduced.
Wider implications of the findings: These findings confirm that multinucleation should be employed as an exclusion criteria for embryo selection in 4 cells stage embryos, while it should not be consider detrimental for 2 cells stage embryos, enhancing embryo selection in those circumstances when it should be done on early stage.
Study funding/competing interest(s): Funding by hospital/clinic(s), IVI.
Trial registration number: Not applicable.

O-213 Vitrified embryo transfer (VFET) success of euploid blastocysts (BL) is similar among morphologic quality grades

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Study question: Does the morphological size or quality grade of Day5 or Day6 euploid BL at the time of biopsy significantly influence pregnancy outcomes?
Summary answer: There was no difference in the euploid status or implantation rate of Day5 versus Day6 biopsied embryos. Furthermore, blastocoele size and quality grade had no significant effect. However, a trend toward lower implantation and higher spontaneous miscarriages was observed among Day6 Full BL and BL with 'B' quality trophectoderm (TE).
What is known already: The integration of array CGH-PGS technology into an establish BL culture program is best achieved by vitrification mediated delayed implantation of euploid embryos into normal, unstimulated uterine conditions. The relative importance of TE or inner cell mass (ICM) grades influencing fresh non-PGS cycles outcomes have been mixed. A recent report (ESHRE 2013) contested that an 'A' quality TE grade is more predictive of pregnancy success than an 'A' quality ICM.
Study design, size, duration: 244 PGS cycles and 196 VFET embryo transfer cycles were evaluated between mid-2011–2013. The objective was to determine whether BL development and ICM/TE quality of Day5 or Day6 embryos correlated to implantation success. Differences were determined by Chi-square analysis ($P < 0.05$).
Participants/materials, setting, methods: Embryos underwent Day3 laser zona ablation, followed by Day5/6 BL dissection of protruding TE. Biopsied cells were analyzed by array-CGH (Genesis Genetics). Prior to biopsy, BLs were morphologically graded (Gardner method: 3–6/A or B ICM/TE grade), and then vitrified post-biopsy by MicroSecure vitrification using non-DMSO solutions (Innovative Cryo Enterprises).
Main results and the role of chance: BL development (65%) resulted in 82% being of sufficient quality for biopsy (grade 3–6 and AA, AB, BA, or BB),

and 99.2% cryosurvival upon VFET. There was no difference in the euploidy, implantation or miscarriage rates, respectively, of Day5 (50.6%, 79.4%, 2.6%) or Day6 (49.8%, 77.5%, 8.1%) BL. Spontaneous miscarriage rates were highest among cycles transferring Day6 BB (15.3%, n = 17 VFET) or AB (33.3%, n = 10 VFET) quality BL. The majority of VFET cycles (n = 139) used AA quality BL with high implantation potential (82%) and low loss rates (3.5%).
Limitations, reason for caution: A patients' highest quality, euploid BL were selected for VFET, with Day5 (n = 117) preferred over Day6 (n = 79), resulting in a disproportionately low number of non-AA quality BL cycles (n = 57) to interpret the significance of suboptimal trends.
Wider implications of the findings: Extremely high implantation and pregnancy success can be achieved incorporating a PGS/VFET treatment plan, independent of the developmental progress and quality of the euploid BL vitrified. It is anticipated however, that a larger database will further reveal that Day 6 euploid BLs with fair quality TE will have less ability to sustain a healthy pregnancy. Overall, these data suggest that time-imaging analysis alone, without euploidy determination, is unlikely to attain similar pregnancy outcomes.
Study funding/competing interest(s): Funding by hospital/clinic(s), SCCRM.
Trial registration number: Not applicable.

O-214 Analysis of morphology, morphokinetic and ploidy status in 1035 biopsied blastocysts

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Study question: The aim of this study was to evaluate possible relationships among blastocyst ploidy status, standard morphology evaluation and time-lapse kinetics, in Preimplantation Genetic Screening (PGS)-array comparative genomic hybridization (aCGH) cycles.
Summary answer: A statistically higher percentage of top quality inner cell mass and trophectoderm cells was found in euploid compared to aneuploid blastocysts. In addition, euploid blastocysts showed higher expansion grades than aneuploid ones. Finally, euploid blastocysts started to blastulate, expand and hatch faster than aneuploid ones
What is known already: Embryo quality has been always considered an important predictor of implantation and pregnancy. Nevertheless, knowledge of the relative impact of each morphological parameter at the blastocyst stage needs to be increased. Recently, with the introduction of time-lapse technology morphokinetic parameters can also be evaluated.
Study design, size, duration: The morphology of 1035 blastocysts obtained in 307 PGS cycles performed from September 2012 to October 2013 with trophectoderm biopsy and aCGH were retrospectively analyzed. Mean female age was 36.6 ± 4.20 years old; 726 blastocysts were cultured in a time-lapse incubator allowing the morphokinetic parameters to be analyzed.
Participants/materials, setting, methods: For standard morphology, expansion grade (EXP, 1–6), quality of inner cell mass (ICM, A–C) and trophectoderm cells (TE, A–C) were analyzed. The morphokinetic parameters observed were: IIPB extrusion, 2PN appearance, pronuclear fading, onset of 2- to 8-cell divisions, time between 3- and 4-cell stage (s2), blastulation, expansion and hatching timing.
Main results and the role of chance: Euploid blastocysts with grade-A ICM were 179/432 = 41.4%, grade-B ICM were 115/360 = 31.9% and grade-C ICM were 43/179 = 24.0% (A vs. B: $p < 0.01$; A vs. C $p < 0.001$). Euploid blastocysts with grade-A TE were 171/408 = 41.9%, grade-B TE were 95/297 = 32.0% and grade-C TE were 71/266 = 26.7% (A vs. B: $p < 0.01$; A vs. C: $p < 0.001$). Sixty-four blastocysts were not expanded enough. Regarding expansion, percentages of euploid blastocysts were 18/64 = 28.1% for grade-2, 10/52 = 19.2% for grade-3, 32/116 = 27.6% for grade-4, 25/764 = 33.9% for grade-5 and 18/39 = 46.2% for grade 6 (2–3 vs. 5–6; 4 vs. 6 $p < 0.05$). Blastulation, expansion, hatching and s2 timing were 109.6 ± 10.4, 115.8 ± 10.3, 121.1 ± 12.8 and 1.6 ± 2.8 in euploid and 111.4 ± 10.6, 119.3 ± 12.4, 124.6 ± 13.5 and 3.4 ± 4.9 in aneuploid blastocysts ($p < 0.001$ for s2 $p < 0.05$ for the rest). No statistical differences were found for the remaining morphokinetic parameters.
Limitations, reason for caution: The main limitation of morphology assessment is that it is a static system and can be operator-dependent. The main limitations of the time-lapse technology is that it is impossible to rotate the embryos making it very difficult to observe in case of blastomere overlapping or elevated cytoplasmic fragmentation.
Wider implications of the findings: Although there seems to be a relationship between the ploidy status and blastocyst morphology/development dynamics, morphological and morphokinetics evaluation cannot replace PGS. However time-lapse monitoring could be used in conjunction with PGS to choose, within a cohort, the blastocysts to analyze. It could also represent a valid alternative to genetic analysis when PGS is not applicable for legal or personal reasons.
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 declared.
Trial registration number: Not applicable.

O-215 Next generation sequencing reveals distinct differences in global gene expression profiles in non-implanted blastocysts and blastocysts resulting in live birth

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Study question: Do global gene expression profiles differ between non-implanted embryos and embryos resulting in live birth?
Summary answer: From the set of 181 genes that were the most differentially expressed, 145 genes were upregulated in trophectoderm (TE) biopsies from embryos that did not implant ($TE^{(-)}$ biopsies), whereas only 36 genes showed an upregulation in the TE samples from embryos resulting in live birth ($TE^{(+)}$ biopsies).
What is known already: Results obtained from animal models point towards the existence of a gene expression profile that is distinguishably different in viable embryo compared with non-viable embryos. Knowledge of human embryo transcripts is limited, in particular with regard to a relation between gene expression and clinical outcome.
Study design, size, duration: Embryos from infertile couples presenting for infertility treatment at the Fertility Clinic Aarhus University Hospital. TE biopsies were obtained on Day 5 and the embryos transferred on Day 6
Participants/materials, setting, methods: RNA from three biopsies ($TE^{(+)}$) obtained from blastocysts resulting in live birth and three biopsies ($TE^{(-)}$) obtained from non-implanting blastocysts was subjected to genome wide sequencing. qPCR analyses was performed on a set of independently collected TE biopsies.
Main results and the role of chance: All reads were mapped to the human reference genome (hg19) and filtered. RPKM values were calculated and analysed. Using a set of filtering criteria, we obtained a list of 181 genes that were the most differentially expressed. When these genes were grouped as specific to either $TE^{(+)}$ or $TE^{(-)}$ biopsies, it showed that almost 80% (145 genes) of the genes were upregulated in $TE^{(-)}$ biopsies, whereas only 20% (36 genes) showed an upregulation in the $TE^{(+)}$ samples compared to the $TE^{(-)}$ biopsies. The RNA sequencing results were supported by qPCR of three selected genes (*DNAJC1*, *SPEN* and *CALR*) that were upregulated in the $TE^{(-)}$ biopsy compared to the $TE^{(+)}$ biopsy.
Limitations, reason for caution: Results need to be confirmed on a larger set of biopsies.
Wider implications of the findings: The distinct differences between expressed genes in implanted and non-implanted embryos that were morphologically similar suggest the presence of functional, but visually undetectable differences. Our study contribute with new knowledge of which gene-families plays an important role in implantation
Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), Funding by commercial/corporate company(ies), the study was conducted with support from Aarhus University, The Danielsen Foundation, AP Møller Foundation, Lipperts Foundation, Segels Foundation, The Toyota Foundation. Research at the Fertility Clinic, Aarhus University Hospital is supported by an unrestricted grant from MSD and Ferring.
Trial registration number: The study was part of a study registered at ClinicalTrial.gov with accession number NCT01139268.

SELECTED ORAL COMMUNICATION SESSION

SESSION 56: STEM CELLS & TRANSLATIONAL RESEARCH

Wednesday 2 July 2014 **10:00 - 11:45**

O-216 Assessment of cellular heterogeneity in the level of mitochondrial DNA heteroplasmy in mouse embryonic stem cells

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Study question: Is there any heterogeneity in the level of mitochondrial DNA (mtDNA)-heteroplasmy among individual colonies (intercolony) and at the single cell level (intracolony) in mouse embryonic stem cells (ESCs), both in pluripotent and differentiated states?, and is there any correlation between ESCs and cells from pre-implantation embryos, in terms of mtDNA-heteroplasmy?
Summary answer: ESCs exhibit variability in the level of mtDNA-heteroplasmy between individual colonies and also at the single cell level, both in differentiated and undifferentiated states. Intracolony heterogeneity is more divergent than intercolony heterogeneity. mtDNA-heteroplasmy in ESCs is closer to trophectoderm (TE) than to second polar body (2PB) of the founder embryo.
What is known already: MtDNA-heteroplasmy is a condition when more than one type of mtDNA co-exists within a cell. Intercellular variation of heteroplasmic load is frequently seen in individuals with mtDNA mutations. However, it is unresolved how these differences between somatic cells arise and how heteroplasmic mtDNA segregates between daughter cells. To our knowledge, this is the first report showing heterogeneity in mtDNA-heteroplasmy level between and within ESC colonies before and after differentiation and their correlation with parent embryos.
Study design, size, duration: Intercolony heterogeneity was analyzed in ESCs at different passages until 40th passage (n = 137) and also in differentiated cells after retinoic acid (RA) stimulated differentiation (n = 42). Heterogeneity in 100 single cells from both ESCs and embryoid bodies was investigated. MtDNA-heteroplasmy levels were compared between ESCs and their corresponding 2PB and TE.
Participants/materials, setting, methods: *In-vivo* fertilized zygotes were recovered from heteroplasmic BALB/cOlaHsd mice. ESCs were derived from blastocysts previously subjected to 2PB biopsy and TE biopsy. The coefficient of variation (CV) in the level of mtDNA heteroplasmy was determined in ESC colonies and in single cells before and after differentiation. $P < 0.05$ was considered significant.
Main results and the role of chance: Heteroplasmic ESCs were derived from five embryos previously subjected to second PB and TE biopsy. The CV in pluripotent ESC colonies was 3.7%, 11.6%, 12.5%, 14.7% and 7.4% in lines 1 through 5, respectively. After RA mediated differentiation, the CV in these lines was 2.8%, 21.9%, 12.0%, 0.6% and 35.7% respectively. At the single cell level, bigger divergence was observed, before (L1 = 16.0%, L2 = 25.2%, L3 = 27.3%, L4 = 12.5%, L5 = 14.6%) and after differentiation (L1 = 38.0%, L2 = 12.4%, L3 = 26.9%, L4 = 39.1%, L5 = 36.4%) compared to the colonies. In line 3, level of mtDNA heteroplasmy in undifferentiated and differentiated single cells was significantly lower compared to that in the colonies. The level of mtDNA heteroplasmy in ESCs was closer to the TE (r = 0.6) than to the second PBs (r = 0.3) of the founder embryo.
Limitations, reason for caution: These results in a neutral polymorphic mouse model should be extrapolated to mtDNA mutation disorders in humans only with caution.
Wider implications of the findings: Our results support a large degree of heterogeneity at the single cell level in ESCs in terms of mtDNA heteroplasmy. Unbalanced mtDNA segregation in pluripotent cell populations during subsequent cell divisions may lead to alterations in the level of mtDNA heteroplasmy in future somatic tissues. Our research may serve to gain insight into the functional regulation of mtDNA heteroplasmy in humans with mitochondrial disorders.
Study funding/competing interest(s): Funding by University(ies), This research was funded by the Special Research Fund (Doctoral mandate, JN-01D05611) from Ghent University. No competing interests declared.
Trial registration number: Not applicable.

O-217 Stable non-random X chromosome inactivation in human embryonic stem cells: culture artefact rather than a parent-of-origin specific event

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Study question: In this study, we investigated the X chromosome inactivation (XCI) pattern in a large cohort of human embryonic stem cells (hESC) during long-term culture.
Summary answer: A stable XCI pattern was detected during long-term undifferentiated culture and after differentiation to somatic and trophoblast lineages: all fifteen lines displayed stage III XCI, with major loss of XCI marks, and those lines which were informative for the microsatellite markers we studied (11/15) showed a completely non-random XCI pattern.
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 origin of this non-random pattern in hESC remained unresolved.
Study design, size, duration: The XCI pattern of fifteen female hESC lines was analyzed in the undifferentiated state, at different passages during long term culture, and after differentiation into somatic osteoprogenitor-like cells and towards trophoblast lineage. The observed XCI patterns were correlated to the DNA of the donors to identify the inactivated X chromosome.
Participants/materials, setting, methods: To analyze DNA methylation, we applied methylation-sensitive restriction, followed by PCR for two microsatellite markers, and bisulphite sequencing. Expression of *XIST* and *TSIX* was investigated using real time PCR, while minisequencing was used to determine mono- or biallelic gene expression. Histone modifications were analysed by immunostaining.
Main results and the role of chance: Methylation analysis revealed XCI in all 15 hESC lines. One line was non-informative for both markers; eleven lines displayed a completely skewed, non-random methylation pattern. From ten lines for which donor DNA was available, six lines displayed non-random methylation, and thus inactivation, of the male donor allele; four of the female donor allele. The remaining three lines did not display DNA methylation at the microsatellite markers, but further bisulphite sequencing analysis revealed partially methylated profiles, suggesting erosion of methylation rather than absence of XCI. Strikingly, in none of the lines the presence of repressing histone marks or *XIST*/*TSIX* expression could be observed. The same methylation pattern was observed at low and high passages and in undifferentiated as well as in differentiated samples in all lines.
Limitations, reason for caution: We investigated the methylation status of the X chromosome using two 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: We excluded a parent-of-origin-related mechanism for the non-random XCI appearance. Whether this pattern reflects the epigenetic status of the embryonic donor cells or an XCI-related culture advantage remains to be investigated.

The presence of the culture-adapted XCI pattern, already at early passages, and its persistence in differentiated progeny cautions for the applicability of hESC. As this seems to result from suboptimal culture conditions, future efforts should focus on the optimization of hESC derivation and culture.
Study funding/competing interest(s): Funding by University(ies), Research Foundation - Flanders (FWO-Vlaanderen) [grant number 1502512N], Methusalem grant of the Research Council of the Vrije Universiteit Brussel.
Trial registration number: N/A.

O-218 Endoderm formation improves germ cell differentiation potential of human embryonic stem cells

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Study question: Is there a relationship between endoderm and germ cell development *in vitro* upon differentiation of human embryonic stem cells (hESCs), similar to mice *in vivo*?

Summary answer: Endoderm-directed differentiation of hESCs induces upregulation of primordial germ cell markers and facilitates the formation of postmigratory germ cells.
What is known already: Activin A and BMP4 are known to synergistically induce efficient definitive endoderm formation in hESCs. BMP4 is known to promote robust germ cell differentiation of hESCs *in vitro*. In addition, derivation of hESCs in the presence of Activin A predisposes them towards the germ cell lineage due to inherent increased expression of primordial germ cell markers. Hence, we investigated whether there is an association between endoderm and germ cell differentiation *in vitro* from hESCs.
Study design, size, duration: Two standard and two Activin A-derived hESC lines were differentiated as embryoid bodies (EBs) in either 1) differentiation medium (DM), 2) DM with 10 µM SB431542 (SB, Activin signaling inhibitor), 3) DM with 20 ng/ml Activin A, 4) DM with 50 ng/ml BMP4 and 5) DM with both Activin A and BMP4. EBs were analysed by qRT-PCR and immunocytochemistry.
Participants/materials, setting, methods: EBs differentiated from four hESC lines were analysed for germ cell-markers STELLA, cKIT, DAZL,VASA after differentiation in five conditions for 7 days. One hESC line showing highest germ cell predisposition was differentiated again and checked for ecto-, meso- and endodermal markers to determine which condition showed a concomitant increase in endodermal genes.
Main results and the role of chance: EBs from all hESC lines, in the presence of both Activin A and BMP4, exhibited significant upregulation of germ cell markers compared to all other conditions ($p < 0.05$). The Activin A-derived hESCs showed highest expression of the pre-meiotic gene VASA by a 20-fold difference. At the protein level, combination of Activin A and BMP4 resulted in cytoplasmic VASA expression in EBs differentiated from Activin A-derived hESC lines only. DM supplemented with SB completely downregulated both early and late PGC-specific genes in all hESCs, indicating that their expression is dependent on signaling via Activin A. In Activin A-derived hESCs, expression of endodermal markers GATA4 and GATA6 increased 8- and 9-fold ($p < 0.05$) respectively in the presence of both Activin A and BMP4, while markers of ecto-and mesoderm remained low.
Limitations, reason for caution: Our results demonstrate the correlation between endodermal and germ cell differentiation of hESCs. To further validate these results, functional tests such as implementing siRNA knock-down against the endodermal gene GATA6 need to be carried out to determine whether germ cell differentiation continues to proceed to meiosis.
Wider implications of the findings: Although significant progress has been made in inducing *in vitro* gametogenesis in the mouse model, the mechanism underlying germ cell development from hESCs still needs in-depth investigation. Our results underscore the importance of mimicking the processes involved in early embryo development to an *in vitro* environment in order to facilitate efficient induction of germ cell differentiation.
Study funding/competing interest(s): Funding by University(ies), this research is funded by the Research Foundation - Flanders (FWO, grant number FWO-3G062910).
Trial registration number: N/A.

O-219 Inhibition of DMRTA2 impairs human female germline development in xeno-grafted ovaries

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Study question: We identified 3 DMRT genes sexually differently expressed in the human fetal ovaries at the time of meiotic initiation. Those were also retrieved in murine embryonic female germ cells. We focused on DMRTA2 to clarify its implication in human fetal ovaries using an original xenograft model.
Summary answer: This study reveals for the first time the requirement of DMRTA2 for the normal development of human female embryonic germ cells. DMRTA2 appears required for proper oogonia differentiation shortly prior their entry into meiosis.
What is known already: DMRTA2 belongs to a gene family coding for proteins with a DM-domain that are widely involved in gonadal differentiation. The most well known member is DMRT1 a transcription factor required for testis and male germ cell differentiation and meiosis regulation in both sexes in mice. To our knowledge, no role for Dmrta2 has been reported in gametogenesis though its expression has been detected in mouse fetal ovary and adult testis.
Study design, size, duration: Experimental study conducted on 26 human early fetal ovaries (8 to 11 weeks post fertilization) harvested following legally induced abortions.
Participants/materials, setting, methods: Human fetal ovaries were grafted in two immunodeficient mice. Some recipient mice were treated 4 weeks after the graft with siRNA targeting specifically the human sequence of DMRTA2. Graft were retrieved and analyzed by immunohistochemistry and RT-qPCR.
Main results and the role of chance: Grafted ovaries developed properly with no overt change compared to ungrafted ovaries of the equivalent stage, displaying a correct germ cell density, the mitotic /meiotic transition, evidences of meiotic progression and follicle formation. DMRTA2 inhibition by siRNA triggered an increase of undifferentiated FUT4-positive germ cells and a decrease in the percentage of meiotic gH2AX-positive germ cells compared to untreated mice without altering germ cell density or apoptosis. Interestingly, RT-qPCR based analysis of the expression of many markers associated with pre-meiotic (DAZL, DDX4, STRA8) and meiotic (SPO11) germ cell differentiation were impaired as was the expression of DMRTB1 and DMRTC2.
Limitations, reason for caution: The main limitation of the study was the few human material available.
Wider implications of the findings: We provided and characterized a potent model of xenograft coupled to RNA interference to allow genetic investigations to study human germline development. We show that several DMRT family members are specifically expressed during the mitosis/meiosis transition and the relationships among them needs further elucidations.
Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), INSERM U967 - CEA/DSV/iRCM/ LDG- Université Paris Diderot-Paris 7, Fontenay aux roses, France.
Trial registration number: Our study was approved by the Biomedecine Agency (trial registration number PFS12-002) and all women gave their informed consent.

O-220 The human sperm reservoir – first proof and characterization by a new real-time imaging technique

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Study question: Is a sperm reservoir formed in the human uterine tube?
Summary answer: Using probe-based confocal laser endomicroscopy (pCLE) the formation of a human sperm reservoir in the isthmus was documented in real-time movies. Only spermatozoa with high membrane integrity bind with their head to the cilia of the isthmus. They maintain motility during attachment and stay viable for at least 3 days.
What is known already: In animals, spermatozoa form a sperm reservoir in the isthmus by binding with their head to the cilia of the oviduct. Thus they maintain their capacity to fertilize for days (most mammals), months (birds) or even years (reptiles). Due to the lack of methods for investigating the human oviduct under *in vivo* conditions a human sperm reservoir has not been proven in the human uterine tube up to now.
Study design, size, duration: The pre-clinical randomized study was designed to characterize the human sperm reservoir under near *in vivo* conditions. Thus, the sperm-oviduct interaction was analysed in a) 7 transsexual females, b) 2 transsexual patients who had had intercourse 2.5 days before surgery and c) 9 premenopausal women undergoing hysterectomy.
Participants/materials, setting, methods: The uterine tubes of the transsexual and premenopausal women were investigated immediately after surgery. Ampulla and isthmus with and without co-incubation with a) native and b) frozen thawed spermatozoa were examined by pCLE (MaunaKea Technologies, France, microprobe ProFlexTM S1500), confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM).
Main results and the role of chance: Human spermatozoa form a sperm reservoir in the uterine tube by binding with their head to the cilia of the isthmus. The binding capacity is not confined to the isthmus – it also takes place in the ampulla. The binding occurs independently from the stage of the cycle. Only sperm revealing intact plasma membranes on their head are capable to bind. Thus, frozen thawed sperm have a distinctly reduced capability to form the

reservoir. After attachment the sperm stay motile. They maintain their vitality for at least 3 days. The long term application of testosterone in transsexuals results in dedifferentiation of oviductal epithelial cells and increased formation of mucus leading to impaired sperm binding.
Limitations, reason for caution: Medications – especially hormones – distinctly influence the formation of the sperm reservoir and have to be taken into account.
Wider implications of the findings: Our studies for the first time prove the existence of a human sperm reservoir under near *in vivo* conditions and in real-time. The fact that only spermatozoa with an intact plasma membrane are able to bind point to the oviduct playing a pivotal role in sperm selection. As motility is maintained during attachment, essential nourishment of spermatozoa has to be locally provided by the tubal epithelium.
Study funding/competing interest(s): Funding by University(ies), University of Munich.
Trial registration number: N/A.

O-221 Pre-IVF analysis of peripheral blood immune cell surface markers predicts pregnancy

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Study question: Can we predict pregnancy by measuring peripheral immune cell (NK and T cells) expression of ST2L, IL-18R (receptors for IL-33 and IL-18 respectively) and CD69 (an early activation marker) before an IVF cycle, and can changes be identified in upon successful implantation?
Summary answer: ST2L expression by peripheral blood NK^{dim} cells and cytotoxic T lymphocytes (CTL), and IL-18R expression by CTL predicts implantation before commencement of an IVF cycle. Maternal systemic immune cell changes occur within 10 days of successful implantation, as significant changes in CTL and T helper (Th) cell ST2L expression were identified.
What is known already: ST2L and IL-18R are dysregulated on the surface of NK cells from women with reproductive disorders (pre-eclampsia and recurrent miscarriage). ST2L and IL-18R expression levels are changed in normal pregnancy by 12 weeks' gestation. CD69 is reported to be associated with implantation failure. Peripheral immune cell measurements are widely offered by ART clinics although treatments for apparent abnormal levels are not proven by randomized control trials to increase live birth rates.
Study design, size, duration: 28 women (mean age 34 years) undergoing a long protocol IVF/ICSI cycle were recruited from the Oxford Fertility Unit between 2010 and 2013.
Participants/materials, setting, methods: We examined absolute counts and cell surface expression of ST2L, IL-18R and CD69 of peripheral blood NK^{bright}, NK^{dim}, CTL and Th cells by flow cytometry at: baseline (day 1–5 of cycle), down-regulation, egg retrieval and 15 days post embryo transfer (24 h before a urine pregnancy test).
Main results and the role of chance: Implantation occurred in 60.7% of recruits. There were no significant differences in age, FSH, AFC, length of subfertility, BMI, number of oocytes or embryos between pregnant and non-pregnant recruits. At baseline, NK^{dim} and CTL expression of IL-18R, and CTL expression of ST2L were predictive of implantation, with ROC analyses (AUC) of 0.81, 0.77 and 0.79 respectively. Other analysed parameters were unchanged.

We detected upregulation of ST2L expression by CTL ($F = 10.74$, $p < 0.0001$), Th ($F = 4.722$, $p < 0.01$), and NK^{dim} ($F = 3.964$, $p < 0.05$) in the pregnant group although cell numbers were unchanged and the cells were not activated (CD69 expression). Correcting for multiple comparisons, ST2L expression was significantly increased on CTL ($p < 0.01$), Th ($p < 0.05$) and NK^{dim} ($p < 0.05$) cells 24 h before the pregnancy test.
Limitations, reason for caution: A larger cohort of patients is required to confirm these results and we are currently recruiting to this study. As yet we do not know if there is cycle-to-cycle variation in these biomarkers. Further investigations are required to understand why the phenotype described predicts pregnancy.
Wider implications of the findings: IL-18R and ST2L expression by peripheral CTL and NK^{dim} cells are, to our knowledge, the first immunological parameters that predict IVF success. If a larger study confirms these data the findings will have significant implications for ART, predicting the likelihood of success and may assist treatment timing. ST2L upregulation within 10 days of implantation is, as far as we know, the earliest evidence of pregnancy altering maternal systemic immunity.
Study funding/competing interest(s): Funding by national/international organization(s), Wellbeing of Women.
Trial registration number: None.

O-222 Endothelial progenitors and multipotent Oct4+ cells from bone marrow contribute to endometrial regeneration in an irradiated mice model

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Study question: The aim of the present study was to investigate the contribution of different BM stem cell types in the restoration of the endometrium in a mice model after total body irradiation.
Summary answer: Our results suggest that EPC and MAPCsDDD are the best candidate population for endometrial regeneration in this irradiated mice model. The enhanced endometrial regeneration, the presence of donor-GFP cells in all endometrial compartments and the percentage of apoptosis observed, demonstrates the important function of these in the murine endometrial reconstruction.
What is known already: In humans and mice, endometrial regeneration is thought to be dependent of resident somatic stem cells (Cervelló et al., 2007). Also, it has been reported the contribution of bone marrow (BM) using the model of BM transplantation (Du and Taylor, 2007; Mints et al., 2008; Cervelló et al., 2012). Nevertheless BM is a heterogeneous population of cells and the specific subpopulations contributing to endometrial regeneration has not been solved.
Study design, size, duration: B6.SJL-PTPRCA-CD45.2GFP^+ mice were used to collect the Lin^- unfractionated BM cells by MACS. Additionally, FACS was done to sorted out: Mesenchymal Stem Cells (MSC, $Lin^-CD45^-CD31^-Sca\text{-}1^+$), Endothelial Progenitor Cells (EPC, $Lin^-CD45^-CD31^+$), Hematopoietic Progenitor Stem Cells (HSC, Lin^-CD45^+), and unfractioned BM (UBM). Also, three murine established GFP^+ cell lines were used: MAPCs2slowclon, MAPCsDDDclon and MSC.
Participants/materials, setting, methods: All were injected into vein tail of irradiated 9 Gy C57BL/6J female mice 8 weeks age ($n = 21$). Mice were sacrificed 12 weeks after and uteri were formalin fixed-paraffin embedded. Histology was examined by HE staining whereas presence of engrafted cells was determined by immunohistochemistry. Finally, TUNEL staining determined presence of apoptotic cells.
Main results and the role of chance: HE staining revealed that MSC, EPC, HSC, and UBM subpopulations as well as MAPCsDDD line reconstituted properly the irradiated uterus, whereas MAPCs 2slow and MSC lines did not show any regenerative capacity. The presence of BM injected cells was assessed by GFP staining (MSC, EPC, HSC, and UBM as well as MAPCsDDD line), and were located in all endometrial compartments, stroma, epithelium and mostly in the endothelium of blood vessels.

Determination of apoptotic cells by TUNEL revealed that the percentages of apoptotic cells in normal non-irradiated versus irradiated control uteri were 0.69% vs. 0.04%, respectively. All injected uteri with the 7 BM subpopulations and cell lines induced higher apoptotic percentages ranging from 0.26% to 0.82%, suggesting the contribution of the BM subpopulations to tissue turnover.
Limitations, reason for caution: Sorted cells GFP^+ corresponding to the following populations: MSC, EPC and HSC were obtained accordingly to their normal expression in mice, limiting the number of injected cells into the recipient female mice.
Wider implications of the findings: Damaged uteri injected with the 7 different cell subpopulations were macroscopically examined and morphological differences were already evident being the MAPCs 2 slow and MSC line poorly developed. Interestingly, EPC and MAPCsDDD provoked an unexpected higher apoptosis ratio compared to non-irradiated uteri (0.82% and 0.81% vs. 0.69%, respectively); suggesting that these subtypes composed by endothelial progenitors (EPC) and enriched multipotent Oct4+ cells (MAPCsDDD) contribute the most to endometrial regeneration.
Study funding/competing interest(s): Funding by national/international organization(s), This work has been financed by grant PhD grant PFIS 09-00404, the grant SAF 2012-31017 from the Spanish Ministry of Science and Innovation (PI: CS), and by PROMETEO II/2013/018 from the Regional Valencian Ministry of Education.
Trial registration number: This is not a clinical trial.

SELECTED ORAL COMMUNICATION SESSION

SESSION 57: FEMALE REPRODUCTIVE PHYSIOLOGY

Wednesday 2 July 2014 **10:00 - 11:45**

O-223 Amino acid concentration of human uterine fluid: effect of age, pathology and lifestyle

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Study question: What is the amino acid composition of human uterine fluid, and is it affected by age, body mass index (BMI), diet or gynaecological pathologies?
Summary answer: The amino acid composition is characterised by high glutamate, glycine and alanine levels. The profile does not appear to vary with phase of the cycle, increasing age, BMI or history of gynaecological pathology. However, a non-prudent diet was associated with higher concentrations of asparagine, histidine, phenylalanine, isoleucine and leucine.
What is known already: Murine, bovine and ovine uterine amino acid content has been reported, but no reliable data on the human exists. Amino acids are routinely included in IVF culture medium and are associated with improved blastocyst formation. Moreover, the turnover of amino acids by individual embryos is a recognised marker of embryo viability *in vitro*. Recent murine studies have demonstrated that the intra-uterine periconceptional nutritional environment affects developmental programming and long-term development.
Study design, size, duration: 56 women aged 18–45, undergoing Hysterosalpingo-Contrast-Sonography had their gynaecological history, body build and diet (using the Southampton Women's Survey Short Food Frequency Questionnaire) assessed. Uterine fluid was sampled at different phases of the menstrual cycle and snap frozen at –80°C.
Participants/materials, setting, methods: The concentration of 18 amino acids present in the uterine fluid samples were calculated from a known standard using reverse phase high pressure liquid chromatography (Agilent 1100).
Main results and the role of chance: Glutamate was present in the highest concentration in uterine fluid followed by glycine and alanine. In contrast, methionine and tryptophan were present in the lowest concentration. In total, human uterine fluid contained an amino acid concentration of 4.92 ± 0.53 mM. Variations in the concentrations of the uterine fluid amino acids with participants' age, BMI and pathology showed no statistical difference. However, in females with a negative prudent diet score, higher concentrations of asparagine (p = 0.010), histidine (p = 0.027), phenylalanine (p = 0.031), isoleucine (p = 0.034) and leucine (p = 0.043) were present compared to those with a positive prudent diet score.
Limitations, reason for caution: In both mice and cows, higher concentrations of amino acids were found in the oviduct than the uterus. In contrast, the uterine fluid amino acid concentrations presented in this current work were greater for all amino acids than those previously reported in the fluid of the human Fallopian tube.
Wider implications of the findings: This is the first study to measure the amino acid concentration of human uterine fluid. Levels were lower in women with a more prudent diet. These data suggest that the in utero environment is affected by diet and therefore further research is required to investigate the impact of periconceptional diet on both the uterine environment and embryo development.
Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), This work was funded by the NIHR, the Medical Research Council (G0701153) and the University of Southampton and is supported by the NIHR BRC in Nutrition.
Trial registration number: Not applicable.

O-224 Altered expression and localization of maternal proteins and changes in histone pattern after preovulatory and postovulatory ageing of mammalian oocytes

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Study question: Does preovulatory (PreOA) or postovulatory ageing (PostOA) of oocytes affect spindle, histone pattern, and the abundance and subcellular localization of maternal effect gene (MEG) products like chromatin remodeling factor Brg1 or germ cell-specific RNA-binding protein Msy2, which is mandatory for mRNA storage and stability during oogenesis and early embryogenesis?
Summary answer: PostOA and PreOA caused alterations in spindle formation, histone H3K9 trimethylation and chromosome alignment. Brg1 transcriptional modulating protein was significantly increased in GV stage oocytes after PreOA. PostOA reduced Msy2 abundance, as well as its spindle and cortical localization in MII oocytes. Glutathione donor prevented some ageing effects.
What is known already: PreOA (intrafollicular overripeness) and PostOA can cause spindle degeneration, altered redox homeostasis and developmental defects in mice. We showed that ageing of mouse oocytes leads to an altered total mRNA content and poly(A) tail length of several maternal effect genes (e.g. Brg1, Dnmt1, Nlrp5). Maternal Brg1 is involved in chromatin remodeling and control of gene expression prior to and past ZGA, while Msy2 contributes to mRNA storage and stability in oocytes and embryos.
Study design, size, duration: GV and MII oocytes were obtained from *in vitro* preantral follicle culture after 12 (control) or 16 days (PreOA), or 18 h (control) or 30 h (PostOA) after the ovulatory stimulus, respectively. *In vivo* matured MII oocytes were collected 15 h after ovulation induction (control) or after additional ageing for 24 h (PostOA).
Participants/materials, setting, methods: Spindle formation, chromosome alignment, Brg1 abundance and localization, histone H3K9 trimethylation, and distribution and abundance of Msy2 were analyzed by quantitative confocal microscopy and Western Blot analysis. Medium was supplemented with glutathione donor glutathionethylester (GEE, 1 mM) during PostOA to normalize redox homeostasis.
Main results and the role of chance: PreOA and PostOA caused a significant increase in spindle aberrations and a reduced trimethylation of histone H3K9 in MII oocytes, while chromosome alignment was only affected after PreOA but not after PostOA for 12 h. Quantitative confocal microscopy revealed that protein level of Brg1 was significantly increased in GV oocytes after PreOA, suggesting a precocious recruitment of maternal mRNAs of this transcriptional regulator of early embryogenesis. Msy2 was enriched in the subcortical RNP domain and, as shown for the first time, in the spindle chromosome complex at metaphase II stage of unaged control oocytes. After PostOA for 24 h, there was a significant reduction of Msy2 protein, and spindle and cortical localization was lost. 1 mM GEE supplementation during PostOA reduced some ageing-induced alterations in Msy2.
Limitations, reason for caution: Further studies have to show whether precocious expression of Brg1 in PreOA affects zygotic gene expression contributing to congenital abnormalities, and whether the changes in Msy2 abundance and distribution are causally related to spindle degeneration, altered mRNA stability and recruitment (e.g., of MEGs), and reduced developmental competence after PostOA.
Wider implications of the findings: The abundance of Brg1 in GV and the H3K9 trimethylation in MII oocytes may be useful markers of appropriate stimulation protocols in ART. Localization of transcription factors appears relevant for stage-specific gene expression. Msy2 in the meiotic spindle and its decreased abundance after PostOA imply that it may be a marker of reduced oocyte competence. GEE supplementation appears promising to prevent oocyte ageing possibly not only in mouse, but also in human oocytes.
Study funding/competing interest(s): Funding by national/international organization(s), The study was supported by the German Research Foundation (DFG; HO949/21-1, GR1138/12-1, EI 199/7-1). Authors declare no conflict of interest.
Trial registration number: N/A.

O-225 Molecular signature of human cumulus cells reveals impact of female age on pathways that are crucial for oocyte development

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Study question: Quality and developmental potential of oocytes decline with patient's age. Which pathways in cumulus cells were affected with aging that may lead to decline of oocyte quality?
Summary answer: CCs from older patients displayed significant differences in the expression of genes involved in angiogenesis, *TGF-β* and insulin signaling pathways comparing with CCs from younger patients.
What is known already: It was reported that maternal aging affects the lipid metabolism and oxidative phosphorylation of human CCs. However, little is known about the impact of female age on pathways that are crucial for oocyte development.
Study design, size, duration: This study includes 10, 10 and 8 CCs isolated from mature MII oocytes collected from patients aged <30 years, 31–36 years and >37 years respectively. All groups of CCs were obtained from patients who underwent COS for ICSI.
Participants/materials, setting, methods: CCs from each MII oocyte were analyzed individually using whole genome U133 Plus 2.0 GeneChip Affymetrix microarrays. Significance analysis of microarray-Multiclass was used to analyze the array data according to age of patients with false discovery rate (FDR <5%). Validation was performed by RT-qPCR.
Main results and the role of chance: 2,170 genes were differentially expressed among the three groups according to age. In CCs collected from patients >37 years, angiogenic genes including *ANGPTL4* (×3.9, FDR = 0), *LEPR* (×2, FDR = 0.004), *TGFBR3* (×2.4, FDR = 0) and *VEGFC* (×2.0, FDR = 0.001) were over-expressed. Conversely, the genes implicated in *TGF-β* signaling pathway such as *TGFB1*, inhibin (*INHA*) and activin (*ACVR2B*) were under-expressed. Whereas, genes related to insulin signaling pathway were over-expressed in CCs of patients (31–36 years), like *INSR* (×2.7, FDR = 0.003), *IGFBP3* (×2.0, FDR = 0.004) and *IGFBP5* (×1.7, FDR = 0). The inflammatory response genes such as *SERPINA1* (×2.2, FDR = 0.002) and *IL18R1* (×2.2, FDR = 0.019) were over-expressed in CC of patients (<30 years). Interestingly, most of the genes that are impacted by maternal age and that are needed for oocyte development are predicted targets of microRNAs.
Limitations, reason for caution: Further investigations with larger number of patients are needed to confirm these results.
Wider implications of the findings: This study presents a comprehensive resource to constitute a base for future investigations on the role of aging on specific genes and miRNAs in CCs.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Funding by commercial/corporate company(ies), University hospital of Montpellier and Ferring pharmaceutical company are partially supported this study. There were no competing interests.
Trial registration number: Not applicable.

O-226 Relation between oocyte transcriptome and competence

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Study question: Is the competence of the mature human oocyte reflected in the mRNA stockpiled during oogenesis? Does the expression profile differentiate between human MII oocytes classified as euploid and aneuploid, respectively? Can bioinformatic analysis identify enriched functions and cellular networks correlated to competence?
Summary answer: 1871 genes were differentially expressed of which 236 had fold-change >2 between oocytes classified as euploid and aneuploid, respectively. Genes involved in pluripotency and DNA methylation showed the highest fold-changes. The biofunctions 'DNA Replication, Recombination, and Repair', 'RNA Post-Transcriptional-Modification', 'microtubule-dynamics' and 'cell-viability' were enriched in the differentially expressed genes.
What is known already: The mRNAs contributed to the oocyte during oogenesis support the machineries sustaining fertilization and initial embryo-development, however, little is known about how the composition of the transcriptome relates to oocyte developmental competence.

Presently, euploidy is the strongest indirect measure of oocyte competence. Cumulus Cell (CC) gene-expression has recently been correlated to ploidy of the associated oocyte Fragouli et al., 2013. Using this tool, a set of 12 CC has been used to categorize (euploid/aneuploid) the associated MII oocytes.
Study design, size, duration: *In-silico* bioinformatic comparisons between transcriptomes of human MII oocytes categorized as euploid and aneuploid by marker genes Fragouli et al., 2013 expression in the connecting CC. The transcriptome data originate from 12 women aged 25–39 years receiving IVF treatment following the standard long agonist protocol. Oocyte and CC were isolated immediately after retrieval.
Participants/materials, setting, methods: Classification of the 12 data-set based on CC expression level of 58 published marker genes Fragouli et al., 2013 resulted in three categorized as euploid and three as aneuploid, while six failed to be classified. The transcriptomes of the MII oocytes classified as euploid and aneuploid were compared and the difference bio-informatically analyzed.
Main results and the role of chance: 1871 genes were differentially expressed ($p < 0.05$) of which 236 had fold-change >2. Function enrichment analysis of the differentially expressed genes showed increase in 'microtubule-dynamics' ($p < 0.001$), 'cell-viability' ($p < 0.01$) and 'RNA-transactivation' ($p < 0.01$) activity in euploid as compared to aneuploid. Top-networks represented in the 236 transcripts were 'DNA Replication, Recombination, and Repair' (network score 30), 'Cell-To-Cell-Signalling' and 'RNA Post-Transcriptional-Modification' (network score 22). Among the genes with highest positive fold-change in the euploid oocytes as compared to the aneuploidy were *LIN28A* (fold = 4.6; $p = 0.01$) and *LIN28B* (fold = 4.5; $p = 0.009$). *LIN28A* + B are regulators of miRNAs and mRNAs and involved in pluripotency. The gene with the highest down-regulation in euploid as compared to aneuploid oocytes was the imprinted maternally expressed *H19* (fold = –11.8; $p = 0.04$). Moreover, *MTHFR, MBD1 and MTRR*, all involved in DNA-methylation, were differentially expressed.
Limitations, reason for caution: Based on published CC marker genes Fragouli et al., 2013 we succeeded in categorizing six of the 12 samples into aneuploid and euploid, while six clustered in between. Hence the number of observation in the two groups is limited and the reported findings need to be validated in a larger scaled study.
Wider implications of the findings: The present use of microarray technology allows us to characterize the mRNA profile of oocytes in relation to the highly relevant competence measurement: ploidy. These preliminary data suggest multiple functional categories and genes reflecting competence; genes central for DNA-replication, RNA-translation and DNA-methylation – all central functions in the early development after fertilization and before the genome activation. Improvement of our understanding of the mechanisms underlying oocyte competence could lead to improved techniques in ART.
Study funding/competing interest(s): Funding by hospital/clinic(s), Copenhagen University Hospitals: Herlev Hospital and Rigshospitalet, Denmark.
Trial registration number: None.

O-227 SIRT1 acts in the mechanisms preventing oocyte damage from methylglyoxal, a reactive dicarbonyl increasing oxidative stress and AGEs (advanced glycation end-products)

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Study question: Is SIRT1 involved in cellular pathways against AGEs in mouse oocytes?
Summary answer: In the present study, we demonstrated for the first time that glyoxalases and SIRT1 participate in the cellular pathways activated by the oocyte to counteract methylglyoxal (MG), a highly reactive dicarbonyl promoting AGE accumulation and oxidative stress.
What is known already: It is known that AGE and glyoxalases dysfunctions are associated with ovarian aging and polycystic ovarian syndrome (PCOS), and that MG, a glycolysis by-product, negatively affects oocyte mitochondria, spindle assembly and maturation kinetics. Against these effects, the oocyte activates specific mechanisms: it can rely on glyoxalase 1 (Glo1) and glyoxalase 2 (Glo2) genes to detoxify MG, and on SIRT1, a redox-sensitive deacetylase, to sense oxidative stress and activate antioxidant genes including superoxide dismutase (MnSod).
Study design, size, duration: Ovarian oocytes from CD1 mice were exposed to 75 μM MG and processed following different times for the evaluation of Glo1, Glo2, Sirt1 and MnSod gene expression. A group of oocytes were matured *in vitro* in M16 medium with/without 75 μM MG, in the presence/absence of the SIRT1 inhibitor Ex527.
Participants/materials, setting, methods: Real time RT-PCR was employed to investigate whether mouse oocytes activate Glo1, Glo2, Sirt1 and MnSod gene

expression in response to treatment with MG. In IVM experiments, different Ex527concentrations (0, 5, 10 μM) were employed and polar body extrusion was recorded after 16 h of *in vitro* culture.
Main results and the role of chance: We found that Glo2, Sirt1 and MnSod genes were up regulated in response to MG, whereas transcript levels of Glo1 remained constant. These results suggest that the oocyte is capable to respond to MG challenge by activating pathways promoting MG detoxification and antioxidant defences. Upregulation of Sirt1 and MnSod genes is an evidence for the role of SIRT1-MnSOD axis in the adaptive response to MG-induced oxidative stress. When oocytes were exposed to Ex527, a dose-dependent reduction of maturation rates was observed. When oocytes were challenged with both Ex527 and MG, effects of MG on maturation kinetics were increased. This synergistic effect is an indirect evidence for the protective role of SIRT1.
Limitations, reason for caution: The main limitation of this study was the absence of direct quantification of glyoxalase 1, glyoxalase 2 and SIRT1 enzymatic activity due to the lack of an appropriately sensitive method in mouse oocytes.
Wider implications of the findings: From recent literature on animal and clinical studies, a role for MG and AGEs has emerged in female infertility and aging. For this reason, it is important to understand the mechanisms activated by the oocyte to counteract toxic effects exerted by MG and AGEs. In this context, SIRT1 and glyoxalases may be considered potential pharmacological targets to improve ovarian function and oocyte quality under conditions hampering female fertility.
Study funding/competing interest(s): Funding by University(ies), University of L'Aquila.
Trial registration number: Not required.

O-228 MicroRNAs upregulated in follicular fluid are carried by exosomes: new actors in the communication between oocyte and somatic follicular cells?

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Study question: Characterize human follicular fluid (FF) exosomes, detect their microRNA cargo specifically synthesized by somatic follicular cell and/or by oocyte and finally assess if their expression profiles changes with aging.
Summary answer: We identified 37 miRNAs significantly more expressed in FF than in plasma samples from 15 women underwent IVF program, most of them carried by exosomes. Exosomal FF miRNAs are involved in WNT, MAPK, and TGFβ signaling pathways, which are critically important for follicle growth, oocyte maturation, and early embryo development.
What is known already: MicroRNA presence has been described in several biological fluids and recently in human FF, although the comparison of their expression profiles with plasma is to be yet performed. Moreover studies on animal models have demonstrated the presence of exosomes in FF but their molecular characterization in human is not been performed. It has been demonstrated for intracellular ovarian miRNAs that their deregulation could cause alterations in the pathways involved in follicle growth and oocyte maturation.
Study design, size, duration: FF and plasma were collected from 15 women. Aspiration of follicles was performed 34-36 h after HCG injection. To collect the FFs in which MII oocytes had been identified, we kept separated FF of individual follicles until oocyte decumulation; the samples successively were pooled and exosome vesicles were purified.
Participants/materials, setting, methods: 384 miRNAs purified from total FF and from FF exosomes were compared with those from plasma. Analysis consisted of RNA retrotranscription, preamplification and TaqMan Low Density Array technology. By Nanosight and FACS the phenotypic characterization of FF exosomes was performed. Gene Ontology, Pathway, Target Analysis were executed by bioinformatics software.
Main results and the role of chance: We identified 37 highly expressed miRNAs in human FF compared to plasma. In particular we found 22 miRNAs detectable only in exosomes, 10 miRNAs highly expressed in both compartments and 5 miRNAs in total FF. We demonstrated that the majority of them are carried by FF vesicles, which we phenotypically and molecularly characterized as exosomes. They had an average diameter of 40 nm and were positive for CD63 and CD81, known to be enriched in exosomes. Exosomal FF miRNAs are involved in WNT, MAPK, ErbB and TGFβ signaling pathways, critically important for follicle growth, oocyte maturation, and early embryo development. 9 exosomal FF miRNAs could negatively regulate genes encoding inhibitors of follicle maturation and meiosis resumption as PTEN.
Limitations, reason for caution: We speculate that FF exosomes carrying miRNAs could be involved in communication among somatic follicular cells and also between somatic follicular cells and oocyte, but we don't give any prove that exocitosis and endocytosis occur in human follicle.
Wider implications of the findings: We propose that FF-miRNAs are synthesized by ovarian follicle cells and that they can be transferred to recipient cells through exosomes. Our data could contribute to clarify the complex signaling pathways involved in follicular growth and oocyte maturation and further could provide noninvasive molecular markers of oocyte quality in ART. The discovery that FF exosomes carry microRNAs could open up the possibility of a novel mechanism of cellular communication among follicular cells.
Study funding/competing interest(s): Funding by University(ies), Funding by commercial/corporate company(ies), Ministero dell'Università e della ricerca scientifica e tecnologica Italy. Farmitalia SRL and LJ Pharma SRL.
Trial registration number: Not clinical trial.

O-229 Menstrual cycles affect vascular permeability due to an alteration of the endothelial glycocalyx

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Study question: The endothelial glycocalyx is pivotal for a healthy vascular barrier. Normal pregnancy and HELLP syndrome are associated with pronounced shedding of the glycocalyx. Healthy women tend to have slight premenstrual edema. Is there a loss in the integrity of the glycocalyx during normal menstrual cycle?
Summary answer: In contrast to male and postmenopausal female controls, ovulatory women show significant shedding of endothelial glycocalyx components during the late luteal phase.
What is known already: The endothelial glycocalyx controls fluid extravasation, cell adhesion, microvascular perfusion, and is involved in a multitude of acute and chronic diseases related to the vascular system. As an example, shedding of the endothelial glycocalyx has a strong negative effect on outcome in patients with sepsis and septic shock. Recent data showed an increase of glycocalyx components in the circulating blood during normal pregnancy and HELLP syndrome. Little is known about the physiological control mechanism.
Study design, size, duration: This is a basic, non-interventional study, 37 healthy volunteers (21 ovulatory, 6 postmenopausal females, 10 males) were investigated. In ovulatory females blood was taken at cycle days 3–5, 24 h after urinary LH peak and 8 days thereafter. In males and postmenopausal individuals, blood was taken every 8–10 days for 4 weeks.
Participants/materials, setting, methods: In the follicular phase, periovulatory and luteal phase, estradiol, luteinizing hormone and progesterone as well as serum concentration of the three glycocalyx components (syndecan-1, heparan sulfate, hyaluronic acid), cortisol, hemoglobin and hematocrit were measured and compared with male and postmenopausal volunteers.
Main results and the role of chance: During ovulatory cycles, which were precisely characterized by the change in progesterone (cycle days 3–5: 0.5 ± 0.1 ng/ml, ovulation: 1.4 ± 0.2 ng/ml, 8 days thereafter: 13.4 ± 1.6 ng/ml) syndecan-1 increased from 11.1 ± 2.4 ng/ml at ovulation to 12.6 ± 2.3 ng/ml at progesterone peak and heparan sulfate increased from 636 ± 45 ng/ml at ovulation to 751 ± 60 ng/ml at progesterone peak ($p < 0.05$, respectively). In controls there was no change in syndecan-1 (men: 19.6 ± 3.2 ng/ml, 18.8 ± 3.7 ng/ml, 18.8 ± 3.4 ng/ml; postmenopausal females: 13.1 ± 3.9 ng/ml, 11.5 ± 4.1 ng/ml, 11.1 ± 3.4 ng/ml) and heparan sulfate (men: 618 ± 58 ng/ml, 652 ± 63 ng/ml, 635 ± 73 ng/ml; postmenopausal females: 814 ± 81 ng/ml, 928 ± 44 ng/ml, 841 ± 107 ng/ml; $p > 0.05$, respectively).
Limitations, reason for caution: Shedding of the endothelial glycocalyx during normal menstrual cycle is far less than during sepsis and HELLP syndrome,

which are diseases with extreme detrimental effects on the vascular barrier. However, even small changes of the integrity of the endothelial glycocalyx can have relevant impact of edema formation.
Wider implications of the findings: This is the first study suggesting that ovulation and sex hormones affect vascular permeability. Symptoms of severe ovarian hyperstimulation syndrome comprise fluid- and electrolyte imbalance, severe edema formations, haemoconcentration and hypercoagulation. Our results might provide new insights in the pathophysiology involved (follow-up study). Furthermore hormonally based interventions should be explored in diseases which lead to an extreme shedding of the endothelial glycocalyx (sepsis, septic shock, HELLP syndrome).
Study funding/competing interest(s): Funding by University(ies), Department of anaesthesiology, University of Munich, supported by Bavarian State Ministry of Science, Research and the Arts, Munich, Germany.
Trial registration number: Basic non-interventional study, no trial registration number needed.

SELECTED ORAL COMMUNICATION SESSION

SESSION 58: MALE AND FEMALE FERTILITY PRESERVATION

Wednesday 2 July 2014 **10:00 - 11:45**

O-230 Assessment of spermatogenesis recovery and sperm DNA damages after chemotherapy or radiotherapy for testicular cancer

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Study question: To prospectively evaluate the consequences of BEP (bleomycin, etoposide, cis-platinum) chemotherapy or radiotherapy used for the treatment of testicular cancer (TC) on sperm characteristics and sperm nuclear damages 3, 6, 12 and 24 months after the end of treatment.
Summary answer: Semen characteristics and sperm nuclear status recovered pre-treatment values 12 months after 2 BEP cycles, but only after 24 months when more than 2 cycles of BEP or radiotherapy were used. These novel data are useful tools for counseling patients treated for TC who wish to conceive.
What is known already: Orchiectomy is the first step of TC treatment generally followed by either adjuvant chemotherapy consisting of two to four cycles of the BEP (bleomycin, etoposide, cis-platinum) regimen or radiotherapy, Although TC is the most frequent cancer in young men, few prospective studies have been performed to evaluate the impact of chemotherapy or radiotherapy on spermatogenesis and sperm DNA alterations.
Study design, size, duration: A total of 53 TC patients aged 22 to 43 years were included in a multicenter and prospective longitudinal study performed in eight fertility preservation centers. Those patients collected semen samples before TC treatment and 3, 6, 12, and 24 months after the end of treatment.
Participants/materials, setting, methods: Routine semen analyses, sperm aneuploidy (fluorescence in situ hybridization), sperm chromatin condensation (aniline blue staining), DNA fragmentation (TUNEL assay), number and relative telomere length (quantitative fluorescence in situ hybridization) were compared before and after treatment and with the control group (10 fertile sperm donors) using the nonparametric Mann-Whitney test.
Main results and the role of chance: Twenty six patients were treated by radiotherapy, 28 patients by BEP regimen. Total sperm count recovered pre-treatment values at 12 months for patients given less than 2 BEP cycles, and at 24 months after radiotherapy or more than 2 BEP cycles. The rate of sperm chromosome aneuploidy was higher 6 and 12 months after chemotherapy, 3 and 6 months after radiotherapy. About 50% of patients did not recover their pre-treatment sperm chromosome aneuploidy rate. Sperm chromatin condensation was altered 6 months after radiotherapy. Sperm DNA fragmentation did not vary after the end of treatment. The number of telomeres increased at 12 and 24 months after radiotherapy but did not vary after chemotherapy. The relative telomere length was higher 12 months after chemo- or radiotherapy.
Limitations, reason for caution: Patients with azoospermia or cryptozoospermia 3 months after chemotherapy were not included in the study. The data obtained are specific for TC treatment but cannot be used for other types of cancer treatments. This study is the largest prospective study evaluated simultaneously several sperm nuclear parameters after TC treatment.
Wider implications of the findings: Adjuvant treatments for TC have drastic effects on spermatogenesis and sperm chromatin quality. Pre-treatment values were higher than values observed in fertile donors. The type of tumors, the quantitative and qualitative pre-treatment sperm characteristics and the dose of chemotherapy influenced the recovery of a good spermatogenesis. These new data on both the recovery period according to treatment modalities and the post-treatment chromatin status of sperm are useful tools for counseling patients wishing to conceive.
Study funding/competing interest(s): Funding by national/international organization(s), National hospital funding for clinical research.
Trial registration number: PHRC n°20030222.

O-231 Metastatic lesions in cortical ovarian fragments intended for fertility preservation: a new model system to evaluate tumour-purging protocols

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Study question: Is it possible to introduce tumour cells into ovarian cortical fragments to obtain proliferating, metastases-like structures?
Summary answer: Micro-injection of tumour cells in ovarian cortex leads to the formation of growing tumours that form small, metastases-like structures during *in vitro* culture.
What is known already: The major drawback of autotransplanting cryopreserved ovarian cortical fragments from cancer patients is the potential presence of residual malignant disease in the transplant. Cortex fragments containing metastatic cancer cells are essential for the development of efficient tumour-purging protocols to eliminate residual cancer cells. Patient derived cortical fragments containing cancer cells are hardly ever available for research purposes. We therefore developed a model system for generating contaminated cortex fragments to test tumour-purging protocols.
Study design, size, duration: Bovine and human ovarian cortical fragments were micro-injected with human Ewing's sarcoma, leukaemia, breast cancer or lymphoma cell lines. Injected fragments were cultured for 4, 7 or 10 days. At each time point the formation of metastases-like structures was monitored.
Participants/materials, setting, methods: Human ovarian tissue was obtained from patients carrying the BRCA mutation. Cell lines were used as a source of Ewing's sarcoma, leukaemia, breast cancer or lymphoma cells. Each fragment was injected 5 times with 10^4 cells. Formation of metastases was assessed by conventional staining and by tumour cell-specific immunohistochemistry.
Main results and the role of chance: All types of cancer cells were able to form tumour and metastases-like structures in ovarian cortex *in vitro*. Formation of small metastases was not yet apparent at day 4 but became prominent at day 7 of culture. Proliferation of cancer cells in the ovarian cortex was confirmed by staining with Ki-67, an antigen that is found in dividing cells. Immunohistochemical staining using cancer cell-specific antibodies revealed single neoplastic cells migrating through the ovarian tissue.

Limitations, reason for caution: For the induction of tumours we used cancer cell lines that might behave differently than cancer cells directly derived from patients.
Wider implications of the findings: Our results indicate that it is possible to efficiently induce metastases-like structures from different types of cancers in ovarian cortical fragments. These contaminated fragments are essential for developing protocols for tumour-purging of ovarian tissue. An efficient purging protocol will increase the safety of autotransplantation of cortical fragments and make it available for all young female cancer patients seeking to preserve their fertility, regardless of their type of primary tumour.
Study funding/competing interest(s): Funding by University(ies), Radboud University Medical Centre.
Trial registration number: None.

O-232 Fertility preservation of oocyte for women with ovarian and lung cancers: dysregulation in gene expression profile of cumulus cells

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Study question: Does the transcriptome of individual cumulus cells (CCs) of mature MII oocytes differ between healthy women and patients with cancer disease?
Summary answer: Comparing with CCs from healthy women, the gene expression profile of CCs isolated from patients with ovarian and lung cancer present alterations that could lead to reduction in oocyte competence.
What is known already: The transcriptional profiles of human CCs are known. However, non available data about the molecular signature of CCs from women with cancer included for fertility preservation before cancer treatment.
Study design, size, duration: This study includes 16 CCs from healthy women and 16 CCs isolated from mature MII oocytes collected from patients with lung (n = 10) and ovarian (n = 6) cancers for fertility preservation.
Participants/materials, setting, methods: CCs from each MII oocyte were analyzed individually using whole genome U133 Plus 2.0 GeneChip Affymetrix microarrays. Significance analysis of microarray was used to analyze the array data with 2-fold cut-off and false discovery rate (FDR <5%). Validation was performed by RT-qPCR.
Main results and the role of chance: The molecular signature of CCs from patients with ovarian and lung cancers was significantly different from the healthy women. Specifically, CCs with cancer were characterized by over-expression of genes related to *HER2* signaling including *ERBB3* (×2.3, FDR = 0.0005), *ERBB4* (×2.8, FDR = 0.005), *PARD3* (×2.2, FDR = 0.005) and *EGFR* (×2.7, FDR = 0.002). These gene products may elevate the risk for breast, endometrial and other non-gynecological cancers. In addition, mRNA transcripts of factors controlling cell cycle and proliferation such as *MCM10* (×8.1, FDR = 0.002), *CCNE2* (×4.1, FDR = 0.0001), *CDK6* (×2.9, FDR = 0), *MDM2* (×3.2, FDR = 0.003) and *CCND3* (×5.7, FDR = 0) were up-regulated in CC of ovarian and lung cancers. Functional annotation of the differentially expressed genes suggests that dysregulation in the steroidogenesis process, estrogen receptors and TGF-β signaling cascades may contribute to the reduced of oocyte developmental competence in patients with ovarian and lung cancers.
Limitations, reason for caution: Further investigations with large number of patients are needed to confirm these results.
Wider implications of the findings: This study opens a new perspective for understanding the pathogenesis of ovarian and lung cancer patients' candidate for fertility preservation before chemotherapy treatment.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Funding by commercial/corporate company(ies), University hospital of Montpellier and Ferring pharmaceutical company are partially supported this study.
Trial registration number: Not applicable.

O-233 When is it safe to conceive after lymphoma treatment?

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Study question: What is the evolution of sperm quality (quantitative and qualitative aspects including DNA fragmentation, chromatin compaction and aneuploidy rates) after radiotherapy or chemotherapy in the case of Hodgkin (HL) or non Hodgkin lymphoma (NHL)
Summary answer: Sperm count and motility, are significantly decreased during the first year after treatment, while DNA fragmentation and aneuploidy rates are significantly higher in the same period. The values recover normality 24 months after treatment and some are even better than those observed before the beginning of the radiotherapy and/or chemotherapy.
What is known already: Hodgkin or non Hodgkin lymphoma is a frequent pathology of young males. The treatment protocols are based on radiotherapy and/or chemotherapy and known to potentially induce sterility. Sperm cryopreservation is thus often proposed. When addressing the kinetics of sperm recovery, and the quality of the gametes, retrospective studies are mainly available and the few prospective ones concern only a very small number of patients, offering thus a low predictive power.
Study design, size, duration: Prospective analysis of sperm parameters, chromatin and DNA quality and aneuploidy rates in a cohort of 75 patients treated for Hodgkin or non Hodgkin lymphoma, before and 3, 6, 12 and 24 months after the end of the treatment. The data are also compared to those obtained in fertile men (controls C).
Participants/materials, setting, methods: All participants signed an informed consent. Sperm parameters were analysed following the WHO recommendations. Chromatin and DNA quality was assessed by SCSA (DFI) and TUNEL, aneuploidy rates by three color FISH. Sperm recovery was analysed on R software by the Kaplan-Meier method and comparative analyses of sperm quality were assessed using Mann-Whitney test.
Main results and the role of chance: Kaplan-Meier estimates showed cumulative rates of normal recovery for sperm production of 92% [95% CI, 72%–99%] and 90% [95% CI, 76%–98%] respectively in patients with ABVD + Radiotherapy or ABVD alone but only 61% [95% CI, 35%–88%] in patients treated by CHOP (p = 0.017). A statistical difference was observed according to diagnosis (p = 0.036) or to the sperm count before treatment (p = 0.001). Before treatment, patients had significantly higher mean values than controls for DFI (18.9 ± 10.9 vs. 11.4 ± 7.6%, $p < 0.05$) or TUNEL (12.9 ± 6.8 vs. 9.9 ± 5.5%, $p < 0.05$). The proportion of patients with abnormal results increased at T6. Sperm aneuploïdy rate was significantly higher in T0 patients (0.78 ± 0.28) compared to controls (0.58 ± 0.18, $p < 0.01$). The aneuploïdy rate significantly increased at T3 (1.1 ± 0.73, $p < 0.05$) and decreased at T6 and T12. Only T24 values were similar to the controls.
Limitations, reason for caution: Not all the patients underwent all the sampling (i.e., 12 or 24 months after treatment), especially when they had recovered normal sperm analysis. Moreover, the variety of treatments in the non Hodgkin lymphoma group, did not allow analysing separately each type of treatment in this group.
Wider implications of the findings: We show that normal parameters are usually recovered 24 months after treatment. They also point out abnormal quality of sperm even before chemotherapy or radiotherapy has started. Further work is needed to understand this point.
Study funding/competing interest(s): Funding by national/international organization(s), French Programme Hospitalier de Recherche Clinique (PHRC).
Trial registration number: Grant no 20030222.

O-234 Poly(A) tail length of maternal-effect gene mRNAs in oocytes is influenced by postovulatory aging

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Study question: Does postovulatory aging affect poly(A) tail length of maternal-effect gene mRNAs in mouse oocytes matured *in vivo* or grown and matured *in vitro* in preantral follicle culture?
Summary answer: Postovulatory aging of oocytes led to a decrease in total mRNA amount as well as a reduction in poly(A) tail length of specific ME gene transcripts that are developmentally relevant. The postovulatory deadenylation of the poly(A) tail appeared to be progressive and independent on egg activation, and was stronger in *in vitro* matured compared to *in vivo* matured oocytes.
What is known already: The maternal effect (ME) is the influence of the mother's genotype on the phenotype of her offspring. In the zygote and during the first cell divisions after fertilization the embryonic genome is not active and therefore early embryonic development depends on the ME gene mRNAs and proteins stored in the oocyte. It has been shown before that postovulatory aging of oocytes from *Xenopus tropicalis* led to developmental defects which appeared to be mainly due to deadenylation of maternal transcripts.
Study design, size, duration: For each experimental group, total RNA from 3 pools of 20 oocytes was analyzed.
Participants/materials, setting, methods: *In vivo* and *in vitro* matured oocytes were either directly frozen after ovulation or aged *in vitro* for 12 or 24 h. qRT-PCR-analysis was performed with random-hexamer or with oligo(dT)$_{16}$ primed cDNA. Poly(A) tail length was quantified by extension Poly(A) test (ePAT) for two representative genes (*Zar1* and *Dnmt1)*.
Main results and the role of chance: Postovulatory aging of *in vivo* matured oocytes led to a decrease in total mRNA amount as well as in poly(A) tail length of the selected ME genes. While after 12 h of aging only one (*Nlrp5*) of the 10 genes investigated showed a trend in poly(A) tail reduction, additional aging for a total of 24 h resulted in a stronger decrease in total mRNA amount and a reduction of poly(A) tail length of 6 ME-genes (*Tet3, Trim28, Dnmt1, Nlrp5, Nlrp14, Oct4*). *In vitro* grown and matured oocytes from preantral follicle culture appeared more susceptible to postovulatory age-related decrease in mRNA amount and poly(A) tail length of 6 ME genes (*Tet3, Trim28, Zfp57, Dnmt1, Nlrp5, Zar1*) already after 12 h of postovulatory aging.
Limitations, reason for caution: High standard deviations were observed for qRT-PCR products of oocyte mRNA resulting in significant changes only for some of the genes. However, since concordant effects were seen for almost all of the genes investigated, a reliable effect of postovulatory aging on poly(A) tail length can be assumed.
Wider implications of the findings: Since ME genes are essential for oocyte developmental competence, postovulatory aging-induced reduction of poly(A) tail length may lead to developmental defects of the embryo. Postovulatory aging of oocytes may be induced in the course of assisted reproduction techniques as well as in *in vitro* maturation of oocytes. Thus these results could be of clinical relevance.
Study funding/competing interest(s): Funding by national/international organization(s), Founded by DFG: GR 1138/12-1, HO 949/21-1, EI 199/7-1.
Trial registration number: None.

O-235 Gonadal effects of acute leukaemia treatment in prepubertal girls

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Study question: Girls diagnosed of acute leukaemia (AL), who undergo Ovarian Tissue Cryopreservation (OTC) to preserve their fertility, need to receive chemotherapy (ChT) prior to OTC. The aim of this study is to analyse the gonadotoxic effect of these treatments in order to find the right time to perform the OTC.
Summary answer: Oncology treatment cannot be ascribed to direct ovarian apoptotic cell-death. The decrease of Foxo3a-phosphorylation found in myeloid-AL treatment samples, followed by an increase of growing follicles average, could suggest that this ChT-regimen has induced a dormant follicles activation prior OTC. Lymphoid-AL ChT-regimens do not appear to affect the ovarian reserve.
What is known already: Dormant primordial follicles are a nonrenewable population representing the 'ovarian reserve' of potential fertility available to each patient. OTC is the only Fertility Preservation option, in girls with AL who need a stem-cell-transplant (SCT).However, is unavoidable that these patients haven't been ChT-treated prior OTC procedure due to their clinical instability and because it's unknown which patients will need this technique until see the treatment response. There's no literature regarding to the AL-treatments effects on dormant follicles.
Study design, size, duration: Thirteen ovarian cortex from girls (6.90 ± 2.69 years, range: 3–10) were included in this study and divided according to the intensity and ChT-regimen prior to OTC. G1(lymphoid-AL, n = 4): CPM:8400 mg/m^2, ADE(5000 mg/m 2,380 mg/m^2, 500 mg/m^2), VCR:33 mg/m^2, MTX:20 g/m^2); G2(lymphoid-AL, n = 3): CPM:4000 mg/m^2, Ara-C:16000 mg/m^2, DNR:240 mg/m^2, VCR:18 mg/m^2); G3(myeloid-AL, n = 3): Ara-C:9000 mg/m^2, Idarubicin:60 mg/m^2, Mitoxantrone:36 mg/m^2, ETOP:900 mg/m^2); G4(Myelodysplastic, Medulloblastoma and Ewing sarcoma, n = 3): without ChT. The mean time between the last dose of ChT and OTC was 40 days.
Participants/materials, setting, methods: Histological/morphometric analysis was carried out to evaluate the primordial, primary, secondary and the total follicular density (follicles/mm^2). Follicular activation was evaluated by Foxo3a phosphorylation (pFoxo3a) immunohistochemistry, a follicle activation suppressor that can be deactivated by phosphorylation. TUNEL-assay was developed to study the cell-damage expressed as nº TUNEL+ cells/nº total cells (DAPI).
Main results and the role of chance: No structural alterations were detected in stroma or follicular structure samples. There were no significant differences in total follicular density between G1(6.92 ± 3.85), G2(14.90 ± 18.13), G3(5.31 ± 6.72) or G4(13.28 ± 10.28). The main population was composed of primordial follicles (G1: 89.13 ± 11.68%, G2: 85.76 ± 10.19%, G3:80.86 ± 18.19%, G4: 88.38 ± 2.23) and no secondary follicles were found in the samples. No significant differences were found in the average of growing follicles, however a trend to decrease the pFoxo3a in dormant follicles and increase in the growing follicles were found in G3 (49.67 ± 9.68%, 19.14 ± 10.29%) compared to G1 (59.17% ± 8.43, 10.87% ± 11.68), G2 (67.36% ± 7.43, 14.24% ± 10.19) or G4 (72.12 ± 12.14, 11.62 ± 2.23) suggesting that a follicle activation has been place. Very little TUNEL-staining was found in all samples and no significant differences between groups were found (G1:0.003 ± 0.001; G2:0.003 ± 0.001, G3:0.001 ± 0.001, G4:0.002 ± 0.001).
Limitations, reason for caution: The main limitations are the small number of patients and obtain control samples from girls without any malignancy/treatment. Girl ovarian biopsies intended for study are very small due to the small ovary size, besides follicle distribution is heterogeneous and follicular density can vary in cortical tissues within the same ovary.
Wider implications of the findings: The fact that the parameters analysed in ChT-treated samples is comparable to those who did not received ChT prior the ovarian tissue extraction, can corroborate perform the OTC just before the SCT, coinciding with other anaesthetic procedures and ensuring the status of complete remission of the underlying disease. However, further studies should be developed including a larger number of patients.
Study funding/competing interest(s): Funding by national/international organization(s), This work was supported by grant PI13/02353 from Instituto de Salud Carlos III, Spanish Government.
Trial registration number: None.

O-236 Counselling performance about fertility preservation in young women facing cancer: a prospective multicentric evaluation of patient's concerns and experiences in 146 women

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Study question: The unique duality involved in confronting a life-threatening diagnosis while simultaneously considering the human desire to have a child presents a struggle both for patients with cancer and for clinicians. The aim of this analysis was to evaluate prospectively the quality of counselling regarding fertility preserving options in young women.
Summary answer: The results of our evaluation strongly suggest that fertility preservation is of great importance to many young women diagnosed with cancer, and that in general the quality of counseling performance regarding fertility preservation is highly valued by our patients.
What is known already: Chemotherapy and radiation therapy often result in reduced fertility, and patients receiving gonadotoxic treatment should be informed of options for fertility preservation and future reproduction prior to such treatment. However, since patients may be focused primarily on their cancer diagnosis, little is known about their fertility concerns, the quality of counselling performance and the effects of counselling on the patients well-being and quality of life.
Study design, size, duration: A survey regarding fertility concerns and counselling performance was prospectively conducted between January 2012 and December 2013 in 5 FertiPROTEKT centres (www.fertiprotekt.com) in Germany and Switzerland.
Participants/materials, setting, methods: A questionnaire was administered to 146 women with newly diagnosed cancer directly after consultation by a fertility specialist. Patients were asked about the quality of counselling performance, the impact of counselling on their individual decision making process, and the effects of counselling on the patients well-being and quality of life.
Main results and the role of chance: The mean age of the patients was 31 years. 93% were sent by their oncologists. Almost all patients rated the overall quality of counselling as very good (62%) or good (50%). In concordance 94% would recommend a counselling to their best friends. After counselling, two-third of the patients (67%) had the impression to be able to make an informed decision due to sufficient information regarding fertility preserving options and their associated chances of becoming pregnant in the future. It is of note, that by using ordinal logistic-regression analyses to identify values that were predictive of higher patient satisfaction, that the only value which was significantly associated with better estimates regarding the overall quality of counselling performance was being counselled in a low volume center ($p = 0.03$).
Limitations, reason for caution: It can be assumed that most of the patients being sent for counselling were already interested in undergoing fertility preservation procedures, which could have caused some bias in the data collection.
Wider implications of the findings: The increasing number of young survivors of cancer with favorable outcomes is defining the need for a more comprehensive approach that will improve the quality of life after cancer, including the preservation of fertility. In this respect clinicians must break through old practice patterns and understand that young patients will no longer be concerned only with preserving their lives in the present but will want to preserve the fullness of their future as well.
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by the participating centers. No competing interests.
Trial registration number: Not applicable.

INVITED SESSION

SESSION 59: DOES DELAYED TRANSFER GIVE BETTER RESULTS (FREEZE ALL POLICY)?

Wednesday 2 July 2014 **12:00 - 13:00**

O-237 Freeze all and transfer later policy-is endometrium more receptive without ovarian stimulation?

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Since the first child was born after frozen embryo transfer (FET) thirty years ago, improved facilities and success rates approaching those in fresh stimulated cycles has encouraged the increasing use of this technique. According to the EIM data the use of FET even exceeds the number of fresh cycles performed in some countries. In this presentation a "*freeze-all-and-transfer-later*" policy with postponement of embryo transfer from the fresh stimulated cycle to the non-stimulated cycle is highlighted.

Endometrial receptivity occurs only during a very short time in the mid-secretory phase of the menstrual cycle. Successful implantation requires synchrony between the blastocyst and the endometrium. Although embryo implantation is a prerequisite for human reproduction, the molecular regulatory mechanism of endometrial receptivity is still largely unclear. Gene expression, immune-regulation and ion channels are developing as important players in the regulatory processes of endometrial receptivity and embryo implantation. However, a valid clinical test to assess biomarkers of endometrial receptivity does not yet exist and no such test can guide us in the question of fresh or frozen embryo transfer.

The obvious pros of FET are the diminished risk of ovarian hyperstimulation syndrome and multiple pregnancies. Further endometrial receptivity is shown to be better in some trials comparing FET vs. fresh embryo transfer in stimulated cycles. This indicates that the endometrial function is better in non-stimulated FET cycles and hence improves implantation, early embryo development and embryogenesis. Trials including cumulative pregnancy rates show a clear advantage of FET when adding the surplus FET cycles. Further singletons conceived by FET have lower perinatal risks compared to singletons from fresh embryo transfer.

Cons of FET are the requirements of a well-functioning freezing program to obtain similar implantation rates in FET and stimulated fresh embryo transfer cycles. Further several studies have shown that the risk of being large-for-gestational age is higher in FET singletons also compared to singletons from natural conception. This indicates that the foetal growth potential differs after cycles with transfer of cryopreserved/thawed embryos, which may be associated with epigenetic modification. This can have detrimental effect on the long-term health in the children with regard to diabetes and cardiovascular disease. A "freeze-all" strategy is a paradigm shift in ART and has some logistic and financial consequences, which make it less feasible to health care professionals. Further we need to acknowledge the wishes of the patients, as FET with many repeated short cycles and pregnancy tests may put more emotional and financial burden to patients than fresh embryo transfer. In the context of patient-centred-care these factors should not be overlooked.

Future research should focus on large randomized trials measuring not only implantation rates but also live birth rates after a freeze-all strategy compared to a conventional fresh embryo transfer policy addressing also different methodologies i.e. vitrification vs. slow freeze techniques and cleavage stage vs. blastocyst transfer. Further we need to explore epigenetic differences between cryopreserved and fresh embryos as such changes indicate if cryo techniques may play a role in the subsequent growth and long-term health of the children.
Conclusion: There is no clear evidence of higher endometrial receptivity in FET cycles although existing randomized trials seem to favour a freeze all strategy, larger trials are needed before a major change in daily clinical practise can be approved. Further epigenetic modification after cryopreservation should be explored and the long term health of FET children should be considered before shifting towards a freeze-all-and-transfer later policy. A future approach for a freeze-all strategy is to pin-point the appropriate patients however the emotional and financial burdens to these patients should also be acknowledged.

O-238 Endometrial receptivity: lessons learned form oocyte donation cycles-the effect of ovarian stimulation

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Embryo Implantation is dependent on multiple factors, which can be classified into three main categories: Embryo Quality, Endometrial Receptivity, and Transfer Efficiency. When implantation is successful, we infer that all

categories of factors were satisfactory, but when it fails, we do not know which factor played the largest role, or if multiple factors were involved. The advent of oocyte donation separated for the first time the source of the oocytes from the uterus, and thus made comparisons of implantation rates possible. It was noted early on that embryos derived from IVF had a higher chance of implanting in a recipient than in the women who produced the oocytes themselves. A number of theories for this difference exist. The most appealing observation is that of advanced luteinization in women undergoing controlled ovarian stimulation. Progesterone levels are often elevated prior to HCG administration, and are clearly much higher at the time of oocyte retrieval. Endometrial histology has been shown to be advanced in stimulated cycles. This lecture will focus on endometrial receptivity in IVF cycles and in artificial cycles associated with frozen embryo transfer and oocyte donation.

INVITED SESSION

SESSION 60: SOCIETY OF REPRODUCTIVE SURGEONS SYMPOSIUM - "PREVENTION AND MANAGEMENT OF LAPAROSCOPIC COMPLICATIONS"

Wednesday 2 July 2014 **12:00 - 13:00**

O-239 Avoiding neurologic injuries due to patient positioning; safe trocar entry; complications related to robotic surgery

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Complications during laparoscopic surgery may occur during points of the surgical procedure. The aim of this presentation is to review the injuries that occur during the critical part of access to the peritoneal cavity. Patient positioning during surgery has long been associated with potential nerve injury. Although the most common nerve initially reported was compression of the common peroneal nerve as it is closely associated with the head of the fibula others are reported. For example, compression of the femoral nerve with acute flexion at the hip can cause femoral nerve palsy. The use of braces to prevent sliding of the patient in steep Trendelenburg position can cause a brachial plexus palsy. The anterior abdominal wall nerves that can be injured during port site closure are the ilioinguinal/iliohypogastric nerves that are located between the external and internal oblique aponeurosis. Keeping trocars above the anterior superior iliac spine will prevent these injuries. The anterior abdominal port placement requires knowledge of the relative anatomy of the blood vessels. The umbilicus is related to the bifurcation of the aorta but in fact, the most caudal large retroperitoneal vessel is the left common iliac vein that can injured during insertion of the primary trocar. The relative anatomy is altered with increasing body fat in such a way that the umbilicus may be caudal to the bifurcation in obese women. Insertion of the accessory trocars must be performed under direct visualization. The main structures to avoid with lateral ports are the superficial epigastric vessels and the inferior epigastric vessels. The superficial epigastric vessels are branches of the femoral system and can be Trans illuminated in thin women. They are injured during port placement. The superficial circumflex iliac vessels can be injured with the lateral placement of the robotic ports. The inferior epigastric vessels are branches of the external iliac and cannot be Trans illuminated but must be directly visualized. They are seen medial to the insertion of the round ligament in the deep inguinal ring. If they are not visualized because of obesity they can be indirectly observed by following the course of the round ligament. The most common reason why robotic surgery is terminated is anesthesia inability to ventilate the patient in steep Trendelenburg. The unique complication with robotic surgery for hysterectomy is Vaginal Cuff Dehiscence. Laparoscopic hysterectomy with vaginal closure has a prevalence of 0.18% (0.09–0.44) versus 0.64 5 (0.47–0.79) with laparoscopic closure and 1.64 % (1.16–2.32) with robotic closure.

O-240 Bowel and urologic injuries; complications from morcellation

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INVITED SESSION

SESSION 61: INFERTILITY TRIALS: ROADMAP TO SUCCESS

Wednesday 2 July 2014 **12:00 - 13:30**

O-241 Clinical trials in infertility with traditional Chinese medicine: lessons in infrastructure and team building

X. Wu[1]
[1]*Heilongjiang University of Chinese Medicine, Harbin, China*

O-242 Resource intensive multicenter trials in infertility: Strengths and weaknesses

R. Legro[1]
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Study question: What is the role of resource intensive multicentre randomized clinical trials (RCTs) vs. pragmatic trials?
Summary answer: Resource intensive clinical trials offer the opportunity to incorporate testable secondary hypotheses as well as the systematic collection of specimens from male, female, foetuses, and infants for repositories for additional hypothesis driven research. This requires however additional financial and personnel resources. The collection of additional data increases the chance for missing data and protocol deviations.
What is known already: Pragmatic trials in infertility are performed more commonly than resource intensive trials. The more focused nature of the trial combined with the lesser burden of participant and investigator participation make this the logical first choice. There are several examples of resource intensive infertility trials, mainly supported by the NIH. Such trials require the establishment and maintenance of infrastructure not usually supported by pragmatic trials including a data coordinating center, a specimen repository, written policies for access to materials and data, and incorporation of multidisciplinary teams to design and analyse data related to secondary hypotheses.
Main results and the role of chance: There is a limited role for resource intensive studies, usually within the context of more extensive extramural funding, who supports the broader reach and additional costs of such trials. Involving stakeholders and future collaborators in the design of the study and the collection of specimens, increases the ultimate utility of the study, but can delay start up. Similarly written policies governing access to data, specimen sharing, and authorship should be established in advance. These large multi-center trials offer the opportunity to explore secondary and mechanistic hypotheses among a large and diverse cohort of patients. Such studies also lend themselves to linked studies of developmental origins, both in foetuses and infants. Finally the design allows for multiple pre-specified secondary analyses where authorship can be shuffled to address the varying contributions of study investigators. This allows for greater academic credit and greater individual investigator buy in for multi-center trials.
Limitations, reason for caution: Resource intensive RCTs should be the exception and not the rule. Most research questions can be answered by simpler studies.
Wider implications of the findings: Resource intensive studies may allow for the broader participation of basic scientists, social scientists, and clinical investigators in RCTs. This may increase the enthusiasm of a broader swath of researchers and ultimate funding agencies in supporting RCTs. Ultimately this may serve to transform RCTs into multi-disciplinary platforms with enhanced research productivity.

O-243 Pragmatic multicenter clinical trials in infertility: Streamlining the trial enterprise

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O-244 Clinical trials and treatment of infertility in low resource settings

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Worldwide, individuals present with in/subfertility problems regardless of socio-economic standing. Should low resource settings be managed differently from

other settings in clinical trials and treatments for in/subfertility care? Is clinical research required within low resource settings in order to generate knowledge or evidence for best practice for fertility care? Evidence-based best practice should drive clinical decision-making when addressing in/subfertility problems, worldwide. Globally, differences exist in clinical presentation and causal factors for in/subfertility across regions. Research is needed to explore and address these differences within populations, while respecting cultural norms. In addition, quality standard-of-care proven clinical treatments for fertility care need to be introduced through pilot studies in a cost-effective manner in low resource settings. Once proven safe and effective within these settings, the interventions need to be scaled-up following implementation studies that either inform or are cognisant of existing policies and regulations, and address systems and service delivery mechanisms. From patient and provider perspectives, this will require assurance of acceptability, accessibility and quality-of-care with accountability.

Contrary to current assumptions, the major burden of in/subfertility is highest in developing countries and countries in transition, not in developed countries. And, similar to other neglected diseases, in/subfertility is least studied within countries presenting with the highest burden. Furthermore, there is a lack of prioritization to conduct research on topics and treatments that address in/subfertility, fertility awareness and pre-pregnancy care, not only in low resource settings. This becomes apparent during allocation of limited health and research funds, and during development of national level reproductive, maternal and newborn health policies and strategies. Fertility issues and risk factors affecting both sexes, impact a woman's ability to become and remain pregnant, and ultimately influences her newborn child's health and future life. Critically, public health in low resource settings often neglect in/subfertility issues despite: 1) the critical importance of these early interventions that impact future maternal/child outcomes; and, 2) affordable interventions that are able to address fertility awareness issues and non-tubal factor in/subfertility. A broad spectrum of affordable assisted fertility care interventions exist. However, higher costs persist in association with more advanced technologies, despite the fact that certain components of these technologies are already decades old. Nevertheless, cost-effectiveness of time-intensive sub/infertility interventions need to be balanced against cumulative costs of inexpensive interventions, while maintaining a risk assessment of not only pregnancy, but maternal and child outcomes.

Low resource settings are not restricted to developing countries but exist worldwide, as a country-specific construct. Lower and higher socio-economic settings may provide access to fertility treatments, but may result in individual catastrophic expenses. Perhaps unintentionally and not exclusively, private practice or for-profit clinics can create conflict-of-interest in research trial design and/or treatment choice. Treatment and clinical research are inescapably distinct. Research with participants who contribute to costs of clinical trials and desire a birth outcome are understandably problematic. Providing a quality "standard-of-care" control can be ambiguous, but should represent what "should be done" based upon best evidence, not necessarily what currently exists. Thus, treatment costs, therapeutic misconception and provider preferences can result in biased clinical studies with risk of undue inducement of child-seeking participants. However, to ensure principles of respect for persons, beneficence/non-maleficence and distributive justice, as with other neglected global diseases, in order to address in/subfertility: 1) Clinical trials are justified within low resource settings in order to understand global and regional differences in disease parameters and develop an evidence-based medicine culture with strengthening of country-level clinical research capacity; and, 2) Components in quality interventions and treatments that have been proven efficacious, require sensitive negotiations to ensure affordability, and to be defined as "standard of care" when researching newer innovations.

SELECTED ORAL COMMUNICATION SESSION

SESSION 62: A LOOK INSIDE THE OCCYTE

Wednesday 2 July 2014 **14:00 - 15:15**

O-245 Metaphase II spindle and cortical actin characteristics in morphologically normal and dysmorphic human oocytes

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Study question: Are morphological anomalies in mature human oocytes, as revealed by transmitted light microscopy, correlated to intrinsic damage to the meiotic spindle and actin cytoskeleton?

Summary answer: Specific morphological anomalies of human oocytes can reflect an intrinsic damage to the cytoskeleton, namely perturbation of spindle disorganization, chromosomes misalignment and cortical actin disorganization.

What is known already: In preparation for *in vitro* fertilization, oocytes are usually assessed by morphological criteria, but the predictive value of this practice is controversial. Currently, very little information, if any, is available on the relationship between morphological features of oocytes intrinsic cellular characteristics, such as the actin cytoskeleton, meiotic spindle and chromosome alignment.

Study design, size, duration: Oocytes used in this study were donated from consenting IVF patients. A total of 81 metaphase II (II) oocytes were analyzed: 32 classified as good quality oocytes (control), 18 MII with smooth endoplasmic reticulum cluster (SER^+MII), 24 with large central dark granulations (Dark-MII) and 7 with large perivitelline space (LPVS-MII).

Participants/materials, setting, methods: Surplus MII oocytes, were fixed within 2 h from recovery and prepared for confocal microscopy. Spindles were analysed for one- and two-dimensional characteristics. Chromosomes were classified as scattered or aligned and the intensity of cortical actin was evaluated.

Main results and the role of chance: The spindle major axis length was significantly increased in Dark-MII (13.3 ± 2.8 µm; p = 0.046) and LPVS-MII (13.9 ± 2.9 µm; p = 0.035) but not in SER^+MII (13.1 ± 2.7 µm; p = 0.088) compared to control (12.2 ± 2.2 µm). Dark-MII and LPVS-MII showed a significantly higher percentage of compromised alignment than controls (29.2% vs. 3.1% p = 0.008; 57.1% vs. 3.1% p = 0.002 respectively). No differences were observed in SER^+MII (5.6% vs. 3.1% p = 0.595). The continuity of subooolemmal cortical actin was lost in 62.5% of Dark-MII ($p < 0.0001$), in 33.3% of LPVS-MII (p = 0.034) and in 33.3% of SER^+MII (p = 0.008).

Limitations, reason for caution: These results require confirmation by larger studies and extension of analysis.

Wider implications of the findings: This preliminary analysis suggests that oocytes with large central dark granulations and with large perivitelline space have an impaired geometry of the spindle as well as a compromised chromosome alignment, while oocytes with smooth endoplasmic reticulum clusters appear to maintain a regular architecture of spindle and chromosomes. The Cortical actin meshwork is affected by discontinuities in all types of dysmorphic oocytes. This might have significant implications for embryo development.

Study funding/competing interest(s): Funding by hospital/clinic(s), Biogenesi Reproductive Medicine Centre.

Trial registration number: N/A.

O-246 The effect of smooth endoplasmic reticulum aggregates in human oocytes on calcium signalling and the significance for oocyte collection cycle outcome

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Study question: What is the effect of smooth endoplasmic reticulum aggregates (aSER) in human metaphase II (MII) oocytes on Ca^{2+} signalling and is the occurrence of MII-aSER positive oocytes (MII-$aSER^+$) in a cohort of MII oocytes influencing the oocyte collection cycle (OCC) outcome?

Summary answer: The Ca^{2+} signalling of MII-$aSER^+$ is compromised compared to control MII oocytes. However, the occurrence of MII-$aSER^+$ in a cohort of MII oocytes does not affect the clinical outcome of the OCC

What is known already: It has been reported that embryos derived from MII-$aSER^+$ have a lower chance of successful pregnancy and may lead to congenital anomalies in the offspring. Therefore it has been strongly recommended that MII-$aSER^+$ should not be inseminated. Physiologically, SER plays a pivotal role in Ca^{2+} storage and release, which is mediated by the inositol 1,4,5-trisphosphate receptor (IP_3R1) during oocyte maturation, fertilisation and pre- and post-implantation embryonic development.

Study design, size, duration: Donated MII-$aSER^+$, which were never used clinically, were immunostained (n = 49) or subjected to Ca^{2+}-oscillation pattern analysis (n = 27). Secondly, we retrospectively analysed over a 1.5-year

period the outcome of 158 cycles with at least one MII-aSER$^+$ (aSER$^+$) and 1589 cycles with no MII-aSER$^+$ (control) in the oocyte cohort.
Participants/materials, setting, methods: MII-aSER$^+$ were evaluated with confocal microscopy (IP_3R1, spindle and chromatin) or injected with the same donor sperm. Outcome measures of aSER$^+$ and control cycles were fertilisation rate (FR), embryos transferred and frozen per fertilised oocytes (embryo utilisation rate, EUR), and ongoing pregnancy/transfer. Statistical analysis was done with The Mann-Whitney-U-test and Chi-square-test.
Main results and the role of chance: The spindle-chromosome complexes of MII-aSER$^+$ were comparable to control oocytes. The IP_3R1 showed a localized pattern, but in MII-aSER$^+$ it also formed a central large aggregation (23 ± 5.7 µm) surrounded by a receptor-free ring and occasionally formed smaller aggregates. Compared to control oocytes, the Ca^{2+}-oscillations frequency in MII-aSER$^+$ was lower (1.7 [1.1–2.4] versus 2.4 [2.0–3.8] peaks/h, $P < 0.05$), the first Ca^{2+} peak lasted longer (4.5 [3.5–5.5] versus 4.0 [3.5–4.0] min, $P < 0.05$) and the total amount of Ca^{2+} released was higher (7.2 [5.5–11.9] versus 5.1 [4.7–7.0] AUxmin, $P < 0.05$). The FR (72.7% versus 71.7%) and ongoing pregnancy rate (19.9% versus 20.8%) were similar between aSER$^+$ and control cycles. There was a tendency for lower EUR of aSER$^+$ cycles compared to the control cycles (35.2% versus 38.4%, $P = 0.0518$).
Limitations, reason for caution: The lack of live birth and neonatal outcome data in our study leaves the matter of safety of MII-aSER negative oocytes in a cohort with at least one MII-aSER $^+$ still under question.
Wider implications of the findings: Our results provide novel insight into the abnormalities of MII-aSER$^+$ by showing that Ca^2-signalling during activation is abnormal. Whether this is associated with compromised embryo development and implantation of the embryos derived is unknown. Although clinical outcome of cycles with at least one MII-aSER$^+$ in which the other oocytes are used is encouraging, the tendency for lower EUR supports the idea that the intrinsic developmental capacity of the entire oocyte cohort may be reduced.
Study funding/competing interest(s): Funding by national/international organization(s), This work was supported by a fundamental clinical research mandate from the Flemish Foundation of Scientific Research to PDS. The authors have no competing interests to declare.
Trial registration number: The study was approved by our local ethical committee (2009/130, 2010/182) and the Federal Ethical Committee (Adv020).

O-247 Meiotic spindle and cortical actin configuration in human oocytes following vitrification using open and closed system

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Study question: Cryopreservation may damage the organization of the metaphase II (MII) spindle and other cytoskeletal attributes of human oocytes. The goal of the present study was to compare two different carriers (open and closed) used for oocyte vitrification, in terms of spindle architecture, chromosome alignment and cortical actin.
Summary answer: Vitrification influences the MII spindle organization, chromosome alignment and cortical actin integrity.
What is known already: Vitrification can alter the organization of the oocyte MII spindle. Different cryopreservation protocols can influence the cytoskeleton response to cryopreservation. So far, open devices have been preferentially chosen due to their capacity to generate high cooling and warming rates. Recently, closed devices have been introduced to avoid cross-contamination during vitrification and liquid nitrogen storage, because they are impermeable to pathogenic agents. It is unknown how different devices impact cytoskeleton stability.
Study design, size, duration: Seventy-five MII oocytes were donated from consenting IVF patients. We analyzed spindle morphological parameters (major, minor axis and area of maximum projection) of fresh (32) and cryopreserved oocytes, of which 23 vitrified with cryoleaf (open system) and 11 with high security straw (closed system).
Participants/materials, setting, methods: Oocytes were fixed after retrieval or vitrified on cryoleaf or high security straw system, within 2 h. After warming oocytes were prepared for confocal microscopy. Spindles were analysed according to one- and two-dimensional characteristics. Chromosomes were classified as scattered or aligned and the intensity of cortical actin signal was evaluated.
Main results and the role of chance: The MII spindles of oocytes vitrified with open and closed systems were morphologically comparable to fresh oocytes (major axis: 12.3 ± 2.0 µm, 14.0 ± 2.2 µm and 12.2 ± 2.2 µm; minor axis: 8.6 ± 0.9 µm, 9.3 ± 1.4 µm and 9.2 ± 1.4 µm; spindle area: 82.6 ± 17.6 µm^2, 100.1 ± 21.8 µm^2 and 92.9 ± 23.5 µm^2, respectively). The percentage of oocytes with chromosome misalignment was significantly higher in vitrified compared to fresh oocytes: 45.5% in closed system, 34.8% in open system and 3.1% in control ($p = 0.001$). Only oocytes vitrified with open system (39.1%) presented a irregular dotted actin signal in the cortex.
Limitations, reason for caution: Data regarding oocytes cryopreserved with high security straw are numerically limited and require further confirmation.
Wider implications of the findings: During vitrification, the geometry of meiotic spindle of oocytes is maintained, but chromosome alignment is compromised, especially in oocytes vitrified with closed system. Moreover, this study demonstrated for the first time that the actin is sensitive to vitrification. The subooolemmal meshwork displays areas of discontinuity in oocytes vitrified with an open system. This could have significant implications for embryo development.
Study funding/competing interest(s): Funding by hospital/clinic(s), Biogenesi Reproductive Medicine Centre.
Trial registration number: Not applicable.

O-248 Global DNA methylation in *in vitro* produced bovine zygotes is affected by the oxygen concentration during *in vitro* maturation

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Study question: The aim of the present study was to investigate the effect of different oxygen tensions during *in vitro* maturation of bovine oocytes on global DNA methylation of resulting *in vitro* produced zygotes.
Summary answer: Results indicate a reduction of the global DNA methylation level of the maternal genome in *in vitro* produced bovine zygotes derived from oocytes matured under 5% oxygen.
What is known already: *In vitro* maturation (IVM) of bovine COC has commonly been performed using an oxygen concentration which is around 20% (atmospheric oxygen concentration), whereas the oxygen concentration in follicular fluid ranges between 3–13%. Thus, the gaseous environment varies considerably between *in vitro* and *in vivo* conditions. Oxygen concentrations during IVM can contribute to oxidative stress which can be associated with various types of DNA damage, including hyper- and hypomethylation of DNA.
Study design, size, duration: Bovine zygotes were generated employing a standard protocol. COC were matured under 5% and 20% oxygen tension. After IVF, presumptive zygotes were denuded and fixed for immunocytochemistry. Quantitative analysis of fluorescence signals was assessed in 1261 zygotes from 9 IVP sessions.
Participants/materials, setting, methods: Global DNA methylation was determined using an anti-5MeC-antibody. DNA was stained with Hoechst 33342. The fluorescence signal of DNA was used for normalization of 5MeC-fluorescence signal. Total fluorescence intensity of the female and male nuclei was measured via NIH ImageJ software and data were analyzed by ANOVA.
Main results and the role of chance: Normalized 5MeC signals were significantly higher in paternal compared to those of maternal pronuclei of zygotes stemming from oocytes matured under 5% oxygen ($p \leq 0.05$). No differences were detectable in both parental pronuclei of zygotes from oocytes matured under 20% oxygen ($p \geq 0.05$). Following IVM under 20% oxygen, maternal pronuclei of zygotes displayed a significant higher fluorescence signal compared to those stemming from oocytes matured under 5% oxygen ($p \leq 0.05$). Maturation at either 5% or 20% oxygen did not cause an effect on normalized fluorescence signals of paternal pronuclei ($p \geq 0.05$).
Limitations, reason for caution: No data regarding the *in vivo* situation are available. Further studies are necessary to analyze gene-specific DNA methylation profiles.
Wider implications of the findings: Results indicate for the first time that the global DNA methylation level of maternal genome can be affected due to the environment. This might have implication for fertilization and further development.
Study funding/competing interest(s): Funding by hospital/clinic(s), Clinic for Cattle, University of Veterinary Medicine Hannover.
Trial registration number: No.

O-249 Prostasomes from the prostate gland attach to the sperm and transfer DNA to the oocyte at fertilization

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Study question: Is DNA from prostasomes transferred to the oocyte at fertilization?
Summary answer: DNA-containing prostasomes fuse with sperm and the DNA is transferred to the oocyte at fertilization
What is known already: Microvesicles are produced from most secretory cells. They transport microRNA, mRNA, proteins and in some cases also DNA. Thereby they are able fuse with distant cells and alter the action of these cells. Our previous studies show that micro vesicles secreted from the prostate gland, prostasomes, contain DNA. They have the ability to attach and fuse with the sperm. Therefore, it can be hypothesized that the DNA enters the oocyte at fertilization.
Study design, size, duration: This is an *in vivo* mouse study of a new mechanism, the transfer of prostasomal DNA at fertilization.
Participants/materials, setting, methods: Human prostasomes were allowed to adhere to mouse sperm. After thorough washing, mouse oocytes were fertilized and the presence of human DNA detected in 2-cell embryos. Prostasome DNA were labeled with acridine orange to be able to detect the prostasomes attached to the fertilized oocyte. Fluorescence microscopy using "apotome", was used to detect prostasome in the fertilized oocyte. Cell-to Ct PCR was used to detect human DNA in the fertilized mouse oocyte.
Main results and the role of chance: Fluorescence microscopy show faint expression of acridine orange labelled DNA in the early mouse embryo. PCR using a human specific probe, show the presence of DNA in the 2-cell mouse embryo.
Limitations, reason for caution: The prostasomes are small microvesicles and the DNA transferred is limited. Therefore, the data is close to detection limit.
Wider implications of the findings: The transfer of DNA, not only from the sperm but also from prostasomes from the prostate gland gives a new concept of DNA transfer at fertilization. The role of the extra DNA is not clear, but it might play a role in DNA repair.
Study funding/competing interest(s): Funding by University(ies), Uppsala University, Family planning foundation.
Trial registration number: N/A.

SELECTED ORAL COMMUNICATION SESSION

SESSION 63: BASIC ENDOCRINOLOGY

Wednesday 2 July 2014 **14:00 - 15:15**

O-250 Gonadotropin hyperstimulation leads to an influx of proinflammatory cells into the mouse ovary and to the expression of proangiogenic genes

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Study question: We sought to determine whether ovarian hyperstimulation in mice leads to an influx of proinflammatory cells in a similar fashion to other inflammatory states.
Summary answer: In close similarity to changes observed in the colonic mucosa of mice with ulcerative colitis, ovarian hyperstimualtion is associated with an influx of inflammatory effector cells and to the expression of key proangiogenic genes.
What is known already: Ovarian hyperstimulation syndrome (OHSS) is associated with increased angiogenesis and vascular leakage. We have previously shown that gonadotropin stimulation and OHSS are associated with proangiogenic-myeloid cell alterations, reflected by a dose dependent increase in ovarian immature myeloid cells and a parallel decrease in dendritic cells. Nevertheless, the role of inflammatory cells and the expression of angiogenic genes that may contribute to the pathophysiology of OHSS remain unexplored.
Study design, size, duration: Animal experiment (4-week old pre-pubertal CX3CR1$^{GFP/+}$ transgenic female mice), treatment- versus controls: Group 1: Stimulation with 20 U pregnant mare serum gonadotropins (PMSG) for 2 days; Group 2: Sham injected controls. On day 3 human chorionic gonadotropin (HCG) (5U) was injected to induce ovulation.
Participants/materials, setting, methods: 48 h after HCG mice were sacrificed and the ovaries were harvested, weighed and enzymatically digested. Single cell suspensions were immunostained using fluorescently labeled: anti-CD11b and CD45 and analyzed by flow cytometry. Histological sections were similarly stained along with PECAM1+ endothelial cells and examined by confocal microscopy. qPCR was performed to determine gene expression.
Main results and the role of chance: When compared to non-stimulated controls, gonadotropin stimulation led to a 3–4 fold increase in the ovarian CD45^{+}CD11b^{+}CX3CR1^{-} neutrophil population. Interestingly, the balance between proinflammatory monocyte derived CD45^{+}CD11b^{+}CX3CR1^{int} effector cells to CD45^{+}CD11b^{+}CX3CR^{high} noninflammatory resident macrophages was significantly increased toward the former. This pattern of inflammatory cell distribution strictly resembles that observed in the colonic mucosa in a mouse model of ulcerative colitis. Finally, we examined the expression of key genes that were previously shown to play roles in angiogenesis. Our results indicated that the expression of S100a8, S100a9, Dmbt1 and Vcan was significantly upregulated in stimulated ovaries compared to controls. On the other hand, the expression of Mmp12 was significantly down regulated.
Limitations, reason for caution: This is an animal experiment and its findings need to be further validated also in humans.
Wider implications of the findings: OHSS is associated with increased inflammation, vascular permeability and angiogenesis. Our study indicates that OHSS is accompanied by ovarian influx of inflammatory cells such as neutrophils and monocyte-derived effector cells, in close resemblance to that observed in other inflammatory states such as ulcerative colitis. This myeloid population shift is associated with differential expression of key angiogenic genes. Studies testing therapies that target these myeloid cells for the prevention and treatment of OHSS are warranted.
Study funding/competing interest(s): Funding by national/international organization(s), Israel Science Foundation (ISF 142/09).
Trial registration number: None.

O-251 Effect of estetrol administration on brain and serum allopregnanolone in intact and ovariectomised rats

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Study question: How Estetrol (E4) administration effects allopregnanolone (AP) synthesis in specific brain structures and whether E4 has a synergic or antagonistic effect on estradiol-mediated AP synthesis.
Summary answer: E4 increases the CNS and peripheral level of AP, behaving as a weak estrogen-agonist in ovariectomised (OVX) rat. The antagonist effect evidenced when E2 valerate (E2) was co-administrated, further profiles E4 as natural SERM.
What is known already: E4, a naturally occurring estrogen only produced by the human fetal liver, is under evaluation in human studies for potential applications in contraception and menopause care. In well-validated and predictive rat models, E4 behaves as a weak estrogen agonist in most tissues investigated (except the breast) and was found to prevent hot flushes in an experimental rat model. Thus, there is evidence for its definition as a SERM. Allopregnanolone is a metabolite of progesterone produced by the CNS, adrenals and ovaries with important neurotrophic/neuroprotective actions.
Study design, size, duration: In vivo model study containing adult female Wistar rats of 3 months old. A total number of 96 rats was used. The duration of the study was approximately 45 days.
Participants/materials, setting, methods: Intact female adult rats received different doses of E4 and OVX rats received different doses of E4, E2V or combinations of them. The concentration of AP was assessed in the frontal and parietal cortex, hippocampus, hypothalamus, anterior pituitary, and serum.
Main results and the role of chance: E4 does not modify AP in intact animals at any site. E4 at the dose of 5 mg/kg/day increased AP content in different brain areas and in the serum of OVX animals, whereas, in presence of estradiol, E4 showed an estrogen-antagonist effect on brain and serum levels of AP. E4 modulates hypothalamic thermoregulatory center.
Limitations, reason for caution: Animal model study with preliminary results. Further human studies are needed.

Wider implications of the findings: The present study evidenced that E4 affects brain and serum AP content with different effects depending the brain area analyzed, the dose of E4 administrated and the hormonal status of the animals receiving E4 treatment. Since the crucial role of AP in brain development, adaptation and senescence, these findings characterize additional E4 roles in human physiology with interesting clinical implications in contraception and menopause hormonal treatment.
Study funding/competing interest(s): Funding by hospital/clinic(s), University Hospital of Pisa.
Trial registration number: None.

O-252 The effects of testosterone on the functional integrity of the human oviduct

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Study question: How do elevated testosterone levels alter the morphology and function of the human oviduct?
Summary answer: Long term treatment with testosterone induced distinctly increased secretory activity in the ampullar cells, accumulation of cell detritus in the lumen and alterations in the ratio of secretory and ciliated cells in the ampulla. Additionally isthmic epithelial breakdown occurred with cases of epithelial attenuation and lumen collapse.
What is known already: Supraphysiological quantities of androgens play a decisive role in the pathogenesis of the polycystic ovary syndrome (PCOS). PCOS is characterized by the presence of cystic ovaries and chronic anovulation and is a major cause for infertility in young women. To date, the effects of long-term testosterone treatment have not been fully evaluated. Precise knowledge on the effects of testosterone in the female genital tract is the prerequisite for developing new strategies for PCOS treatment.
Study design, size, duration: Samples of ampulla and isthmus were obtained postoperatively from transsexual patients (n = 9, age 23–34; BMI 22–34) who had received preoperative testosterone treatment for 1–3 years. Two patients received additional hormonal treatments (goserelin acetate and ethinylestradiol/chlormadinone acetate, respectively). Control samples (n = 11) were taken postoperatively from women (cycle day 1–28) undergoing hysterectomy.
Participants/materials, setting, methods: The study was designed to compare functional morphology of the oviduct in female transsexuals having received androgen treatment for years with that of normal premenopausal women. For this purpose, samples were processed for light microscopy (Hematoxylin and Eosin, HE), histochemistry (Periodic Acid Schiff Reaction, PAS) and scanning electron microscopy (SEM).
Main results and the role of chance: The ampullar epithelial cells of the transsexual patients revealed abundant secretory activity and a unique secretory cell distribution pattern as compared to the controls. The ampullar lumen was regularly filled up with secretions and accumulated cell detritus. However, synthesis of glycoproteins was not affected by testosterone application. Thus, glycoproteins were proven primarily in ciliated cells (CCs) with some apical accumulations which were sporadic to absent in the secretory cells (SCs). In the controls, at day 14 of the cycle (time of ovulation), a distinct increase in glycoprotein synthesis both in CCs and SCs occurred. The isthmus of the transsexuals was characterised by partial to complete epithelial breakdown, attenuation of epithelial cells and closure of lumen.
Limitations, reason for caution: Some patients received additional medications, the effects of which have to be considered. Thus, the treatment with salbutamol (bronchodilatator) caused re-opening of the isthmic lumen, whereas additional treatment with goserelin and ethinylestradiol/chlormadinone acetate induced distinct variations in ampullar epithelial cell size and form resulting in a dedifferentiated appearance.
Wider implications of the findings: Our studies show that excess androgens severely alter secretory activity and cellular breakdown in the human oviduct. Additionally closure of isthmic the lumen implies that PCOS patients with ovulations reveal reduced fertility due to inadequate nutrition and lack of transport of the early embryo. As shown in one case, e.g. a bronchodilatator can induce re-opening of the isthmus pointing to new strategies for increasing success rates of assisted reproductive technologies in PCOS patients.
Study funding/competing interest(s): Funding by University(ies), School of Medicine & Medical Science, University College Dublin.
Trial registration number: N/A.

O-253 The hypothalamic hormone neurokinin B: a novel therapeutic target for menopausal hot flushes

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Study question: Does administration of the hypothalamic hormone Neurokinin B (NKB) elicit menopausal-like hot flushes in healthy pre-menopausal women?
Summary answer: NKB administration to healthy pre-menopausal women results in symptoms and physiological changes analogous to those observed in menopausal hot flushes.
What is known already: Hot flushes affect millions of women worldwide yet their aetiology remains elusive limiting the development of improved therapies.

Hypothalamic NKB expression is markedly increased in post-menopausal women. Furthermore, the NKB receptor is strongly expressed in hypothalamic thermoregulatory centres. In rodents, ovariectomy (as a model of menopause) results in thermoregulatory tail-skin vasodilatation, while ablation of hypothalamic NKB receptor-expressing neurones blocks this effect. Pre-clinical studies therefore suggest that NKB is a potential mediator of menopausal flushing; however no studies have been performed in humans to investigate this.
Study design, size, duration: Design: Translational, randomised, placebo-controlled, double-blinded, 2-way cross-over study comparing administration of NKB to vehicle. The study was performed in a temperature-controlled (24°C) and humidity-controlled (50%) clinical research facility.
Size: n = 9.
Duration: Each participant attended a single 6 h study visit (commencing 9am) during the follicular phase of their menstrual cycle.
Participants/materials, setting, methods: Participants and Methods: Nine healthy pre-menopausal women (age 34 ± 1.4 years) received two 30 min intravenous infusions (randomised to either NKB or vehicle) separated by a 90 min washout interval. Symptoms, skin temperature, skin conductance (sweating), heart rate, blood pressure, and reproductive hormone levels were recorded frequently throughout the study visit (minutely peri-infusion).
Main results and the role of chance: Hot flushes were reported in 7/9 women during NKB administration, and 0/9 during vehicle administration ($p < 0.01$ vs. vehicle). In total there were 12 episodes of flushing during NKB administration with a mean duration of 3.7 ± 0.6 min. Flushing symptoms were localised to the face and upper torso in all women who reported hot flushing (7/7). During hot flush episodes, elevations in mean heart rate (+6.1 ± 2.1 bpm, $p < 0.01$ vs. mean pre-symptoms), mean skin temperature (+0.2 ± 0.08°C, $p < 0.05$ vs. mean pre-symptoms), and alterations in skin conductance suggestive of menopausal flushing were observed. There were no significant changes in BP or reproductive hormone levels (luteinising hormone, follicle stimulating hormone, and oestradiol) during NKB or vehicle administration.
Limitations, reason for caution: Inter-subject variations in stress and background sex steroid levels may have influenced their predisposition for flushing in response to NKB - to minimise this all women attended studies during their follicular phase. However it remains clear that administration of NKB elicited menopausal-like hot flushing in the majority of healthy pre-menopausal women (7/9).
Wider implications of the findings: This is the first-in-human study to investigate symptoms and physiological changes in response to NKB.

We demonstrate that NKB administration to healthy pre-menopausal women results in symptoms and physiological changes analogous to menopausal hot flushes. These data have important implications for the better understanding of the elusive aetiology of menopausal flushing and for the future clinical development of improved targeted therapies for hot flushes.
Study funding/competing interest(s): Funding by national/international organization(s), Wellcome Trust, National Institute for Health Research, Medical Research Council.
Trial registration number: N/A.

O-254 Increased incidence of mitochondrial cytochrome C-oxidase 1 gene mutations in patients with primary ovarian insufficiency

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Study question: This study was aimed to uncover the underlying mitochondrial genetic defect on primary ovarian insufficiency.
Summary answer: There are increased incidence of mitochondrial cytochrome C-oxidase 1 (mt-CO1) gene mutations in patients with primary ovarian insufficiency (POI), mt-CO1 gene mutation may be causal in POI.
What is known already: Mitochondrial dysfunction has been associated with a wide range of human pathology including atherosclerosis, age-related neurodegenerative disease and human aging and infertility. Mitochondrial energy production plays an important role in oogenesis and follicle maturation. Oocytes of women with ovarian insufficiency have been reported to contain a lower mitochondria DNA (mtDNA) copy number than women with a normal ovarian life.
Study design, size, duration: Case-control study, our study was composed of 63 POI patients and 63 age matched health women.
Participants/materials, setting, methods: The entire region of the mitochondrial genome was amplified in POI and age-matched controls using 9 pair sets of primers. According to SureSelectXT Target enrichment system for illumina paired-end sequencing library, Pool samples for multiplexed sequencing. High-throughput sequence was performed using Illumina MiSeq.
Main results and the role of chance: A total of mitochondrial 96 nonsynonymous variations were observed in POI and 93 nonsynonymous variations in control. 21 (9 in POI and 12 in control) nonsynonymous variations have not been reported previously. 8 mitochondrial cytochrome C-oxidase 1 (mt-CO1) misenses variants were identified in POI patients whereas 4 missense mutations in controls. High incidence of CO1 gene missense variants were identified in POI patients compared with controls and the difference was statistically significant (13/63 vs. 5/63, $P = 0.042$).
Limitations, reason for caution: Limitation of this study lies in the small sample size.
Wider implications of the findings: We found high incidence of mt-CO1 missense mutations in idiopathic primary ovarian insufficiency and two novel missense mutations in mitochondrial CO 1 gene were predicted to be probably damaging. Thus, mt-CO1 gene mutation may be an important causal in POI.
Study funding/competing interest(s): Funding by national/international organization(s), Ministry of health.
Trial registration number: 201002013.

SELECTED ORAL COMMUNICATION SESSION

SESSION 64: HOW TO GET THE MOST OUT OF YOUR EMBRYOS

Wednesday 2 July 2014 **14:00 - 15:15**

O-255 Effect of the quality of the additional embryos on the pregnancy and multiple pregnancy rates in IVF cycles: predictions using mathematical formulae and nomograms

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Study question: To assess the influence of adding embryos with different embryo quality (EQ) on pregnancy rate (PR) and multiple pregnancy rate (MPR) in IVF programmes.
Summary answer: The addition of additional embryos, irrespectively of their quality follows a collaborative pattern and always results in an increase in PR and MPR, depending on their respective implantation rates (IR).
What is known already: In IVF both the PR and MPR are related with the number of transferred embryos and their quality. We have previously described a mathematical model which predicted PR and MPR based on mean IR and the number of transferred embryos, but the influence of the different combinations of embryo quality has not been addressed.
Study design, size, duration: A total of 1891 consecutive fresh embryo transfers corresponding to two different centres with different IR and different embryo transfer policies were analysed over a 30-month period.
Participants/materials, setting, methods: PR and MPR observed in the aforementioned programmes were compared with those obtained following three mathematical models: independent model, ground model and collaborative model. We developed a mathematical model and two nomograms to predict the influence of the different combinations of number and embryo quality on PR and MPR.
Main results and the role of chance: The model based on embryo independence at implantation was completely incompatible with the observed data, while both the ground and collaborative models provided an excellent fit. The collaborative model, however, predicted multiple pregnancies, especially triplets, much more accurately. The subsequent analysis based on the number of transferred embryos and their quality found that the additional embryos always increased both PR and MPR. When IR were low the increase in PR was remarkable and the increase in MPR relatively small. On the other hand, with higher IR the increase in PR was mainly due to the increase in MPR, with the same single PR. Two nomograms were developed to visually represent the different outcomes based on embryo quality and the number of transferred embryos.
Limitations, reason for caution: In centres performing no transfers of more than two embryos, the ground model would be similarly consistent with observed data as the collaborative model.
Wider implications of the findings: Our model was tailored according the different IR (from 10% to 50%) and for transfers from 1 to 3 embryos. Our nomograms accurately predicted the influence of the different combinations of number and embryo quality on PR and MPR. From our data, it can be observed that for IR ≥40% including an additional embryo increases PR exclusively by increasing multiplets, the number of singletons remaining constant or even falling for the highest IR.
Study funding/competing interest(s): Funding by hospital/clinic(s), I confirm that I have no conflict of interest in relation to this work.
Trial registration number: Unnecessary.

O-256 Potential of single blastocyst transfer in combination with vitrification of surplus blastocysts in good prognosis patients

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Study question: What is in good prognosis patients, the cumulative ongoing pregnancy rate of single day 5 blastocyst transfer in combination with vitrification of surplus blastocysts (SBT cycles) compared to single day-3 cleavage-stage embryo transfer in combination with freezing of surplus embryos (SET cycles)?
Summary answer: In good prognosis patients, the cumulative ongoing pregnancy rate is similar for SBT cycles and SET cycles.
What is known already: Advances in culture media have led to a shift from cleavage stage to blastocyst stage transfer. The most recent Cochrane database evaluation found a slight benefit for blastocyst transfer however, cumulative pregnancy rates after cleavage stage transfers were far more efficient. This was attributed to the fact that less surplus embryos are cryopreserved in SBT cycles and that slow freezing procedures for surplus blastocysts are suboptimal.
Study design, size, duration: Retrospective analysis of SET and SBT from women <36 years in their first IVF/ICSI cycle with >9 zygotes. The study period started on July 1st 2010 till December 31st 2011 for SET and from January 1st 2012 till June 30th 2013 for SBT. Thawing/warming cycles were evaluated for another 6 months.
Participants/materials, setting, methods: The cumulative ongoing pregnancy rate was defined as the ongoing pregnancy rate per OCC combining ongoing pregnancies after fresh transfer cycles with ongoing pregnancies in cryopreserved embryo transfer cycles from those patients that did not become pregnant in the fresh cycle. Outcome parameters were compared using χ^2 with a 5% significance level.
Main results and the role of chance: Ongoing pregnancy rate was similar after fresh SBT (37.8% 56/148)) as compared to fresh SET (30.3% (27/89)). The embryo cryopreservation rate was significantly higher for SET cycles as compared to SBT cycles (41.7% (535/1282)) versus 31.9% (627/1966) ($p = 0.0001$). The ongoing implantation rate per thawed cleavage-stage embryo was significantly lower than the ongoing implantation rate per warmed blastocyst (10.7%

(22/215) versus 20.0% (31/155)) ($p = 0.0104$). When cumulative ongoing pregnancy rates were calculated per OCC, there was no difference between SET cycles and SBT cycles (52.8% (47/89) versus 58.8% (87/148)).
Limitations, reason for caution: This study is retrospective and the study period for the SET cycles and SBT cycles are different. Cryopreservation of blastocysts was done using vitrification method while cleavage-stage embryos were cryopreserved with freezing method.
Wider implications of the findings: Although there is no benefit of SBT cycles as compared to SET cycles in terms of cumulative ongoing pregnancy rates, to obtain similar cumulative ongoing pregnancy rates less embryos needed to be vitrified and warmed in SBT cycles as those needed to be frozen and thawed in SET cycles. This indicates the superior efficiency of vitrification of blastocysts compared to freezing of cleavage-stage embryos.
Study funding/competing interest(s): Funding by hospital/clinic(s), Ghent University Hospital.
Trial registration number: Not applicable.

O-257 Selection of embryos on day 3 by time-lapse technology or on day 5? The paternal factor brings the answer

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Study question: Knowing that late paternal effects may not be visible before onset of the embryonic genome, the question is if selection of embryos on day 3 by analysing kinetic markers (KMs) is predictive enough to choose precisely those embryo(s) having the capacity to develop to the blastocyst stage and to implant?
Summary answer: Even though the establishment of algorithms based on retrospective analysis of morphology and kinetics of cell divisions until day 3, it is not yet possible to select accurately cleavage stage embryos only on the basis of those that fulfill all KMs without considering the type of injected spermatozoa.
What is known already: Recent publications on time-lapse indicate that the dynamic observation of cleavage stage embryos increase the prediction of which one are most likely to form good quality blastocysts while maintaining transfer on day 3.

However, a negative effect of spermatozoa with large nuclear vacuoles on embryo development was reported which might be noticeable only after day 3 and could contribute to the observation, that not all good quality embryos on day 3 reach the blastocyst stage.
Study design, size, duration: After intracytoplasmic morphologically selected sperm injection (IMSI) on 403 oocytes, embryos were maintained in a time-lapse incubator (EmbryoScope) until embryo transfer on day 5. A retrospective cohort study of KM until day 3 was undertaken on 321 embryos from 36 patients with external previous failure after transfer on day 3.
Participants/materials, setting, methods: Three KMs, including the length of the second cycle (cc2:t3–t2:5 h–12 h), the time to 3 (t3:35.6 h–40.6 h) and 5 (t5:49.5 h–56.7 h) cells were recorded. Top quality blastocyst development on day 5 was related to the number of KM that each embryo fulfills and to the type of injected spermatozoon (degree of nuclear vacuolization).
Main results and the role of chance: The percentage of blastocysts originating from day 3 embryos which met all 3 KM (KM3) was 51.8% and drop significantly to 35.7% ($P < 0.001$) when at least one marker was not achieved (KM <3). Similarly, the rate of top blastocysts decreased significantly from 24.1% to 11.3% in the KM < 3 group ($P < 0.01$).

However, top blastocyst development was additionally influenced by the type of injected spermatozoa. In the KM3 and KM < 3 groups, the rate of top blastocysts was negatively affected when IMSI class II or III spermatozoa was injected. In the KM3 group, the top blastocyst rate dropped from 35.1% to 15.2% ($P < 0.05$), respectively, after IMSI with a normal or with a spermatozoon carrying vacuoles. For KM < 3 embryos, a reduction from 17.2% to 7.6% ($P < 0.05$) was observed.
Limitations, reason for caution: Due to severe teratozoospermic semen samples in our patient group it was not possible to inject all oocytes with morphologically normal spermatozoa. Our observations have to be confirmed in patients with milder form of teratozoospermia and in non-teratozoospermic samples.
Wider implications of the findings: The study demonstrates that if embryo selection is performed on day 3 based only on time-lapse analysis, a non-negligible proportion of embryos will not be properly selected because the KMs did not reflect the paternal factor.

As consequence, in order to take into account the paternal effect, IMSI selection is one option. Finally, selection of embryo(s) on day 5 for transfer is at the present the best and most simple strategy.
Study funding/competing interest(s): Funding by hospital/clinic(s), IVF-Centers Prof. Zech.
Trial registration number: Informed consent was signed by all patients. All works performed were in concordance with the principles for medical research according to the WMA declaration of Helsinki.

O-258 What is the total pregnancy potential per oocyte aspiration after assisted reproduction – in how many cycles are biological competent oocytes available?

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Study question: What is the total pregnancy potential per oocyte aspiration after assisted reproduction – in how many cycles are biological competent oocytes available?
Summary answer: For each live birth there were transferred 5, 26 embryos in either a fresh or a frozen/thawed cycle. The total number of fresh oocytes needed per live birth was 20. In 64 % of the cycles there were no oocytes capable of developing into a child.
What is known already: While stimulation of the women prior to assisted reproduction is associated with increased success rates the full biological pregnancy potential per stimulation cycle remains unknown. Often the failure of establishing a pregnancy is attributed to wrong selection among the embryos and importantly much effort have been put into accessing the developmental competence of individual embryos either morphologically, via genetics, kinetics or through 'omics' of various kinds in order to improve selection.
Study design, size, duration: Retrospective sequential cohort study of cumulative birth rate in 1148 first cycle IVF/ICSI cycles during 2004–2006, including 5-year follow up of frozen embryo replacement (FER) cycles, as under Danish law fertilised embryos can be stored in up to 5 years after cryopreservation.
Participants/materials, setting, methods: All births resulting from 1148 fresh IVF/ICSI-cycles (1028 transfers) and subsequent 634 FER-cycles (604 transfers) were registered. Oocyte number, number of transferred/cryopreserved/thawed and transferred in a FER-cycle were registrered and the subsequent totals were calculated. Children per oocyte and per transferred embryo and percentage of cycles with births were calculated.
Main results and the role of chance: From the 1148 cycles we obtained 9529 oocytes, resulting in 4227 (44% of oocytes) embryos of sufficient quality for either transfer or cryopreservation (GQE). In total 1416 (33% of GQE) were transferred in 1028 fresh cycles and 2811 (67% of GQE) were cryopreserved. In 634 FER-cycles 1867 embryos (66,4%) were thawed, 1091 were transferred and 944 frozen embryos (33,6%) were not used prior to the 5 years expiry date. In total 2507 embryos were transferred resulting in 412 births and 476 live born children.

In conclusion, 36% of the cycles ended with a live birth while in 64% of the cycles no oocytes were retrieved capable of developing into a child. Of the retrieved oocytes only 5% had the competence to develop into a child.
Limitations, reason for caution: One of the limitations of the study is that we included first cycles only, which may have a slightly better outcome than subsequent cycles. Further, 1/3 of the cryopreserved embryos were never used suggesting a marginal residual pregnancy potential not captured by the data.
Wider implications of the findings: In the light of enormous focus on selection of the best embryo to select, we argue that 5% of the oocytes are competent and in 64% of all cycles none of the embryos had the potential to result in the birth of a child.

While we should always strive to select the most competent embryos it is important to realize that we cannot select for something that is not there.
Study funding/competing interest(s): Funding by hospital/clinic(s), Rigshospitalet Copenhagen, Denmark.
Trial registration number: None.

O-259 Cumulative live birth rates (CLBR) according to the number of vitrified oocytes consumed in an ovum donation (OD)/ egg-banking program

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Study question: How does the CLBR increase in OD cycles using vitrified oocytes according to the number of oocytes consumed?
Summary answer: The probability of achieving a baby increases progressively according to the number of oocytes consumed, rapidly until oocyte number 10–12, with a slower pace from the 20th onwards, reaching a plateau close to 100% when 40 vitrified oocytes have been used.
What is known already: So far the efficiency of ovum donation trough egg banking has been measured considering ongoing pregnancy or delivery rates per cycle. Cumulative rates have been considered lately as a much more accurate measure to evaluate the probability of success, allowing the precise description of the rhythm at which live births are attained. Additionally, CLBR calculated according to the number of vitrified oocytes used, provides valuable information on the number of oocytes to be donated.
Study design, size, duration: Retrospective study, January 2007–March 2013.
Participants/materials, setting, methods: University affiliated infertility center. Survival curves and Kaplan-Meier methods were employed to analyze CLBR with respect to the number of oocytes in a retrospective cohort of OD subjects (N = 3446 cycles) who received vitrified oocytes (N = 40,741).
Main results and the role of chance: CLBR increased as the number of vitrified oocytes augmented. Kaplan-Meier survival curve showed a rapid increase of CLBR between 12 (39.4%; 95% CI = 37.5–41.3) and 20 (75.9%; 95% CI = 37.5–41.3) vitrified oocytes used. The increase in CLBR was slower up to 30 oocytes (88.7%; 95% CI = 37.5–41.3) and got close to 100% when 40 oocytes were consumed (97.3%; 95% CI = 94.9–99.7). From then on, a plateau was reached and practically no increase was attained.
Limitations, reason for caution: The strategy applied allows the description of the probability of achieving a live birth according to the number of vitrified oocytes used-up with in a large date set, however this approach cannot predict if the patient will finally succeed and when.
Wider implications of the findings: The measurement of success of egg-banking in OD based on CLBR per number of consumed oocytes provides pragmatic and useful information for physicians and centers in order to manage egg donation programs and for counseling patients not only for OD but for young fertile women who are seeking for fertility preservation.
Study funding/competing interest(s): Funding by hospital/clinic(s), IVI.
Trial registration number: N/A.

SELECTED ORAL COMMUNICATION SESSION

SESSION 65: ORIGIN AND DIAGNOSIS OF MEIOTIC ERRORS

Wednesday 2 July 2014 **14:00 - 15:15**

O-260 Age-related decrease of meiotic cohesins in human oocytes

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Study question: Aneuploidy of fetal chromosomes is one of the causes of pregnancy loss or congenital birth defects. It is known that the frequency of oocyte aneuploidy increases with maternal age in humans. However, the etiology of maternal age-related increases in chromosomal segregation errors remains unclear.
Summary answer: Recent data have highlighted the contribution of cohesin complex in the correct segregation of meiotic chromosomes. It is possible that the long meiotic arrest of oocytes facilitates the deterioration of meiosis-specific cohesion leading to the age-related increase in aneuploidy.
What is known already: In oocytes, cohesions are established during pre-meiotic DNA replication followed by entry into meiosis I during the fetal stage. After homologous chromosomes are connected at the crossover via homologous recombination, the oocytes enter a meiotic arrest until ovulation after sexual maturation. During this prolonged period, the homologous chromosomes are kept together by sister chromatid cohesion distal to the chiasma. It has been shown that meiotic cohesins do not undergo turnover after birth in female mice.
Study design, size, duration: We examined the cohesin levels in dictyate oocytes from different age groups.
Participants/materials, setting, methods: Samples were obtained from the ovarian tissues of 8 women (age range: 19–49 years) and also from 2- and 10-month-old female mice. Ovarian tissue sections were immunostained using cohesin antibodies and the cohesion levels were determined by immunofluorescence.
Main results and the role of chance: The levels of the meiosis-specific cohesin subunits, REC8 and SMC1B, were found to be decreased in women aged above 40 compared with those aged around 20 ($P < 0.01$). An age-related decrease in meiotic cohesins was also evident in mice. Interestingly, SMC1A, the mitotic counterpart of SMC1B, was readily detectable in human oocytes but only barely in mice. The mitotic cohesion levels increased with age.
Limitations, reason for caution: Besides the age-effect, we observed a variation among individuals in the rate of cohesin decrease. This might be due to the differences in genetic background and/or lifestyle.
Wider implications of the findings: The mitotic and meiotic cohesins may act in a coordinate manner in humans to maintain the levels of this protein. The decreased meiotic cohesion subunit levels with age impairs sister chromatid cohesion and increases the rate of segregation error.
Study funding/competing interest(s): Funding by national/international organization(s), KAKENHI funded by JSPS.
Trial registration number: N/A.

O-261 First reporting of aneuploidy rates in preimplantation embryos from fertile couples undergoing preimplantation genetic diagnosis (PGD) for single gene disorders

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Study question: What is the level of aneuploidy in embryos from fertile couples undergoing PGD for monogenic disorders? How does day 3 versus day 5 biopsy and maternal age affect the level of aneuploidy in PGD embryos?
Summary answer: High levels of aneuploidy were identified in preimplantation embryos from monogenic PGD cycles from fertile couples. Over half (405/712) of the monogenic PGD embryos studied (57%) were found to be aneuploid. Aneuploidy was more common in day 3 versus day 5 biopsies and it increased with maternal age.
What is known already: Aneuploidy is a common, well described phenomenon in human preimplantation embryos, associated with failure of pregnancy. Up to date, most data has come from the analysis of Preimplantation Genetic Screening (PGS) embryos from infertile couples, quoting up to 75% aneuploidy in cleavage stage embryos and up to 48% in blastocysts. Combined PGD and PGS diagnosis has been reported clinically but there is no detailed investigation of aneuploidy levels in preimplantation embryos from fertile couples.
Study design, size, duration: Retrospective study. A total of 126 couples were referred for PGD for single gene disorders and PGS for aneuploidy screening between January and November 2012. Overall 845 embryos were biopsied: 355 on day 3 and 490 on day 5. The average maternal age was 35 years of age.
Participants/materials, setting, methods: The biopsied material was subjected to whole genome amplification (WGA). The same WGA product was analysed by PCR using STR markers for the monogenic disorder and by array-CGH for aneuploidy testing. The array-CGH results were analysed in order to identify the level of aneuploidy in this set of embryos.
Main results and the role of chance: Overall 845 embryos were tested, out of which 47 were not tested for aneuploidy and 86 did not produce a result. From the remainder 712 embryos, 307 (43.1%) were euploid and 405 (56.9%) aneuploid.

Overall 419/490 embryos biopsied on day 3 and 293/355 on day 5 had results. On day 3 there were significantly more aneuploid embryos ($p < 0.01$): 149 (35.6%) were euploid and 270 (64.4%) were aneuploid, compared to 158 (53.9%) euploid and 135 (46.1%) aneuploid embryos on day 5.

Aneuploidy varied with maternal age (<35, 35–37, 38–40, 41–42, >42 years) and ranged from 36 to 90%. Women younger than 35 years had significantly less aneuploid embryos than women of 35–37 years ($p < 0.01$), who had significantly less aneuploid embryos than women of ≥41 years ($p < 0.01$).
Limitations, reason for caution: The embryos analysed here came from fertile couples, but they were generated in vitro, after following a stimulation protocol so they do not provide a faithful representation of the embryos generated naturally by a fertile couple.
Wider implications of the findings: A high number of monogenic PGD preimplantation embryos were found to be aneuploid. This prevalence of chromosomal abnormalities could explain cases where transferred unaffected PGD embryos do not lead to a pregnancy. Combining PGD with PGS for aneuploidy screening can help improve pregnancy rates as it allows the selection of unaffected euploid embryos. Karyomapping is a new approach to PGD allowing PGD and aneuploidy analysis to be performed on the same platform.
Study funding/competing interest(s): Funding by hospital/clinic(s), The data analysed in this study came from clinical PGD/PGS cycles.
Trial registration number: N/A.

O-262 Single nucleotide polymorphism (SNP) genotyping and karyomapping of trophectoderm biopsies for the investigation of recombination events in the human preimplantation embryo

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Study question: Can SNP arrays be used to determine recombination events in human blastocysts and if so, how do these events relate to factors of biological significance such as the level of recombination in individual chromosomes, parent of origin of inherited chromosomes and parental age?
Summary answer: Through genotyping parental and grandparental genomic DNA and whole genome amplified products from trophectoderm biopsies, karyomapping identified recombination events across each parental chromosome in human blastocysts enabling correlations to be made between the number of recombinations and various factors of clinical significance.
What is known already: In humans, recombination is important for the successful completion of meiosis, helping in the alignment of homologous chromosomes and errors in the process can lead to aneuploidy which has deleterious effects on the corresponding embryo. The use of karyomapping to detect recombination events across the entire chromosomal complement of euploid and aneuploid human embryos is an important tool for the investigation of its role in normal and abnormal chromosome segregation.
Study design, size, duration: Whole genome SNP genotyping of 32 blastocyst embryos from 9 different couples undergoing preimplantation genetic diagnosis for single gene disorders by karyomapping was carried out. Recombination events were calculated for each of 22 autosomes inherited from father and mother and for X chromosome inherited from mother.
Participants/materials, setting, methods: A single trophectoderm biopsy from each embryo was amplified (multiple displacement amplification) and genotyped for approximately 300 K SNPs genome-wide using a beadarray, according to the manufacturer's instructions (Human CytoSNP-12; Illumina, San Diego, USA) and beadarray data imported directly into dedicated software for karyomapping (Bluefuse Multi v4; Illumina, San Diego, USA).
Main results and the role of chance: In total, 991 chromosomes and 1,528 recombination events were investigated. More recombination events were observed in female meiosis than in male meiosis with the female:male ratio being 1.6. The average number of recombinations was 39.6 ± 1.6 (±standard error of the mean) for female meiosis and 25.1 ± 0.9 for male meiosis. Crossovers for chromosomes inherited from the father were found to range from 0.5 to 1.9 per chromosome. Maternal meiosis crossovers ranged from 0.8 to 3.2. Interestingly, chromosomes 21 and 22 which are found to be aneuploid at high frequencies in human blastocysts, had the lowest rate of recombination, averaged 0.68 and 0.65, respectively. No association of maternal or paternal age with recombination rate could be identified.
Limitations, reason for caution: Study only investigated the rate of recombination events but it is known that other factors also might play a role in human meiosis such as recombination positions. The exact position of recombination cannot be determined with the current version of the analysis software.
Wider implications of the findings: Recombination studies carried out at the single cell level often concentrated on the investigation of specific chromosomes. Karyomapping technology offers the potential of investigating the entire chromosomal complement of embryos and gametes at once and it is expected to offer new insight into the recombination process. The findings of this study endorse the important role of recombination in human meiosis, indicating an inverse correlation of recombination rate with aneuploidy.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Part of the study was funded by Illumina, Inc. A.H.H. and S.K.A.N. are employees of Illumina, Cambridge, UK.
Trial registration number: N/A.

O-263 From polar bodies to blastocysts: meiotic errors and chromosome segregation

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Study question: How chromosome segregate at meiosis and at initial mitoses to define the chromosomal status of blastocysts? In other words, are polar bodies (PBs) and blastomeres predictive of the blastocyst aneuploid condition?
Summary answer: Except for one case, results of PBs or blastomeres were predictive of the chromosome constitution of the blastocysts. In some cases, new aneuploidies were detected in blastocysts, both in the blastocoelic fluid (BF) and trophectoderm (TE) cells, which were either of paternal origin or derived during the first mitoses.
What is known already: Aneuploidy is one of the main factors affecting embryo implantation. According to lately published data, blastocysts represent the stage providing the most reliable results for PGS. Biopsy at previous stages, PBs and blastomeres, seem to be inadequate due to the additional abnormalities contributed by sperm and initial mitoses (PB testing), and to mosaicism (blastomere analysis). More recently, it was reported that the BF contains DNA possibly representing another source of DNA for PGS.
Study design, size, duration: Prospective study including 27 supernumerary blastocysts, both euploid and aneuploid, from 12 couples (maternal age 39.3 ± 2.9 years) undergoing PGS by array-CGH in March–December 2013. The study aim was to verify whether the chromosomal status predicted by PBs or blastomeres corresponded to the ploidy condition in BFs and TE cells.
Participants/materials, setting, methods: Chromosome analysis had been performed by array-CGH on PBs or blastomeres of the 27 blastocysts. From each blastocyst, the BF was aspirated and TE cells were biopsied, and submitted to array-CGH. The results were compared to those obtained by the analysis done at the previous stages to track chromosome segregation.
Main results and the role of chance: DNA was detected in 18 BFs (67%) providing 18 complete sets with the chromosome status available for PBs or blastomeres, BFs and TE cells. Nine cases showed full correspondence between the status predicted by PBs ($n = 5$) and blastomeres ($n = 4$), and that found in BFs and TE cells. In 5 cases, the anomalies predicted by PBs ($n = 4$) or blastomeres ($n = 1$) were found in BFs and TE cells with the addition of new ones. In 3 cases, several aneuploidies were detected in BF and TE cells, some of which were anticipated in the previous stages. In the last case, no correspondence was found between the different stages. In all cases, the euploid/aneuploid condition of BFs and TE cells corresponded; 13 blastocysts showed correspondence for all chromosomes.
Limitations, reason for caution: The testing of PBs provides information on the maternal side only. The limited proportion of BFs in which DNA was demonstrated does not make BF a potential alternative for DNA source in PGS.
Wider implications of the findings: The present data confirm the relevance of the oocyte in determining the aneuploidy of the resulting embryo. Even when in the blastocyst new aneuplodies were found compared to the predictions made by PBs and blastomeres, the anomalies detected at initial stages were still present in the blastocyst. Importantly, the 5 blastocysts predicted to be euploid were actually confirmed to be chromosomally normal. Therefore, initial stages of embryogenesis can provide reliable results for PGS.
Study funding/competing interest(s): Funding by hospital/clinic(s), S.I.S.Me.R.
Trial registration number: Not applicable.

O-264 NGS vs. aCGH for the detection of segmental aneuploidies in human blastocysts

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Study question: To evaluate the capability of next-generation sequencing (NGS) to detect pure and mosaic segmental aneuploidies in trophectoderm biopsies and the concordance rate with results from the current platform of array comparative genomic hybridization (aCGH).
Summary answer: NGS allows the detection of pure segmental aneuploidies in human blastocyst with the same efficiency as aCGH. NGS platform software could be trained to establish new thresholds for the detection of segmental aneuploidies in mosaic blastocysts.
What is known already: Comprehensive chromosomal screening (CCS) has become a must in every fertility center around the world. Nowadays, aCGH is the most used method for this purpose. Recently, NGS has emerged as a promising platform for the aneuploidy detection in the human embryo.
Study design, size, duration: Amplified DNA from trophectoderm biopsies in which segmental aneuploidies (Range: 12.4-187.8Mb) were detected by aCGH in a CCS cycle were selected. A total of 50 segmental aneuploidies were reanalyzed by NGS. In addition, blastocyst containing 17 segmental events were disassembled into single cells and analyzed by fluorescent *in situ* hybridization (FISH).
Participants/materials, setting, methods: Samples from each embryo underwent whole genome amplification. For aCGH, DNA was labeled, co-hybridized in 24 sure arrays and analyzed by BlueFuse Multi software. For NGS, a library was generated from dsDNA and loaded into a MiSeq instrument. Finally, FISH analysis was performed by using telomeric probes for affected chromosomes.
Main results and the role of chance: Segmental aneuploidies were classified into pure and mosaic according to $\log_2$ ratio values in the aCGH experiments. In pure segmentals a concordance rate of 97.1% (34/35) was found with NGS. In the mosaic ones the concordance rate was 80% (12/15). FISH validation for pure segmentals in disassembled blastocysts confirmed the results in 8 out of 10 cases (39.0 ± 18.7 analyzed cells per blastocyst). FISH was performed for 7 mosaic segmentals, showing a mosaic pattern in 4 of them (54.2 ± 34.5 cells), with an average of 39.7 ± 22.5% aneuploid cells per blastocyst.

In total, only 4 discrepancies out of 50 were observed between aCGH and NGS. FISH analysis was performed in 2 of them resulting in a concordance with aCGH in one of them, and with NGS in the other.
Limitations, reason for caution: This study was limited by the sample size. Beyond that, extremely low mosaicism levels in the blastocyst could have not been detected. Finally, we assumed that mosaicism degree in biopsied cells was the same that in whole embryo, but no necessarily.
Wider implications of the findings: Studies like this are essential for the development of appropriate software that allows the efficient translation of NGS into the CCS programs.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), IVIOMICS S.L., ILLUMINA Inc.
Trial registration number: N/A.

SELECTED ORAL COMMUNICATION SESSION

SESSION 66: FEMALE INFERTILITY: DIAGNOSIS AND TREATMENT

Wednesday 2 July 2014 **14:00 - 15:15**

O-265 Does extending luteal support with progesterone beyond positive pregnancy test following IVF treatment improve pregnancy outcome?

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Study question: Does extending luteal support with progesterone following IVF treatment beyond confirmation of biochemical pregnancy test until 12 weeks gestation improve ongoing clinical pregnancy and live birth rates?
Summary answer: Extending luteal support beyond confirmation of biochemical pregnancy does not improve the clinical or live birth rate after IVF.
What is known already: Luteal support following IVF is a compulsory component of IVF treatment. Down-regulation of the pituitary gland, aspiration of granulosa cells and supraphysiological oestradiol levels produced, reduce the implantation and ongoing pregnancy rate following IVF. Supplementation of the luteal phase with progesterone after embryo transfer significantly improves pregnancy outcome. The optimum duration of luteal support has not been defined. Worldwide 15% clinics use progesterone until pregnancy test and 85% up to and beyond 12 weeks gestation.
Study design, size, duration: A prospective randomised double blind placebo controlled trial. The study allocated women with a confirmed pregnancy test result following IVF to receive additional progesterone for 8 weeks or placebo. 467 women were randomised between November 2008 and May 2012. Allocation concealment was revealed after completion of data analysis.
Participants/materials, setting, methods: The trial was performed in a large UK IVF unit. Women received vaginal progesterone (Cyclogest 400 mg BD) or placebo until 12 weeks gestation.

Pregnancy ultrasound including uterine artery doppler analysis and serum biochemical profiles were performed at 7 and 12 weeks. 228 women received progesterone and 233 women received placebo.
Main results and the role of chance: Viable pregnancy at 12 weeks gestation was 167/228 patients (73.3%) in patients exposed to prolonged luteal support compared to 167/223 (71.7%) in patients exposed to placebo; adjusted risk ratio 0.97 (95% CI 0.87 to 1.09). There was no difference in clinical pregnancy or live birth rates between the study interventions.

There was no difference in the incidence of chromosomal abnormalities, doppler indices, pregnancy complications or birth and neonatal outcomes.
Limitations, reason for caution: We believe the results can be universally extrapolated to all patients undergoing IVF treatment.
Wider implications of the findings: Worldwide 85% of IVF clinicians use luteal support beyond confirmation of biochemical pregnancy. Our study reports no advantages of increasing duration of luteal support in terms of clinical pregnancy or live birth rate, incidence of pregnancy complications or adverse neonatal outcome.

By reducing exposure of luteal support to two weeks following IVF treatment, the pregnancy rate is successfully maintained whilst reducing the treatment burden for patients and reducing the financial implications of unnecessary medicines.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Actavis UK Ltd, Moulton Charitable Foundation.
Trial registration number: ISRCTN Registration Number: 05696887, Eudract No: 2006-000599-33.

O-266 The effectiveness of an IUI program compared to no treatment. A matched cohort study

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[3]*Jeroen Bosch Ziekenhuis, Department of Obstetrics and Gynaecology's Hertogenbosch, The Netherlands*
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Study question: What is the effectiveness of an intrauterine insemination (IUI) program compared to no treatment in subfertile couples with unexplained subfertility and a poor prognosis for natural conception?
Summary answer: Treatment with IUI was not effective over no treatment. Only when in vitro fertilization (IVF) was added after IUI failure there were additional pregnancies in the couples treated.
What is known already: Treatment with IUI, with or without hyperstimulation, is in many countries a first-line treatment in couples with unexplained subfertility. This treatment has been compared to other treatments, like expectant management and IVF, in different settings and different patients groups. Currently, the effectiveness of IUI for couples with unexplained subfertility and a poor prognosis on natural conception remains unknown as this intervention has not been tested against expectant management over a considerable period of time.
Study design, size, duration: We performed a retrospective cohort study among couples who started IUI between 2000 and 2007. We matched couples who voluntarily stopped IUI (first or second cycle) to couples who continued IUI for six cycles followed by IVF if necessary (comparable female age, duration of subfertility, diagnosis, primary/secondary subfertility).
Participants/materials, setting, methods: We matched 72 couples that stopped treatment with 144 couples that continued treatment. Couples were censored at the moment they were lost to follow up, when their child wish ended, when IVF was started or if 'no treatment' couples started treatment. Primary outcome was ongoing pregnancy after 3 years.
Main results and the role of chance: The mean female age at inclusion was 32 years, while the median duration of subfertility was 32 months in the no treatment group versus 30 months in the treatment group. After three years, there were 18 pregnancies in the no treatment group (25%) versus 41 pregnancies in the treatment group (28%) ($p = 0.4$). When we also considered the couples from the moment they were treated with IVF, there were 75 (52%) ongoing pregnancies in the treatment group ($p < 0.01$) after 3 years.
Limitations, reason for caution: There is a risk of selection bias due to the retrospective design of our study. We aimed to correct for this by matching the couples on important prognostic factors for obtaining an ongoing pregnancy.
Wider implications of the findings: Our study showed that treatment with IUI is not effective over no treatment on the long run. The time has come to perform a randomized clinical trial comparing IUI with expectant management in couples with a poor prognosis for treatment independent pregnancy.
Study funding/competing interest(s): Funding by University(ies), Academic Medical Center, Amsterdam, the Netherlands, Vrije Universiteit Medical Center, Amsterdam, the Netherlands.
Trial registration number: Not applicable.

O-267 Singleton pregnancy after in-vitro fertilization and factors influencing abnormal placentation

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Study question: Which clinical and laboratory parameters potentially influence occurrence of placenta praevia and other abnormal placentation forms in singleton IVF pregnancies?
Summary answer: Placenta praevia is more common in singleton IVF pregnancies following fresh embryo transfer (ET), ET in a stimulated cycle and ET in good prognosis patients having 2 blastocysts transferred. These factors seem to influence placentation in IVF treated population more than generally accepted risk factors.
What is known already: Placenta praevia and other placental abnormalities occur more often in the IVF pregnancies and the causes are still poorly understood. Placentas derived from assisted reproduction techniques (ART) differ substantially from other placentas irrespective of the ART technique used. The changes in morphology, metabolism and growth were noticed in humans and animals. The proposed reasons were ET technique involving insertion through cervix, the vanishing twin syndrome, hormonal stimulation, media used, higher maternal age and others.
Study design, size, duration: In our retrospective cohort study we analyzed the placental abnormality occurrence in 1126 singleton pregnancies after IVF – ET performed at the Reproductive Unit, University Medical Centre Ljubljana in the time period between January 2004 and December 2011.
Participants/materials, setting, methods: Data on 211 frozen-thawed and 915 fresh IVF- ET cycles with following pregnancies were included in the analysis. Abnormal placentation rates were analysed according to the clinical and laboratory data. Complete Slovenian maternity data were collected using National Perinatal Information System.
Main results and the role of chance: We found no connection of abnormal placentation in 99 mothers (placenta praevia, accreta, retained placenta) to the maternal age ($p = 0.846$, OR = 0.995, 95% CI 0.943–1.050), multiparity ($p = 0.293$, OR = 0.751, 95% CI 0.459–1.235), uterine surgery ($p = 0.490$, OR 1.132, 95% CI 0.718–1.784), fertilization procedure ($p = 0.671$) or supernumerary embryos ($p = 0.275$, OR 1.269, 95% CI 0.827–1.946).

The connection of abnormal placentation to stimulated cycle ET was of borderline significance ($p = 0.064$, OR 0.417, 95% CI 0.150–1.164) and significant to day 5 ET ($p = 0.016$, OR 1.608) as well as to ET of 2 embryos ($p = 0.043$, OR 1.952, 95% CI 0.020–3.735).

There were no cases of placenta praevia following frozen-thawed ET or spontaneous cycle ET. All 33 (incidence of 3.5%) cases of placenta praevia developed after fresh day 5 ET in a stimulated cycle.
Limitations, reason for caution: In our study 94.7% of embryos were transferred at the blastocyst stage on day 5, therefore the influence of embryo development stage to placentation is less certain.
Wider implications of the findings: Placenta praevia is probably connected to the hormonal stimulation or other factors, present only in the fresh stimulated ET cycles. Good prognosis patients are more exposed to placenta praevia. In our study, other known risk factors for the placenta praevia (maternal age, previous uterine surgery, caesarean section, parity) were less important than the IVF factors, and this has not yet been established elsewhere.
Study funding/competing interest(s): Funding by hospital/clinic(s), Department of Obstetrics and Gynecology, University Medical Centre Ljubljana, Lajmerjeva 3, 1000 Ljubljana, Slovenia.
Trial registration number: Not registered.

O-268 Predicting criteria for freeze all policy in high-risk patients after GnRH agonist triggering combined with low dose hCG

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Study question: Can we predict the cases of freeze all due to severe early onset OHSS in high-risk patients after GnRH agonist triggering combined with 1500 IU HCG?
Summary answer: Severe early OHSS can occur even after the GnRH agonist trigger and plus 1500 IU hCG for luteal phase support. The use of GnRH agonist trigger will prevent OHSS even in extreme cases if a freeze-all policy is adopted.
What is known already: Current status of the literature suggests that severe early onset OHSS can be completely prevented, and that late OHSS occurs sporadically with this protocol. A prior study including 182 women who received the GnRH agonist trigger and 1500hCG for luteal support protocol has reported complete prevention of severe early OHSS. Only a few late onset OHSS cases have been reported and this protocol has been recommended for a safe application to any women under risk.
Study design, size, duration: This retrospective cohort study included all women who were at high risk of OHSS and were given a GnRH agonist trigger followed by one bolus of 1500 IU hCG 1 h after oocyte retrieval between August 2010 and January 2013.
Participants/materials, setting, methods: There were 38 women as defined by baseline characteristics [median (interquartile range)]: age 30.5(25–36) years, BMI 26.4 ± 5.2 kg/m^2, AFC 25(18–34), total FSH dosage 1450 IU (1050–2100), AMH 6.8 ng/ml (4.2–9.7 ng/ml), mean number of oocytes retrived 24.4(19–34) and peak serum estradiol level 5090 ± 2899 pg/ml, mean number of 11–14 mm follicles 20(10–28) on the day of ovulation triggering.
Main results and the role of chance: Overall 5 of the 38 (13%) women developed severe early onset OHSS. Five women had severe early OHSS requiring hospitalization and none of these women did undergo embryo transfer, a freeze all strategy was employed. The number of follicles measuring

11–14 mm on the day of triggering was significantly different between women who developed severe early OHSS and those who did not. There was no significant difference between other parameters. The clinical pregnancy rate was 51.5%, the miscarriage rate was 11.7% and the live birth rate was calculated as 45.5% per embryo transfer. Single embryo transfer was performed in all patients.

Limitations, reason for caution: Women who may develop severe early OHSS were defined before administration of hCG. We recommend a freeze-all policy to completely avoid OHSS in this group of high responder patients.

Wider implications of the findings: Although the GnRH agonist plus 1500 IU hCG luteal support protocol significantly decreases the risk of severe OHSS, this life threatening complication can still occur in high-risk patients. It would be necessary to avoid hCG for luteal support and freeze all embryos for future transfer in such women, particularly when there are ≥20 follicles with 11–14 mm intermediate diameter.

Study funding/competing interest(s): Funding by hospital/clinic(s), Istanbul Medical Park Bahçelievler Hospital.

Trial registration number: None.

O-269 Self-operated endo-vaginal tele-monitoring versus traditional monitoring of ovarian stimulation in ART: prospective randomized trial

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Study question: Does self-operated endo-vaginal tele-monitoring (SOET, S) (patients perform sonograms themselves at home) of the ovarian stimulation phase in IVF/ICSI produce similar laboratory, clinical, patient reported and health-economic results as traditional monitoring (non-SOET, NS) (patients come to the centre for sonograms)?

Summary answer: S is not inferior to traditional monitoring (NS) because the numbers of (metaphase-II) oocytes (=primary outcome), good quality embryos, total pregnancies, ongoing pregnancies and embryos to freeze are similar and because S patients feel more contented, less stressed and more empowered than NS patients at a lower societal cost.

What is known already: Monitoring the follicular phase is needed to adapt gonadotrophin dose, detect threatening hyperstimulation and plan HCG-administration. In current practice, patients pay visits to care providers, entailing transportation costs and productivity loss. It stresses patients, partners, care providers and the environment. Patients living at great distance from IVF centres have more difficult access to treatment. Logistics and stress of the follicular phase of ART is often an impediment for treatment. Home monitoring provides a patient-centered alternative.

Study design, size, duration: Non-inferiority RCT between S and NS. Sample size calculations based on n of metaphase-II oocytes at retrieval showed 100 patients needed in each study arm. This also allows comparing patient reported outcomes and health-economic data. Running time: 20 months. Block randomization; allocation concealment through electronic clinical research files.

Participants/materials, setting, methods: Inclusion criteria: <41 years, ICSI, no poor response, two ovaries. Small PC with USB connected vaginal probe; specific web site application. Sonographic training at the centre. 185 eligible patients recruited in four centres: 123 randomized; 121 completed S (n = 59) or NS (n = 62); 62/185 (33%) eligible patients declined participation for various reasons.

Main results and the role of chance: Patient characteristics comparable.

Clinic: Positive acceptance by 2/3 of eligible study patients. No clinical complications. Similar conceptions (P = 0.47) and ongoing pregnancies (P = 1.00).

Laboratory: Similar n of follicles >15 mm at OPU, ova at OPU, metaphase II oocytes (S:9.6 ± 5.5; NS:9.4 ± 6.7)(p = 0.27), log2 n metaphase II oocytes, embryos available at ET, excellent embryos and embryos frozen.

PRO: S (n = 56) showed significantly higher contentedness in patients and partners, higher feeling of empowerment, discretion, and partner participation; and a trend towards less stress than NS (n = 61). Comparing S patients with previous NS attempts (n = 39) showed significant differences in favour of S for all outcome variables ($p < 0.001$).

HE analysis: S resulted in less productivity loss ($p < 0.001$), lower transportation cost ($p < 0.001$), lower sonograms and consultation costs ($p < 0.001$), but higher personnel cost than NS.

Limitations, reason for caution: Non-inferiority of S versus NS can be concluded. Study ended before sample size was reached due to external circumstances (end of financial support) but conclusions are statistically valid. Higher personnel cost artificially induced by study setting (time for randomization, explanation).

Wider implications of the findings: The concept of SOET works. ART sonograms can be made and sent, received and analysed anytime and anywhere wifi is available. This approach offers advantages: similar results with less logistical stress, at lower cost, decreasing environmental impact, empowering patients in urban western settings, increasing and facilitating access to treatment in large countries, allowing disconnection between monitoring and laboratory facilities, improving the role of reproductive midwives in ART, bringing care towards patients in poor resource settings.

Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), IOF (industrial research fund) of Ghent University, Demonstration project of technological innovation in care (Flanders Care; Flemish Government).

Trial registration number: EC/2011/669 (Ethical Committee Ghent University Hospital) and **B670201112232** (Belgian registration).

SELECTED ORAL COMMUNICATION SESSION

SESSION 67: GENETICS IN ANDROLOGY

Wednesday 2 July 2014 **14:00 - 15:15**

O-270 Exome sequencing in TESE children: a pilot study

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Study question: What is the *de novo* exonic mutation rate in children born from fathers that have been diagnosed with the most extreme form of male infertility, non-obstructive azoospermia (NOA), after intracytoplasmic sperm injection with testicular spermatozoa (ICSI-TESE)?

Summary answer: Our pilot study does not show an increased number of *de novo* mutations in the coding sequence in children born after ICSI-TESE compared to the average number reported in healthy controls. In this pilot study the number of *de novo* mutations in children born after ICSI-TESE seems not increased.

What is known already: *De novo* mutations are known to largely originate during male gametogenesis and increase by advancing paternal age. Most genetic diseases caused by such mutations are paternal in origin. In children conceived with epididymal sperm, previous DNA-analysis showed an increase in copy number variation without phenotypic consequences. Yet, NOA males have a theoretical higher risk of producing genetically abnormal gametes, but it remains unclear whether their offspring carry more *de novo* mutations in protein coding sequences.

Study design, size, duration: For this pilot study 10 trios were included between January 2013 and January 2014. Couples were selected based on the degree of spermatogenic impairment upon testicular cytology. We collected a venous blood sample from the child born after ICSI-TESE and both parents, which was analyzed with whole exome sequencing.
Participants/materials, setting, methods: *De novo* mutations were detected by comparison of variants detected in the children to their respective parents. The *de novo* mutation rate of ICSI-TESE children was compared to published data from controls using Student's *t*-test. Also, the distribution of the mutation types were assessed and compared to control data.
Main results and the role of chance: Preliminary data on 5 of 10 trios has so far revealed 2 *de novo* mutations, ranging from 0 to 1 *de novo* mutations per patient. Notably, in three children no *de novo* coding mutation was identified. Importantly, the range of exonic *de novo* mutations per individual is very narrow, varying between 0 and 4 per exome per individual. Our preliminary data may thus suggest that the protein-coding *de novo* mutation rate children conceived by ICSI-TESE is not increased compared to controls. Further analyses to complete this series of 10 trios is still ongoing to validate these results and is expected to be finished by March 2014.
Limitations, reason for caution: It is a pilot study with a limited number of participants and currently incomplete test results. In addition, the exome represents only a small mutational target within the human genome. Whole genome sequencing would be preferred for a better insight in the total number of *de novo* mutations.
Wider implications of the findings: This pilot study will provide a first indication whether or not there is a significantly raised *de novo* mutation rate in children born to NOA fathers, exceeding that of a paternal age effect. Regardless of the results obtained, the outcome will be of societal significance: an increase in mutations will be a warning for the use of ICSI-TESE, while no increase in genetic mutations may reassure the safety of reproductive treatment using testicular sperm.
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: N/A.

O-271 Male reproductive function is genetically determined by polymorphisms in FSHB and FSHR genes

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Study question: Could polymorphisms in FSH receptor gene (*FSHR*) and FSH beta subunit (*FSHB*) gene have a combined effect on FSH levels and male reproductive function?
Summary answer: The −211 G > T polymorphism in *FSHB* gene has a determinant role, slightly modulated by polymorphisms in *FSHR* gene, in FSH plasma levels, sperm count and testicular volume.
What is known already: Recent studies also from our group evidenced that polymorphisms in *FSHR* and *FSHB* genes could modulate FSH plasma levels and could represent valid genetic markers for a pharmacogenetic approach to male infertility treatment. Polymorphisms rs6166 (c.2039 A > G, Asn680Ser) and rs1394205 (c. –29 G > A) in *FSHR* have been better characterized in women, whereas rs10835638 (–211 G > T) in *FSHB* seems to have a determinant role mainly in the male. However, no studies have been performed analyzing the combined effect of these three polymorphisms.
Study design, size, duration: Cross sectional study including 365 consecutive males over a period of 1 year.
Participants/materials, setting, methods: Subjects were: 39 with azoospermia, 177 with oligozoospermia, and 149 with normozoospermia. Main parameters examined were: seminal analysis, FSH, LH, and testosterone levels, testicular volume, rs6166, rs1394205 and rs10835638 (through RFLP and direct sequencing).
Main results and the role of chance: The polymorphism –211 G > T in *FSHB* is significantly associated with FSH plasma levels (9.3 ± 8.2, 7.4 ± 7.2 and 3.0 ± 2.5 IU/L, respectively for subjects GG homozygotes, GT heterozygotes, and TT homozygotes, $P < 0.001$). Polymorphisms −29 G > A and Asn680Ser in *FSHR* considered separately were not associated with different FSH levels. Combined analysis of the three polymorphisms underlined better that the major determinant of FSH plasma levels is polymorphism –211 G > T, but the effect is modulated by the –29 G > A polymorphism, so that subjects with the –211 GG/–29 GG genotype had the highest levels of FSH. No effect was evident for LH and testosterone, whereas, in agreement with these findings, the total sperm count and testicular volume are modulated by the genotype. Subjects TT homozygotes for polymorphism –211 in *FSHB* are invariably azoo-oligozoospermic with low testicular volume and FSH always <8 IU/L. The combined effect of Asn680Ser polymorphism is marginal.
Limitations, reason for caution: Increasing the number of subjects would strengthen the results.
Wider implications of the findings: This is the first study analyzing the combined contribution of the most interesting polymorphisms in *FSHR* and *FSHB* gene to male reproductive function. We showed that the –211 G > T polymorphism in *FSHB* has a determinant role, slightly modulated by polymorphisms in *FSHR*, in FSH plasma levels, sperm count and testicular volume. This polymorphism influences the transcriptional activity of the *FSHB* gene, causing isolated deficiency of FSH and azoo-oligozoospermia, therefore representing the ideal marker for a pharmacogenetic approach to treatment with FSH.
Study funding/competing interest(s): Funding by University(ies), University Hospital of Padova.
Trial registration number: None.

O-272 Does the male factor influence in the embryo aneuploidy rate

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Study question: Investigate the influence of gamete aneuploidy in the chromosome alterations in embryos from egg donors.
Summary answer: Differences in aneuploidy rates are detected in oocytes and embryos from eggs donors. The cause of the high aneuploidy rate in embryos from egg donors are caused mainly by male factor.
What is known already: The data obtained from previous studies show that high aneuploidy rate are present in embryos generated during oocyte donation cycles. It could be due to aggressive stimulation, male factor or other issues, but a definite contributing cause remains to be defined. A better approach for examining the incidence of aneuploidy in young fertile women is to analyse oocytes directly, removing male-derived confounding factors.
Study design, size, duration: Retrospective study. We included the array-CGH results of 53 polar bodies from oocyte egg donors and 56 embryo biopsies from egg donor Comprehensive Chromosome Screening cycles carried out in 2013 from January to December at Instituto Bernabeu. The main outcome measures were aneuploidy, chromosome alteration and monosomy and trisomy rates.
Participants/materials, setting, methods: Oocyte donors were selected according to Instituto Bernabeu egg donation program requirements and ASRM and ESHRE guidelines for oocyte donation. We examined the chromosome content of polar bodies and embryos by array-CGH using the Agilent technology platform (SurePrintG3) according to manufacturer recommendations applied to single-cell.
Main results and the role of chance: Significant differences were reported in the aneuploidy rate between polar bodies (22.6%) and embryos (46.4%) ($p < 0.05$). As for each chromosome, alteration on chromosome 16 (42%) and 22 (18%) were the most frequently involved in aneuploidy in oocytes, followed by chromosomes 20, 10 and 11. Chromosomes 1, 3, 4, 7 were not involved in aneuploidy in oocytes. Alteration on chromosome 16 (24%) was the most frequently involved in aneuploidy in embryos, followed by chromosomes 19 and 5. All the chromosomes were involved in aneuploidy. In order to show the origin of the aneuploidy in embryos, sperm FISH was performed in the recipients males. The incidence of pathological sperm segregation in recipients male was 51.2%.
Limitations, reason for caution: In order to provide more information about the male factor effect in the embryo aneuploidy, haplotyping studies in embryos could definitively identified the origin of the chromosome alteration. The present work suggests that male factor could be responsible of a high percentage of aneuploidy rates, mainly in pathological sperm segregation.
Wider implications of the findings: This investigation reveals that a high percentage of the aneuploidy rate of embryos from egg donor program is caused

by the male factor because a low aneuploidy rate is shown in the oocyte from young egg donors. Comprehensive Chromosome Screening in embryos from an egg donor program could be benefit for the euploid selection especially in pathological segregation males.

Study funding/competing interest(s): Funding by hospital/clinic(s), Instituto Bernabeu.

Trial registration number: Not clinical trial.

O-273 Impairment of DAZ-AZFc gene expression in human male germ cells of men with severe hypospermatogenesis

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Study question: Functional analysis of *DAZ* (*Deleted in Azoospermia*) gene cluster in AZFc during postmeiotic human male germ cell development. Development of expression kit for human genes expressed only in germ cells including the *DAZ* gene copies, to mark the heterogeneity of postmeiotic male germ cell development (spermiogenesis) in men with azoospermia

Summary answer: RT-PCR assays analysing germ cell specific expression of human genes including major AZF genes (AZFa: *DDX3Y*; AZFb: *RBMY*; AZFc: *DAZ1/2/3/4)* were performed in testicular tissue samples gathered from TESE biopsies of men with azoospermia. We identified impairment of *DAZ* gene copy specific transcript splicing processes putatively disrupting their translation control.

What is known already: Some major human spermatogenesis genes are clustered in three distinct regions on the human Y chromosome (Yq11). These have designated as AZFa, AZFb, AZFc because their deletion caused a distinct disruption of germ cell development. AZFa deletion causes complete absence of germ cells, AZFb deletion causes their meiotic arrest. AZFc deletions reduce sperm numbers significantly eventually resulting in complete absence in the semen fluid (i.e. azoospermia). Each AZF interval includes a major AZF candidate gene.

Study design, size, duration: Development of RT-PCR assays for expression analysis of a set of human genes known to be expressed solely in germ cells. Focus on specific expression assays for AZF candidate genes: *DDX3Y* (major AZFa gene); *RBMY* (major AZFb gene); *DAZ1/2/3/4 (*major AZFc gene) to mark presence/absence of spermatogonia, spermatocytes or spermatids.

Participants/materials, setting, methods: Collection of 150 testicular tissue samples from TESE patients displaying with different pathologies classified with Sigg grade 1–5 and corresponding blood samples to isolate DNA, RNA, and proteins. Specific exon bridging primer pairs (to repress genomic DNA amplification) were designed for RT-PCR assays of germ cell specific transcript variants.

Main results and the role of chance: *FGR3* and *DDX3Y* expression was found to be diagnostic for the presence of spermatogonia. Expression of *MAEL* and *RBMY* was found to be diagnostic for the presence of all pre-meiotic germ cells including spermatocytes. Expression of *BRDT* and *DAZ* was found to diagnostic for the presence of postmeiotic germ cells (spermatids). Since expression of the 4 *DAZ* gene copies could be distinguished by specific lengths of their amplification products we revealed dominant expression for *DAZ1* and *DAZ3* in testis tissue with normal spermatogenesis. Instead of this, testicular tissue samples of men with severe disruptions in postmeiotic germ cell development in most tubules displayed significant reproducible impairment of the *DAZ* gene copy expression pattern due to the use of alternative splice sites in the *DAZ* repeat.

Limitations, reason for caution: Testicular histology is highly variable in men with azoospermia also depending on stochastic parameters from the environment like the patient age and life style. Therefore, to reach statistical significance of each variant *DAZ* gene copy expression pattern RT-PCR assays of testicular transcripts in more TESE samples are required.

Wider implications of the findings: All AZF genes are expressed in human testicular tissue. However, specific mutations in these genes causing impairment of human spermatogenesis are not yet known. Expression studies of AZF genes in human testis tissues with distinct testicular pathologies in parallel with some X-chromosomal/autosomal genes known to be expressed solely in a specific germ cell will therefore help to reveal their functional contribution to the phase specific development of human male germ cells.

Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Faculty of Medicine, University of Heidelberg; University Women's Hospital.

Trial registration number: Not necessary.

O-274 β-defensin 126 gene (DEFB126) alteration is associated with male infertility and assisted reproduction technique outcomes (ART)

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Study question: Does two cytosine nucleotide deletion in second exon of β-defensin 126 gene (DEFB126) is associated with unexplained male infertility and the outcomes of assisted reproduction technique (ART)?

Summary answer: Our results revealed a significantly higher rate of homozygote mutation in unexplained infertile compared with control fertile men. The frequency of this mutation was also higher in men with failed IUI compared with successful IUI. Although IVF and ICSI result did not show any association with this mutation.

What is known already: High expression of β-defensin 126 is reported in epididymis, which coats the plasma membrane of sperm during epididy-mal transit. This protein is considered as an important component of the primates and human sperm glycocalyx and supplies sperm for penetrating into the cervical mucus. Previous studies were found DEFB126 variation would affect the sperm function and male fertility rate.

Study design, size, duration: DEFB126 variation was investigated in 40 fertile men and 190 men with unexplained infertility including 35 patients who did not undergo any ART cycles, 76 male partners of unexplained couples whose wives had undergone IUI and 79 male partners of unexplained couples who had tried IVF and ICSI procedures.

Participants/materials, setting, methods: Standard PCR and Single-strand conformation polymorphism (SSCP), Tetra PCR and Sequencing were used to confirm the results of gene mutation. ELISA and Immunochemistry were performed for the assessment of this protein expression on sperm cells.

Main results and the role of chance: Analysis of genetic data revealed 28.8% homozygote deletion in unexplained infertile men while this deletion was detected in 7.5% of controls. The deletion frequency was statistically higher in infertile patients than that of in control group ($P < 0.05$). Results of the IUI showed that 24.4 % of men, whose wife showed a negative result for IUI, were homozygote for this mutation ($P \leq 0.05$) while couples with a positive IUI result showed no mutation. However, no significant differences were found between homozygote mutation and wild type carriers in fertilization rates, implantation rates and clinical pregnancy of IVF and ICSI. The protein expression was less in men with del/del genotype in compare to other genotypes ($P < 0.005$).

Limitations, reason for caution: Sample size was relatively small.

Wider implications of the findings: Results of the present study suggest that common sequence variation of *DEFB126* takes part an important role in impairment of male reproductive function. This could be considered as a critical factor in the success rate of IUI procedure, as men with a homozygote mutation for *DEFB126* are less fertile compared to those with wild type *DEFB126*.

Study funding/competing interest(s): Funding by national/international organization(s), Royan Institute for Reproductive Biomedicine, ACECR.

Trial registration number: None.

Posters Presentations

POSTER VIEWING

ANDROLOGY

P-001 Single cell telomere length assay of spermatozoa showed positive correlation in men's age, no significant difference in sperm morphology and considerable variation between sperm populations

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Study question: In men undergoing in vitro fertilization treatment (IVF), what is the relationship between sperm telomere length, men's age and sperm morphology and how variable are telomere lengths among individual sperm within sperm populations?
Summary answer: Sperm telomere length (STL) increased with men's age, but not with sperm morphology. STL varied considerably among individual spermatozoa from the same men.
What is known already: Reactive oxygen and absent telomerase activity in sperm should reduce telomere length (TL) and contribute to segregation errors, apoptosis, reduced motility and low fertility. While leukocyte telomere length decreases with age, sperm telomere length increases. Some evidence suggests shorter STL in infertile versus fertile men and in oligozoospermic versus normozoospermic men.
Study design, size, duration: To perform this prospective pilot study, we collected 20 sperm single cell from each individual (n = 10).
Participants/materials, setting, methods: 10 semen samples were collected from men undergoing IVF treatment at NYU Fertility Center. A micromanipulator was used to collect 20 individual sperm from each sample. Clinical information included age and sperm morphology. A novel single-cell telomere length assay (SCT-pqPCR) measured telomere to reference gene (T/R) ratio in individual spermatozoa.
Main results and the role of chance: STL in individual sperm increased with advancing age, p = 0.03 (Spearman's correlation coefficient) but did not differ between sperm with normal (12.4 ± 7.86) versus abnormal (13.11 ± 12.6) morphology (p = 0.85, Wilcoxon). STL measured in individual sperm varied markedly within the same sample.
Limitations, reason for caution: The modest sample size in this pilot study should be expanded to further study the effects of aging on mean and variation in STL.
Wider implications of the findings: Older men have longer STL when compared to younger men. TL among individual sperm within an ejaculate varies markedly, perhaps explaining the poor correlation between STL and sperm morphology. No data have yet reported variance in TL within populations of sperm. Study of variation in STL may shed light on the mechanisms of sperm elongation with age in men.
Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), CAPES Foundation Brazil, Dept Ob/Gyn NYU and NIH1UL1RR029893.
Trial registration number: No trial registration number.

P-002 Increased incidence of aneuploidy in severe oligozoospermia samples from carriers of chromosomal rearrangements

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Study question: Is aneuploidy rate increased in spermatozoa from carriers of balanced structural rearrangements presenting with severe oligoasthenoteratozoospermia (OAT)?
Summary answer: The analysis of our results indicates that the frequency of aneuploidy in chromosomal rearrangement carriers is significantly increased in severe OAT samples in comparison to moderate OAT samples. Of the studied 9 chromosomes, only chromosome 21 had a comparable frequency of aneuploidy in both the groups.
What is known already: Structural reorganization carriers usually present compromised fertility due to an increased risk of producing unbalanced gametes with severe abnormalities that can be transmitted to the offspring. During meiosis, the pairing of the chromosomes involved in the translocation may cause disturbances in the proper segregation of the other chromosomes pairs. This phenomenon has been called interchromosomal effect (ICE). Significant increases of gametes with numerical abnormalities have been detected in all types of reorganization carriers.
Study design, size, duration: Until December 2013, a prospective study included 31 male carriers of balanced structural rearrangements. The analysis of sperm cells was performed by Fluorescence In Situ Hybridization (FISH).
Participants/materials, setting, methods: Of the 31 carriers of balanced structural rearrangements, 25 were moderate OAT and 6 were severe OAT. Multicolour FISH was used to estimate the incidence of aneuploidy for the chromosomes X, Y, 13, 15, 16, 17, 18, 21 and 22. Aneuploidy was calculated only for the chromosomes not involved in the translocation.
Main results and the role of chance: In severe OAT, the frequency of aneuploidy for the chromosomes not involved in the rearrangements was significantly higher (7.33 ± 5.09%, range 2.33–16.13%) when compared to moderate OAT (2.43 ± 1.59%, range 0.96–6.50%) (P < 0.01). In normal karyotype patients, the mean frequency of aneuploidy was 4.19% (range 0.53–50.0%) in severe OAT and 1.8% (range 0.41–16.55%) in moderate OAT.

Regarding specific aneuploidies in translocation carriers, severe OAT showed increased incidence for gonosome aneuploidy (3.29% vs 1.37% in moderate OAT), chromosome 13 (2.47% vs 1.06%), chromosome 15 (3.11% vs 1.13%), chromosome 16 (1.44% vs 0.97%), chromosome 17 (3.13% vs 1.45%), chromosome 18 (0.90% vs 0.52%) and chromosome 22 (1.70% vs 1.34%) (P < 0.01). The frequency of aneuploidy for chromosome 21 was comparable between severe and moderate OAT samples (2.08% vs 1.95%).
Limitations, reason for caution: Even if higher aneuploidy levels were detected in OAT translocation carriers compared to normal karyotype OAT samples, the paternal contribution to aneuploidy remains greatly lower compared to the oocyte. Unfortunately, he invasivity of the FISH tests does not permit its use as a selection technique at the time of ICSI.
Wider implications of the findings: These results support the hypothesis of an ICE in translocation carriers, especially in those with severely abnormal semen parameters. The association between severe OAT and increased aneuploidy in conjunction with structural rearrangement probably reflects important disturbances at the time of meiotic segregation. This should be taken into consideration at the time of counselling infertile couples with a male partner carrying a chromosomal rearrangement.
Study funding/competing interest(s): Funding by hospital/clinic(s), SISMeR.
Trial registration number: Not applicable.

P-003 Prevalence of and characteristics of metabolic syndrome in Caucasian-European men presenting for secondary couple's infertility – results of a cross-sectional survey

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Study question: To assess prevalence of and clinical impact of metabolic syndrome (MetS) in European Caucasian men presenting for primary couple's infertility.
Summary answer: MetS accounts for roughly 9% in men presenting for primary couple's infertility and is responsible for a lower general male health status, though not affecting semen parameters.

What is known already: Though diabetes, obesity and single MetS components are known to impact on male reproductive function, MetS has not been comprehensively analyzed in this setting.
Study design, size, duration: Cross-sectional study involving 1483 European Caucasian men presenting for primary couple's infertility.
Participants/materials, setting, methods: Complete clinical and laboratory data from 1169 consecutive infertile men were analyzed. Comorbidities were scored with the Charlson Comorbidity Index (CCI; categorized 0, 1, ≥2); NCEP-ATPIII criteria were used to define MetS. Semen analysis values were assessed based on 2010 WHO reference criteria. Statistics: descriptive and logistic regression analyses.
Main results and the role of chance: MetS was found in 101 (8.6%) of 1169 men. Patients with MetS were older ($p < 0.001$), had a higher BMI ($p < 0.001$), a greater rate of CCI ≥ 1 (χ^2:44.205; $p < 0.001$) as compared with those without MetS. Moreover, MetS patients had a higher level of LH ($p = 0.001$), and were hypogonadal in a higher rate (χ^2:6.958; $p = 0.008$) than patients without MetS. Conversely, no differences were found between groups in terms of FSH, inhibin-B and 17b-estradiol levels and semen parameters. At multivariate logistic regression analysis, FSH (OR: 1.36; $p < 0.001$) and testicular volume (OR: 0.59; $p < 0.001$) achieved independent predictor status for WHO normal semen concentration; conversely, age, CCI scores, MetS, and inhibin-B values did not. No parameters predicted normal sperm morphology or total progressive motility.
Limitations, reason for caution: This is a cross-sectional study, not including healthy controls.
Wider implications of the findings: Casting lights on MetS impact over male reproductive function, prompting further research towards this direction.
Study funding/competing interest(s): Funding by hospital/clinic(s), Department of Urology, University Vita-Salute San Raffaele, Milan, Italy.
Trial registration number: None.

P-004 Age blunts clinically-significant differences between men with primary or secondary infertility - results of a real-life cross-sectional study

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Study question: We sought to assess whether age may differently impact over clinical, laboratory and seminal parameters in Caucasian-European men presenting for either primary or secondary couple's infertility.
Summary answer: The findings of this real-life cross-sectional study showed that hormonal and laboratory values capable of segregating younger primary from secondary infertile men lose relevance with aging. Conversely, older secondary infertile men suffered from a significant poorer general health status than age-comparable primary infertile men.
What is known already: A significant shift toward advanced paternal age has been observed over time. Controversies exit regarding an age-dependent impoverishment of semen parameters. Moreover, clinical and laboratory differences between patients with primary or secondary infertility have been poorly analyzed.
Study design, size, duration: Complete demographic, clinical and laboratory data from 1684 consecutive Caucasian-European infertile men were analyzed.
Participants/materials, setting, methods: Data were collected at an academic outpatient clinic for Couple's Medicine. Male primary and secondary infertility were defined according to the WHO definition criteria. For both groups, median age values were used to segregate patients into younger and older men. Descriptive statistics tested age-related differences in the identified groups.
Main results and the role of chance: Of all, primary and secondary infertility was found in 1483 (88.1%) and 201 (11.9%) patients, respectively. Secondary infertile men were older than those with primary infertility [mean (SD) 39.8 (8.5) vs 36.4 (5.33) yrs; $p < 0.001$]. Younger primary infertile patients showed a lower testicular volume ($p = 0.04$), lower circulating inhibin B levels ($p = 0.01$), higher FSH levels ($p < 0.05$), and a greater rate of individuals with concomitant pathological features at semen analysis ($\chi^2 = 7.85$, $p < 0.05$), as compared with same age-range secondary infertile men. These differences were lost when comparing older primary and secondary infertile patients. Conversely, a greater proportion of older secondary infertile men had CCI ≥ 1 (c2 = 12.02, $p = 0.002$), MetS ($\chi^2 = 9.73$, $p = 0.002$), hypertension ($\chi^2 = 7.27$, $p = 0.007$), and NIH grade 1 obesity ($\chi^2 = 7.57$, $p = 0.02$).
Limitations, reason for caution: Cross-sectional, retrospective analyses
Wider implications of the findings: A better understanding of the pathophysiology and the clinical characteristics of secondary infertility is urgent.
Study funding/competing interest(s): Funding by University(ies). None.
Trial registration number: None.

P-005 Length of infertility affects seminal parameters - findings of a cross-sectional survey in Caucasian-European men presenting for primary couple's infertility

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Study question: To assess if a delay in seeking reproductive medical help [defined as length of a couple's infertility (LI) at first presentation] may negatively impact over male reproductive function.
Summary answer: LI decreased throughout the last 10 years. In this context, LI accounts for a negative impact on semen parameters of men with primary couple's infertility.
What is known already: Controversial data suggested that semen parameters worse with aging, however no evidence claims detrimental effect of delaying in seeking reproductive medical help over semen characteristics.
Study design, size, duration: Cross-sectional study involving 1132 European Caucasian men presenting for primary couple's infertility between 2003 and 2013.
Participants/materials, setting, methods: Complete clinical and laboratory data were analysed. Comorbidities were scored with the Charlson Comorbidity Index (CCI; categorized 0 vs 1 vs ≥2); NCEP-ATPIII criteria were used to define MetS. Complete reproductive history was recorded in order to assess LI. Semen analysis values were based on 2010 WHO reference criteria. Statistics: descriptive/logistic regression analyses.
Main results and the role of chance: Mean (SD; range) patient age was 36.3 yrs (5.3; 20–62). Age at presentation significantly increased over time when comparing 2003–2007 with 2008–2013 period, along with the educational status (all $p = 0.01$). LI was 28.4 mos (23.5; 12–228) at first presentation, whereas it significantly decreased over the analyzed time frame (F: 2.13; $p = 0.02$). When grouping LI into 12-mo time frames (0–12, 13–24, 25–36, 37–48, 49–60, >60mos), patients and partners age, increased over time (all $p < 0.01$). Conversely, sperm concentration, sperm progressive motility, and testis volume (all $p = 0.01$) significantly decreased. No difference was observed in terms of hormonal milieu and CCI. At multivariable linear regression analysis, LI emerged as an independent predictor of sperm concentration (Beta: –0.12; $p < 0.001$) and sperm progressive motility (Beta: –0.12; $p = 0.002$), after adjusting for patients age, educational status, and CCI.
Limitations, reason for caution: This is a cross-sectional study, not including healthy controls.
Wider implications of the findings: This study would suggest that a greater public awareness in the field of reproductive difficulties should be promoted to protect male reproductive health.
Study funding/competing interest(s): Funding by hospital/clinic(s), Department of Urology, University Vita-Salute San Raffaele, Milan, Italy.
Trial registration number: None.

P-006 Low amounts of mitochondrial reactive oxygen species define human sperm quality

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Study question: With this study we intent to understand if sperm cells with different levels of mitochondrial reactive oxygen species (mitochondrial

ROS or mROS; obtained with the probe MitoSOX™ Red) are related with sperm quality and functionality. With this, we want to select a better sperm subpopulation.

Summary answer: We were able to select one (MitoSOX-) of the three subpopulations (MitoSOX-, MitoSOX+ and MitoSOX++) as the one with better characteristics. We found that this subpopulation is related with lower sperm apoptosis, higher viability, sperm parameters quality and pregnancy success.

What is known already: ROS are important in term of both cell signaling and pathology, and mROS are especially known to have major roles. In sperm, besides other sources, the endogenous production of ROS has been linked with the activity of mitochondrial respiratory chain complexes I/III.

In general, both intracellular and extracellular ROS have been associated, not only with sperm damage but also with a positive role in terms of cell functionality, depending on timing/amount of ROS production.

Study design, size, duration: In this study, native sperm samples, samples separated by density gradient centrifugation and sperm separated by swim-up were used. In all these samples, mROS content was analyzed to compare with sperm parameters, apoptosis, viability and pregnancy outcome. Samples were obtained from 100 patients between September 2012 and June 2013.

Participants/materials, setting, methods: Samples were obtained from patients of University Hospitals of Coimbra. Native sperm samples, samples separated by density gradient centrifugation and sperm separated by swim-up were evaluated by flow cytometry for mROS content (MitoSOX™ Red), viability (Sytox) and apoptosis (Annexin-V). Data were compared with sperm parameters, preparation techniques and pregnancy outcome.

Main results and the role of chance: We show that human ejaculates are heterogeneous in terms of mROS production, with three subpopulations clearly detectable, comprised of sperm that produce increasing amounts of mROS (MitoSOX-, MitoSOX+, MitoSOX++). The sperm subpopulation producing the lowest amount of mROS represented the most functional subset of male gametes within the ejaculate, as it was correlated with the highest amount of live and non-apoptotic sperm, and increased both in samples with better semen parameters, and in samples processed by both density gradient centrifugation and swim-up, both known to select for higher quality sperm. Importantly the MitoSOX- subpopulation was clearly more prevalent in samples that gave rise to pregnancies following Assisted Reproduction.

Limitations, reason for caution: Not applicable.

Wider implications of the findings: Our work therefore not only describes discreet human sperm heterogeneity at the mROS level but also suggests that mROS may represent a strategy to both evaluate sperm samples, and isolate the most functional gametes for Assisted Reproduction.

Study funding/competing interest(s): Funding by national/international organization(s), Center for Neuroscience and Cell Biology (CNC) funding is supported by FCT (PEst-C/SAU/LA0001/2011).

Trial registration number: Not applicable.

P-007 Assessment of semen quality by flow cytometry: a new concept of advanced semen analysis

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Study question: Which additional information can we obtain systematically by analyzing semen by flow cytometry?

Summary answer: Advanced semen analysis gets immediate results, and it is very useful in assessing iatrogenic damage. Moreover, by flow cytometry we are able to evaluate the capacitated sperms cells just before treatment in a reasonable time, allowing to detect samples with high dynamic fragmentation.

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.

Study design, size, duration: This is a retrospective observational study with a total of 267 advanced semen analysis from different patients undergoing infertility treatment.

Participants/materials, setting, methods: After a basic seminograma (determining concentration, motility and morphology), the following tests were performed by flow cytometry (MACSQuant Analyzer, Miltenyi Biotec): DNA fragmentation test (SCSA), % of apoptotic cells and % diploid cells. Results were grouped according to the etiology of male factor: normozoospermic (116), oligozoospermic (47), asthenozoospermic (76) and teratospermic (74).

Main results and the role of chance: The group of patients with increased DNA fragmentation (>30%) and higher percentage of diploid cells (>3.4%) were asthenozoospermics followed by oligozoospermics and teratospermics.

Oligozoospermics and asthenozoospermics obtained the highest values ?? of cell apoptosis (>15%).

A total of 8% of normozoospermic patients obtained more than 30% of sperm with DNA fragmentation.

Limitations, reason for caution: None.

Wider implications of the findings: Male factor can be much better studied using flow cytometry than a simple semen analysis. 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.

Study funding/competing interest(s): Funding by hospital/clinic(s), CIRH Foundation.

Trial registration number: None.

P-008 Detection of polymorphic elements in seminal fluid: potential regulators of maternal anti-paternal immune response

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Study question: Although multiple deficiencies and abnormalities can be attributed to sperm inability to achieve pregnancy upon compilation with a fertile female, the role of seminal fluid in stimulating the maternal organism for fertilization, implantation and pregnancy progression has been poorly studied.

Summary answer: The present study demonstrates the presence of soluble major histocompatibility complex (MHC) class I and class II antigens as well as soluble T cell receptor (TCR) αβ and γδ in seminal fluid and shows that at least soluble MHC class II antigens could be produced by sperm.

What is known already: Female mice exposed to seminal plasma show evidence of T cell activation and hyporesponsiveness to male MHC antigens. Candidate immune polymorphic elements of paternal origin mediating maternal immunostimulation and immunosuppression necessary to pregnancy successful outcome include MHC class I and class II antigens as well as TCR molecules, which recognize the complex Class I/antigen or class II/antigen and have been detected in a soluble form as part of the immune suppressor pathway.

Study design, size, duration: Male mice that have given at least one successful pregnancy upon compilation with a female were considered fertile and were included in the study. Sperm and seminal fluid was collected from 8–10 fertile mice 2–4 months old and tested as to sperm quality and polymorphic element content of seminal fluid.

Participants/materials, setting, methods: The expression of classical and non-classical MHC class II antigens in sperm was tested by flow cytometry, confocal and RT-PCR analysis. The presence of cytokines, soluble MHC class I, class II, TCRαβ and TCRγδ in seminal fluid separated by centrifugation at 2000 rpm for 15 min was tested by ELISA.

Main results and the role of chance: Flow cytometry analysis of fertile sperm fulfilling the criteria of physiological cell number, mobility and morphology defined two separate cell populations (A and B) with distinct class II expression. Population A mainly expressed intracellular I-A (42 ± 4%), while population B expressed surface/ intracellular I-A, I-O (17 ± 4/34 ± 5, 33 ± 4 respectively) and to a minimal degree I-M (4 ± 2%). Confocal microscopy analysis confirmed these results. RT-PCR analysis detected transcripts of *Aα-Aβ*, *?α-?β*, *?α-Mβ* but not *CD74* genes. ELISA experiments detected class I, class II, TCRαβ, TCRγδ as well as Interleukin-2 and CD4 in the seminal fluid of all animals tested (n = 8). These results demonstrate the presence of novel elements in the sperm and seminal fluid which could play an important role in sperm-mucus interaction, fertilization and maternal immune responsiveness.

Limitations, reason for caution: Due to the inbred nature of the mouse model used provides the opportunity to examine various parameters in an easily repetitive way. However, it is difficult to evaluate sperm deficiencies and complications similar to human sperm. The significance of the presented findings could be evaluated only when applied to humans.

Wider implications of the findings: Although the detection of HLA-DRB1 on spermatozoa surface has been associated with a reduction of sperm hyperactivity and alterations in sperm kinematic parameters, while the presence of HLA-II antigens on spermatozoa affects sperm count, linearity of movement and sperm-head dimensions, it seems that intracellular MHC class II molecules

as well as immune polymorphic elements including soluble forms of MHC antigens and TCRs in the seminal fluid exert positive signals to fertility and maternal sensitization.
Study funding/competing interest(s): Funding by University(ies), Department of Biology, University of Crete, Heraklion, Crete Greece.
Trial registration number: Not applicable.

P-009 Prevalence of and impact of health-significant comorbidities in Caucasian-European men presenting for primary couple's infertility – results of a cross-sectional survey

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Study question: We sought prevalence of, clinical and seminal impact of comorbidities in Caucasian-European men presenting for primary couple's infertility.
Summary answer: A general lower health status - as assessed with the Charlson Comorbidity Index (CCI) - appears to be associated with a reduced testicular volume, metabolic and hormonal abnormalities, and pathological sperm progressive motility in men presenting for primary couple's infertility.
What is known already: Male factor infertility has been shown to account for a higher CCI, which may be considered a reliable proxy of a lower general health status, regardless of the etiology of pure male infertility.
Study design, size, duration: Complete demographic, clinical and laboratory data from 1435 consecutive infertile men were cross-sectionally analyzed.
Participants/materials, setting, methods: Data were collected at our academic outpatient clinics for Couple's Medicine. Health-significant comorbidities were scored with the CCI. Semen analysis values were assessed based on 2010 WHO reference criteria. Descriptive statistics and logistic regression models tested the association between semen parameters and clinical characteristics and CCI score.
Main results and the role of chance: Of all, 1328 (92.5%) patients had no comorbidities (CCI = 0); conversely, CCI = 1 and CCI ≥ 2 were found in 54 (3.8%) and 53 (3.7%) men, respectively. Patients with CCI ≥ 2 were older ($p = 0.004$), had a lower left testicular volume ($p < 0.001$), and a higher FSH levels ($p < 0.001$). Presence of health-significant comorbidities (namely, CCI ≥ 1) was associated with higher prevalence of hypertension (χ^2: 52.9; $p = 0.005$), obesity (NIH, χ^2: 28.33, $p = 0.002$), Melts (χ^2: 10.77; $p = 0.005$), and pathological sperm progressive motility (χ^2: 8.50, $p = 0.01$). Conversely, CCI scores did not achieve independent predictor status for pathological semen parameters.
Limitations, reason for caution: Cross-sectional, retrospective analyses.
Wider implications of the findings: Patients with primary infertility should undergo a comprehensive assessment of comorbid conditions which may be linked to both the pathogenesis of infertility and the overall male health status.
Study funding/competing interest(s): Funding by hospital/clinic(s). None.
Trial registration number: None.

P-010 Cryoprotectant-free vitrification versus conventional cryopreservation: a comparison of freezing methods on post-thaw sperm quality

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Study question: Can cryoprotectant-free vitrification be used as an alternative method to cryopreserve purified spermatozoa from assisted reproductive technologies?
Summary answer: Conventional cryopreservation and cryoprotectant-free vitrification had equivalent outcomes with respect to sperm motility. The latter method yielded superior results in terms of mitochondrial membrane potential ($\Delta\Psi$) and DNA fragmentation post- thawing.
What is known already: Vitrification allows for the rapid cooling of tissues and cells without ice crystal formation. The birth of a healthy offspring was reported after using spermatozoa that were cryopreserved via a novel aseptic cryoprotectant-free vitrification method, allowing for the preservation of larger volumes of spermatozoa.
Study design, size, duration: During this prospective cross-sectional study, semen samples from thirty HIV-negative patients from the Steve Biko Academic Hospital were collected. Semen analyses were performed according to the WHO (2010) criteria and all samples were normozoospermic. The duration of the sampling and analyses was throughout the period of June-August 2013.
Participants/materials, setting, methods: Semen samples were processed using a discontinuous gradient centrifugation method. Samples were divided and cryopreserved using either cryoprotectant-free vitrification (sucrose + 1% albumin) or conventional slow freezing (TYB). Post-thawing, the motility and kinetic parameters (CASA, MTG-MedeaLAB), $\Delta\Psi$ (flow cytometry; MitoTracker® Red CMXRos) and DNA fragmentation (flow cytometry; APO-DIRECT™) were compared.
Main results and the role of chance: For statistical analysis, a random effect generalized least squares regression (GLS) i.e. a mixed model approach was employed. The average values of the fresh semen samples included in this study were: volume 2.98 ml (±1.071), concentration 41.87×10^6 sperm/ml (±17.81), total motility 74.47% (±9.02) and morphology 9.77% (±2.46). No significant differences were observed in the velocity or kinetic parameters ($p > 0.05$) post-thawing. Preserving spermatozoa by means of cryoprotectant-free vitrification resulted in significantly higher percentages of $\Delta\Psi$ (11.99% ± 4.326% vs. 6.58% ± 1.026; $p < 0.001$) and significantly lower percentages of DNA fragmentation (2.79% ± 1.017% vs. 3.86% ± 1.38%; $p < 0.01$) compared to conventional cryopreservation.
Limitations, reason for caution: This study was only performed on diagnostic samples. Cryoprotectant-free vitrification should be used to preserve sperm samples for therapeutic procedures and determine the outcome on fertilization rates, and subsequently, pregnancy rates.
Wider implications of the findings: Cryoprotectant-free vitrification is an easy, rapid and more affordable technique and requires no special equipment. The use of vitrification for purified sperm samples could potentially result in a superior post-thaw quality of spermatozoa for ART procedures, with positive economic and time implications.
Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), Research Committee of the Faculty of Health Sciences (RESCOM), University of Pretoria, National Research Foundation (NRF).
Trial registration number: Not applicable.

P-011 Are urinary phthalate levels in men related to semen quality and embryo development after medically assisted reproduction?

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Study question: Does environmental exposure to phthalates influence semen quality parameters (sperm count, motility, morphology, vitality, ability to bind hyaluronan) and embryo development (oocyte fertilisation, day 3 embryo quality, blastocyst formation, blastocyst quality) after medically assisted reproduction (MAR) procedure?
Summary answer: Urinary concentrations of several phthalates and their metabolites, reflecting environmental exposure, adversely influence semen quality parameters and could hamper embryo development after medically assisted reproduction.
What is known already: Due to their extensive use in the industry, phthalates are ubiquitously present in the environment. Developmental toxicity of phthalates in laboratory rodents has been well established. Several epidemiologic studies have also addressed the adult male reproductive toxic effects. Although most of them suggest the possible negative influence of some phthalate metabolites on sperm quality, the results are not conclusive. The possible influence of male phthalate environmental exposure on embryo development remains to be studied.
Study design, size, duration: Prospective cohort study including 149 couples in first or second IVF or ICSI attempt at university-based tertiary care centre. The data collected from February 2011 until June 2012. Single-spot urine and

sperm samples collected at oocyte retrieval. Sperm quality analysed according to 2010 WHO criteria. Embryos followed until blastocyst stage.

Participants/materials, setting, methods: Gas chromatography/mass spectrometry was used to measure: di(2-ethylhexyl)-phthalate (DEHP), dibutyl-phthalate (DBP), diethyl-phthalate (DEP) and their metabolites. The molar sums of parent compounds and their metabolites were calculated. To account for urinary dilution, urinary creatinine concentration was measured. For statistical analysis, linear regression and generalized linear models were used.

Main results and the role of chance: Phthalates and their metabolites were detected in >95% of the urine samples with 0.3 ng/mL limit of detection. After adjusting for confounders, an increase in natural logarithm (ln)-transformed urinary sum-DEHP metabolites was associated with lower ln-transformed total sperm count (β = –0.300, 95% confidence interval [CI] –0.523 to –0.078), ln-transformed sperm concentration (β = –0.335, 95% CI –0.540 to –0.130), progressive motility (β = –2.689, 95% CI –5.119 to –0.259), lower ability to bind hyaluronan (β = –4.227, 95% CI –8.225 to –0.229) and sperm vitality (β = –3.111, 95% CI –5.103 to –1.119). Increasing urinary concentrations of DEP metabolite mono-ethyl-phthalate (MEP) and sum-DEP were associated with a lower ratio of optimal day 3 embryos (for MEP; β = –0.036, 95% CI –0.061 to –0.012) and the rate of optimal blastocysts (β = –0.035, 95% CI –0.063 to –0.007).

Limitations, reason for caution: Urinary phthalate exposure measurements are subject to temporal variability. However, single urine sample has been shown to be predictive of individual's longer term exposure. Our study could not account for female phthalate exposure influence on embryo development.

Wider implications of the findings: Our study suggests that the environmental exposure to a number of phthalates can affect semen quality parameters. These results are in concordance with several published studies, suggesting possible negative influence of certain phthalates or their metabolites on male reproductive health. Moreover, according to our results, embryo development after MAR could be disturbed. However, because couples facing subfertility are specific in their characteristics, the results may not be generalized to all men and naturally conceiving couples.

Study funding/competing interest(s): Funding by national/international organization(s), This work was supported by the Slovenian Research Foundation (P3-334-0327).

Trial registration number: None.

P-012 Sperm mitochondrial function in men with normozoospermia and asthenozoospermia

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Study question: One of causes of male infertility can be reduced sperm motility. The reduced efficiency of the mitochondrial respiratory activity may play a role in the development of this disorder. The aim of our study was to comprehensively determine mitochondrial respiratory activity of sperm with normal and reduced motility.

Summary answer: Our data provided by extremely sensitive high-resolution oxygraphy confirm that reduced efficiency of the mitochondrial respiratory activity contributes to the reduced sperm motility.

What is known already: More than 90% of male infertility cases are due to low sperm counts, poor sperm motility, abnormal sperm morphology, or all of these together. In asthenozoospermatic patients, morphological and functional changes in sperm mitochondria have been described. Recent experimental data provided by less sensitive methods then high-resolution oxygraphy suggest that reduced efficiency of the mitochondrial respiratory activity may contribute to the reduced sperm motility.

Study design, size, duration: Prospective study**.** Ejaculates of all 14 men were obtained from IVF Center Prof. Zech, Pilsen. According to the World Health Organization classification, samples were divided into normospermatic (n = 7) and asthenozoospermatic (n = 7) groups.

Participants/materials, setting, methods: In our study, we measured mitochondrial respiratory activity of human sperm, permeabilized by digitonin, by high-resolution oxygraphy, which allows the determination of oxygen consumption from the smallest possible number of germ cells. Respiratory activity of sperm was measured on two-chamber oxygraph Oroboros.

Main results and the role of chance: In asthenozoospermatic samples, significantly reduced activity of complex I (p = 0.007) and increased respiration after application of ATP-synthase inhibitor oligomycin (showing increased uncoupled oxidation and phosphorylation, p = 0.046) were found. Inhibition of complex I by rotenone showed that complex I contribution to the total capacity of oxidative phosphorylation of healthy sperm was relatively lower than it is typical for somatic cells.

Limitations, reason for caution: We did not analyze intact sperm, the spermatozoa cell membrane was permeabilized with digitonin.

Wider implications of the findings: Better characterization of male germ cells, either completely healthy or with affected motility, will help us to understand better the physiological process of fertilization and also to choose the most viable sperm for infertility treatment by methods of assisted reproduction.

Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), Supported by the project ED2.1.00/03.0076 from European Regional Development Fund, the Charles University Research Fund (project number P36), the Specific Student Research Project no. 266 802 and by the grant from Charles University Grant Agency no. 969212.

Trial registration number: None.

P-013 Polyvinylpyrrolidone versus hyaluronic acid: which is more efficient in ICSI procedures with sibling oocytes - a prospective study

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Study question: Does hyaluronic acid (HA) improve the results in intracytoplasmatic sperm injection (ICSI) cycles compared to polyvinylpyrrolidone (PVP) in sibling oocytes from donors, thus avoiding oocyte factor?

Summary answer: HA shows a lower fertilization rate compared to PVP in ICSI procedures with donor oocytes and normozoospermic and no fragmented semen samples. However, there are no significant statistical differences in embryo quality between these two media.

What is known already: It has been reported that spermatozoa able to bind HA "in vitro" are those that have completed plasma membrane remodeling, cytoplasmic extrusion and nuclear maturation. In addition, these spermatozoa seem to have lower chromosomal aneuploidies and DNA fragmentation.

Data about fertilization rate and embryo quality are controversial: some authors haven't found any differences between HA and PVP, while others show better results with HA and claimed that this is a more physiological medium.

Study design, size, duration: Prospective blind study of oocyte donation cycles. Semen samples were normozoospermic with normal levels of DNA fragmentation (≤20%). Sibling oocytes were randomized to be injected with AH or PVP. One or two blastocysts were transferred without knowing the ICSI method used.

Participants/materials, setting, methods: 18 donation cycles were included and ICSI was performed in 205 oocytes. Sibling oocytes were injected 4 h after retrieval.

Spermatozoa were treated with density gradients system or Swim-up.

Embryos were cultured in a time-lapse incubator until blastocyst stage was reached. Fertilization, embryo development and clinical results were assessed.

Main results and the role of chance: 104 PVP-ICSI oocytes were performed compared with 101 HA-ICSI.

Statistical analysis was calculated using the χ^2-method.

There was a significant difference in fertilization rate between PVP-ICSI (80.77%) and HA-ICSI (64.36 %), (p = 0.0084).

Embryo quality was evaluated following ASEBIR criteria (A, B, C, D). There were no statistical differences on day 3 (p = 0.39) and day 5 (p = 0.59).

Furthermore, the percentage of transferred plus vitrified blastocysts was similar in both groups (54.5% HA vs 55.3% PVP).

Pregnancy rate of the entire group was 66.6%.

There were no statistical differences in implantation rate between groups (60% HA vs 64.7% PVP).

Limitations, reason for caution: Differences in fertilization rate could be explained because of the good results obtained with PVP (our average fertilization rate is about 70%).

Regarding to pregnancy and implantation rates, sample size was too small to achieve any conclusion.

Wider implications of the findings: There are no differences in terms of embryo quality in sibling donated oocytes with normal sperm samples (in terms of count and fragmentation).

Perhaps the use of HA could improve the outcome of ICSI cycles with pathological semen. Further studies are necessary to check this hypothesis.

Study funding/competing interest(s): Funding by hospital/clinic(s), IVI Zaragoza.

Trial registration number: This is not a RCT.

P-014 Thalassaemia major, intermedia and sickle cell disease patients have higher incidence of sperm DNA damage compared with infertile men without haemoglobinopathies and controls

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Study question: Firstly, to compare sperm parameters in sickle cell disease (SCD), thalassaemia major (TM) and thalassaemia Intermedia (TI) and to assess sperm DNA damage in these patients by using Sperm-Chromatin-Structure-Assay (SCSA) and flow-cytometry. Secondly, to determine whether hydroxycarbamide (HU) therapy aggravates DNA damage in SCD and TI?

Summary answer: Azoospermia and DNA damage were significantly higher in TM and SCD compared to controls. But sperm parameters and DNA damage in TI were comparable to controls. There was no difference in sperm parameters before and after HU therapy in SCD. Sperm dysfunction in TM may be due to transfusional haemosiderosis.

What is known already: Sperm dysfunction is now a recognised entity in haemoglobinopathies, which is multifactorial in origin (iron load, chelators, hydroxyl urea, disease itself). But there are no studies on comparative evaluation of defective spermatogenesis in different types of haemoglobinopathies or prevalence of sperm DNA damage in these patients.

Study design, size, duration: In a 2 years prospective study 2011–2013, semen parameters of 49 SCD, 49 TM, 21 TI and 203 healthy controls were collected from fertility Laboratory and Reproductive Medicine Unit (RMU) at UCLH. SCSA, flow cytometry and TUNEL were used for quantification of sperm DNA damage in patients and controls.

Participants/materials, setting, methods: Semen parameters of 49 SCD, 49 TM, 21 TI, and 203 normal controls were analysed. Clinical data were obtained from case records. SCSA, flow cytometry and TUNEL were used for quantification of sperm chromatin DNA damage (DFI and HDS) in patients, controls and male infertility samples**.**

Main results and the role of chance: Most sperm parameters were significantly lower in SCD and TM compared to controls. 38% of the TM ($P < 0.001$) and 18% of SCD were azoospermic ($P < 0.01$) compared to only 1% of controls. Sperm parameters in TI were similar to controls. There was no difference in sperm parameters before and after HU therapy in SCD.

Controls had significantly lower DNA damage compared to male infertility subjects as evident from SCSA ($P < .0.01$) and TUNEL ($P < .01$). TM had significantly higher SCSA (20.8 ± 4.4) and TUNEL ($15.2 \pm 9.1\%$) compared to controls ($P < 0.001$) and male infertility subjects ($P < 0.05$). DNA damage was higher in SCD patients following HU therapy compared to pre-HU ($P < 0.05$) and controls ($P < .05$).

Limitations, reason for caution: A larger sample size was not feasible due to time constraints of 2 years of the MSc project. Collection of samples from patients and establishment of new laboratory techniques of DNA damage time were time consuming which was partly obviated by use of multiple techniques for DNA damage.

Wider implications of the findings: Evidence of sperm DNA damage in haemoglobinopathies is an important finding for planning fertility preservation strategies including IVF, as spermatozoa with damaged DNA might be transferred to the offspring, due to loss of natural barriers to fertilization in assisted reproduction. Whether HU induces significant sperm DNA damage with impaired spermatogenesis in SCD and TI needs further work up with longitudinal data and a larger sample size.

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

Trial registration number: None.

P-015 Parenting decision-making and motivations of couples confronted with NOA and failed TESE-ICSI

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Study question: What are the parenting choices and motivations of couples diagnosed with non-obstructive azoospermia (NOA) that had unsuccessful TESE or TESE-ICSI?

Summary answer: Most couples started artificial insemination with donor sperm (AID) after unsuccessful TESE or TESE-ICSI. The main motivation to choose for AID was genetic parenthood of the female partner. Physicians should be aware of the motivations that influence couples decisions to facilitate counseling and decision making.

What is known already: For couples confronted with non-obstructive azoospermia (NOA) testicular sperm extraction (TESE) in combination with intracytoplasmatic sperm injection (ICSI) is currently the only option to have children. Only about one in four of these couples conceive with TESE-ICSI. After failure of TESE-ICSI, these couples could give up on their child wish, opt for artificial insemination with donor sperm (AID), adoption or foster care. Studies on treatment choices and motivations of these couples are not available.

Study design, size, duration: A retrospective observational survey was conducted in 2012–2013 in all 921 couples who had been diagnosed with NOA in the Netherlands between January 2007 and July 2012.

Participants/materials, setting, methods: Couples completed a self-developed questionnaire that covered demographic characteristics, whether they were still trying to fulfill their wish for a child and on chosen options and motivations for these choices. Coded questionnaires and two reminders were sent by mail.

Main results and the role of chance: Out of 494 respondents (54% response rate), 255 couples had an unsuccessful TESE and 67 failed to achieve a pregnancy with ICSI after successful TESE. Most couples had started AID ($n = 163$; 54%) while 21% gave up on their child wish, 13% were indecisive, 9% moved on to adoption and 2% to foster care. The main motivations for AID were genetic parenthood of the female partner (50%) and/or experiencing pregnancy (46%). In case of AID, most relied on sperm from a sperm bank donor (82%) instead of a known donor (18%). The most common motivations for this decision were, respectively, not wanting to trouble relatives and friends (53%), wanting to keep the donation a secret (16%).

Limitations, reason for caution: We did not include couples with NOA who refrained from undergoing TESE. Motivations on why couples gave up on their child wish were not examined.

Wider implications of the findings: Physicians presenting couples with their options after unsuccessful TESE or TESE-ICSI should know that the majority of couples opt for AID. Insight of physicians into the motivations that influence couples decision-making may enable them to facilitate counseling and decision-making. Further research should explore whether decision-making could be facilitated with a decision aid, for which information on the consequences of each option on couples' psychosocial wellbeing should be gathered.

Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), Funding from the Center for Reproductive Medicine of the University of Amsterdam and The Netherlands Organisation for Health Research and Development (ZonMw). No conflict of interest.

Trial registration number: None.

P-016 Chemotaxis assays for human sperm on microfluidic devices

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Study question: By inducing chemotaxis along a longitudinal chemical gradient in a microfluidic devices composed of a biocompatible polydimethylsiloxane

(PMDS) layer and a glass substrate, we present a novel method for the separation of progressive motile sperm from non-progressive motile and immotile human sperm.
Summary answer: The results of a human sperm chemotaxis assay show that progressive motile sperm moved predominantly toward the outlets at an optimal chemical gradient ranging from 0.31 to 0.62 (mg/ml)/mm of acetylcholine.
What is known already: Sperm are known to exhibit chemotaxis, an oriented cell motion, in the presence of hormones, the oocyte microenvironment, and follicular and oviductal fluids; however, the chemicals that actually attract the sperm have not been identified. More recently, a few chemicals that influence the migration of sperm have been identified suggesting that some might have a significant impact on the fertilization process.
Study design, size, duration: Ejaculates were collected from 20 males with normal semen characteristics. The device used allowed us to test the response of sperm to different acetylcholine concentrations. The attractant concentration could be varied at the outlets and compared with the outlet in which no acetylcholine was present.
Participants/materials, setting, methods: Prior to the sperm separation experiment, we performed a simulation using the software COMSOL Multiphysics to verify that a longitudinal chemical gradient was generated in the microchannel.

The microchip was made of polydimethylsiloxane and glass because both substrates are biocompatible and preserve sperm cell functionality.

Based on simulation results, 2 µL of phosphate buffer saline, 100 mg/ml of acetylcholine solution, and 1/2, 1/4, 1/8, 1/16, 1/32 and 1/64 acetylcholine dilution series were carefully dropped into each outlet; each mixture was incubated for 5 min prior to addition of 1 µL human semen sample into the inlet. After 10 min concentration and motility of sperm that reached the outlet were counted under a microscope using a cell counter.
Main results and the role of chance: Simulation with COMSOL software shows that acetylcholine dropped in the outlet spontaneously diffused toward the inlet within 5 min, and the fluorescence intensities of each area of the microchannel decreased from outlet to inlet.

Twenty sperm samples were used. The results showed a statistically significant difference between the control and the acetylcholine dilution series with *p*-value less than 0.05. Moreover, the number of sperm counted at a 1/8, 1/16 dilutions was significantly different from that of the other dilution ratios ($p < 0.05$). The results of statistical analyses indicated that the chemical gradient generated in the microchip had a significant effect on sperm mobility. The progressive sperm tended to swim towards the outlet as a response to the specific chemical gradient. Furthermore, we found that a chemical gradient generated by 1/8 and 1/16 dilutions of 100 mg/ml acetylcholine was the optimal concentration.
Limitations, reason for caution: Microfluidic devises provide a simple, convenient, and disposable platform for separation of motile sperm. Moreover, an important feature is that progressive motile sperm can be selectively separated in the microchip in an environment that mimics that of the female oviduct. More experiments are necessary to demonstrate that the proposed microchip may be a useful tool for ICSI for sperm selection.
Wider implications of the findings: Microchips based on microfluidic technologies provide one of the most suitable methods for rapidly mixing and separating samples, as well as handling small amounts of sample and reagent, and integrating components for reaction and detection.

With these advantages, microfluidic-based microchips have been applied to several areas of biological research such as analysis or separation of DNA, proteins, and cells. Moreover, many researchers are studying the separation or culture of living cells using microfluidic-based microchips, since the microenvironment of the microchip can be formulated to more closely resemble *in vivo* conditions, such as those required for fertilization and development, than conditions in a culture dish. Moreover understanding how and when sperm cells are attracted to the egg could have profound effects on reproduction and contraception.
Study funding/competing interest(s): Funding by hospital/clinic(s), Centro Fecondazione Assistita, Via Tasso 480 - 80123 - Napoli, Italy.
Trial registration number: No trial registration number.

P-017 Angiotensin II (AngII) regulates human testicular peritubular cell functions via AT1 receptor

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Study question: Is AngII involved in the regulation of human peritubular cells and could it thereby contribute to male (in)fertility?
Summary answer: The receptor for AngII, AT1R, is present in the cells of the wall of seminiferous tubules in men and in cultured peritubular cells it causes contractions and increases IL-6 expression, suggesting that AngII is involved in sperm transport and possibly local inflammatory events and male infertility.
What is known already: Endocrine and locally derived factors regulate testicular peritubular cell function, including a recently identified prostaglandin metabolite, which modifies contractile and morphological features of testicular peritubular myoid cells. AngII is known to regulate rodent peritubular cells but whether it plays a role in human testicular peritubular cell function was unknown, as was the possibility that mast cells, via their product chymase (CHY), may generate a local AngII-enriched microenvironment.
Study design, size, duration: Cultured testicular peritubular cells (passages 5–12) from 9 different patients with normal spermatogenesis (HTPCs) or impaired spermatogenesis/testicular fibrosis (HTPC-Fs) were used. Cell contractions were tested for 20 min, IL-6 expression was measured after 6 h of stimulation. As negative control, medium alone was added to the cells instead of stimulants.
Participants/materials, setting, methods: Cell-contractions were performed for 20 min and IL-6 mRNA induction was monitored for 6 h. These drugs were used: 1 µM AngII, 0.1 µM AngII, 0.1 µM losartan, 0.1 µM losartan/0.1 µM AngII. IL-6 was quantified by qPCR, AT1R and CHY-expressing mast cells were localized immunohistochemically in testicular biopsies.
Main results and the role of chance: In testicular biopsies, only peritubular cells and blood vessels were AT1R immunopositive. Cultured cells expressed AT1R but not ATR2 mRNA. Cells contracted upon 0.1 and 1 µM AngII ($p < .05$) compared to control or losartan alone. Pretreatment with losartan neutralized the AngII effect ($p < .05$). IL-6 mRNA was induced by 0.1 and 1 µM AngII after 6 h compared to control.

1 µM losartan 15 min prior to AngII blocked this effect ($p < .05$) while losartan (1 µM) alone did not alter IL-6 mRNA. Few CHY-positive mast cells were seen in samples with normal spermatogenesis. However, they statistically significantly ($p < .05$) increased in infertility patients suffering from MA and SCO syndrome particularly within and/or around the walls of tubules of patients.
Limitations, reason for caution: Functional data of human testicular peritubular cells could be obtained in cell culture experiments, only.
Wider implications of the findings: The results imply that increased numbers of CHY-positive mast cells may generate a pro-inflammatory microenvironment in the tubular compartment of the human testis and via IL-6 may foster inflammatory changes often seen in male infertility patients. Mast cell blockers and AT1R antagonists may be novel avenues to treat sub-/infertile in men.
Study funding/competing interest(s): Funding by national/international organization(s), Deutsche Forschungsgemeinschaft (DFG) MA 1080/21-1 to A. Mayerhofer.
Trial registration number: Not needed.

P-018 Variations in sperm fatty acids profiles after capacitation process in normal and pathological semen samples

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Study question: Is the variation of sperm fatty acid levels during capacitation process related to semen quality?
Summary answer: Pathological samples showed a significant decrease of polyunsaturated fatty acids (PUFA), especially n-3 PUFA and DHA, content as result of swim up procedure. However n-3 PUFA were preserved in normal samples.
What is known already: PUFA of sperm phospholipids have been suggested to be important for the viability, maturity and functions of those cells and are one of the main targets of the lipoperoxidative process. Their degree of unsaturation is therefore an essential parameter in the ability of spermatozoa to maintain

equilibrium in an oxidative environment. The proportion of sperm PUFA could be a good biochemical index of semen quality.
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.
Participants/materials, setting, methods: Semen samples were classified according to WHO criteria in normal (≥15 mill/ml; ≥32% progressive motility and ≥4% normal forms) and pathological samples. Capacitation was performed by swim up. Sperm fatty acids were analysed by capillary gas-liquid chromatography. Results were expressed as the variance percentage of each fatty acid after capacitation.
Main results and the role of chance: In normozoospermic samples, spermatozoa showed a significant decrease of saturated fatty acids (SFA) (−13.5%; $p < 0.05$) and n-6 polyunsaturated fatty acids (PUFA) (−10.3%; $p < 0.05$), and a significant increase of monounsaturated fatty acids (MUFA) (37.3%; $p < 0.05$) after capacitation process. Total PUFA, n-3 PUFA and docosahexaenoic acid (22:6 n-3, DHA) content did not suffer variations as result of swim up procedure. As in normal samples, SFA and n-6 PUFA declined significantly (−17.9% and −10.4% respectively, $p < 0.001$), and MUFA experienced a very significant increase (62.3%; $p < 0.001$). However, total PUFA (−14.8; $p < 0.001$), n-3 PUFA (−20.5%; $p < 0.001$) and DHA (−22.7; $p < 0.001$) showed a very significant reduction of their levels.
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.
Wider implications of the findings: High PUFA content preservation, especially n-3 PUFA and DHA, is associated with optimal semen quality required for proper fertilization in capacitated sperm.
Study funding/competing interest(s): Funding by national/international organization(s), Basque Country Government.
Trial registration number: Not applicable.

P-019 Effect of sperm treatment with myoinositol on in vitro fertilization outcomes: a prospective, randomized sibling-oocyte study

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Study question: Is the treatment of sperm with myoinositol (myo) effective to improve in vitro fertilization outcome?
Summary answer: In ICSI cycles, fertilization rate (FR) and percentage of best quality day 2 embryos was significantly higher in the group of oocytes inseminated with myo treated spermatozoa.
What is known already: Myo is synthesized from glucose-6-phosphate, it is a precursor of second messengers in the cell signaling pathways, it is involved in the regulation of intracellular calcium. It has been demonstrated that, in oligoasthenoteratozoospermic patients, in vitro incubation with 2 mg/ml of myo increases significantly the number of spermatozoa with high mitochondrial membrane potential (MMP). Moreover, it has been found that the in vitro fertilization rate is correlated with the percentage of high MMP spermatozoa.
Study design, size, duration: This is a prospective randomized study on sibling-oocyte performed between March and December 2014 to demonstrate the effectiveness of myo treatment of spermatozoa on FR. Based on a difference of 15%, power of 80% and confidence of 95%, a minimum of 151 oocytes per group were required. Fifty-six couples contributed.
Participants/materials, setting, methods: Fresh ICSI treatment with ejaculated spermatozoa and ≥2 MII-oocyte were included. Oocytes were randomly divided in two groups at ovum pick up step. Mature oocytes of one group (MYO) were injected with spermatozoa treated with 2mg/ml of myo, mature oocytes of other group (CTR) with spermatozoa treated with placebo.
Main results and the role of chance: Primary outcomes were fertilization rates (FR) in MYO and CTR-group. Secondary outcomes were embryo grade. The mean age of the 56 patients was 38.7 ± 3.5. A total of 325 oocytes were involved. FR in MYO-group was 74.7% (127/170), in CTR-group was 61.3% (95/155) which is significantly lower ($p = 0.012$). The percentage of best quality embryos on day 2 was significant higher in MYO than in CTR-group: 59.8% (76/127) vs 44.2% (42/95), $p = 0.029$. Any significant difference was observed in further embryo development. Embryos for transfer were selected irrespective of myo treatment. Twenty-two transfers involved only embryos from MYO-group (implantation rate -IR- 22.2%, 8/36), 8 transfers involved only embryos from CRT-group (IR 25%, 3/12), 24 transfers involved embryos from both group. Overall IR was 22.7% (22/97).
Limitations, reason for caution: In-depth analysis of the classes of patients that could better benefit of myo treatment should be done. Long-term follow up of children by embryo obtained with spermatozoa treated with myo is recommended.
Wider implications of the findings: According to ours results, the finding that in vitro myo treatment increases the percentage of spermatozoa with high MMP could have concrete implications. Indeed, myo treatment can lead to more embryos per cycle. It is known that sperm mitochondria are ubiquitinated inside the ooplasm leading to selective proteolysis during early embryonic development. This mechanism might explain why the functionality of sperm mitochondria better influence the morphology of early than late embryos as we observed.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), This study has been in part funded by Lo.Li.pharma s.r.l.
Trial registration number: NCT02050672.

P-020 Comparison of prestained Cell-Vu® slides and Diff-Quik® staining for assessment of normal/abnormal sperm morphology

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Study question: WHO 2010 recommends the use of Diff-Quik® for sperm morphology assessment. This requires the use of compounds that generate Volatile Organic Compounds (VOCs) which have the potential to compromise embryo development within a clinical laboratory. We asked whether pre-stained Cell-Vu® slides are comparable to Diff-Quik® staining for normal/abnormal morphology assessment?
Summary answer: Comparison of a basic normal/abnormal assessment was equivalent between the two methods. However Cell-Vu® slides showed a much wider variation when scoring individual abnormalities compared to Diff-Quik® staining. This was particularly evident for midpiece defects.
What is known already: Pre-stained morphology slides are popular among IVF units, however they have not been included in the WHO 2010 guidelines for sperm morphology. Previous publications have shown that Testsimplets®, another commercially available pre-stained slide, gave significantly lower numbers of normal forms and an overestimation of head defects when compared to Diff-Quik® staining. No published data is available on the effectiveness of Cell-Vu® slides.
Study design, size, duration: Twenty samples were used in this study. For each sample 200 sperm were analysed, this was replicated for each sample and for each method. Sperm morphology was categorised into normal/abnormal and the abnormalities were further classified into: head, midpiece, tail and excess residual cytoplasm (ERC).
Participants/materials, setting, methods: Slides were prepared according to manufacturers instructions for both Diff-Quik® and Cell-Vu®. All sperm were assessed under oil immersion at ×1000 using bright field optics. Methods were compared using regression analysis and the data was visualised using a Bland Altman plots.
Main results and the role of chance: The use of pre-stained slides eliminates the requirement for handling VOC generating liquids in the laboratory. Furthermore, pre-stained slides also reduced process time within the laboratory. Regression analysis demonstrated that basic normal/abnormal morphology was comparable between the two methods used ($p < 0.0001$). Detailed analysis of the abnormality classification showed that head and tail defects were also comparable between methods ($p < 0.05$). However this was not the case for midpiece and ERC categories; Cell-Vu® slides resulted in an overestimation of both defects and these results were confirmed in the Bland Altman plots.
Limitations, reason for caution: Cell-Vu® slides had more debris present and air drying of smears using Diff-Quik® has been reported to result in reduced head size and loss of cytoplasmic droplets. Further validation is therefore required to determine the best method for detailed analysis of abnormal sperm.

Wider implications of the findings: Pre-stained slides provide a quick method for analysing sperm morphology without the need for VOC generating chemicals. This is particularly appealing to IVF units performing diagnostic semen analysis in the same laboratory as sperm preparation for treatment or freezing. The results of this study have demonstrated that Cell-Vu® pre-stained slides can be used for routine basic sperm morphology. However, these methods should not be considered equivalent for sub-categories of abnormal sperm.
Study funding/competing interest(s): Funding by hospital/clinic(s), The authors declare they have no competing interests.
Trial registration number: N/A.

P-021 Stem cell based therapies for non-obstructive azoospermia: key treatment characteristics according to infertile couples

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Study question: What is the relative importance of treatment characteristics in current and potential future stem-cell based treatments for couples diagnosed with non-obstructive azoospermia (NOA)?
Summary answer: Most couples would prefer autotransplantation of spermatogonial stem cells over testicular sperm extraction with intra-cytoplasmic sperm injection (TESE-ICSI), while artificial sperm from somatic cells constitutes a last resort option for most couples. Characteristics determining treatment choice are treatments' safety for children, effectiveness, curability, resemblance to natural conception, burden and costs.
What is known already: Couples with NOA that undergo TESE followed by ICSI have an overall success rate of 25%. Potential future treatments are 'assisted conception with artificial sperm formed from somatic cells' and 'natural conception after autotransplantation of in vitro proliferated spermatogonial stem cells (SSCs)'. Known treatment characteristics valued by couples are effectiveness, safety, costs and burden. Qualitative interviews with NOA-patients have added safety of offspring, 'resemblance to natural conception' and 'curability'.
Study design, size, duration: A cross-sectional observational survey conducted in 2012–2013, disseminated to all 921 couples confronted with NOA and treated with TESE-ICSI in Dutch fertility clinics between January 2007 and July 2012. Recruitment was via coded questionnaires sent by mail and followed by two reminders if necessary.
Participants/materials, setting, methods: A questionnaire based on a previous qualitative study and literature review was designed containing six treatment characteristics, including 23 specific aspects with Likert scales. Characteristics' reliability was assessed and treatment characteristics were ranked by importance. Linear regression and analysis of variance examined demographic and medical determinants of attached value.
Main results and the role of chance: Our 494 respondents (response rate 54%) had unsuccessful TESE (53.6%), successful TESE with ICSI ongoing (11.1%), successful TESE but unsuccessful ICSI (12.3%) or achieved pregnancy after TESE-ICSI (22.9%). Most patients (67.1%) preferred SSC autotransplantation over TESE-ICSI. If other treatments failed, 75.2% would consider artificial sperm. The order of importance of treatment characteristics was safety for children, effectiveness, curability, resemblance to natural conception, burden and costs. Males' education level and duration of infertility were positively associated with mean importance ratings; past consideration to quit treatment was negatively associated. Current treatment success determined importance ratings: effectiveness was most important to couples undergoing ICSI, limiting the women's burden was most important to couples with unsuccessful ICSI and limiting cell manipulations was most important to couples having achieved pregnancy.
Limitations, reason for caution: Couples dropping out prior to TESE-ICSI were not included and minorities (e.g. immigrants) were underrepresented. Patients' hypothetical choices for preferred future treatments might change when safety and effectiveness data of these new treatments become available.
Wider implications of the findings: The interest of patients into future stem-cell based treatments, encourages further pre-clinical research. Deciding when to test these new treatments in humans should mainly be based on expected safety and effectiveness, but also on the four other treatment characteristics valued by patients. All six treatment characteristics should be assessed in trials comparing new treatments to TESE-ICSI. This study shows the relevance of involving patients while developing new treatments.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Center for Reproductive Medicine of the University of Amsterdam.
Trial registration number: Not applicable.

P-022 Sperm conservation: comparative evaluation of storage in dry and liquid phases of liquid nitrogen

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Study question: To compare sperm motility, vitality and DNA fragmentation after 6 months of storage in "dry phase" (DP) or "liquid phase" (LP). "Dry phase" is the phase of a dewar where liquid nitrogen (LN2) is completely separated from the central location dedicated to conservation, avoiding straw microorganism contamination by LN2.
Summary answer: Our results show that there are no significantly differences between the 2 types of dewars for sperm conservation: sperm motility, vitality and DNA fragmentation after 6 months of storage were similar in "dry phase" dewar (DP) and liquid phase dewar (LP). Temperatures in DP are closed to –196°C.
What is known already: The current reference technique for the cryopreservation of spermatozoa is storage into LN2. However, there is a risk of viral or bacterial contamination of straws stored in LN2. Some research groups have evaluated the storage on vapor phase above LN2 and reported that semen quality was not affect by this type of storage, when compared with storage in LN2. But falling straws in LN2 can occur in this situation, with hypothetic risk of contamination.
Study design, size, duration: This prospective experimental study had included 52 patients between Junes and August 2012. Informed consent for participation was obtained. Half of the frozen straws were stored in LP and the other one in DP of a special dewar (Cryo Diffusion, France). Investigators were blinded to the type of storage.
Participants/materials, setting, methods: All samples were frozen by standard slow freezing method. 6 months after storage, sperm samples were treated by density gradient centrifugation. Afterwards, the sperm quality was assessed by the measurement of standard semen parameters (WHO, 2010) and sperm DNA fragmentation (Terminal Uridine Nick end-Labeling assay) by flow cytometry.
Main results and the role of chance: Initial standard parameters of the frozen samples were normal or altered.

Overall, storage at 6 months in DP or LP did not affect post thaw progressive motility (respectively 14.4% ± 2.1% vs. 14.2% ± 1.8%, $p = 0.08$, 41 patients analyzed) neither vitality (respectively 24.9% ± 2.7% vs 25.1% ± 2.8%, $p = 0.09$, 41 patients analyzed). Moreover, no difference was shown between the percentage of sperm DNA fragmentation between the sperm samples stored in DP (26.6% ± 2.7%) and in LP (26.9% ± 2.6%, $p = 0.41$, 23 patients analyzed).

In the dry phase tank (2 canisters), supervision every minutes showed a mean temperature of –187° C at the higher level and –193°C at the lower one.

Motility and vitality was not affected by the level of storage (higher or lower).
Limitations, reason for caution: Even if the duration of our study was 6 months, assessment of longer conservation in DP is needed to take into account usual sperm storage life.

The fertilizing ability of the spermatozoa after storage in DP need to be measured in IVF program and compared with sperm stored in LP.
Wider implications of the findings: Cryopreservation of human semen is routinely used as an integral part of assisted reproductive technologies. Current reference is the storage of straws in liquid nitrogen at –196°C. Our results show that human sperm conservation in dewar where LN2 is completely separated

from the central location dedicated to conservation could be an alternative to storage in liquid nitrogen, avoiding viral and microbial cross contamination.
Study funding/competing interest(s): Funding by hospital/clinic(s), Centre Hospitalier Universitaire Estaing.
Trial registration number: CPP Sud-Est 6, France, DC2008 - DC58.

P-023 Is Liver X Receptor alpha1 interesting to predict successful testicular sperm recovery in men with hypospermatogenesis?

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Study question: Could the testicular expression of the nuclear receptor LXRα1 be a predictive factor of positive testicular sperm recovery in patients with hypospermatogenesis?
Summary answer: The expression of LXRα1 was lower in testicular biopsies from men with hypospermatogenesis (HS) in which no spermatozoa was extracted than in testes from men showing an active spermatogenesis (obstructive azoospermia, OA). This difference was not observed if sperm testicular extraction was positive in HS biopsies.
What is known already: LXRs (Liver X Receptors) are transcription factors of the nuclear receptor superfamily. Previous studies have shown in mouse models that LXRs regulate several testicular functions: steroidogenesis, lipid homeostasis and proliferation-apoptosis balance in germ cells. However, the expression of LXRs have never been investigated in testes of infertile men with hypospermatogenesis.
Study design, size, duration: Twenty azoospermic patients aged 26–44 years were enrolled between 2011–2013 and signed an informed consent. Testicular samples from HS men in which sperm extraction was either positive (HS+, $n = 8$) or negative (HS-, $n = 4$) were analyzed. Biopsies from OA patients ($n = 8$) were used as controls.
Participants/materials, setting, methods: The accumulations of *LXR* and their downstream genes were measured in testis biopsies by qPCR. Steroidogenesis, the intratesticular lipid content and the proliferation-apoptosis balance of germ cells were evaluated.
Main results and the role of chance: *LXR*α1 mRNA levels were significantly decreased in HS- patients compared to HS+ and OA patients (*ANOVA*, $p < 0.05$). The accumulations of LXR downstream genes encoding the steroidogenic proteins StAR and CYP17A and intratesticular testosterone levels were higher in HS- patients than in HS+ and OA patients. No difference in the accumulations of LXR downstream genes involved in cholesterol capture and lipogenesis and in the amount of testicular lipids were observed between HS and OA patients. The expression of proliferation, meiosis and germ cell-specific markers and the number of spermatozoa were reduced in testes of HS vs OA men. TUNEL analyses revealed a trend towards an increased number of apoptotic germ cells in testes of HS- patients compared with HS+ and OA patients.
Limitations, reason for caution: *LXR*α1 mRNA levels need to be measured in isolated germ and somatic cells in order to determine in which cell types its down-regulation occurs in HS- testes. Higher number of testicular samples need to be analyzed to determine the predictive threshold of a positive sperm recovery.
Wider implications of the findings: Our findings may help to better understand the physiopathology of testicular failure in HS patients. The measurement of *LXR*α1 transcript levels could be used in combination with histopathology to detect more reliably the presence of germ cells and to possibly better predict testicular sperm retrieval outcomes for HS patients.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Funding by national/international organization(s), This study was supported by the university, Foundation pour la Recherche Médicale and regional funding. The authors declare no conflict of interest.
Trial registration number: CPP Sud-Est 6 ethical committee (DC-2008-558).

P-024 Visible light promote sperm capacitation via reactive oxygen production and epidermal growth factor receptor activation

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Study question: What is the mechanism by which light irradiation affects human sperm capacitation and fertilization
Summary answer: Irradiation of sperm with visible light, enhances their capacitation and fertilization capacity via a mechanism requiring ROS, EGFR, actin polymerization, and HAM.
What is known already: To acquire fertilization competence, spermatozoa must undergo several biochemical and motility changes in the female reproductive tract, collectively called capacitation. Actin polymerization and development of HAM occur in the capacitation process. In a recent study we show that irradiation of human sperm with visible light stimulates HAM through a mechanism involving mitochondrial ROS production and activation of protein-kinase A (PKA) and sarcoma-protein-kinase (Src). We show elsewhere that PKA and Src mediate EGFR activation in bovine sperm.
Study design, size, duration: Suspension of washed human sperm in capacitation medium (1×10^7 cells/ml) were placed in culture dish and irradiated for 3 min with 40 mW/cm^2 visible light 400–800 nm with maximum energy at 600 nm. Samples were taken for motility determination by computer-assisted-sperm-analysis (CASA) and western blot analysis.
Participants/materials, setting, methods: EGFR activation was determined by western blot analysis using anti-p-antibody against tyr-845. Actin polymerization was determined by phalloidin-FITC and analyzed using a fluorescence microscope. IVF in mouse; Oocytes librated from superovulated females were incubated for 24 h with light treated epididymal mouse sperm, and the number of 2-cell embryos was counted.
Main results and the role of chance: The effect of light on HAM is mediated by ROS and activation of the EGFR. The stimulation of HAM by paraquat, an inducer of mitochondrial super-oxide production, was blocked by inhibiting the activity of the EGFR. Light irradiation stimulated ROS-dependent actin polymerization, and this effect was abrogated by PBP10, a peptide which activates the actin-severing protein, gelsolin, and causes actin-depolymerization in human sperm. Light stimulated tyrosine phosphorylation of Src-dependent gelsolin, resulting in enhanced HAM. Thus, light irradiation stimulates HAM through a mechanism involving Src-mediated actin polymerization. The effect of light on fertilization rate was further tested in mice under *in vitro*-fertilization (IVF) conditions. Light stimulated HAM and IVF rate in mouse sperm, and these effects were mediated by ROS and EGFR.
Limitations, reason for caution: The effect of light should be shown in human IVF as well.
Wider implications of the findings: Treatment of sperm presenting low capacitation ability and may be low egg-penetration capacity.
Study funding/competing interest(s): Funding by University(ies), Bar-Ilan University.
Trial registration number: Irrelevant.

P-025 Genetic study of Genes in CREM-signaling pathway in non-obstructive azoospermia and testicular CREM expression in maturation arrest azoospermia patients

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Study question: To evaluate the association of genes variants in cyclic AMP-responsive element modulator (CREM) signaling pathway with susceptibility

to idiopathic male infertility and detect the mRNA expression of CREM gene in the NOA patients.
Summary answer: Our findings indicated that the polymorphisms of CREM gene (rs4934540, rs2295415 and rs11592356) were associated with NOA in the Chinese population and altered CREM expression may be a symptom or pathogenesis of spermatid maturation arrest.
What is known already: Previous functional investigations of genes in CREM pathway have proved the essential role in spermatogenesis.
Study design, size, duration: Blood samples were obtained from 361 NOA patients and 368 fertility controls for genotype analysis. Additionally, we collected 30 maturation arrest NOA patients' testicular tissue and 30 men with obstructive azoospermia for quantitative RT–PCR.
Participants/materials, setting, methods: This study genotyped 17 single nucleotide polymorphisms (SNP) in CREM, ACT, KIF17b and SPAG8 by Sequenom iplex technology and detected the relative mRNA expression of CREM in sperm maturation arrest testis and normal controls by quantitative RT–PCR.
Main results and the role of chance: The results showed that 3 SNPs of CREM (rs4934540, rs2295415 and rs11592356) were significantly associated with NOA and played a protective role against the disease (P = 0.000049, OR = 0.624; P = 0.003, OR = 0.686 and P = 0.037, OR = 0.672, respectively). The dominant model (variant-containing genotypes) of the 3 SNP were confirmed to protect against the occurrence of NOA (P = 0.001, OR = 0.786; P = 0.004, OR = 0.750; P = 0.044, OR = 0.705). Haplotype analysis of CREM gene suggested that haplotype CGTG exhibited significant protective effect against the occurrence of NOA (P = 0.001, OR = 0.659), while haplotype TATG conferred a significantly increased risk of NOA (P = 0.011, OR = 1.317). Furthermore, we demonstrated by quantitative RT–PCR that relative mRNA expression of CREM in sperm maturation arrest testis were almost 4-fold lower than in normal spermatogenesis controls (P = 0.000011).
Limitations, reason for caution: The exact biological effect of the 3 SNPs on spermatogenic failure remained unclear, further functional analysis with large scale clinical samples to validate the biological mechanisms of CREM polymorphisms in the spermatogenesis impairment are needed.
Wider implications of the findings: This is the first study addressing the association of gene variants in CREM signaling pathway in the strictly recruited Chinese male infertile population and detected the CREM expression in the testes of NOA in the mRNA level. These findings would be valuable for a better understanding the etiology of male infertility.
Study funding/competing interest(s): Funding by national/international organization(s), Project supported by the National Science Foundation for Distinguished Young Scholars of China.
Trial registration number: Basic Science.

P-026 DNA fragmentation index and its association with the parameters of the conventional semen analysis

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Study question: Are there statistically significant associations between the sperm DNA fragmentation index (DFI) and the conventional seminal parameters?
Summary answer: In semen samples above the reference ranges ('normal'), DFI was negatively correlated with the progressive motility and positively correlated with the percentage of immotile spermatozoa; in 'abnormal' semen samples, DFI was negatively correlated with sperm concentration, progressive motility and normal morphology, whereas positively correlated with immotility.
What is known already: Over the last years, several studies have attempted to investigate the possible correlations between sperm DNA fragmentation and the main conventional seminal characteristics, leading to ambiguous conclusions. Some of the studies report an inverse correlation between the DNA fragmentation rate and sperm quality, however, several others have not established a significant correlation between traditional seminal variables, such as sperm concentration, motility, morphology according to the strict criteria and DNA fragmentation indices.
Study design, size, duration: This is a retrospective study of 858 basic semen analyses combined with the evaluation of DNA fragmentation performed during 2013.
Participants/materials, setting, methods: 789 abnormal and 69 normal semen samples, produced from equal numbers of subjects, were examined by conventional semen analysis. The normality of samples was evaluated according to the World Health Organization 2010 recommendations. DNA fragmentation was evaluated according to the Sperm Chromatin Dispersion assay, with a reference limit of ≥30%.
Main results and the role of chance: In normal semen samples, DFI was negatively correlated with progressive motility and positively correlated with the percentage of immotile spermatozoa. 17.4% of normal semen samples had a DFI ≥30%. Within normal semen samples, those with low DFI had a statistically significant higher progressive motility than those with high DFI, but there was a considerable overlap among them. In abnormal semen samples, DFI was negatively correlated with concentration, progressive motility and morphology, while it was positively correlated with the percentage of immotile spermatozoa. 37.1% of abnormal semen samples had a DFI <30%. Within abnormal semen samples, those with low DFI had a statistically significant higher concentration, progressive motility and normal morphology than those with high DFI, but again considerable overlap was observed.
Limitations, reason for caution: Although DFI is significantly correlated with basic seminal parameters and samples with DFI <30% exhibit at least one statistically significant correlation with higher progressive motility, in contrast to samples with DFI ≥30%, the results of the basic semen analysis cannot provide a clear prediction for the integrity of sperm DNA.
Wider implications of the findings: DNA fragmentation is associated with the conventional semen parameters in both normal and abnormal semen samples. However, this association is not strong enough to predict the DNA fragmentation status of a given sample. Hence, DNA fragmentation assessment remains a valuable diagnostic test in the evaluation of male factor infertility.
Study funding/competing interest(s): Funding by University(ies), Democritus University of Thrace.
Trial registration number: The study was not a clinical trial.

P-027 Differences of seminal plasma proteome in normospermia and nonobstructive azoospermia men

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Study question: The aim of the study was to analyze proteome differences in men with nonobstructive azoospermia and normospermia.
Summary answer: Several proteins were found to be missing in men with non-obstructive azoospermia. These results could provide an important basis for comparative proteomics of male infertility.
What is known already: Seminal plasma is a potential source of biomarkers of many disorders of male reproductive organs. Identification and characterization of individual proteins in seminal plasma could be a useful tool for estimating male infertility.
Study design, size, duration: Case-control study, 16 patients with non-obstructive azoospermia undergoing testicular surgery for sperm retrieval, 16 controls, duration 2011–2013.
Participants/materials, setting, methods: Sixteen non-obstructive azoospermia and 16 normospermia samples were analyzed in university IVF unit. Standard procedures were used to collect, freeze and store samples. Two dimensional gel electrophoresis was used to separate protein isolates. Software PDQuest was used for analyzing. Selected proteins were identified by MALDI-MS/MS or LC-MS/MS and software Mascot.
Main results and the role of chance: Minimal differences in proteomic profile were found among normospermia samples. Obvious quantitative and qualitative differences were detected between men with normospermia and non-obstructive azoospermia. Differences of epididymal secretory protein E12, lactate dehydrogenase B, prostatic binding protein, annexin A1, annexin A4, glutathione transferase T1, alpha-1-acid glycoprotein, apolipoprotein D, and prolactin – induced protein seem to be most important. Some of them were not described in the literature.
Limitations, reason for caution: The technique has limitations in identifying proteins with pH values that are too low or too high and molecular masses that

are too small or too large. Size of the study group was small to find correlations of the biopsy results and proteome differences.
Wider implications of the findings: These results could provide an important basis for comparative proteomics of male infertility. The role in the pathogenesis of azoospermia and the origin of the different proteins is unclear. Differential proteomics integrated with functional analysis may help in searching potential biomarkers of non-obstructive azoospermia.
Study funding/competing interest(s): Funding by national/international organization(s), Supported by IGA of the Ministry of Health of the Czech Republic.
Trial registration number: No. NS 9661-4.

P-028 Sperm selection after T.E.S.E., a new approach

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Study question: May we reduce time occurring to prepare of a semen sample suitable for an Intracytoplasmic Sperm Injection (ICSI) cycle, using testicular extract from testicular biopsy as starting material?
Summary answer: By mean a new method involving the use, in the final step of the procedure, of a Cell Strainer device (Cell Strainer, 40 micron) is possible to reduce the time necessary to process a testicular sample (TESE or TESA) from surgical retrieval until use of final spermatozoa for ICSI.
What is known already: The primary goal of TESE or TESA sperm processing is the recovery of a clean sample containing motile sperm. Laboratory techniques should be carried out with great caution to avoid jeopardizing the sperm fertilizing potential. TESE sperm processing may be incredibly labor intensive, and the searching process may miss the rare spermatozoa within a sea of seminiferous tubules and other cells. This sometimes involves the necessity to replace several times the injection pipette which is usually used to isolate and recover the spermatozoa.

Actually TESE is processed in the Laboratory mincing and shredding the whole tissue; the Cell suspension from the dish is transferred to a centrifuge tube. The supernatant is discharged and the pellet is resuspended.
Study design, size, duration: In this retrospective study, the effectiveness of the isolation method of sperm from TESE was evaluated. A Cell Strainer was applied in 124 azoospermic patients from February 2008 to June 2013: 48 of them were affected from non-obstructive azoospermia (NOA) and 76 from obstructive azoospermia (OA).
Participants/materials, setting, methods: All patients were subjected to testicular biopsy under general anesthesia. A fragment of tissue of about 4 mm^3 was taken from each testis.

The fragment was then divided into two equal parts (A and B). Each part has undergone to a different type of treatment. At first samples were mechanically disrupted with two 25 gouge needles. Than sample A, after filtration and several washing cycles through Cell Strainer 40 micron (BD Falcon), was centrifuged. This allowed to separate the fragments of testicular tissue from the liquid part. Sample B was instead, as it is normally used to in the normal procedure, just centrifuged.

On one hand, the total time necessary to isolate the same number of motile spermatozoa from the same sample, treated with the two different modes A and B, was measured.

On the other hand, the reliability of the new filtration method to recover the spermatozoa from a qualitative and quantitative point of view, was compared with that of the traditional one.
Main results and the role of chance: Sample A appeared extremely clean and totally free of microfragments, almost simulating a PESA or MESA. In addition, by evaluating sample A on Makler chamber, a concentration of motile sperm of 30% higher than the sample treated in the traditional way, was found.

To obtain the same concentration of motile sperm in sample B, it was necessary to incubate the sample at 37°C for about an hour. So way it was possible to allow the release of a greater number of motile sperm, otherwise trapped in the fragments.

In the patients affected with NOA, the sensitivity of the two methods of processing was the same. We had no false-negative results with the method Cell Strainer, to testify the fact that the strainer quietly let the sperm to pass through and that several washing cycles can allow an optimal separation of spermatozoa from fragments.

Furthermore it allows a considerable saving of time compared to the traditional (on average 40 min less).

The spermatozoa selection with injection needles, is much easier and avoids the usage of multiple pipettes.
Limitations, reason for caution: This study needs further cases to confirm our preliminary data.
Wider implications of the findings: If further studies will confirm this trend, this new method, which employs Cell strainer device, non expansive tool, could be applied routinely in Andrology Lab.
Study funding/competing interest(s): Funding by hospital/clinic(s), IVF Unit Momofertilife, Bisceglie – Italy.
Trial registration number: no trial n. is needed, cause in was a retrospective study.

POSTER VIEWING

CROSS BORDER REPRODUCTIVE CARE

P-029 Surplus embryo destination in Spain according to patient nationality

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Study question: To study the differences in the elected destinations for surplus embryos between patients from different nationalities.
Summary answer: 47.9% of patients didn't answer to the letters sent by the centres, 38.8% keep their embryos, 4.7% donated them to other patients, and 4.7% destined them to cessation of conservation, and 3.9% donated them to research projects.
What is known already: Following our Assisted Reproduction Law (RD14/2006), patients sign an informed consent for IVF treatment in which they choose the destination for the untransferred surplus embryos. In this consent all the possible options the law provides are stated and every year they receive notice to validate or change their decision. However, many of these embryos are considered abandoned by not answering to the notifications sent by these centres and received by the patients.
Study design, size, duration: Retrospective review of a total of 4,695 IVF (including oocyte and sperm donation) cycles with surplus frozen embryos performed between 2008 and 2011 and the responses given to notifications until 2013.
Participants/materials, setting, methods: Patients answers to the consent informed and final destinations of the surplus embryos were studied focusing on the differences between countries of residence of the patients.
Main results and the role of chance: Four years after the freezing of their embryos, 47.9% of the patients didn't answer to the letters sent by the centres with regards to the destination of their embryos. 38.8% decided to continue keeping their embryos for themselves. 4.7% decided to destroy them, 4.7% donated them to other patients, and 3.9% donated their embryos to investigation. These are global results without considerations by country, but there were important differences within them. A comparative study is presented with the differences between patients from Spain, Germany, United Kingdom, Italy, Netherlands, and Ireland.
Limitations, reason for caution: The limitations of this study are the ones inherent to every observational retrospective study. Our population can be subject to socio-economic decision taking bias when deciding on the destination of the embryos.
Wider implications of the findings: There are considerable cultural differences between countries within Europe. These differences are even bigger when you consider people from Asia or the Americas. It is important to

understand these differences to be able to adapt to the sensibilities of these different patients.
Study funding/competing interest(s): Funding by hospital/clinic(s), Instituto Marques.
Trial registration number: N/A.

POSTER VIEWING

DEVELOPING COUNTRIES AND INFERTILITY

P-030 Infertility factors in couples with temporarily migrant male partner – an analytical review

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Study question: To assess the basic infertility factors in couples with temporary migrant male partner vs. couple staying together with view to study effect of migration on couple infertility.
Summary answer: Study showed 40% incidence of temporarily migrant husband in couples attending infertility clinic, emphasizing large scale human migration for various regions. Temporary migration status of male partner effects fertility adversely in male as well female partner by compromising multiple factors. Reproductive tract infection causing compromised tubal patency, endometritis in female partner was significantly higher in migrant partner (study) group. In male infertility factor oligoasthenospermia and pyospermia were significantly higher in couple with migrant male partner. Ovulation factor in control and study group were not significantly different. Psychosexual problem, marital disharmony was higher but not statistically different in study group.
What is known already: Steep rise of interstate and international migration in last two decades has posed multi dimensional challenges in reproductive health. Migration is usually associated with knowledge and accessibility barriers for health services.
Study design, size, duration: The 2000 couples attending infertility clinic were offered basic infertility investigation and divided into two groups-Study group (*n*-800) included couple with temporary migrant male partner and control group (*n*-1200) included couple staying together permanently. Various male and female factors were analyzed and compared by chi square and z test.
Participants/materials, setting, methods: Reports of all couples were subjected to detailed history and physical examination. The investigation offered – husband semen analysis, serial ultrasound examination and hormonal estimation for ovulation, Sonosalpingography/Hysterosalpingography and or Hysterolaparoscopy for tubal evaluation. Record of evaluation and follow up treatment was made for a period of 12–18 months.
Main results and the role of chance: In the study male partner with migration status were found to have significantly higher incidence of moderate to severe oligoasthenospermia and semen infection ($p < 0.01$) as compared with couple staying together. Among female factors chronic pelvic inflammatory disease and tubal compromise was significantly higher in study group ($p < 0.01$). Ovulation and hormonal factor were not statistically different in two groups ($p > 0.05$). Higher incidence of psychosexual problem and drop out from infertility treatment were also co-factors for infertility in study group. Break up of continuous follow up largely due to migration status cause difficulty in interpretation of results in few cases.
Limitations, reason for caution: Individual variation in profile may affect the result to some extent.
Wider implications of the findings: Human migration for various reasons is on the steep rise. It is causing new and unrecognized reproductive health challenges. Infertility is one of the important reproductive health issues in migrant population. These couples require tailored approach in infertility management. Sensitivity and awareness of these issues and preventive and promotive intervention is an urgent need of health delivery system.
Study funding/competing interest(s): Funding by hospital/clinic(s), Jeevan Jyoti Hospital & Medical Research Centre, Bobina Road, Gorakhpur 273001, Uttar Pradesh, India.
Trial registration number: Not required as it is clinical study for basic evaluation.

POSTER VIEWING

EARLY PREGNANCY

P-031 Lack of FcRn binding in vitro and no measurable levels of ex vivo placental transfer of certolizumab pegol

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Study question: Does certolizumab pegol (CZP), the only PEGylated Fab' anti-TNF without an Fc region, bind to FcRn *in vitro* and is it transported across a human placenta *ex vivo*? Can placental transfer of a PEGylated Fab' be measured in mice with surrogate reagents?
Summary answer: The lack of an Fc region indicates that CZP does not bind to FcRn *in vitro* and no measurable levels of placental transport are seen in human placentas *ex vivo*. In mice only very low levels of placental transfer of a PEGylated Fab' surrogate were seen.
What is known already: Antibodies are transported one way across the placenta mediated by binding to the neonatal Fc receptor (FcRn) as part of the transfer of immunity from mother to baby. Chronic inflammatory diseases have been successfully treated with anti-TNFs such as CZP for years. Research suggests that patients with high disease activity during pregnancy have poor pregnancy outcomes, and consequently anti-TNFs are often used to treat patients during pregnancy.
Study design, size, duration: A surrogate PEGylated Fab' and IgG, which bind to mouse TNF, were used to study placental transfer in mice. CZP, infliximab, adalimumab and etanercept (all anti-TNFs) were used in the FcRn binding and transcytosis studies. CZP was compared with a control Ig in the *ex vivo* placental transfer model.
Participants/materials, setting, methods: Surrogate PEGylated Fab' and IgG in plasma of mice foetuses and mothers dosed with different forms were measured by ELISA. FcRn binding affinity was measured by Biacore and transcytosis in FcRn-transfected cells. Transfer across placentas, taken from healthy women by elective caesarean, was measured using *ex vivo* perfusion.
Main results and the role of chance: The level of surrogate PEGylated Fab was more than 100-fold lower than IgG in the foetuses of pregnant mice dosed with the different forms of anti-TNF. Binding affinity of infliximab, adalimumab and etanercept (which all have an IgG_1 Fc) to FcRn was 132 nM, 225 nM and 1500 nM, respectively; there was no measurable affinity of CZP binding. The level of FcRn-mediated transcytosis across a cell layer was 249.6, 159.0 and 81.3 ng/mL for infliximab, adalimumab and etanercept, respectively. The CZP transcytosis level (3.2 ng/mL) was less than a negative-control antibody (5.9 ng/mL), which cannot bind to FcRn. In the *ex vivo* perfusion study there was no measurable placental transport to the foetal circulation in 5 out of 6 placentas when transfer of Ig could be measured.
Limitations, reason for caution: A relatively low number of placentas were analysed in the *ex vivo* study and the other anti-TNFs were not studied in this model.
Wider implications of the findings: PEGylated Fab biologics, including CZP, are not actively transported across the placenta because they do not have an Fc region and hence do not bind FcRn. In contrast, antibodies with an IgG Fc region are transported actively by the FcRn molecule. This data corroborates the immunological dogma relating to FcRn binding of IgG and the functional consequence regarding placental transfer. These results have potential implications for the treatment of pregnant women with inflammatory diseases.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), UCB Pharma.
Trial registration number: Not applicable.

P-032 Immunotherapy of patients with repeated implantation failures in randomized controlled design and its impact on luteal progesterone synthesis

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Study question: Effect of PBMC immunotherapy on clinical outcomes and luteal progesterone synthesis of patients with repeated implantation failure (RIF).

Summary answer: Improvement of implantation and clinical pregnancy rate of treated patient with PBMC showing an increase on luteal progesterone synthesis.
What is known already: Repeated implantation failure can be defined as inability to implant following at least two embryo transfers especially because of inadequate immune/hormonal uterine environment which influences cross-talk between embryo and maternal interface. Th1/Th2 balance held by immune system in part, with progesterone intervention, is reassuring key for embryo implantation and maintenance of pregnancy knowing that active inflammatory response called Th1 occurs during embryo implantation while humoral response called Th2 is involved in uterine receptivity to maintain pregnancy.
Study design, size, duration
Hypothesis: Our hypothesis is based on supposing that patients with at least 2 RIF have immune deficit necessary for implantation can be treated by PBMC immunotherapy to stimulate immune maternal system and increase progesterone synthesis in luteal cells through interactive response.
Study design: The study has a randomized controlled design over 1 year including 98 patients with at least two RIF (49 for Control and 49 for PBMC-test).
Participants/materials, setting, methods: PBMCs were isolated from patients included in PBMC-test on the day of ovulation induction, maintained 3 days in culture and then combined with PBMCs freshly isolated from patients the day of oocyte pick-up in order to be administered to the intrauterine cavity of patients 2 days before the embryo transfer.
Main results and the role of chance:
Results: Embryo implantation and clinical pregnancy were significantly increased in PBMC-test versus Control (34%, 57% vs. 14%, 29%). In the other hand, this strategy shows an increase of 63% of luteal progesterone synthesis analysed at day 14 after embryo transfer ($p = 0.0004$).
Limitations, reason for caution: There was no limitation factor.
Wider implications of the findings:
Conclusion: These findings suggest that embryo implantation is controlled by maternal immune cells in utero and that intrauterine administration of autologous PBMC may be an effective therapy to improve the clinical outcomes of patients with RIF with a positive effect on luteal progesterone synthesis during implantation.
Study funding/competing interest(s): Funding by hospital/clinic(s), ANFA Fertility Center.
Trial registration number: AFC05/2013.

P-033 How indicative are the standard investigations in recurrent pregnancy loss?

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Study question: In women suffering from recurrent pregnancy loss (RPL), how indicative is the standard practice screening for chromosomal abnormalities, thrombophilia, auto-immunity, or endocrinological and anatomical anomalies?
Summary answer: Structural chromosomal abnormalities as well as (sub)clinical hypothyroidism are more prevalent in women suffering from RPL, whereas the prevalence of thrombophilia as well as auto-immunity is not increased in these women.
What is known already: Screening women suffering from RPL for chromosomal abnormalities, thrombophilia, auto-immunity, endocrinological and anatomical anomalies is standard practice. However, that might just be one side of the story. Compelling evidence suggests that decidualized endometrial stromal cells act as biosensors of embryo quality, adding a new dimension to the complex pathology of RPL. In some RPL patients impaired decidualization might predispose to late implantation and negate embryo quality, thus failing to timely recognize an unviable pregnancy.
Study design, size, duration: A retrospective cross-sectional analysis was performed on patient files of patients who presented themselves within a 36-month period (2011–2013) at the Department of Reproductive Health at UZ Ghent.
Participants/materials, setting, methods: One hundred ninety women aged 20 to 40 who upon presentation at the hospital had already suffered 3 or more miscarriages were included in the study. We extracted data on obstetrical history, characteristics and test results from their patient files.
Main results and the role of chance: The frequency of abnormal investigations as compared to the general population was as follows: chromosomal abnormality in one of the partners 4.6% (7/153) versus 0.4%; factor VIII:C 11.1% (17/153) versus 11%; protein S: 0.6% (1/158) versus 0.03–0.13%; factor V Leiden mutation: 3.8% (6/156) versus 3–7%; factor II mutation: 2.6% (4/155) versus 0.7–4%; MTHFR C677T mutation: 9.3% (14/150) versus 10–15%; homocystein: 0.6% (1/155) versus 5%; lupus anticoagulant: 1.9% (3/158) versus 1–8%; anticardiolipin antibody: 4.9% (8/162) versus 5%; anti-β2 glycoprotein: 2.5% (4/160) versus 3.4%; indirect MAR: 3.8% (6/158) versus 12–15%; antinuclear factor: 15.6% (25/159) versus 13.3%; thyroid stimulating hormone: 15.6% (25/160) versus 4.3%. In 9.6% (15/157) a diagnostic hysteroscopy revealed abnormalities.
Limitations, reason for caution: Since our study is retrospective cross-sectional and carried out at a tertiary centre, our results must be interpreted with caution. Approximately 16% of patients showed some missing variables.
Wider implications of the findings: It is clearly useful to karyotype these women and their partners, as well as to check for thyroid dysfunction, but thrombophilia and autoimmune screening seems less useful. Furthermore, RPL patients will often show normal results on these investigations, confirming the likelihood of an unknown, perhaps endometrial, factor. A better understanding of implantation and its pathologies could lead to more targeted investigations and treatments, which would improve overall clinical care.
Study funding/competing interest(s): Funding by hospital/clinic(s), Ghent University Hospital.
Trial registration number: Not applicable.

P-034 The use of Intralipid in IVF cycles in poor responders with repeated implantation failure who were positive for Natural Killer (NK) cells

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Study question: Is the use of intralipid as an intervention/treatment in repeated implantation failure safe and efficacious.
Summary answer: Intralipid appears to be a safe and effective option for women with NK cells and multiple implantation failure.
What is known already: There is preliminary evidence that intralipid may be an effective treatment for repeated implantation failure in IVF. Intralipid is relatively inexpensive and easily administered over 30–60 min. However, there are few reports on safety and efficacy.

Currently randomized controlled trials to investigate the effectiveness are underway. We decided to evaluate retrospective data in women where it had been used for the last 3 years in order to analyze its safety and efficacy.
Study design, size, duration: A retrospective study of analysis of an intervention (intralipid) by comparing the before and after cycles, success and safety. Data collected over last 3 years and 76 women with repeated implantation failure who received intralipid were identified.
Participants/materials, setting, methods: 76 women who had prior repeated implantation failure (at least 5 embryo transfers) and were investigated and found positive for NK Cells and who had been consented and treated with a 20% intralipid infusion given over 1 h, the characteristics of the cycle were compared to the cycle previous to intervention for change in pregnancy rate. The intralipid infusion was monitored by physician and an intravenous infusion registered nurse.
Main results and the role of chance: The average age (Mean) was 39 years (SD 4.19), the Mean number of attempted IVF cycles was 9. The overall success rate, defined as having a pregnancy, was 27.6% for those given intralipid, and 6.6% in the cycles without intralipid (p, 0.0009). There were no untoward side effects.
Limitations, reason for caution: A retrospective analysis. the need of having a randomized control trial which proves an effective outcome of the use of intralipid in women with repeated implantation failure.
Wider implications of the findings: The identification of an intervention in women with repeated failure would help improve the clinical pregnancy rate.
Study funding/competing interest(s): Funding by hospital/clinic(s), Monash IVF.
Trial registration number: Nil.

P-035 Clinical significance of biochemical pregnancy and its predictive value in the subsequent cycles in assisted reproduction

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Study question: It is not well established what are the possible reasons associated with biochemical pregnancy and its predictive value for subsequent cycles.

Summary answer: A previous biochemical pregnancy does not affect the chance of getting pregnant, but it worsens the probability of having an initial ongoing pregnancy in the next cycles.
What is known already: Biochemical pregnancy occur when beta hCG levels are rising post embryo transfer, but later decrease and no gestational sac is detected.
Study design, size, duration: Retrospective cohort study performed at the reproductive medical center of a tertiary hospital. It contained 11751 assisted reproductive cycles, and the duration of study lasted more than 4 years.
Participants/materials, setting, methods: We divided them into four groups: group 1, lack of pregnancy (n = 6651); group 2, biochemical pregnancy (n = 520); group 3, miscarriage (n = 427) and group 4, ongoing pregnancy (n = 4153). The characteristics of all groups were compared, and the reproductive outcome of the next three cycles of the first three groups was analyzed.
Main results and the role of chance: 520 patients were ended as biochemical pregnancy (4.43%). The primary infertility proportion, BMI, numbers of occyte retrieval and average ET numbers were similar between groups. Group 2 had highest basal androstenedione level than other groups. Group 2 and 3 had higher LH levels. Group 3 patients were elder than other groups and had highest basal FSH level. Group 4 patients had thickest endometrium on hCG administration day. In subsequent cycles, the biochemical pregnancy rate (4.73%), clinical pregnancy rate (37.28%) and miscarriage rate (8.21%) were similar compared with the first attempt. However group 2 still had the highest biochemical pregnancy rate (%) (7.97 vs. 4.00 and 5.28), and group 2 and 3 had higher miscarriage rate (%) than group 1 (11.90, 14.75 vs. 7.43).
Limitations, reason for caution: Retrospective study**.**
Wider implications of the findings: In terms of clinical characteristics, biochemical pregnancy resembles miscarriage. If biochemical pregnancy recurs, patients should be given a complete screening as in the case of recurrent spontaneously abortion or recurrent implantation failure.
Study funding/competing interest(s): Funding by hospital/clinic(s), Peking University Third Hospital.
Trial registration number: None.

P-036 Sera of patients with recurrent miscarriages containing anti-trophoblast antibodies (ATAB) reduce secretion of hCG and progesterone in trophoblast cells in vitro

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Study question: To investigate potential mechanisms how ATAB may interfere with early gestation, we studied the effects of ATAB-positive and -negative sera of patients diagnosed with recurrent miscarriages (RM) on the secretion of human chorionic gonadotropin (hCG) and progesterone by choriocarcinoma cells JEG-3 *in vitro*.
Summary answer: ATAB positive sera of women suffering from RM significantly inhibit hCG and progesterone secretion by JEG-3 cells *in vitro.*
What is known already: Reproductive failure including RM has been suggested to correlate with antibodies that crossreact 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]. Cells of the choriocarcinoma cell line JEG-3 retain many characteristics of normal trophoblasts. Furthermore, they express hCG and progesterone. The mechanism, how ATAB interfere with early gestation is not known.
Study design, size, duration: *In vitro* study to investigate effects of patient sera with and without ATAB on hCG and progesterone secretion of JEG-3 cells. A total of 3 independent experiments were set up with duplicate wells for each serum additive (ATAB-positive, ATAB-negative and control) and time point (12 and 24 h).
Participants/materials, setting, methods: JEG-3 cells were plated in 24-well-plates and cultured with RPMI1640 + Glutamax medium for 24 h at 37°C and 5% (v/v) CO_2. Cell numbers were established. Culture media were refreshed and supplemented with 10% (v/v) serum additives. Supernatants were analysed for hCG and progesterone production after 12 h and after 24 h.
Main results and the role of chance: Sera of ATAB-positive RM patients significantly inhibit hCG secretion of JEG-3 cells for 12 h compared to sera of healthy controls [7.19 ± 2.91 mIU/mL vs. 13.9 ± 0.14 mIU/mL, controls; p = 0.019]. Sera of ATAB-negative RM patients do not affect hCG secretion of JEC-3 cells. Incubation times over 24 h have no effect on hCG production.

Sera of ATAB-positive RM patients significantly decrease progesterone secretion for 12 and 24 h compared to the sera of healthy controls [12 h: 0.72 ± 0.13 ng/mL vs. 1.68 ± 0.11 ng/mL, controls (p = 0.046); 24 h: 2.39 ± 0.16 ng/mL vs. 2.81 ± 0.23 ng/mL, controls (p = 0.027)]. Sera of ATAB-negative RM patient show no significant effect.
Limitations, reason for caution: The cell line JEG-3 is a model for trophoblast cells at various stages of differentiation. This *in vitro* cell system might differ from the *in vivo* situation.
Wider implications of the findings: HCG and progesterone both play fundamental roles in supporting human pregnancy. Inhibition of either or both hormones might point to a mechanism how ATAB interfere with early pregnancies.
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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: Not required.

P-038 Downregulation of ten-eleven translocation 1–3 and 5-hmC in the human villi of normal pregnancy at 6, 7 and 8 weeks

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Study question: Are there any differences in the expression of the ten-eleven translocation (TET) family and 5-hydroxymethylcytosine (5-hmC) in human villus of normal embryo at 6, 7 and 8 gestational weeks (GW)?
Summary answer: TET1, TET2, and TET3 expression and DNA global demethylation levels (5-hmC) were significantly down-regulated in villi with the increase of gestational weeks (6 GW, 7 GW, and 8 GW).
What is known already: Studies in embryonic stem cells (ESC) investigated whether the TET proteins were involved in active DNA demethylation during early development. Our previous studies suggested that an abnormal methylation pattern of imprinted genes might affect the stability of normal pregnancy.
Study design, size, duration: Villus samples were collected from women undergoing selective pregnancy termination during the first trimester from September 2012 to May 2013, which were divided into three groups according to gestational age (6 GW, 7 GW, and 8 GW) (n1 = 10, n2 = 10, n3 = 10).
Participants/materials, setting, methods: TET mRNA was analysed by means of quantitative real-time polymerase chain reaction, and TET protein was examined in the same samples by western blot. Immunohistochemistry analysis highlighted the localisation of TET protein. The absolute amount of 5-hmC were detected by using the MethylFlash™ Hydroxymethylated DNA Quantification Kit.
Main results and the role of chance: There was a decrease in the expression of TET1, TET2, and TET3 mRNA with increasing gestational age (6 GW, 7 GW, and 8 GW). The TET1, TET2, and TET3 mRNA were expressed at a relatively higher level in the villus at 6 GW (TET1 (6 GW: 0.73 ± 0.19, 8 GW: 0.31 ± 0.05 P = 0.017), TET2 (6 GW: 0.65 ± 0.10, 8 GW: 0.35 ± 0.09 P = 0.027), TET3 (6 GW: 0.76 ± 0.17, 8 GW: 0.25 ± 0.05 P = 0.002)). The TET1, TET2 and TET3 protein expressions were also the greatest in the 6W group compared with the others. Furthermore, immunohistochemistry analysis indicated that TET1–3 protein was present in the cytoplasm of the trophoblast. The 5-hmC in the total DNA of the 6 GW groups (0.13 ± 0.01) was higher than that in 7 GW (0.07 ± 0.004, P = 0.001) and 8 GW (0.06 ± 0.004, P = 0.000) groups.
Limitations, reason for caution: We proved that TET1, TET2 and TET3 proteins existed in the villi at 6, 7 and 8 GW, however, it is hard to get other stages

of specimen and difficult to evaluate a more comprehensive trends at different developmental stages.
Wider implications of the findings: TET1, TET2, and TET3 serve as DNA demethylation regulatory enzymes at the fetal-maternal interface during early embryo development, which alterations in the villi suggested that the function of TET may be weaken with increasing gestational age. These proteins may also act as critical factors for embryo epigenetic reprogramming by the generation of 5-hmC.
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by national/international organization(s), National Natural Science Foundation of China (81170574), National Natural Science Foundation of China (31371517), and Foundation of Nanfang Hospital, Southern Medical University.
Trial registration number: We do not have a trial registration number.

P-039 Insufficient maintenance ten-eleven translocation 1–3 is associated with early pregnancy loss

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Study question: Whether the ten-eleven translocation (TET) proteins are involved in active DNA demethylation during early development in human villus and lead to early pregnancy loss (EPL) with any change of 5-methylcytosine (5-mC) or 5-hydroxymethylcytosine (5-hmC)?
Summary answer: Our results indicate that a reduction of TET1, TET2, and TET3 in the villi may be associated with human EPL.
What is known already: Our previous studies suggested that an abnormal methylation pattern of imprinted genes might affect the stability of normal pregnancy and that abnormal methylation might also be another consequence of the defect leading to spontaneous abortion. Others studies discovered that DNMT1 expression and global DNA methylation levels were significantly down-regulated in the villi of EPL.
Study design, size, duration: Villus samples were collected from September 2012 to May 2013, divided into two groups: EPL group ($n = 30$) and the control group ($n = 31$). The duration of pregnancy ranged from 6 to 8 weeks during the first trimester.
Participants/materials, setting, methods: TET mRNA was analysed by quantitative real-time polymerase chain reaction, and TET protein was examined in the same samples by western blot and immunohistochemistry analysis. The MethylFlash™ Methylated and Hydroxymethylated DNA Quantification Kit was used to detect the absolute amount of methylated DNA (5-mC) and hydroxymethylated DNA (5-hmC).
Main results and the role of chance: ANOVA revealed there were some overall significant differences in TET mRNA expression among the EPL groups and control groups (TET1 (control: 0.46 ± 0.07, EPL: 0.18 ± 0.03, $P = 0.001$), TET2 (control: 0.42 ± 0.06, EPL: 0.11 ± 0.01, $P = 0.000$), TET3 (control: 0.44 ± 0.07, EPL: 0.24 ± 0.04, $P = 0.040$)). We examined the TET1, TET2, TET3 protein level in the same samples and discovered there were nearly statistically significant differences between groups (TET1 $P = 0.000$, TET2 $P = 0.000$, TET3 $P = 0.000$). Immunohistochemistry analysis indicated that TET1, TET2 and TET3 protein was present in the cytoplasm of the trophoblast. There was no statistical difference between EPL group and control group in the expression of 5-hmC (control: 0.09 ± 0.01, EPL: 0.08 ± 0.01, $P = 0.243$); however, the expression of 5-mC of the control groups was slightly lower than that of EPL group (control: 1.33 ± 0.07, EPL: 1.53 ± 0.09, $P = 0.041$).
Limitations, reason for caution: We proved that TET1, TET2 and TET3 proteins existed in the villi during early pregnancy. However, the importance of this expression is unknown. Further studies are required to evaluate changes in the expression of these hydroxylases.
Wider implications of the findings: Our findings suggested that the insufficiency of persistent demethyltransferases of embryo was associated with abnormal embryonic development in human early pregnancy. These proteins may also act as critical factors for embryo epigenetic reprogramming by initiating the global conversion of 5 mC to 5 hmC and possibly other oxidation forms.
Study funding/competing interest(s): Funding by hospital/clinic(s), funding by national/international organization(s), National Natural Science Foundation of China (81170574), National Natural Science Foundation of China (31371517), and Foundation of Nanfang Hospital, Southern Medical University.
Trial registration number: We do not have a trial registration number.

P-040 Expression of vascular endothelial growth factor a and its receptor in gdm-affected placental tissues

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Study question: Is there a link between the expression of vascular endothelial growth factor A (VEGFA) and receptor-2 ($VEGFR_2$) in gestational diabetes mellitus (GDM) and placental tissue and their functional effects on the villi microvascular?
Summary answer: The results confirm that the expression of VEGFA and $VEGFR_2$ in GDM is associated with placental tissue and their functional effects on the villi microvascular.
What is known already: VEGFA is a major angiogenic factor and a prime regulator of the vascular endothelial cells proliferation, vasculogenesis and vascular permeability.
Study design, size, duration: Collection of placentas from GDM and normotensive term deliveries ($n = 20$ per group) was approved by Lianyungang Maternity and Child Health Care Hospital, and followed the recommended guidelines for using human subjects. All the normal and GDM placentas were obtained immediately after cesarean section delivery. The diagnostic criteria for normotensive and GDM pregnancies were based on American College of Obstetricians and Gynecologists guidelines.
Participants/materials, setting, methods: The real-time PCR analysis of VGFA and $VEGFR_2$, immunohistochemistry and western blots were completed. The effects of VEGF ligands on microvascular of terminal villi in placentas were determined on the transmission electron microscopy (TEM).
Main results and the role of chance: Expression of VEGFA and $VEGFR_2$ genes on mRNA and protein level were significantly decreased in the GDM group. Immunohistochemical analysis showed reduced expression of VEGFA and $VEGFR_2$ protein. The degenerative alterations of the terminal villi vascular appeared in the GDM-affected placenta.
Limitations, reason for caution: The current study was limited by sample size.
Wider implications of the findings: The placental mRNA expression and protein production of VEGFA and $VEGFR_2$ is reduced in GDM. There is evidence of a biologically plausible mechanism by which VEGF family may contribute to the etiology of this important pregnancy disorder of GDM.
Study funding/competing interest(s): Funding by University(ies), 1. The State Key Laboratory of Reproductive Medicine, Center of Clinical Reproductive Medicine, First Affiliated Hospital of Nanjing Medical University, China. 2. Lianyungang Maternity and Child Health Care Hospital, China.
Trial registration number: None.

P-041 Performance of nine home urinary hCG (human chorionic gonadotropin) pregnancy tests

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Study question: Do currently available at home pregnancy tests (HPTs) meet their claimed sensitivity for hCG in early pregnancy testing?
Summary answer: Performance claims ranged from plausible (consistent with hCG rise), to unrealistic. Products from manufacturers that also made USA marketed tests (cleared by Food and Drug Administration (FDA)), made realistic claims and laboratory testing found claims were likely to be valid. Whereas,

many tests marketed only in Europe, made improper claims that testing found to be invalid.

What is known already: HPTs exploit the exponential rise in urinary hCG during early pregnancy. It is important that manufacturers are clear regarding the performance of tests when used in early pregnancy. Commonly, manufacturers state a test's sensitivity and whether a test can be used early (before the period is due). FDA guidance states that tests which are intended for use before the period is due must be validated with clinical data, there is no compulsory European definition. Some products make claims such as "8 days early," or "can detect 10 mIU/ml", which appear inconsistent with both assay performance and hCG rise, and therefore provide misleading information.

Study design, size, duration: Nine HPTs available in Germany (Clearblue Digital Pregnancy test with Conception Indicator (CBD), Clearblue Plus Pregnancy Test (CBP), Cyclotest (C), Cyclotest supersensitive (CSS), MedVec International (MI), Presence (P), Prima Sicher (PS), Testamed diagnostics Digital (TDD), Testamed diagnostics Sensitive (TDS)), were tested using 5 hCG standards (representative of not pregnant and early pregnancy (0, 5, 10, 25 and 50 mIU/ml)), which were tested in triplicate. Testing was conducted in a randomised, blinded fashion. The tests were read by 3 technicians and the majority decision for each test recorded.

Participants/materials, setting, methods: Results were expressed as the number of "Pregnant" results over number of tests returning a result. hCG standards (in pooled hCG negative urine) were prepared from an initial stock solution calibrated to the WHO (World Health Organisation) 4th International standard. The standards were all measured by AutoDELFIA (Perkin Elmer) to ensure they were within ±5% of target value. All test results were photographed.

Main results and the role of chance: Important differences in the laboratory performance of urinary hCG tests was found. Four tests (CBD, CBV, PS, T) were all able to detect 25 mIU/ml, consistent with their respective manufacturers claimed sensitivity of 25 mIU/ml and test up to 4 days before the period is due. TDD had an extremely high error rate of 40% and was also unable to reliably detect 25 mIU/ml hCG. Although test C has a claimed sensitivity of 25 mIU/ml, it gave negative results for all standards tested. MI and CSS made claims of 10 mIU/ml sensitivity and test up to 8 days early but neither of these tests had performance consistent with these claims. Of the C and CSS HPTs only 1/6 tests was able to detect 50 mIU/ml and these results suggest false negative results may be obtained when testing early or on days around expected period.

Limitations, reason for caution: The number of tests run on each standard was low so could introduce bias. All products tested were within expiry date, but as only one batch was tested per product, batch and product stability effects are not considered. Although technicians were blinded to the standard being tested, product blinding was not possible, so could introduce bias. However, all results were photographed to permit external assessment if required.

Wider implications of the findings: In the USA FDA regulations have ensured only home pregnancy tests which meet their performance claims can be sold. The only tests in this study that are sold in the USA are the CBD and CBP HPTs and they met their specifications in this study. In Europe, products appear to be being sold with unrealistic claims, and there is no standard by which performance can be assessed. Having claims inconsistent with performance could have serious consequences if using these tests to confirm/rule out pregnancy before introducing lifestyle changes for pregnancy. Therefore, a European standard for home pregnancy test performance evaluation should be set in order for women to be certain of the performance of the product.

Study funding/competing interest(s): Funding by commercial/corporate company(ies), SPD Development Company Ltd., Priory Business Park, Stannard Way, Bedford, Bedfordshire, MK44 3UP, United Kingdom.

Trial registration number: N/A.

P-042 Genotyping analysis for the 46C/T polymorphism of coagulation factor XII and the involvement of factor XII activity in patients with recurrent pregnancy loss

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Study question: Is the 46C/T SNP of coagulation factor XII (FXII) or low activity of FXII a risk factor for recurrent pregnancy loss (RPL)?

Summary answer: The C/T genotype, but not low FXII activity was confirmed to be a risk factor for PRL. However, neither the C/T genotype nor low FXII activity had any predictive effect on the for subsequent pregnancy outcome.

What is known already: We previously found that low FXII activity, but not an associated common genetic polymorphism, 46C/T, was linked to PRL, although sample size was small, and these results could be attributable to the inclusion of patients with lupus anticoagulant (LA) in the previous study. There is no reported study until date indicating the influence of the FXII SNP on further pregnancy outcome.

Study design, size, duration: Cross-sectional study and cohort study. The study group consisted of 279 Japanese women with two or more unexplained RPL and 100 fertile women. All patients were seen at the Nagoya City University Hospital between September 2008 and July 2012.

Participants/materials, setting, methods: The frequency of the C/T genotype and the FXII activity were compared between the 279 patients with PRL and 100 control women. Subsequent miscarriage rates were compared among the C/T genotype and according to the FXII activity. The association between LA and FXII activity was also examined.

Main results and the role of chance: The FXII activity in patients with LA was significantly lower than in patients without LA (60.7 ± 17.9% vs. 83.4 ± 29.3%; $p = 0.005$). The CT, but not the TT, genotype was confirmed to be a risk factor for PRL after excluding patients with LA in the cross-sectional study (OR, 2.96; 95% CI, 1.44–6.07; $p = 0.003$). The plasma FXII activity in the patients was similar to that in the controls. Neither low FXII activity nor the CT genotype predicted the subsequent pregnancy outcome in the cohort study. On the other hand, and intermediate FXII activity level of 88–113% was predictive of subsequent miscarriage.

Limitations, reason for caution: The mean age of the fertile controls was higher than that of the patients. Thus, multivariate logistic regression analysis was performed in the cross-sectional study.

Wider implications of the findings: The CT genotype of the FXII gene was found to be a risk factor for PRL. However, the presence of CT did not have any predictive effect on the subsequent pregnancy outcome. Low FXII activity associated with LA appeared to have predictive value for the subsequent pregnancy outcome in the previous study.

Study funding/competing interest(s): Funding by national/international organization(s), the Ministry of Health, Labour and Welfare of Japan, the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Trial registration number: None.

P-043 Determination of clinically significant tests for anti-phospholipid antibodies and cutoff levels for obstetric antiphospholipid syndrome

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Study question: Which tests for anti-phospholipid antibodies are clinically significant for patients with recurrent miscarriages?

Summary answer: LA-aPTT StaClot and aPS/PT IgG were clinically significant that treatment could improve live birth rate in positive cases. There was no difference of live birth rate between with and without treatment in positive patients with CL IgG and IgM.

What is known already: The antiphospholipid syndrome (APS) is the most important treatable etiology of recurrent miscarriages. There are many methods to tests anti-phospholipid antibodies (aPL), but only a few tests proved the evaluation of the clinical performance for recurrent pregnancy loss.

Study design, size, duration: We studied 560 patients with a history of recurrent pregnancy losses (defined as two or more consecutive pregnancy losses) prospectively. Subsequent pregnancies were established between April 2005 and May 2013.
Participants/materials, setting, methods: We examined 11 assays for aPL, including LA-aPTT, phosphatidylserine-dependent antiprothrombin (PS/PT) IgG, IgM, classical aCL-IgG, IgM, and aCL-IgG, IgM, IgA and anti-β2GPI-IgG, IgM, IgA. We examined associations among each aPL and conventional aPL, and obstetric significance comparing live birth rate between with and without treatment in positive cases.
Main results and the role of chance: Live birth rate of patients with treatment tended to be higher that of patients with no medication in positive patients with LA-aPTT StaClot and aPS/PT IgG. Cutoff value of the 98th percentile was more appropriate for LA-aPTT StaClot. There was no difference of live birth rate between with and without treatment in positive patients with CL IgG and IgM. All tests had high specificity and relative risk for APS. It was found that LA-aPTT StaClot and aPS/PT IgG had obstetrical significance that treatment could improve live birth rate in positive cases.
Limitations, reason for caution: We could not determine the obstetric significance of CL IgA, b2GPI IgG, IgM and IgA because of the small number of single-positive cases. Further studies to determine the relevant titers and the antiphospholipid score for obstetric APS are needed.
Wider implications of the findings: Tests of CL-IgG and IgM might be unnecessary for RPL though these tests were included criteria for APS. LA-aPTT StaClot using the 98th percentile value as cutoff and LA-aPS/PT IgG should be added in clinical practice for patients with RPL. This study may be able to standardize of the measurement for antiphospholipid antibodies in recurrent miscarriages.
Study funding/competing interest(s): Funding by national/international organization(s), The Ministry of Health, Labour and Welfare of Japan.
Trial registration number: Not applicable.

P-044 Does the depth of embryo transfer affect the chances of pregnancy and risk of ectopic pregnancy?

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Study question: To determine if the incidence of pregnancy and specifically ectopic pregnancy, changes when the distance between the tip of the transcervical transfer catheter to the uterine fundus was increased from 10 mm to between 15–20 mm.
Summary answer: Results demonstrate that when the tip of the transcervical transfer catheter was placed lower in the uterine cavity between 15–20 mm from the uterine fundus there was no reduction in ectopic pregnancies from 2.29% to 1.63%, however pregnancy rates increased significantly from 33.30% to 40.09% ($p < 0.05$).
What is known already: The rate of ectopic pregnancies after assisted reproduction is reported between 2 and 5%. Tubal disease is the most significant risk factor. Studies have reported no difference in ectopic rates between fresh/frozen and day 3/blastocyst embryo transfers (ET). ET itself could be a cause specifically with the use of tenaculums, deep fundal or traumatic transfers. These may elicit strong random endometrial waves or junctional zone contractions that could relocate the embryo towards the fallopian tubes.
Study design, size, duration: A retrospective cohort study of all fresh/frozen IVF and ICSI cycles performed between the 1st of January 2008 and 31st of December 2013 at the Hull IVF unit. 778 patients attained a clinical pregnancy throughout this period and no patients were lost to follow up.
Participants/materials, setting, methods: From 2008 to 2010 ETs were performed 10 mm from the uterine fundus. From 2011 to 2013 ETs were performed between 15–20 mm from the uterine fundus. All patients had a mock embryo transfer to measure uterine cavity length prior to down-regulation; USS was not used to guide the transfer catheter.
Main results and the role of chance: During this time there were 1844 fresh and 274 frozen IVF/ICSI cycles. 778 clinical pregnancies and 15 (1.9%) ectopic pregnancies occurred. Fresh cycles produced 707 clinical pregnancies with 10 (1.41%) ectopic pregnancies. Frozen cycles produced 71 clinical pregnancies with 5 (7.04%) ectopic pregnancies.

Of the 349 clinical pregnancies were the ET was performed within 10 mm from the uterine fundus there were 8 ectopic pregnancies (2.29%) and only 7 (1.63%) ectopic pregnancies of 429 clinical pregnancies in the 15–20 mm population. All procedures were documented as easy to perform, and been undertaken by the same practitioners. Tenaculums were not used. These results showed that our rate of ectopic pregnancies had not changed with embryo transfer performed lower in the uterine cavity, but clinical pregnancy rate had significantly increased.
Limitations, reason for caution: Despite this analysis including 2118 cycles, we were unable to power the data to detect a difference between the low level of ectopic pregnancy in both the 10 mm and 15–20 mm ET populations.
Wider implications of the findings: Increasing the ET distance from the fundus resulted in no change of ectopic pregnancy rates. This would disagree with previous studies that report higher risks of ectopic pregnancy with ET performed ≤10 mm from the uterine fundus. We believe a meticulous mock ET gives greater reliability to uterine depth and problems that could be encountered thus reducing uterine contractions resulting in a possible lower ET transfer failure or ectopic pregnancy rate and significantly increasing pregnancy rates.
Study funding/competing interest(s): Funding by hospital/clinic(s), The Hull IVF Unit. No Competing interests.
Trial registration number: Not required.

P-045 ICSI did not reduce spontaneous abortion of women with recurrent miscarriage

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Study question: Can ICSI be helpful in resolving paternal chromosomal abnormality in women with recurrent miscarriage and yielding a better clinical outcome?
Summary answer: In women with recurrent miscarriage with normal sperm, administration of ICSI for reducing paternal chromosomal abnormality did not have positive effect in the pregnancy and abortion rate.
What is known already: RM (recurrent miscarriage) is characterized by a heterogeneous population, and in more than 50% of the couples with RM, the cause of miscarriage remains unexplained. One of the RM risk factor is embryonal chromosomal abnormality. Due to the fact that ICSI can select sperm that shows a normal morphology, we supposed that it might possibly reduce paternal chromosomal abnormality. In our study, we aimed to determine whether ICSI can help women with recurrent miscarriage.
Study design, size, duration: All fresh, non-donor in vitro fertilization (IVF) cycles performed from January 2010 through December 2013 ($n = 223$) were included in this study. A total of 141 patients underwent conventional IVF while 82 patients underwent IVF with ICSI.
Participants/materials, setting, methods: All patients had two or more spontaneous abortions, male factors were considered normal based on WHO standards. Outcome measures included method of fertilization, pregnancy rate, ongoing pregnancy rate and abortion rate.
Main results and the role of chance: Patient characteristics were similar in the two groups. In each group, age (IVF: 35.3 ± 4.5; IVF with ICSI: 35.7 ± 4.3 $P = 0.06$), basal FSH (6.9 ± 3.0; 6.2 ± 3.2 mIU/ml $P = 0.18$), duration of rFSH (10.0 ± 2.2; 9.3 ± 2.0 $P = 0.18$), and endometrial thickness (10.3 ± 1.3; 9.9 ± 2.0 $P = 0.58$) did not show significant differences. There were no significant differences between two groups in the number of MII oocytes (11.8 ± 8.0; 10.6 ± 7.3, $P = 0.51$), fertilization rate (92.3 ± 13.3; 88.6 ± 16.5, $p = 0.07$), pregnancy rate (47.5%; 47.6% $P = 0.93$), ongoing pregnancy rate (41.8%; 41.5% $P = 0.90$) and abortion rate (11.9%; 12.8% $P = 0.90$).
Limitations, reason for caution: In our study, we were not able to select only unexplained RM patients. We believed ICSI can be helpful, although not complete, just like the pre-implantation genetic diagnosis (PGD) method. However ICSI was not able to decrease abortion rate.

Wider implications of the findings: ICSI fertilization method was carried out on women with recurrent miscarriage in order to reducing paternal chromosomal abnormality. However, the results showed that there was no positive effect in both pregnancy rate and abortion rate. It seems that the ICSI would not be necessary for such patients. Our study shows chromosomally abnormal sperm cannot be detected by ICSI alone and various causes can lead to unexplained recurrent miscarriage.
Study funding/competing interest(s): Funding by hospital/clinic(s), Mamapapa & Baby Obstetrics Gynecology Clinic.
Trial registration number: None.

P-046 Vaginal progesterone is inferior to intramuscular progesterone in inducing a rise in the serum of the immunomodulatory protein, progesterone induced blocking factor (PIBF)

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Study question: What is the effect of the route of progesterone administration in causing an increase in a unique 34 kDa protein known as the PIBF which, in turn, plays a vital role in tolerance to the fetal semi-allograft?
Summary answer: Progesterone given by vaginal, intramuscular, or oral routes all cause an increase in serum PIBF over baseline. However, the degree of increase is far superior by the intramuscular and oral routes.
What is known already: Various studies have demonstrated approximate equal efficacy of vaginal micronized progesterone and intramuscular progesterone on inducing appropriate histologic changes in the endometrium of anovulatory women with estrogen deficiency taking estrogen followed by estrogen and progesterone. However, oral micronized progesterone has been found not to advance endometrial change nearly so well probably because it is metabolized in the liver before reaching the endometrium. PIBF suppresses natural killer cell cytotoxicity by stabilizing perforin granules.
Study design, size, duration: Prospective comparison study. Seven participants. Endogenous production of progesterone blocked by either graduated estradiol (6 women transferring embryos from donor oocytes or frozen embryos) and one compensated volunteer. Monitoring up to 7 days. Serum PIBF levels measured according to taking intramuscular, vaginal or oral micronized progesterone.
Participants/materials, setting, methods: Six women preparing for embryo transfer given 100 mg/day intramuscular progesterone. One paid volunteer had 1 course of 7 days vaginal progesterone gel 90 mg daily and 5 days of oral micronized progesterone 400 mg/day. Serum PIBF levels obtained at various intervals on progesterone and compared to baseline.
Main results and the role of chance: Two women had serum PIBF levels measured times two on the fourth day of intramuscular progesterone 1 h before and 1 h after embryo transfer. The serum PIBF levels were 737.9, 703.1, 222.4, and 228.7 ng/mL, respectively. Four women (2 donor oocytes and 2 frozen-thawed embryos had PIBF after 7 days of 100 mg intramuscular daily progesterone and the PIBF levels were >800 in 3 women and 502 in the fourth. The first baseline serum PIBF was 32.7 ng/mL for the paid volunteer which increased to 73.2 after 7 days of Crinone® vaginal gel 8%. Her baseline prior to starting oral micronized progesterone was 10.2 and increased to greater than 618 after 5 days of progesterone.
Limitations, reason for caution: Small size. PIBF assay not commercial and not perfected as yet.
Wider implications of the findings: One study found superior pregnancy rates following intramuscular progesterone supplementation vs. vaginal in women without corpora lutea preparing for frozen embryo transfer. This could possibly be explained by superior immune suppression by increased PIBF when progesterone is given intramuscularly. If subsequent studies find a discriminatory level of PIBF below which correlates with reducing fertility potential, perhaps additional oral progesterone could be supplemented with vaginal estrogen to better suppress immune surveillance.
Study funding/competing interest(s): Funding by hospital/clinic(s), Cooper Institute for Reproductive Hormonal Disorders, P.C.
Trial registration number: Approved by a Western IRB (protocol number 20121249, CIR110).

P-047 Intramuscular injection of 17-hydroxyprogesterone in contrast to intramuscular progesterone fails to induce a clear increase in the immunomodulatory protein, progesterone induced blocking factor (PIBF)

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Study question: Since pre-term labor or first trimester miscarriage could be in part related to abrogation of immune tolerance causing an immunological rejection phenomenon, the question arises as to which type of progesterone supplementation – progesterone or 17-hydroxyprogesterone will produce higher levels of the immunosuppressive protein, PIBF.
Summary answer: Daily intramuscular injection of progesterone does produce a precipitous rise in serum PIBF. However, 17-hydroxyprogesterone injection, which is supposed to require less frequent injection does not increase the PIBF level over baseline. Thus progesterone could prove more effective in preventing pre-term delivery or first trimester miscarriage than 17-hydroxyprogesterone.
What is known already: PIBF has been shown to inhibit natural killer cell cytotoxicity. Studies using less accurate immunocytochemistry techniques nevertheless found a correlation of low PIBF expression by white cells and preterm delivery and miscarriage. Though controversial some studies show that injection of 17-hydroxyprogesterone can inhibit pre-term deliveries. A new highly sensitive ELISA assay for PIBF has now been produced related to the development of monoclonal antibodies with a purified antigen.
Study design, size, duration: Prospective comparison study. Seven total participants – 4 receiving intramuscular progesterone in preparation for frozen embryo transfer (ET), 2 preparing for ET from donor oocytes, and 1 compensated volunteer. Measurement of serum PIBF occurred over a 1 week period. Corpus luteum function suppressed by high dose estrogen or oral contraceptives.
Participants/materials, setting, methods: Corpus luteum function suppressed by either a graduated estrogen protocol in women preparing for frozen ET or donor oocytes or oral contraceptives in a paid volunteer. Progesterone injected daily in those preparing for ET, and 17-hydroxyprogesterone injected once into a paid volunteer. ELISA measurement of PIBF using non-commercial assay.
Main results and the role of chance: Two women had serum PIBF levels measured times two on the fourth day of intramuscular progesterone 1 h before and 1 h after ET. The serum PIBF levels were 737.9, 703.1, 222.4, and 228.7 ng/mL, respectively. Four women (2 donor oocytes and 2 frozen-thawed embryos) had PIBF after 7 days of 100 mg I.M. daily *P* and the PIBF levels were >800 in 3 women and 502 in the fourth. In the paid volunteer the baseline serum PIBF was 32.7 ng/mL and it was 22.7 1 day after and 11.7 3 days after the 17-hydroxyprogesterone (250 mg) injection.
Limitations, reason for caution: The PIBF assay is not commercially available and its accuracy and coefficient of variation have not been fully established. One could not state for sure that some defect in the paid volunteer precluded her from generating high levels of PIBF (but subsequently a steep rise followed progesterone).
Wider implications of the findings: There has been conflicting data as to the efficacy of injection of 17-hydroxyprogesterone in preventing pre-term delivery. Pre-term delivery has been associated with lower levels of serum PIBF. Progesterone and possibly 17-hydroxyprogesterone seem to inhibit smooth muscle contractibility and this may in part be the mechanism of thwarting preterm delivery. However, progesterone seems to be the better choice because it is far superior in generating this immunomodulatory protein and thus better to prevent miscarriages also.
Study funding/competing interest(s): Funding by hospital/clinic(s), Cooper Institute for Reproductive Hormonal Disorders, P.C.
Trial registration number: Approved by a Western IRB (protocol number 20121249, CIR110).

P-048 A randomised study on the use of acupuncture for pain relief during the first trimester abortion by suction evacuation

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Study question: Will the use of acupuncture significantly reduce the pain level during the first trimester abortion by suction evacuation (SE) done under local anaethesia (LA)?
Summary answer: Acupuncture significantly reduced the pain level during SE done under LA.
What is known already: SE is a common simple procedure done under LA but many women still report significant procedural pain. Pharmacological methods for pain relief are often associated with adverse effects and may not be acceptable to all women. Acupuncture has been shown to be an effective pain relief for a number of painful conditions including dysmenorrhea, chronic low back pain, migraine and osteoarthritis of the knee and hip.
Study design, size, duration: This is a prospective randomized study of 60 nulliparous women undergoing SE before 10 weeks of gestation carried out between November 2011 and April 2012.
Participants/materials, setting, methods: Subjects were randomized into the standard, acupuncture and combined groups. In the standard group, women received oral diazepam and intramuscular pethidine injection. In the acupuncture group, women received diazepam, normal saline injection and acupuncture. In the combined group, women received diazepam, pethidine and acupuncture. Pain scores were compared.
Main results and the role of chance: No significant differences were found in the age of women, body mass index, gestational age and the smoking habit, anxiety trait, anxiety level, the sedation level and satisfactory level were observed among the three groups. The pain level during SE in the standard group was significantly higher than that of the acupuncture group ($P = 0.026$) and that of the combined group ($P = 0.024$). There was no difference in the corresponding pain levels between the acupuncture and combined groups. Pain levels at the intramuscular injection, at the operating theatre prior to SE, cervical dilation and 1 h after SE were similar for the three groups.
Limitations, reason for caution: This is not a double blind study and the perception of pain levels by subjects may be affected. We studied nulliparous women who are likely to have a higher pain level during SE and the results of the present study may not be applicable to multiparous women.
Wider implications of the findings: Acupuncture significantly reduced the pain level during SE done under LA with little side effects. It can be used alone or in combination of standard conscious sedation.
Study funding/competing interest(s): Funding by University(ies), The University of Hong Kong.
Trial registration number: HKClinicalTrials.com--HKCTR-1397.

P-049 Expression profile of micro-RNA in human chorionic villi from early recurrent miscarriage

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Study question: This study was designed to investigate the miRNA expression profile in the chorionic villi from early recurrent miscarriage, and to understand the biological functions of some miRNAs.
Summary answer: Expression profile of miRNA is related with the early miscarriage. Further study on those target genes will enrich our knowledges on the diverse pathophysiological processes of recurrent abortion.
What is known already: MiRNAs are small non-protein coding RNA molecules that interact with their target genes by inhibiting translation or/and cleavaging the targets. Aberrant expression of miRNAs has been reported in placenta from women with compromised pregnancies, few investigators have studied chorionic villi global miRNA profiling in relation to early recurrent miscarriage.
Study design, size, duration: No.
Participants/materials, setting, methods: The expression profiles of chorionic villi tissues collected from 5 early recurrent miscarriage cases and 5 controls were analyzed using the miRNA microarray. Target genes of down-regulated miRNAs were predicted by bioinformatic analysis and analyzed by Gene Ontology (GO) classifications.
Main results and the role of chance: We found 155 miRNAs exhibiting over 2-fold changes ($P < 0.05$), including 98 up-regulated and 57 down-regulated. The significant up-regulation of miR-149-3p, miR-4417, miR-4497, miR-3651 and down-regulation of miR-181d, miR-29b-1-5p, miR-24-1-5p were confirmed by qRT–PCR ($p < 0.05$), while there were no significances in miR-625-3p, miR-10b-5p, let-7e-5p between two groups ($p > 0.05$). The biological and cellular process of those target miRNAs will be predetermined and further tested.
Limitations, reason for caution: Further studies are required to validate that the synregulated target genes were associated with miRNAs regulation, as well as investigate the changes not only in mRNA level but also in protein levels.
Wider implications of the findings: The present study demonstrated an altered expression of a number of miRNAs with potential regulatory functions on the expression of specific target genes. The target genes enrich in multiple biological process for laying the foundation for the further study in early recurrent miscarriage.
Study funding/competing interest(s): Funding by University(ies), State Key Laboratory of Reproductive Medicine of Nanjing Medical University, China.
Trial registration number: No.

P-050 Association between miscarriage and premature birth and assisted reproductive technologies (ART)

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Study question: To identify the relationship between different methods of ART, etiopathogenesis of reproductive disorders, miscarriage and premature birth in induced pregnancy.
Summary answer: Miscarriage and premature birth are mainly connected with genesis of infertility rather than using different techniques of ART. Most often spontaneous interruption of induced pregnancy occurred in women with a history of endocrine infertility.
What is known already: Despite the fact that the problem of infertility is successfully solved by ART duration of induced pregnancy is more complicated than a spontaneous one. There is evidence that complications of pregnancy are associated with ART.
Study design, size, duration: Retrospective and prospective study of duration of 1331 induced pregnancies in families with impaired reproduction (293 of them were after controlled stimulation of ovulation (CSO), 337 were after intrauterine insemination (IUI) + CSO, 701 were after in vitro fertilization (IVF)/IVF + intracytoplasmic sperm injection (ICSI)) and 1026 spontaneous pregnancies in reproductively healthy women for 10 years were held.
Participants/materials, setting, methods: Among 1331 infertile families 464 had female endocrine, 251 had tubal-peritoneal, 295 had male and 321 had idiopathic infertility. Statistical analysis was performed by *t*-test, $\chi 2$ (Statistica, Statgraf), reliable differences were considered in values of $P < 0.05$.
Main results and the role of chance: The frequency of miscarriage in I trimester after ART (CSO, IUI + CSO, IVF/IVF + ICSI) was higher than in spontaneous pregnancies (respectively 38 (12.9%), 36 (10.7%), 75 (10.7%) and 65 (6.3%)) ($P < 0.01$); in II trimester respectively 10 (3.4%), 7 (2.1%), 17 (2.4%) and 65 7 (0.7%) ($P < 0.001$); preterm labor in III trimester respectively 9.9% (29), 10.4% (35), 11.9% (84) and 5.8% (59) ($P < 0.01$). The frequency of preterm termination of pregnancy between groups with different methods of ART had no differences ($P > 0.05$). Frequency of miscarriage in I trimester in families with history of female endocrine, tuboperitoneal, male, idiopathic infertility was respectively 15.3% (71), 11.2% (28), 9.2% (27), 7.2% (23); in II trimester respectively 4.7% (22), 2.0% (5), 0.7% (2), 0.9% (3); preterm labor in III trimester respectively 15.7% (73), 11.2% (28), 7.8% (23), 7.5% (24). Thus, the highest frequency of preterm interruption of pregnancy was at endocrine ($P < 0.01$), then at tubal-peritoneal infertility ($P < 0.05$), while families with the male and idiopathic infertility did not differ from spontaneous pregnancy ($P > 0.05$). When comparing the frequency of preterm interruption of pregnancy in different types of infertility

depending on ART techniques we have not found statistically significant differences ($P > 0.05$).

Limitations, reason for caution: We excluded from the study couples who used donor cells, as well as cases where the pregnancies were carried by a surrogate mothers.

Wider implications of the findings: The data on the association between etiopathogenesis of infertility and complications of pregnancy will enable a more complex correction of disorders and improve the outcome of induced pregnancy.

Study funding/competing interest(s): Funding by commercial/corporate company(ies), Supported by private IVF clinic "Sana-Med".

Trial registration number: This study does not an RCT, no registration number.

P-051 Uterine NK cells at gestational day 12 contained CXCR4 or IL-15 receptor-bearing cells and interferon-gamma producing cells

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Study question: Uterine NK(uNK) cells support pregnancy-associated vascularization and decidualization. CXCL12-CXCR4 chemokine signaling is essential for NK cell development. The present study demonstrates that the migration of NK cell precursors into the uterus is mediated through chemokine, CXCL12, and chemokine receptor, CXCR4.

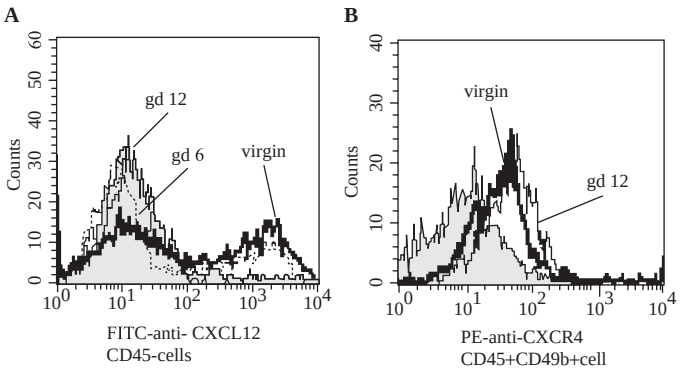
Summary answer: CXCR4 was detected on uNK cells at virgin and gd 12. CXCL12-CXCR4 chemokine signaling is essential for migration of uNKcell.

What is known already: The number of uNK cells increases dramatically after implantation in the mouse uterus, peaks at gd 10 to 12, and decreases thereafter. After implantation, NK progenitor cells have been shown to migrate into the uterus and proliferate in response to IL-15. The interaction of CXCR4 on blood NK cells or their precursors with CXCL12 was shown to play a role in their migration into peripheral tissues and activate them.

Study design, size, duration: Female BALB/c mice, virgins and pregnant at gd 6 and 12 were obtained from Charles River Laboratories. The uteri were carefully removed, excluding tissue from the trophoblast, developing fetus, or embryonic sac. The cells were analyzed on a flow cytometer. One embryo was randomly selected and frozen for histologic analysis.

Participants/materials, setting, methods: For staining cells expressing intracellular IL-15, either CXCL-12 or IFN-γ uterine cells were serially treated with FITC-anti-CD45 and APC-anti-CD49b. The treated cells were incubated with an appropriate anti-cytokine antibody in the permeabilization buffer. The treated sections were stained with biotin-anti-IL15 rabbit antibody and horseradish peroxidase-conjugated streptavidin, and developed with diaminobenzidine.

Main results and the role of chance: CXCL12 expression was observed on CD45- uterine cells at virgin or gd 6, but not at gd 12, whereas CXCR4 was detected on CD45+/CD49b+ uNK cells at virgin and 12 (Figure). A much higher expression of IL-15 in uterine cells expression in uNK cells was observed at gd 12 than at virgin. IL-15 receptor alpha chain was detected on uNK cells at gd 12, but not at virgin. The expression of IL-15 was histologically confirmed to be higher at gd 12 than in the virgin uteri. The CD45+/CD49b+ uNK cells from the virgin mice evidently expressed IFN-γ, and higher expression was observed at gd 12.



Limitations, reason for caution: Our result raised the question of whether CXCL12 is involved in the generation of mature NKs from immature NK cells. Further investigation will be required.

Wider implications of the findings: The results of the present study were consistent with our interpretation that NK progenitor cells migrate into the uterus by the interaction of CXCR4 expressed on the surface of the progenitor cells, with CXCL12 expressed on the uterine cells after gestation, and proliferate in response to IL-15 produced in the uterus. NK cells or their precursors migrate into the uterus, mature, and produce IFN-γ to support pregnancy.

Study funding/competing interest(s): Funding by University(ies), Project Research Grants of Toho University School of Medicine to A. Takashima (24–27).

Trial registration number: No.

P-052 Systemic immune responses in women with Chlamydia trachomatis infection: the difference between tubal ectopic pregnancy and early intrauterine pregnancy

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Study question: Is there a difference in the systemic immune response to *Chlamydia trachomatis* infection in women with tubal ectopic pregnancy (EP) compared to women with early intrauterine pregnancy (IUP) or to non-pregnant women at various stages of their menstrual cycle?

Summary answer: This study suggests that IL-8 is likely involved in the development of tubal EP in women who have asymptomatic and undiagnosed *Chlamydia* infections.

What is known already: Excessive or chronic inflammation of the Fallopian tube is the primary cause of tubal disease. Genital *C. trachomatis* infection induces the host immune response that is normally mounted against intracellular bacterial pathogens, but it also leads to immunopathological conditions and can cause tubal damage if it is not treated or if reinfection occurs. Although the altered regulation of various proinflammtory cytokines in the Fallopian tubes and in circulation is linked to the onset of EP, we have noticed that conflicting results have been reported in the literature.

Study design, size, duration: A total of 225 blood samples were obtained (139 in the non-pregnant menstrual cycle group, 50 in the IUP group, and 36 in the tubal EP group). Clinical information on the patients was collected and entered into a dedicated database.

Participants/materials, setting, methods: The serum levels of the interleukin (IL)-1β, IL-4, IL-6, IL-7, IL-8, IL-10, tumor necrosis factor α (TNFα), and interferon-γ (IFN-γ) cytokines were quantified by high sensitivity human cytokine magnetic bead array. Epidermal growth factor (EGF), a marker of trophoblast invasion and placental development, was also measured. Receiver operating characteristic (ROC) curves were used to assess the specificity and sensitivity of the cytokine levels in response to *C. trachomatis* infection for discriminating tubal EP from IUP and non-pregnant conditions. The *C. trachomatis* IgG and HSP60 human ELISA kits were used for screening and diagnosis of *C. trachomatis* infection.

Main results and the role of chance: The rates of *C. trachomatis* infection in the women with tubal EP, IUP, and no pregnancy were 82.76%, 58.33%, and 55.80%, respectively. Although there was no significant difference in IL-8 levels between IUP and non-pregnancy, IL-8 levels were significantly higher in *C. trachomatis*-positive women with tubal EP than in women with IUP and in non-pregnant women regardless of *C. trachomatis* infection status. Moreover, the ROC analysis showed that the IL-8 level had excellent discriminative validity in positively identifying tubal EP (concomitant with *C. trachomatis* infection) from IUP and non-pregnant conditions regardless of *C. trachomatis* infection. Moreover, an increase in IL-1β levels and a decrease in IL-10 levels were observed in *C. trachomatis*-positive women with tubal EP compared to *C. trachomatis*-positive women with IUP and *C. trachomatis*-positive non-pregnant women. There were no significant differences in IL-4, IL-6, IL-7, IL-8, TNFα, or IFN-γ levels among *C. trachomatis*-positive or negative women under pregnant and non-pregnant conditions. We also found that individual measurements of serum EGF

levels were strongly related to early pregnancy outcomes for women with tubal EP and IUP.

Limitations, reason for caution: The stage of the *C. trachomatis* infection is difficult to establish in patients. In the present study, there are no groups of women with pregnancy of unknown location – including spontaneous miscarriage – either with or without *C. trachomatis* infection. It is possible that the expression of circulating cytokines is different between a tubal EP and an abnormal IUP.

Wider implications of the findings: Although the exact events leading to tubal EP are still poorly defined, *C. trachomatis* infection has been suggested to be a main risk factor for tubal EP. The immune response to *C. trachomatis* infection is dynamic, and an array of cytokines actively participates in the pathogenesis of *C. trachomatis* infection. IL-8 might be a critical cytokine in response to *C. trachomatis* infection in women with tubal EP. In order to understand the mechanism leading from *Chlamydia* infection to tubal EP, it is necessary to determine whether increased IL-8 in the circulation is a cause or a consequence of tubal EP.

Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), this work was supported by grants from the Swedish Medical Research Council (Grants 5859 and 10380) and the Swedish federal government under the LUA/ALF agreement (ALFGBG-147791) as well as by the National Natural Science Foundation of China (81001544 and 81102668) and the "ZuoXue" Foundation of Fudan University, China, to YF. No competing interests are declared.

Trial registration number: This was a retrospective clinical study.

P-053 Association of bacterial vaginosis and vitamin D deficiency in the first half of pregnancy

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Study question: Bacterial vaginosis (BV) is a highly prevalent vaginal infection that is associated with adverse pregnancy outcomes. Our question: is there any association between Bacterial vaginosis and Vitamin D deficiency in the first half of pregnancy?

Summary answer: We found the higher prevalence of Vitamin D deficiency in pregnant women with BV and concluded that Vitamin D deficiency is a modifiable risk factor for BV among pregnant women.

What is known already: Among non pregnant women some risk factors were found for BV such as douching, smoking and black race, but there are limited studies about the risk factors of BV in pregnancy. Vitamin D may be important for BV, because it influences a number of aspects of the immune system. The few studies have investigated the relation between serum 25(OH) D and BV in pregnancy.

Study design, size, duration: This study was a cross sectional study. The sample size was calculated according to the following formula for descriptive studies:

$$n = Z^2_{1-\alpha/2} \times P(1-P) / d^2$$
$$\alpha = 0.05,\ d = 0.06,\ P = 0.25,\ n = 200$$

Participants/materials, setting, methods: Women (n = 204) seeking care at Ayatollah Moosavi Hospital clinic in Zanjan, at <20 weeks enrolled in the study and underwent a pelvic examination and provided a blood sample for determination of serum 25-hydroxyvitamin D [25(OH)D]. BV was diagnosed using Gram-stained vaginal smears interpreted using the method of Nugent.

Main results and the role of chance: We had 99% response rate, approximately 26.9% of women had BV and 45.1% had a serum 25(OH)D concentration <20 nmol/L. The mean serum 25(OH)D concentration was lower among BV cases (17.2 nmol/L; 95% CI: 12.2, 22.2) compared with women with normal vaginal flora (23.7 nmol/L; 95% CI:18.2, 29.2; $P < 0.001$). BV prevalence decreased as vitamin D status improved ($P < 0.001$).

Approximately 85.5% of the women with BV had a serum 25(OH)D concentration <20 nmol/L compared with 70% of women with normal vaginal flora had a serum 25(OH)D concentration ≥20 nmol/L.

There were 16.3-fold (95% CI: 6, 45.5) increases in the prevalence of BV associated with a serum 25(OH)D concentration of 50 nmol/L compared with 15 nmol/L, after adjustment for BMI and maternal age.

Limitations, reason for caution: The cross-sectional nature of this analysis limited our ability to determine the temporal relation between vitamin D and BV. Although we attempted to control for the many covariates related to vitamin D and BV, our effect estimates may have been further biased by other unmeasured or unknown confounders.

Wider implications of the findings: We found that vitamin D deficiency was strongly associated with BV, at <20 weeks of pregnancy. The other studies didn't find this association in non pregnant women or this relation was found in first trimester of pregnancy and just in black women. Unlike many studies of risk factors for BV, which ascertained symptomatic patients only, we screened all pregnant women in the cohort, which increases the generalizability of our findings to low-income pregnant women.

Study funding/competing interest(s): Funding by University(ies), Zanjan University of Medical Sciences.

Trial registration number: This study was not an RCT.

P-054 Infertility and advanced age women

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Study question: What is the effect of advanced aged in infertile women using assisted reproductive technology on pregnancy outcomes?

Summary answer: The advanced age effects on the rate of miscarriages. Also the cause of infertility, gravida and having PCO were important as the contributing factors.

What is known already: It is well known that miscarriage risk increases with age. The proportion of women giving birth after 35 years of age has risen considerably in industrialized countries. Several studies have concluded that women aged ≥35 years have a higher frequency of various adverse reproductive events: infertility, spontaneous abortion, pregnancy complications (such as Caesarean section, pre-eclampsia), congenital abnormalities, maternal mortality and perinatal mortality, than do younger women.

Maternal age is associated with adverse pregnancy outcomes, but it is not clear how much of the pregnancy risks are involved. The aim of this study was to evaluate the relationship between the use of assisted reproductive technology and the incidence risk of pregnancy outcomes in advanced aged women.

Study design, size, duration: A retrospective approach was chosen to analyze the data of women who referred to Royan Institute from 24 October 2009 to 20 September 2012. In general, this study examined 5,108 women over 3 years.

Participants/materials, setting, methods: In this study pregnancy outcome of women less than 35 years and over 35 years were compared. Chi-square and logistic regression were used to statistical analysis. In this regard, factors such as blood pressure, height, weight, disease, cause of infertility and adverse pregnancy outcomes were reviewed and analyzed.

Main results and the role of chance: It means for every 1 year increase in age the risk of late miscarriage increased 10% from 2 to 20 weeks of gestation age. For every unit increase in the age group, infertility by female cause increase by 1.8 times, ovarian cause 1.4 times, gravida 1.3-fold and body weight increased risk of a miscarriage after checking embryo's heart rate. For each unit increase in male factor infertility and per kg increase in body weight risk of early abortion less than 12 weeks of gestational age increased for 1.95-fold and 1.03-fold respectively. Per unit increase in male factor infertility 52.8 times, ovarian 32.7 times, first gravida 2.95 times and second para 0.6 times increased risk of recurrent abortion. Chi square results showed: in >35 years old women with ovarian cause or PCOs and second gravida there was significant relationship with more missed abortion after heart beat check. Also, there were more premature abortion less than 12 weeks and recurrent abortion in this age range.

Limitations, reason for caution: In this investigation there might be some source of uncertainty in the method used to calculate the outcomes. Some of outcomes might not be registered or some cases may be disconnecting their relationship with the clinic and this was out of researcher reach.

Wider implications of the findings: This combination of findings provides some support for pursuing a guideline in admission day would be very helpful.

Study funding/competing interest(s): Funding by commercial/corporate company(ies), Royan Institute for Reproductive Biomedicine.

Trial registration number: This was not a trial.

P-055 Is anti-Müllerian hormone, a determinant in unexplained recurrent miscarriage

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Study question: Our case-control study aimed to explore the role of ovarian reserve (OR) in unexplained recurrent early miscarriage (UREM) through the compared measurement of the anti-Müllerian hormone (AMH) serum level.
Summary answer: This is the first case-control study with large numbers (564) suggesting an association between the reduction of ovarian reserve and recurrent miscarriage in females older than 25 years and not followed up in fertility centers.
What is known already: Maternal age and abnormalities of OR were often considered in the etiologies of UREM. Studies that looked for a relationship between UREM and abnormalities of OR showed very discordant results. Major bias of most of these studies was in the selection of the studied populations (small number, infertile women, managed or not in fertility centers …). Recent studies have shown that AMH is a reliable marker of ovarian reserve which decay begins at around 25 years.
Study design, size, duration: It was a 1:2 case–control study with age matching, ancillary to the case-control incidence study DÉFI – (in French: determinants of unexplained recurrent fetal losses) (PHRC régional Brestois, 2002), which patient enrollment increased from February, 2003 to October, 2008 in the Brest regional university hospital center.
Participants/materials, setting, methods: 188 cases having suffered from at least 3 UREM were compared to 376 controls from the general population with no history of miscarriage and having already given birth to a living child. Evaluation of OR was carried out through the simultaneous AMH measurement on serum bank made during the inclusion in cases and controls (Immunotech*, Beckman-Coulter France).
Main results and the role of chance: The two studied populations are in all points comparable apart from parity. AMH level did not significantly differ between cases and controls even after adjusting mother's age, tobacco consumption, cycles and body mass index. Analysis of the female subpopulation aging older than 25 years (176 cases vs 358 controls) showed significantly lower AMH levels in cases than in controls (3.51 vs 4.11 ng/ml $p < 0.05$) even after adjusting the variables stated above. Conversely, AMH levels were significantly higher in cases than in controls for the less than 25-year-old female subpopulation (12 cases-AMH: 5 ng/ml- vs. 18 controls-AMH: 2.1 ng/ml, $p < 0.05$). Tobacco consumption and body mass index don't seem to be a risk factors of UREM.
Limitations, reason for caution: Due to the small size of the less than 25-year-old female subpopulation, results should be carefully interpreted in this subpopulation even though they concord with the AMH measurement study in very young Filipino females before and after pregnancy.
Wider implications of the findings: Low AMH serum level seemed to be a determinant in recurrent miscarriage in females older than 25 years. This reduction is possibly an early marker of the deterioration of the oocyte quality independently from age after 25 years. New markers of OR and oocyte quality are to be discovered to help in understanding the role of OR in the mechanisms of UREM. These will better identify patients affected by this issue.
Study funding/competing interest(s): Funding by hospital/clinic(s), CHRU Brest Département de Médecine interne et pneumologie CHRU Brest Service de Gynécologie obstétrique et médecine de la reproduction, 2 avenue Foch 29200 Brest Téléphone: 02 98 34 73 36, Bourse FARO.
Trial registration number: None.

P-056 Polymorphisms in the FOXP3 gene show association with unexplained recurrent spontaneous abortion: a study from Iran

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Study question: Is there any association of the functional polymorphisms of FOXP3 with the occurrence of unexplained recurrent spontaneous abortion (URSA)?
Summary answer: In this study, it was shown that polymorphisms of FOXP3 gene are associated with URSA in Iranian women.
What is known already: A successful pregnancy requires competent immunological tolerance to protect the implanting embryo from maternal immune rejection. URSA is proposed to be associated with the failure of fetal-maternal immunologic tolerance in which the regulatory T cells (Tregs) play an important role. Tregs express the fork head/winged helix transcription factor, FOXP3, which appears to be of key importance in the development and function of Tregs. The number of Tregs is diminished in tissues from URSA women.
Study design, size, duration: This is a case-control study. Hundred and ninety six women with histories of at least three consecutive miscarriages with unexplained etiology before 20th week of gestation and 104 healthy women with at least two normal pregnancies were included as case and control subjects.
Participants/materials, setting, methods: Using PCR-RFLP, four SNPs (rs2232365A/G, rs3761548C/A, rs2280883T/C and rs2232368G/A) were genotyped. The level of antibodies against (phospholipids, cardiolipin, nuclear, ds DNA, thrombin III and sperm) and CD markers (CD3, CD4, CD5, CD8, CD16, CD19, CD56) in peripheral blood of URSA women was determined.
Main results and the role of chance: The results showed that rs2232365A/G ($p < 0.001$), rs2280883T/C ($p < 0.01$) and rs2232368G/A ($p < 0.01$) differed significantly between URSA patients and controls. Women who were homozygous for the G allele and those with GA genotype of rs2232365A/G had a significant increased risk (Odds ratio (OR): 10.425, 95% CI (1.337–81.291) and OR: 4.009, 95% CI (2.183–7.364), respectively) for abortion compared with AA genotype. TC and GA genotypes of rs2280883T/C and rs2232368G/A showed an increased risk for abortion (OR: 4.529, 95% CI (1.708–12.010) and OR: 2.288, 95% CI (1.325–3.948)) compared with TT or GG genotypes. No association was found among the levels of antibodies and different genotypes. Further analysis demonstrated significantly increased frequencies of CD4+ T cells in GG genotype of rs2232365A/G ($p = 0.015$) and in CC genotype of rs2280883T/C ($p = 0.004$).
Limitations, reason for caution: There was no limitation.
Wider implications of the findings: These findings demonstrate that polymorphisms in immunoregulatory gene FOXP3, may influence susceptibility to URSA. This could be through altering Foxp3 function and/or its expression and thereby affecting on proportion of CD4+ T cells leading to immune responses directed against the fetus.
Study funding/competing interest(s): Funding by University(ies), Avicenna Research Institute, Tehran, Iran.
Trial registration number: This study was not a clinical trial.

P-057 Detection of single nucleotide polymorphisms (SNPs) miR-196a2 and interleukin-18 (Il-18) 105G > A and –656C > A in women with recurrent miscarriages

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Study question: Are miR-196a2, Il-18 105G > A and -656C > A SNPs associated with recurrent miscarriages?
Summary answer: No statistically significant difference was detected between women with recurrent miscarriages and control group.
What is known already: The single nucleotide polymorphism (SNP) of miR-196a2, miR-196a2T > C (rs11614913) has been reported to be a possible genetic risk factor for recurrent spontaneous abortion in a Korean population. Additionally, interleukin-18 is a proinflammatory cytokine which

plays a crucial role in immune response and it has been linked with pregnancy complications including recurrent miscarriages in Tunesian and Bachrein population (RMs).
Study design, size, duration: The study includes 91 women with at least one live birth (control) and 88 with recurrent miscarriages. Duration of the study was 18 months.
Participants/materials, setting, methods: Peripheral blood was collected from all women, and subsequently DNA was extracted. Real-time PCR was applied for the detection of all SNPs, using specific primers and hybridization probes. The results from the two groups were evaluated for possible statistical correlations.
Main results and the role of chance: All women were checked for DNA quantity and quality. In the control group, the percentages of women heterozygous for the polymorphisms 105G > A –656C > A and miR-196a2 were 43, 48 and 47.9 while homozygosity was observed in 7%, 23% and 6.25% respectively. In the recurrent miscarriages group, the heterozygosity for 105G > A was 32%, for –656C > A was 49% and for miR-196a2 was 45, 45 while the homozygosity was 6%, 14%, 7.8% respectively. Comparison of the incidence of polymorphisms between the two groups showed no statistically significant association.
Limitations, reason for caution: No genotype combination has been studied, to conclude if a multigenic model may be used as a prognostic marker for recurrent miscarriages.
Wider implications of the findings: A combination of different genotypes of all SNPs may show correlations with the incidence of recurrent miscarriages and may help to identify a certain subgroup of patients with higher risk for miscarriage. Further statistical analyses are required to show if a multigenic model may be used as a prognostic marker. These genotypes may differ for different ethnic populations.
Study funding/competing interest(s): Funding by University(ies), University of Athens.
Trial registration number: No registration number.

P-058 The effect of sildenafil (Viagra™) on live birth and the development of mouse offspring

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Study question: Does the *in vivo* administration of clinically relevant concentrations of sildenafil citrate (Viagra™) exhibit any effect on the outcome of pregnancy (live birth) and health of offspring?
Summary answer: Sildenafil citrate at physiological doses did not exhibit any effect on live birth rate, offspring's sex, total body or individual organ mass.
What is known already: Sildenafil has been suggested as a useful adjuvant therapy in women undergoing ART to increase blood flow to the developing endometrium and ovary. Studies from our laboratory indicated that while sildenafil addition to mouse embryos cultured *in-vitro and in-vivo* had no effect (Safe) on embryo development at normal physiological range doses, higher concentrations had a detrimental effect. This study looks at the effect of viagra administration in-vivo on live births and their health.
Study design, size, duration: Mice were randomly assigned to either controls ($n = 8$) or two different doses of sildenafil (1.25 mg/kg $n = 8$ or 5 mg/kg $n = 8$) administered by oral gavage twice daily over 2 days before mating then sacrifice. In vitro cultured blastocysts from each group were then transferred into 2.5 dpc pseudo-pregnant CD1 female mice.
Participants/materials, setting, methods: Day 1 zygotes from each group were cultured *in vitro* until the blastocyst stage, then transferred into 2.5 dpc CD1 pseudo-pregnant mice. All offspring were counted, sexed then sacrificed at 2 weeks after weaning for organ allometry (total weight, heart, liver and lungs, kidneys, spleen, pancreas).
Main results and the role of chance: The number of embryos obtained from each treatment group were, 1.25 mg/kg: 31, 5 mg/kg: 21 and Control: 21. The development to blastocysts in vitro was not affected by the sildenafil treatment with blastocyst formation rate in different groups being; 1.25 mg/kg: 35%, 5 mg/kg: 28% and Control: 30% ($P > 0.05$). The number or gender of pups was not significantly different compare to the control. In addition, there was no significant difference ($p > 0.05$) in the total body mass and various organ sizes between the two treatments groups and control.
Limitations, reason for caution: This work has been carried out in mice so caution is needed when translating to the human situation as it is known that the pharmacokinetics of sildenafil differs between species.
Wider implications of the findings: The study has shown no negative effects from using Sildenafil Citrate at normal physiological doses on the development of mouse offspring while excluding the beneficial effects on the endometrium as previously reported. However as previous data has indicated negative effects with high doses of sildenafil, caution should be used when utilizing higher doses and/or longer acting phosphodiesterase inhibitors in this context.
Study funding/competing interest(s): Funding by University(ies), 1-My beloved late father: Mohandes Ehsan Asgari, 2-University of Nottingham.
Trial registration number: None.

P-059 Uterine and peripheral natural killer cells do not correlate in RSA and RIF patients

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Study question: DO uterine and peripheral natural killer cells correlate in RSA and RIF patients.
Summary answer: UTERINE and peripheral natural killer cells do not correlate in RSA and RIF patients.
What is known already: There are several studies indicating that uterine and peripheral natural killer cells (NK cells) are elevated in RSA and RIF patients.
Study design, size, duration: This retrospective case control study was performed between April 2013 and January 2014 with a total of 67 patients ($n = 27$ recurrent implantation failure (RIF), $n = 40$ recurrent miscarriage (RM) or RM).
Participants/materials, setting, methods: Blood samples were taken during the luteal phase. Blood levels of pNK cells were determined using FACS (CD16+ CD56+), uterine NK cells were identified by Immunohistochemistry (CD56+, counter staining with hematoxilin). Data are presented as mean ± standard deviation, (minimum-maximum). Statistic was performed with SPSS for windows ($p < 0.05$ significant).
Main results and the role of chance: Mean uNK numbers were 196.0 ± 227.6 (29-1109) and mean pNK levels 226.4 ± 226.4 (66-1267). RM patients showed higher levels of uNK (208.5 ± 249.4) and pNK (250.7 ± 214.7) as compared to RIF patients (uNK 191.0 ± 186.4; pNK:195.1 ± 91.2) without reaching significance. In total, 22/67 (32.8%) patients showed elevated uNK and 15/67 (22.4%) elevated pNK. Only 5 patients showed both, elevated uNK and pNK. No correlation was identified between uNK and pNK. Peripheral and uterine NK did not correlate within our study population. However, the study subgroups were small and different methods were applied to identify pNK and uNK.
Limitations, reason for caution: The study population is small, but the study is ongoing.
Wider implications of the findings: The measurement of uterine and peripheral NK cells should be elevated in a larger study group including a control group and menstrual cycle-specific analysis in the follicular and luteal phase.
Study funding/competing interest(s): Funding by University(ies), Department of Obstetrics an Gynecology, University Hospital, Heidelberg, Germany, Department of Gynecological Endocrinology an Fertility Disorders, University Hospital Heidelberg, Heidelberg, Germany, Department of Transplantation-Immunology, Institute of Immunology, University of Heidelberg, Heidelberg, Germany, Placenta-Lab, Department of Obstetrics, University Hospital Jena, Germany.
Trial registration number: A trial registration number was not required due to the retrospective study design.

P-060 Association of multilocus methylation defects of imprinted genes in spontaneous abortions with recurrent pregnancy loss

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Study question: Does multilocus methylation defects (MLMD) of imprinted genes may led to the recurrent pregnancy loss after natural conception?
Summary answer: Our results show that abnormal development and miscarriage of embryos from women with recurrent pregnancy is affected by multiple epimutations at imprinted genes.
What is known already: Numerous human studies demonstrated that methylation defects in **imprinted** genes might affect subsequent embryonic development and contribute to spontaneous abortion (SA) after assisted reproduction techniques and natural conception. Previously we have reported a tissue-specific multiple epimutations affecting 4 to 12 imprinted genes in first-trimester spontaneous abortions (SA) from women with recurrent pregnancy loss.
Study design, size, duration: Chorionic villus samples (CVS) and extraembryonic mesoderm (EM) were collected from SA from women who underwent abortion procedures. SA with normal karyotype (n = 95) were investigated as an experimental group and induced abortion (IA) (n = 56) as a control group.
Participants/materials, setting, methods: The DNA methylation patterns of one maternally (*MEG3*) and two paternally expressed (*PEG10*, *PEG1/MEST*) imprinted genes were analyzed in both tissues using methylation-specific PCR. Comparative analysis of the distribution epimutations between studied tissues allows the identification its somatic or germinal origin.
Main results and the role of chance: Analyses showed that in the 95 SA samples aberrant DNA methylation of studied imprinted gene were found in 41% (39/95) and multiple epimutations in 24% of cases (23/95), 19 (20%) of them had epimutations in two genes and 4 (4.21%) SA in three genes. Somatic epimutations were found in 91.3% (21/23) SA with MLMD and germinal origin – in 8.7% (2/23). In contrast, no epimutations were found in the 56 IA samples. MLMD of imprinted genes in the SA samples was more frequent than that in the IA samples ($p < 0.001$).
Limitations, reason for caution: The studied loci represent only a small fraction of developmentally important genes. Further studies are needed to evaluate changes in the expression and the methylation status of other genes that may lead to SA.
Wider implications of the findings: The findings provide new insights into the etiology of human SA from couples with recurrent pregnancy loss. Importantly, epimutations in SA weren't associated with errors of imprinting reprogramming in gametes (as would expect), but associated with abnormal maintaining of imprinting in somatic cells of the embryo on the chromosomes of maternal and paternal origin. The possibility that the embryo inherits from parents defective factors that led to the abnormal methylation and SA cannot be excluded.
Study funding/competing interest(s): Funding by University(ies), Institute of Medical Genetics Russian Academy, Tomsk, Russia C.I.S.
Trial registration number: P803.

P-061 Subendometrial blood flow assessment in recurrent unexplained first trimesteric abortion

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Study question: Is there a difference in subendometrial blood flow and vascular flow index (VFI) in women with unexplained recurrent abortion compared to those women with at least one baby.
Summary answer: Subendometrial blood flow is altered in women with recurrent unexplained miscarriage.
What is known already: Transvaginal 3D power Doppler ultrasonography can detect subendometrial blood flow presented by the indices vascularization index (VI), flow index (FI), and vascular flow index (VFI) (Ferreira et al., 2007**).** In an effort to elucidate the vascular changes that occur in women with recurrent abortion, and to identify women with poor uterine perfusion, we compared uterine artery pulsatility index (PI) and resistance index (RI), subendometrial blood flow indices (VI, FI, VFI) and endometrial thickness, between women with no history of abortion and women with recurrent abortion.
Study design, size, duration: The study was performed as a cross sectional design during the period from (December 2011) till (January 2013). We had 100 participants presented to the outpatient clinic in Kasr El Aini maternity hospital, they were classified into two groups: (Group A): 50 participants presented with a history of recurrent pregnancy loss (the case group) and (Group B): 50 participants who had no history of abortion and at least 1 child born at term (the control group).
Participants/materials, setting, methods: Transvaginal 3D power Doppler ultrasonography was performed to all women in the second phase of non-pregnant unstimulated cycle to detect uterine artery pulsatility index (UAPI) and subendometrial area to detect subendometrial blood flow presented by the indices vascularisation index (VI), flow index (FI), and vascular flow index (VFI). The indices between the two groups were compared.

We included women with history of three or more spontaneous, unexplained first trimestric abortions and having regular menstrual cycles for the previous 3 months before the study. Values for activated partial thromboplastin time in these women were normal, no antinuclear antibodies or lupus anticoagulant antibodies were detected. In this group titres were less than 20 GPL for IgG anticardioliplin antibody, and less than 20 MPL for IgM anticardiolipin antibody The endocrine evaluation consisted in measuring thyroid-stimulating hormone, free thyroxin (T4), and progesterone levels on days 19 and 21 of the menstrual cycle, and the results were normal. Results to the glucose tolerance test were also normal. Inclusion criteria included also normal karyotyping and normal Hysterosalpingography.

We excluded women with Systemic diseases that might affect the hemodynamic indices (e.g. thrombocytopenia, thyrotoxicosis…, etc.), history of consanguinity, autoimmune diseases, diabetes or thyroid abnormalities.
Main results and the role of chance: Mean uterine artery pulsatility index (M UAPI), it was higher in case group (2.319 ± 0.5309) than in control group (1.689 ± 0.4832) which was statistically significant (p value 0.000). Vascularity index (VI) was higher in case group (2.726 ± 3.0482) than in control group (2.298 ± 3.0272) which was statistically insignificant (p value 0.295).

As regards flow index (FI), it was higher in control group (23.975 ± 4.1716) than in case group (19.138 ± 6.9013) which was statistically significant (p value 0.002). As regards vascular flow index (VFI), it was higher in control group (1.2048 ± 1.11649) than in case group (0.71254 ± 0.6522) which was statistically significant (p value 0.048).
Limitations, reason for caution: Decline in endometrial receptivity is not always associated with a decrease in uterine perfusion and other immunological factors may cause recurrent unexplained abortion.
Wider implications of the findings: It is an easy, safe evaluation that can be applied to all women with history of even one abortion.
Study funding/competing interest(s): Funding by University(ies), Cairo University.
Trial registration number: Local institutional registration.

P-062 Th17 and treg cells in recurrent miscarriage

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Study question: Do the frequency and balance of Treg and Th17 cells and also expression of cytokines and factors related to these cells change in women with unexplained recurrent spontaneous miscarriage (URSM) compared to normal non-pregnant women (NNP) during window of implantation?
Summary answer: A significant Th17/Treg imbalance and a significant increase in frequency of Th17 cells accompanied with a significant decrease in Th17 cells as well as changes in cytokines expression of mononuclear cells could be considered as parameters which might involve in the pathogenesis of URSM.

What is known already: Inappropriate immunological responses of mother are probably the main cause of unexplained recurrent spontaneous miscarriage (URSM), particularly Th1/Th2, Treg/Th17 and cytokines balances. The Th1/Th2 and Th17 /Treg balances provide a new insight toward understanding of appropriate immune responses and mis-conducting of the immune system toward immune-pathological disorders.
Study design, size, duration: This cross-sectional study was conducted on 20 women with at least three unexplained spontaneous miscarriage and 20 healthy fertile women between December 2011 and October 2012.
Participants/materials, setting, methods: Peripheral blood samples were taken from participants during luteal phase in the window of implantation. A Flow cytometry analysis was used to measure the frequencies of Th17 and Treg cells. Quantitative real-time PCR was performed for expressions of GITR, FoxP3, CTLA-4, TGF-β, IL-10, IL-23, IL-17, IL-6, IL-21 cytokines and markers.
Main results and the role of chance: There were 5.66 ± 0.85% Treg cells in the URSM subjects which was lower than in the NNP (9.5 ± 1.48%; $p = 0.001$). The frequency of Th17 cells in the URSM group (2.8 ± 0.51%) was higher than in the NNP group (1.82 ± 0.41%; $p = 0.018$). Significantly higher expression of IL-23 ($p = 0.0001$), IL-17 ($p = 0.038$), IL-6 ($p = 0.048$) cytokines were observed in URSM subjects compared to NNP group. Furthermore, expression of IL-21 in the URSM group was higher than the NNP group which was not statistically significant ($p = 0.581$). Although, there was significantly less expression of TGF-β ($p = 0.001$), FoxP3 ($p = 0.008$), GITR ($p = 0.005$) and CTLA-4 ($p = 0.015$) cytokines and factors in URSM subjects compared to NNP group. However, expression of IL-10 in the URSM subjects was non-significantly higher than in the NNP ($p = 0.52$).
Limitations, reason for caution: Since cellular and molecular tests are labor intensive, time consuming, and requires processing of samples within a few hours, the study was conducted on a small population. However, further studies with larger sample size are needed for better understanding of the molecular basis of immunological factors which might be implicated in miscarriage.
Wider implications of the findings: As we expected there was a significant imbalance in Treg/Th17 cells. The frequencies of these cells as well as expression of related cytokines and factors were changed significantly in URSM group compared to NNP group which was supported with the results of previous studies. It is important to focus on the Treg/Th17 balance in reproductive biology and immunology to find new strategies for diagnosis and therapies about implantation failure and preterm labor recurrent pregnancy loss.
Study funding/competing interest(s): Funding by University(ies), Research Deputy of Iran University of Medical Sciences, the Department of Anatomy and Molecular Research Center, Iran University.
Trial registration number: 130174.

P-063 Intravenous lipid administration in RPL and RIF patients with elevated uterine natural killer cells

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Study question: Does intravenous lipid treatment in patients with recurrent pregnancy loss (RPL) or recurrent implantation failure (RIF) and elevated uterine natural killer cells improve live birth rate (LBR)?
Summary answer: Preliminary data indicate that treatment with a soya containing lipid solution in RPL and RIF patients might elevate live birth rate.
What is known already: There are several studies indicating that uterine and peripheral natural killer cells (uNK and pNK) are elevated in RPL and RIF patients. However, there is no standardized immunological treatment option so far.
Study design, size, duration: This retrospective case control study started in April 2013 with a total of 14 patients with elevated uterine NK cells included so far ($n = 4$ RPL, $n = 10$ RIF).
Participants/materials, setting, methods: A standardized diagnostic procedure for analyzing uNK cells in the endometrial biopsy was established. Uterine NK cells were identified by immunohistochemistry (CD56+, counterstaining with hematoxylin). Patients with elevated uNK cells were offered to receive intravenous Intralipid (8 ml of a 20% lipid solution containing soya oil in 250 ml NaCl). Patients with a known allergy to soya were excluded. Treatment started at the day of the oocyte collection or embryo transfer in RIF patients and after the positive pregnancy test in RPL patients. Intralipid administration was repeated every 2 weeks until 12 + 0 weeks of gestation.
Main results and the role of chance: Pregnancy was achieved in 9 patients (4 RPL and 5 RIF patients) with one live birth (healthy baby girl), one early miscarriage and 7 currently ongoing pregnancies. Of the remaining 5 RIF patients with unsuccessful assisted reproduction cycles 4 were older-aged (44 years $n = 2$, 42 years $n = 1$ and 40 years $n = 1$) and one patient underwent a natural cycle IVF with only one oocyte. So far, no allergic reactions or side effects were observed within the study population.
Limitations, reason for caution: The study population is small, but the study is ongoing.
Wider implications of the findings: Intravenous lntralipid administration could be a cost-effective treatment to enhance live birth rate in RPL and RIF patients with elevated uterine NK cells.
Study funding/competing interest(s): Funding by hospital/clinic(s), Department of Gynecological Endocrinology and Fertility Disorders, University Hospital Heidelberg, Germany, Placenta-Lab, Department of Obstetrics, University Hospital Jena, Germany.
Trial registration number: A trial registration number was not required so far due to the retrospective study design.

P-064 Increased chromosome 16 disomy rates in human spermatozoa seem to be associated with recurrent miscarriages

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Study question: We investigated, whether unexplained recurrent spontaneous abortions (RSA) are associated with increased rates of aneuploidy in spermatozoa of RSA-partners ('RSA-males').
Summary answer: Our 'sperm-FISH' data on 'RSA-males' showed in more than 70% increased disomy rates for three or more chromosomes. Particularly, for chromosome 16 significantly increased disomy rates were found in more than 80% of cases. We suggest, that aneuploidy screening of sperm samples of 'RSA-males' may be beneficial in a comprehensive clinical work-up of RSA.
What is known already: Numerous fluorescence in situ hybridization (FISH) studies to interphase nuclei of human spermatozoa ('sperm-FISH') have elucidated non-disjunction mechanisms and frequencies in male germ cells [1]. Up to now 'sperm-FISH' data on 'RSA-males' are rare and limited to chromosomes 13, 18, 21, X and Y. However, available data indicate that these patients may have an elevated gonosomal disomy rate as well increased cumulative aneuploidy frequencies in their spermatozoa [2].
Study design, size, duration: We analyzed eleven male partners of an exactly characterized group of patients suffering from unexplained RSA by using a custom designed FISH on spermatozoa.
Participants/materials, setting, methods: Sperm samples were evaluated for elevated diploidy and disomy levels of chromosomes 1–22, X and Y by multicolour 'sperm-FISH'. For the first time, all human chromosomes were screened, as numeric chromosomal aberrations in chromosomes other than 13, 18, 21, X and Y have been shown to be prevalent in miscarriages [3].
Main results and the role of chance: This study revealed mean disomy rates between 0.04% for chromosome 7 and 0.36% for chromosome 16. Comparing with base-line aneuploidy rates from literature and from internal control samples, significantly ($P < 0.05$) elevated mean disomy rates were observed for chromosomes 1, 2, 6, 15 and 16 while chromosomes 3, 7, 8, 14, 17 and X were inconspicuous in all patients. Particularly, for chromosome 16 significantly increased disomy rates were found in more than 80% of cases. Analysing spermatozoa for aneuploidy of chromosomes 1, 6, 21 and especially 16 may help to increase prognostic prediction in 'RSA-males'.
Limitations, reason for caution: However, so far, insufficient information is available to support our findings and additional data on the incidence rate of

aneuploid spermatozoa in the general male population and in 'RSA-males' is required.

Wider implications of the findings: Aneuploidy and diploidy screening in spermatozoa of 'RSA-partners' appear to be an effective prognostic tools that may be useful in reproductive genetic counselling. This notion may offer more detailed insights and a more accurate prognostic prediction for couples with unexplained RSA than traditional karyotype analysis on merely somatic cells.

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Study funding/competing interest(s): Funding by national/international organization(s), Supported by Friedrich-Baur-Stiftung.

Trial registration number: Approved by Human Investigation Review Board of LMU (107-09).

POSTER VIEWING

EMBRYOLOGY

P-065 Sibling embryo study to evaluate the potential impact on morphokinetics of a medium refresh on day 3, during extended culture

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Study question: When comparing sibling embryos, does refreshing of universal culture medium, on day 3 (D3), during extended culture, impact morphokinetics compared to leaving the medium unchanged?

Summary answer: There was no significant difference in the stage of development reached, or the start time of blastulation (tSB) or time to reach full blastocyst (tB) compared to hours post insemination (hpi) whether the medium was refreshed on D3 or not.

What is known already: During embryo culture with universal medium, manufacturer recommendation is to refresh the medium on D3 to avoid the potential build -up of ammonia, VOCs or toxins that may be detrimental to embryo development. EmbryoScope™ (Fertilitech, Denmark) culture utilizes HEPA and active carbon filtered gas, in an enclosed culture environment, which may reduce the requirement for refreshing media on D3.

Study design, size, duration: A total of 232 sibling embryos were annotated for morphokinetic variables including tSB and tB between 04/07/2013 and 12/12/2013 in a private IVF centre. tSB and tB were previously shown to be biomarkers predictive of embryo viability.

Participants/materials, setting, methods: Using the EmbryoScope™ the morphological and morphokinetic development of sibling oocytes, in the same EmbryoScope slide, with and without refreshing the medium on D3 of development were compared. 85 cycles were included in this sibling embryo study for patients undergoing ICSI and electing to use the EmbryoScope.

Main results and the role of chance: There was no significant difference in the proportion of embryos that reached the blastocyst stage in the two groups. In the refresh group, 76.4% and 67% we recorded a tSB and a tB, compared to 72.4% (p = 0.25) and 62.3% (p = 0.22) respectively in the non refresh group. There was no significant difference in the proportion of embryos that reached tSB by 100 h post ICSI (hpi) at 50.3% and 43.6% (p = 0.08) in the refresh and non refresh group respectively. There was no significant difference in the proportion of embryos that reached the full blastocyst stage by116 hpi at 55.1% and 47.6% for the refresh and non-refresh groups respectively (p = 0.06).

Limitations, reason for caution: Whilst refreshing the medium had no impact on morphokinetics we have not studied the potential impact on clinical outcome.

Wider implications of the findings: It may prove beneficial to remove the need to disturb the stable conditions of EmbryoScope culture, with a media change on D3, with no detriment to embryo development or morphokinetic parameters. This will also improve work flow in a laboratory utilizing EmbryoScope technology for embryo selection.

Study funding/competing interest(s): Funding by hospital/clinic(s), non-applicable.

Trial registration number: Non-applicable.

P-066 Comparison of embryonic development by pronucleus diameter and differences in second synchrony using time-lapse monitoring

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Study question: This study compared the transition time from the three-cell stage to the four-cell stage (s2 = second synchrony), differences in size of pronuclei, and subsequent embryonic development.

Summary answer: This study revealed that the rate of embryonic development was lower in uneven embryos than in even ones. Good embryonic development can be determined based on the size of pronuclei alone, but measurement of the time for s2 could predict subsequent embryonic development.

What is known already: There is no study which is divided into the pronucleus diameter difference.

Study design, size, duration: Cohort study (retrospective). This study was performed on 584 fertilized embryos obtained from 172 cycles of 116 subjects who underwent in vitro fertilization or intracytoplasmic sperm injection between March 2012 and November 2013 in this clinic.

Participants/materials, setting, methods: According to the observation of the appearance of pronuclei, they were divided into two groups (even embryos with a diameter difference of <5 μm). The transition time for s2 was classified into the speed of subsequent embryonic development and the rate of good embryonic development between even and uneven embryos.

Main results and the role of chance: The comparison indicated that the rate of good embryonic development was significantly lower in the uneven embryo group (uneven embryos vs. even embryos = 20.1:51.5). For s2, the rate of good embryonic development was significantly lower in the group ≥5 h than in that <5 h, which was the same for both uneven and even embryos (uneven = 25.7:5.6, even = 57.7:17.9). When the uneven and even embryos in the group of s2 <5 h were compared, the rate of good embryonic development was significantly lower in the uneven embryos (uneven vs. even = 25.7:57.7). When comparing the even embryos in the group of s2 <5 h and the uneven embryos in the group of s2 ≥5 h, the former group showed a significantly lower rate of good embryonic development (uneven vs. even = 25.7:17.9).

Limitations, reason for caution: there are no limitations, and no reasons for caution**.**

Wider implications of the findings: This study revealed that the rate of embryonic development was lower in uneven embryos than in even ones. revealed that the rate of embryonic development was lower in the group ≥5 h than in that <5 h.

Study funding/competing interest(s): Funding by hospital/clinic(s), Sakamoto Tomomi clinic.

Trial registration number: No number.

P-067 Impact of morphological assessment of embryo quality on day 2 on reproductive outcomes in day 3 embryo transfers: a randomized study

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Study question: The objective of this study is to investigate whether forgoing the usual developmental and morphological check at day 2 of in vitro development has any impact on pregnancy rates after day 3 embryo transfer (ET) in ICSI cycles.

Summary answer: We found no significant difference in pregnancy rates between embryos transferred on day 3 that were morphologically assessed in day 2 versus those that that were transferred without assessment in day 2.

What is known already: During morphological assessment of embryo development, day 2 evaluation is often used, together with day 3, to select the embryos for transfer, as improved knowledge of developmental speed and cleavage timing should improve embryo selection. However, classical day 2 evaluation implies exposure of the embryos to a non-ideal environment.
Study design, size, duration: Randomized study powered to detect a difference of 10% in biochemical pregnancy rates. Highest scoring embryos on day 3 were selected for ET. If scores were similar, day 2 assessment was taken into account. Exclusion criteria were: development to blastocyst, ET at day 2, testicular biopsy.
Participants/materials, setting, methods: Embryos proceeding from 762 ICSI cycles with either own or donated eggs were classified according to a standardized scoring system (1 to 10) at day 2 and day 3 in the control group (n = 380), and only on day 3 in the study group (n = 382). ET was performed on day 3 in all cases.
Main results and the role of chance: In 93% of cases, 2 embryos were transferred. Univariate and multivariate analyses returned no significant differences in biochemical (46.3% vs. 43.2%), clinical (31.3% vs. 28.5%), and ongoing pregnancy rates (30.8% vs. 24%) between the control and the study group. The average age for oocyte donors was 26.5, while for cycles with own cycles was 36.8; the age of the woman providing the eggs was the only factor found to affect clinical and ongoing pregnancy rates (p = 0.019) however, the source of oocytes (donation of own patient) did not affect the pregnancy rate (p = 0.72) indicating oocyte quality as the main determinant of success in all cycles.
Limitations, reason for caution: This is a prospective, randomized study. All cycles were performed using ICSI as fertilization technique; generalization of these findings to classical IVF cycles should be made with caution.
Wider implications of the findings: Our results indicate that increased knowledge on developmental speed gathered by comparing day 2 to day 3 assessment is offset by the detrimental effect of removal from the culture environment of the developing embryos. Our results are in line with emerging evidence suggesting that undisturbed culture, per se, improves embryo viability.
Study funding/competing interest(s): Funding by hospital/clinic(s).
Trial registration number: N/A.

P-068 Alteration of mitochondrial activity during vitrification of mouse immature oocyte might be recovered by extending gv stage

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Study question: We investigated the possible causes for low development to blastocyst of vitrified/warmed oocytes by evaluating the changes of high-polarized mitochondria redistribution and ROS production.
Summary answer: Alteration of high polarized mitochondria distribution in vitrified oocyte may affect on mitochondria activity including ROS production during fertilization and further development. Pre-incubation in milrinone before in vitro maturation may help the redistribution of high polarized mitochondrial inner membrane potential and ROS production.
What is known already: Mitochondria have a central role for cell viability and in early mammalian embryogenesis. Asymmetric distribution of mitochondria at pronuclear stage has been associated with asymmetric segregation into different blastomeres might be the reason of cell death and abnormal embryonic development. Mitochondrial activity has been known to be altered by vitrification process.
Study design, size, duration: Animal study, September 2012 to February 2013.
Participants/materials, setting, methods: Immature GV oocytes were vitrified in EG + DMSO with an EM grid and slush-nitrogen. To block spontaneous GVBD, we used a milrinone, as phosphodiesterase 3 inhibitor, The thawed/warmed oocyte were cultured in TCM199 for *in vitro* or subjected to test high-polarized mitochondria distribution with JC-1 or ROS with CLSM. In vitro matured MII oocyte were fertilized in vitro and cultured in KSOM for 5 days to analyze embryonic development.
Main results and the role of chance: To confirm the activity and effect of milrinone on oocyte maturation, we cultured mouse GV oocyte in milrinone containing medium for 1, 3, and 5 h and extended culture for oocyte maturation. There is no difference in *in vitro* maturation and fertilization rate between fresh and vitrified/warmed oocyte. However, the development rate to blastocyst in thawed/warmed oocyte was significantly lower than those in fresh oocyte ($p < 0.05$). The development rate to blastocyst was recovered if these oocytes were incubated in milrinone for more than 3 h for recovery of mitochondrial function before oocyte maturation. Thawed/warmed oocyte showed high level of ROS and low mitochondrial membrane potential compare to control oocyte, on the other hand, for 3 h incubated oocyte in milrinone showed similar level of ROS and recovered to high polarized mitochondrial inner membrane potential non-vitrified 3 h in vitro cultured oocyte ($p < 0.05$).
Limitations, reason for caution: None.
Wider implications of the findings: Extended prophase I stage in milrinone before in vitro maturation may help the recovery of antioxidant system from vitrification and facilitate the embryonic development.
Study funding/competing interest(s): Funding by national/international organization(s), Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family affairs, Republic of Korea (A120080).
Trial registration number: None.

P-069 A novel lipidomic strategy for phospholipid biomarker identification during oocyte vitrification

Abstract withdrawn by the author

P-070 Comparison of recovery, survival and clinical pregnancy rate between two different closed vitrification devices (Rapid-i versus Cryotip)

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Study question: Can Rapid-i (Vitrolife) significantly improve the recovery, survival and pregnancy rate in Frozen Embryo Replacement (FER) cycles when compared to Cryotip (Irvine Scientific)?
Summary answer: According to this study Rapid-i was found to significantly improve the recovery and survival rate of warmed embryos. The clinical pregnancy rate (CPR) was higher for patients with embryos vitrified in Rapid-i in comparison to Cryotip. However, no statistical significance was found on the CPR between the two devices.
What is known already: Different devices are used clinically for embryo vitrification in order to achieve high cooling and accurate warming rates. The Rapid-i is a new "closed" vitrification device and only two studies are published regarding its success on human embryo vitrification (Desai et al. 2012; Hashimoto et al. 2013). The findings of this study agree with the present published data.
Study design, size, duration: A total of 289 embryos were thawed from 1st October 2012 to 31st October 2014 in 93 FER cycles and the results of the two devices were compared. The embryos were vitrified at different developmental stages but the majority of the patients had embryos vitrified at the blastocyst stage.
Participants/materials, setting, methods: A total of 93 couples were studied, 41 of them had their embryos frozen with Rapid-i (139 embryos), while 52 on Cryotip (150 embryos). Irvine Scientific Freeze and Thaw kits were used to vitrify and warm embryos irrespective of device. Vitrification and warming were carried out as per protocol.
Main results and the role of chance: The statistical analysis of all the 93 FER cases that were carried out from 1st October 2012 until 31 January 2014 in London Fertility Centre has shown that Rapid-i achieves a significantly higher recovery and survival rate in comparison to the Cryotip ($p < 0.05$).

The CPR was higher after transfer of embryos vitrified on Rapid-i in comparison to those vitrified on Cryotip. However the difference was not significant (p = 0.27, $p > 0.05$). This might be due to the low number of patients that was analysed. A further analysis of additional cases should be carried out.

Limitations, reason for caution: As discussed above, a further analysis of FER cases should take place in order to be more accurate and confident to decide whether Rapid-i significantly improves the success rates.
Wider implications of the findings: Our results agree with the published data (Desai et al. 2013). Further studies on more FER cycles and different developmental stages of embryos should take place. In this way, it will be possible to investigate whether vitrification with the two different devices has different outcomes on embryos of different developmental stages. In addition, improving recovery rates will enhance the chance of an embryo transfer which might give a higher chance of pregnancy, so Rapid-i is highly likely to significantly improve the CPR.
Study funding/competing interest(s): Funding by hospital/clinic(s), London Fertility Centre.
Trial registration number: N/A.

P-071 Evaluation of vitrified/warmed oocyte derived embryos development and morphology

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Study question: Do oocyte vitrification/warming cycles affect embryo morphology and development?
Summary answer: Our results show that embryos obtained using vitrified/warmed oocytes show similar morphological characteristics and do develop the same way as fresh oocytes derived embryos in terms of early cleavage rate, blastomeres size, degree of fragmentation and Morula and Blastocyst development rate.
What is known already: Oocyte vitrification is a worldwide used technique that has proved its interest in fertility preservation and egg donation programs, as well as for infertile couple's management. Although it was shown not to alter oocyte integrity, its impact on derived embryos development has not yet been documented. This study investigates early cleavage rate, blastomeres size, blastomeres fragmentation rate and Blastocyst formation in vitrified/warmed oocyte derived embryos as compared to sibling fresh oocyte derived embryos.
Study design, size, duration: This study includes 90 infertility cases with large oocyte cohort that was divided into 2 groups after denudation. A part of oocytes underwent ICSI while others were vitrified. Oocyte warming cycles were performed when no pregnancy happened using fresh eggs. Zygote to Blastocyst stages were recorded prospectively in an image database.
Participants/materials, setting, methods: 648 oocytes were vitrified using Cryotop (Kitazato) and 537 fresh sibling oocytes were microinjected for 90 infertility couples. 117 oocyte warming cycles after pregnancy failure (when fresh oocytes were used) enabled to compare vitrified/warmed oocytes derived embryo development to that of fresh oocyte derived embryos using image database.
Main results and the role of chance: Vitrified/warmed oocyte derived embryos (VODE) have not shown major differences as compared to fresh oocyte derived embryos (FODE). The evolution of 520 and 451 zygotes obtained after microinjection of fresh oocytes and vitrified/warmed oocytes, respectively, was followed and recorded daily in an image database up to day 5. No statistical difference was observed in early cleavage rate at about 45 h post microinjection (66.7% in FODE *vs* 68.7% in VODE). Blastomeres fragmentation rate and blastomeres size in VODE were the same as in FODE. Our data show that day 4 Morula development rate (43.6% in FODE *vs* 41.1% in VODE) and day 5 Blastocyst development rate (35.4% in FODE *vs* 35.8% in VODE) is to not affected by oocyte vitrification.
Limitations, reason for caution: Large oocyte cohort needed to conduct our study may account for poor oocyte quality and thus low blastocyst *in vitro* development. This study design enables to exclude potential negative effect of oocyte vitrification on embryo development but does not allow comparing the implantation rate of VODE with that of FODE.
Wider implications of the findings: Our results showing that embryo development is not altered by oocyte vitrification emphasize the fact that oocyte vitrification is a reliable technique that could replace embryo cryoconservation. However, children follow up is essential to exclude adverse developmental effect of the technique. Oocyte vitrification was performed using a specific open system based process the only one supported by evidence based medicine, other vitrification systems should be tested based on comparable criteria.
Study funding/competing interest(s): Funding by hospital/clinic(s), Hopital Saint-Jospeh.
Trial registration number: None.

P-072 Detailed time-lapse morphokinetic analysis of early embryo multinucleation: impact on implantation rate

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Study question: Is there any effect of time range in which 2-cell embryos show nuclei on the implantation rate?
Summary answer: By using a large database, we provide an analysis of the number of nuclei present on each of the two blastomeres of the two cell embryo and their times of appearance/disappearance, proving that only the presence of >2 nuclei on at least 1 blastomere decreases implantation rates.
What is known already: Embryo evaluation is mainly performed under light microscope with limitations as subjectivity, prolonged exposure to light, changes in culture conditions and static perception of embryonic development. Evaluating morphological changes using time-lapse, through Embryoscope® allows maintaining conditions and provides useful information to predict embryo implantation. Multinucleation is the presence of more than one nucleus per blastomere; it has been used as a tool to select embryos, as it is considered a marker of poor embryo development.
Study design, size, duration: Observational, retrospective, clinical study. A total of 1602 transferred embryos were cultured and analyzed in Embryoscope® time-lapse incubator after ICSI until day 3 (D3) of development, only embryos when gestational sacs matched with the number of transferred embryos were used to study implantation (n = 1195). Study was performed between 2011 and 2013.
Participants/materials, setting, methods: From 1602 embryos, timing of appearance and disappearance of blastomere nuclei was annotated along embryo development in 2-cell stage, as well as the presence and number of the nuclei seen in 2 and 4-cell-stage embryos.
Main results and the role of chance: From 1602 embryos analyzed, 497 (31.0%) presented at least one multinucleated blastomere, while only 124 (8.2%) of transferred embryos had multinucleated blastomeres at the 4-cell stage.

Implantation rates decreased if multinucleated embryos were transferred, but differences were only significant if >2 nuclei were present in at least one blastomere (25.0 vs. 35.1%) (p = 0.006). Regarding 4-cell stage, the simple presence of one blastomere with more than one nucleus significantly affects implantation rates (34.8 vs. 19.7%) ($p < 0.0001$).

Average timings of appearance of nuclei were 28.5 (CI95% 28.1–28.5) hours post ICSI, being significantly delayed in embryos with multinucleated blastomeres (30.1 h, CI95% 29.4–30.7). Disappearance of nuclei occurs 36.7 h (CI95% 36.4–37.0), as previously multinucleated embryos presented a delayed fading of blastomere nuclei.
Limitations, reason for caution: This research is from a retrospective analysis. Only transferred embryos were analyzed, there is no information of the nuclear dynamics of bad quality un-selected embryos.
Wider implications of the findings: The multinucleation parameters here described are not compatible with the single point morphology observations, then, inclusion of novel kinetic parameters into embryo evaluation may improve current selection criteria. Data here provided show the relevance of multinucleation at the 2-cell stage only in those cases when more than one blastomere present an additional nucleus. Nuclei dynamics (including duration of synthesis phase is longer on multinucleated embryos) and may be applied as a kinetic selection criterion.
Study funding/competing interest(s): Funding by hospital/clinic(s), IVI.
Trial registration number: None.

P-073 Quality enhancement of bovine blastocysts produced in defined media containing an optimised concentration of recombinant human albumin

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Study question: A bovine embryo model was used to compare the yield and quality of blastocysts produced in standard commercial media containing human serum albumin (HSA) with the yield and quality of those produced in chemically defined media containing different concentrations of recombinant human albumin (recHA).
Summary answer: In standard embryo production media, blood-derived HSA (5 mg/mL) can be replaced with a lower concentration of recHA (0.5 mg/mL) to produce good-quality bovine blastocysts; furthermore, blastocyst viability after vitrification was higher in the recHA group than in the HSA group.
What is known already: HSA, a common protein source in assisted reproductive technology (ART), may contribute to biological variation, introduction of toxic residues, and disease transmission. Using recHA as the sole protein source is advantageous in terms of batch-to-batch consistency, biological stability, and minimising viral contamination; however, the extant literature on recHA use is limited. Studies on recHA use, including the determination of the optimal recHA concentration for embryo production, are needed to develop chemically defined ART systems.
Study design, size, duration: Experiment 1: in vitro-matured bovine oocytes (n = 1798) were fertilised/cultured with HSA or recHA and evaluated for blastocyst development (day 8). Experiment 2: the produced blastocyst quality was assessed in terms of cryosurvival (n = 131), cell count (n = 116), and oxygen consumption rate (OCR) (n = 196).
Participants/materials, setting, methods: Experiment 1: oocytes underwent conventional in vitro fertilisation (IVF) in G-IVF medium and were cultured in G1/G2 media. The media contained 5 mg/mL HSA or 0.5, 2.5, or 5 mg/mL recHA (Vitrolife). Experiment 2: blastocysts derived from oocytes fertilised/cultured in 5 mg/mL HSA or 0.5 mg/mL recHA were quality tested.
Main results and the role of chance: Experiment 1: blastocyst rates increased with decreasing recHA concentrations in the defined IVF and in the embryo culture media. In addition, blastocyst rates were similar between oocytes fertilised/cultured in 5 mg/mL HSA (60/239, 25.8 ± 3.2%) or 0.5 mg/mL recHA (60/230, 26.7 ± 3.5%). Experiment 2: hatched blastocyst rates after 24-h cryotop vitrification were higher in the recHA group (28/67, 41.8%) than in the HSA group (14/64, 21.9%) ($P < 0.05$), although all warmed blastocysts re-expanded. Blastocyst cell numbers after nuclear staining were similar in the HSA (108.9 ± 3.1) and recHA (114.0 ± 2.7) groups. Blastocyst OCRs ($F \times 10^{14}$/mol^{-1}s^{-1}) measured by the cell respiration assay system (Clino, Japan) were similar in the HSA (1.16 ± 0.02) and recHA (1.15 ± 0.02) groups.
Limitations, reason for caution: The results of this study are restricted to in vitro data using bovine embryos; however, humans and cattle have similar follicular dynamics and timings of embryo development. Based on the presented results, we are investigating the use of completely defined media for all ART protocols in clinical settings.
Wider implications of the findings: These results suggest that chemically defined media, containing a reduced concentration of recHA, for in vitro embryo production yield good-quality bovine blastocysts, with improved viability after cryopreservation. A defined culture system may be effective for producing embryos with increased cryotolerance. Our findings represent a useful step towards developing standardised and defined ART systems. These investigations will eliminate the inherent variation and potential risks associated with the use of biological products.
Study funding/competing interest(s): Funding by hospital/clinic(s), Kuramoto Women's Clinic.
Trial registration number: N/A.

P-074 Optimization of a closed system for human oocyte vitrification using in vitro matured oocytes and subsequent clinical outcomes from in vivo matured oocytes

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Study question: Can survival rates and subsequent implantation rates similar to those obtained with open oocyte vitrification systems be achieved using a closed system that reduces contamination risks associated with long term storage?
Summary answer: Following optimization of cryoprotectant exposure and modification of the cooling procedure, survival and implantation rates similar to those observed with fresh oocytes can be achieved using a closed vitrification tool.
What is known already: High survival rates and clinical outcomes similar to those from fresh oocytes have been observed with an open oocyte vitrification system using donor oocytes. It has been suggested that the extremely fast cooling rates that are only achieved with open systems are necessary for human oocyte vitrification. However, there is a potential risk of introducing contamination with open systems and this is of concern for fertility preservation patients storing oocytes over long time periods.
Study design, size, duration: Comparison of oocyte vitrification using ethylene glycol (EG) + dimethylsuphoxide (DMSO) + sucrose employing an optimized closed system (Rapid i®) and an open system (Cryolock). Assessment of subsequent application of this closed vitrification procedure in clinical oocyte cryopreservation cases in an ART clinic.
Participants/materials, setting, methods: Comparison of tools and closed vitrification optimization were carried out using donated immature oocytes that were matured in vitro (IVM). Only resultant Metaphase II (MII) oocytes were vitrified. The closed system was subsequently introduced clinically for mature oocyte cryopreservation cases, mainly involving failure to retrieve sufficient sperm for ICSI.
Main results and the role of chance: IVM oocytes were exposed to equilibration solution (ES) for 10 min followed by exposure to vitrification solution and rapid cooling (≤1 min) using either the open or closed system. 92% (73/79) survival was achieved with the open system but initial survival with the closed system was significantly lower (76%; 22/29; $p < 0.05$). Inadvertent exposure to vapour during the closed vitrification was identified as a problem and closed system survival subsequently improved to 89% (34/38).

Due to large variation in re-expansion time in ES for in vivo matured oocytes (re-expansion range 4–12min; n = 1244 oocytes), ES exposure was extended to 12 min. To date, clinical closed vitrification has resulted in 85% (80/94) survival, and an implantation rate of 31% (5/16) from the transfer of day 2 embryos.
Limitations, reason for caution: These are preliminary clinical results for the closed oocyte vitrification system and require confirmation with larger numbers. Further improvements in the system may also be possible.
Wider implications of the findings: A successful closed oocyte vitrification system eliminates potential contamination risks, thereby providing a safer system for long-term storage in cases such as those involving young fertility preservation patients.
Study funding/competing interest(s): Funding by hospital/clinic(s), Melbourne IVF.
Trial registration number: Not applicable.

P-075 Time-lapse imaging demonstrates that human embryos undergoing nuclear envelope breakdown (NEBD) within an optimal time range exhibit enhanced developmental potential

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Study question: Is the precise timing of Nuclear Envelope Breakdown (NEBD) predictive of the ability to form a blastocyst by 115 h post insemination and can it be used in a simple algorithm to select human early cleavage stage embryos with enhanced developmental potential?
Summary answer: Timing of NEBD is predictive of timely blastocyst formation and an optimal window for NEBD was identified. A simple algorithm based on this window and a similar optimal window for cleavage to the 4-cell stage can be used early on day 2 to identify embryos with significantly enhanced developmental potential.
What is known already: The developmental/ implantation potential of a human embryo has been shown to be dependent on whether or not it

has undergone NEBD/syngamy or the first cleavage division by a defined time post insemination (hpi), usually 24–27 hpi. The advent of time-lapse monitoring technology for human embryos in vitro has now allowed us to determine the timing of NEBD/entry into syngamy as a continuous variable in order to establish more accurately its relationship to subsequent development.

Study design, size, duration: Time-lapse imaging of individual human embryos cultured in vitro was used to determine the precise times at which early developmental events occurred. Timing of these events was assessed retrospectively and related to the potential of an individual embryo to form a blastocyst by 115 hpi.

Participants/materials, setting, methods: Fertilised oocytes (n = 432) from patients undergoing ICSI were cultured in individual Embryoscope™ wells in sequential media (SAGE). Timings for NEBD and early cleavage divisions were documented for each embryo by analysis of time-lapse images. Embryos were also assessed for their ability to complete blastulation within 115 h of ICSI.

Main results and the role of chance: Timing of NEBD in fertilised oocytes ranged from 17.5 to 46.4 hpi (median 24.3 hpi). When NEBD timing data was analysed by quartiles, a gradual decrease in the proportion of embryos forming blastocysts by 115 hpi (from 71% to 15%) was observed from the 1st to 4th quartile. However, when the same data was analysed by octiles, optimal blastocyst development (79%) was observed in the 2nd octile (NEBD timing range 20.5–21.9 hpi). Using the same approach, blastocyst development was observed to be optimal (86%) when cleavage to the 4 cell stage occurred between 35.4 and 36.7 hpi. Applying a combination of these 2 optimal developmental windows identified a population of early cleavage stage embryos with a 91% probability of completing blastulation by 115 hpi.

Limitations, reason for caution: Although the optimal timing of NEBD together with other early developmental events may be used to construct a simple algorithm that predicts the probability of timely blastocyst formation, the actual values used to construct the algorithm may be specific to individual culture conditions/ laboratories and may require in-house validation.

Wider implications of the findings: The high predictive accuracy of timing of NEBD suggests that it should be included in any time-lapse based algorithm used to select early cleavage stage embryos for transfer. The results also demonstrate that it is feasible for individual laboratories to construct simple predictive algorithms based on in-house data.

Study funding/competing interest(s): Funding by hospital/clinic(s), Melbourne IVF.

Trial registration number: Not applicable.

P-076 Efficiency comparison of cryopreservation of eggs and embryos in a shared egg donor program

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Study question: The efficiency between egg cryopreservation and embryo cryopreservation has not been comparatively studied, and it is still unknown which can provide better clinical outcomes, especially in donated human eggs.

Summary answer: Transfer of cryopreserved blastocysts from fresh eggs has similar clinical outcomes (pregnancy and implantation) as transfer of fresh blastocysts from frozen eggs. These outcomes are also comparative to those with fresh transfer of blastocysts from fresh eggs, indicating a similar efficiency of the vitrification for both embryo and egg cryopreservation.

What is known already: Acceptable laboratory outcomes (survival, fertilization, cleavage and blastocyst rates) and clinical outcomes (pregnancy and implantation rates) have been reported with the vitrification of human eggs and embryos. However, most of these data are based on the studies with small number of samples, especially on egg cryopreservation. Some studies indicated that comparative outcomes can be obtained by use of cryopreserved embryos and eggs as compared with fresh samples.

Study design, size, duration: Clinical data were collected from 242 recipients who received (2–4 recipients per donor) 941 fresh and 1060 frozen eggs donated from anonymous egg donors during 2012 and 2013. The efficiency was compared between fresh blastocyst transfer from frozen eggs and frozen blastocyst transfer from fresh eggs.

Participants/materials, setting, methods: Donor eggs were used in the study and recipients received either frozen blastocysts from fresh eggs or fresh blastocysts from frozen eggs. As a control, some recipients received fresh blastocysts from fresh eggs. Vitrification was used to freeze both blastocysts and eggs.

Main results and the role of chance: A 96.9% egg survival rate and a 96.6% embryo survival rate were obtained. No differences were observed between frozen blastocyst transfer from fresh eggs and fresh blastocyst transfer from frozen eggs in terms of clinical pregnancy rates (58.8% vs. 60.3%) and embryo implantation rates (42.9% vs. 49.3%). The clinical outcomes are also comparative to those with fresh embryo transfer from fresh eggs (65.3% and 44.9% respectively). The results indicate that egg cryopreservation has the same efficiency as embryo cryopreservation if donor eggs are used. This allows embryology laboratories to freeze either eggs or embryos for recipients if their partner's semen samples are not available, the cycles between donors and recipients are not synchronized, or spare eggs are retrieved.

Limitations, reason for caution: The efficiency was analyzed only in the recipients who had the first embryo transfer, so the case number in the frozen embryo transfer group is limited. More cycles and/or accumulated clinical data is necessary to further evaluate the efficiency between embryo cryopreservation and egg cryopreservation.

Wider implications of the findings: Cryopreserved egg banks have many advantages, especially for egg donor programs if egg freezing has the same efficiency as embryo freezing. Successful cryopreserved egg banks would have more benefits to both patients and clinics than cryopreserved embryo banks if the high efficiency is obtained.

Study funding/competing interest(s): Funding by hospital/clinic(s), There was no external funding for this study.

Trial registration number: N/A.

P-077 Reproductive potential of vitrified in vitro matured (IVM) oocytes obtained from unstimulated IVM cycles

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Study question: What is the different reproductive potential between IVM oocytes with and without vitrification in IVM cycles?

Summary answer: Healthy live births can be achieved from the combination of IVM and vitrification of immature oocytes collected from unstimulated ovaries. However, the embryos derived from the vitrified IVM oocytes have a poorer reproductive potential and resulted in lower clinical outcomes compared to fresh IVM oocytes.

What is known already: Few live births after IVM oocytes cryopreservation have been reported. Embryological aspects and clinical outcomes after IVM oocyte vitrification are also still poorly studied. In our previous studies, IVM oocytes had lower reproductive potential after vitrification compared to IVF oocyte. From the study, however, it was difficult to know whether the lower reproductive potential in the oocytes produced from IVM program is because of low quality of IVM oocytes or/and vitrification procedure.

Study design, size, duration: Fifty-six cycles of IVM oocyte vitrification in polycystic ovaries (PCO) patients were performed between June 2005 and November 2009. The embryological aspects and clinical outcomes of these vitrification cycles were compared in against a control consisting of fresh IVM cycles which were done in the same period (n = 225 cycles).

Participants/materials, setting, methods: PCO patients who agreed IVM oocyte vitrification study and PCO patients who had fresh IVM cycles. Data between two groups were analyzed retrospectively. Study was conducted at a university based IVF center. A 10,000 IU hCG was administered 36 h before oocyte collection and vitrified at mature stage after IVM.

Main results and the role of chance: There was no significant difference in the number of mature oocytes between vitrification- and fresh-IVM cycles (12.6 ± 6.8 vs. 11.7 ± 7.4). The survival rate per IVM oocyte after performing vitrification/warming was 59.3%. The fertilization and cleavage rates per oocyte were significantly lower in vitrification group (57.8%, 66.8%) than those of control fresh IVM group (71.9%, 91.1%) ($P < 0.01$). The clinical pregnancy,

implantation, live birth rates per cycle were also lower in vitrification group (10.7%, 4.4%, 8.9%, respectively) compared to fresh IVM group (37.8%, 15.0%, 27.1%, respectively) ($P < 0.01$). There were 5 healthy live births from IVM vitrification group.

Limitations, reason for caution: The primary limitation of this study is its retrospective study.

Wider implications of the findings: Our results suggest that IVM of immature oocytes combined and vitrification is an option for women who cannot pursue IVF oocyte vitrification for their fertility preservation. However, there are significantly reduced reproductive potential after vitrification/warming compared to control fresh IVM oocytes. Therefore, this procedure will require further advances in IVM- and Cryo-technology.

Study funding/competing interest(s): Funding by hospital/clinic(s), No external funding was obtained for this study. There are no competing interests to declare.

Trial registration number: Not applicable.

P-078 Is the retardance of mitotic spindle a good predictor of embryo development?

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Study question: The aim of this work is to relate the retardance of diploid mitotic spindle in zygotes 2-0 (presenting 2 Polar Bodies (PB) and no pronuclei) with their development capability. We used a polarized light microscope to verify the existence of mitotic (central position) spindle and to measure its retardance.

Summary answer: The direct correlation between mitotic spindle retardance and cells number in day 2 and 3 of embryo development, as well as blastocyst rate and good quality blastocyst formation rate may indicate that this parameter can be useful for predicting embryo quality and blastocyst rate.

What is known already: During the lab routine work we usually find zygotes 2-0 where 2 PB are identified but not pronuclei. Those zygotes are typically discarded to avoid those abnormally fertilized. In previous works in our laboratory we confirmed normal development of some of those zygotes that delivered a healthy newborn.

Study design, size, duration: 5166 IVF cycles were performed from January 2009 to March 2010, The zygotes 2-0 were classified as mitotic spindle in central position and not in central position. G-stat 2.0 statistic program was used to perform the statistical analysis. Informed consent was obtained from all patients included in this group.

Participants/materials, setting, methods: 607 zygotes 2-0 were analyzed using polarized light (Oosight CRI, Research Instruments Ltd). Retardance of mitotic spindle was measured in those zygotes ($n = 202$, 115 patients) and were cultured up to the blastocyst stage and frozen if they met our standards of quality.

Main results and the role of chance: 89.5% of zygotes 2-0 (with mitotic spindle) presented cell division compared with 0.49% with those who didn't have central spindle ($p < 0.0001$).

We have related the number of cells in day 2 and 3 of development with retardance values, and we found a direct correlation in day 3 ($p = 0.0001$).

Splitting the study group into quartiles, according to the mitotic spindle retardance, and relating it with, blastocyst rate, and good quality blastocyst formation rate (blastocele cavity at least more than half the volume of the embryo, inner cell mass with many cells, and trophoectoderm with at least few cells, forming a loose epithelium).

We have found a strong correlation between retardance rate and blastocyst rate ($p < 0.0001$), and with good quality blastocyst formation rate ($p < 0.0001$).

Limitations, reason for caution: To verify these results, it would be advisable to extend the number of embryos analyzed, and to compare these results with embryos that haven't erased their pronuclei before 19 hours post fertilization.

Wider implications of the findings: To our knowledge, there are no described results on mitotic spindle in 2-0 zygotes, and our results might suggest that the measurement of mitotic spindle retardance, could help us to predict the embryo development, using this tool as a complement to embryo morphokinetic.

Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by commercial/corporate company(ies), IVI Madrid.

Trial registration number: B.

P-079 Comparison of genes expression in germinal vesicle oocyte in the GnRH agonist long vs. GnRH antagonist protocol of human ovarian stimulation

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Study question: To compare the expression of Bone Morphogenetic Protein 15 (BMP15), Growth Differentiation Factor 9 (GDF9), Neuronal Apoptosis Inhibitory Protein (NAIP) and ATPase 6 genes in germinal vesicle (GV) oocyte between the gonadotropin-releasing hormone antagonist (GnRH-ant) and gonadotropin-releasing hormone agonist (GnRH-a) protocols.

Summary answer: Germinal vesicle (GV) oocytes were found to be of high quality under the GnRH-ant protocol. This combined with the lesser extent and shorter periods of drug use in the GnRH-ant protocol suggests that the GnRH-ant protocol can be considered as a suitable alternative for the GnRH-a long protocol for women undergoing controlled ovarian stimulation (COS).

What is known already: Several studies have been conducted comparing the efficacy of the two protocols. A recent Cochrane review indicated no evidence of a statistically significant difference between the efficacies of the two protocols. In addition, the incidence of ovarian hyperstimulation syndrome (OHSS) in the GnRH-ant treatment was lower than that of the GnRH-a treatment. Furthermore, the following characteristics of the two protocols have also been compared: the follicular microenvironment, the percentage of granulosa cells with positive DNA fragmentation and apoptosis, genes expression in cumulus cells.

Study design, size, duration: From June 2012 to November 2013, in this cross-sectional study, 100 morphologically normal germinal vesicle (GV) oocytes were donated. The gene expression of the pools of 50 germinal vesicle oocytes from the GnRH-a long protocol group (obtained from 39 patients) and 50 germinal vesicle oocytes from the GnRH-ant protocol group (obtained from 24 patients) were compared.

Participants/materials, setting, methods: After the sampling, pools of 50 germinal vesicle oocytes from the GnRH-a long protocol group (obtained from 39 patients aged 30.7 ± 5.2 years) and 50 germinal vesicle oocytes from the GnRH-ant protocol group (obtained from 24 patients aged 33.1 ± 5.7 years) were separately analyzed by quantitative PCR.

Main results and the role of chance: Age, hormonal profile, number of oocytes, cause of infertility, and infertility duration were similar in the two groups ($P > 0.05$). the serum level of estradiol on the day of human chorionic gonadotropin (hCG) administration was higher in the GnRH-ant protocol group than in the GnRH-a long protocol group; however, this difference was not statistically significant. We analyzed the results of gene expression by the RG mode of REST 2009 (QIAGEN). The expression of *ATPase 6, BMP15and NAIP* were significantly higher in the pooled oocytes of the patients in the GnRH-ant protocol group than those of the patients in the GnRH-a long protocol group ($P < 0.001$). *GDF9* mRNA did not have any expression in the pooled oocytes of the patients in the GnRH-a long protocol group; however, GDF9

mRNA was expressed in the pooled oocytes of the patients in the GnRH-ant protocol group. *ATPase6* is UP-regulated x by a mean factor of 3.990 (S.E. range is 3.069–5.321), BMP15 is UP-regulated by a mean factor of 6.274 (S.E. range is 5.052–7.793), NAIP is UP-regulated by a mean factor of 2.156 (S.E. range is 1.559–3.362) in the pooled oocytes of the patients in the GnRH-ant protocol group than those of the patients in the GnRH-a long protocol group ($P < 0.001$)

Limitations, reason for caution: Due to lack of time and resources, we did not have access to a large number of immature oocytes.

Wider implications of the findings: we did not find any similar study in the literature.

Study funding/competing interest(s): Funding by University(ies), Tehran University of Medical Sciences.

Trial registration number: no IRCT.

P-080 Oocyte developmental competence: an improved predictive model combining morphological criteria with transcripts or proteins biomarkers

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Study question: We developed a predictive model of oocyte developmental competence based on morphological criteria of embryo at day 2 (MCE) combined to transcript and protein levels identified in human cumulus cells from oocytes reaching the blastocyst stage (CCB+) compared to cumulus cells from oocytes arresting their development (CCB–) once fertilised.

Summary answer: The combination of MCE and transcript or protein levels identified in cumulus cells led to a predictive value of 0.78 and 0.81 respectively whereas the prediction based on the morphological criteria only remained under 0.5.

What is known already: The morphological criteria are mostly used in ART laboratories but remain poorly predictive of embryo potential to develop (lower than 0.7). Various approaches involving transcriptomics, proteomics, and metabolomics on the embryo or its cellular environment have been proposed without improving the predictive value of embryo potential to develop. Moreover, most of the studies were focused on a single approach even though their combination in a unified predictive model might increase the predictive value.

Study design, size, duration: This study included 72 patients undergoing intracytoplasmic sperm injection (ICSI) for male infertility from January 2008 to May 2012, in which 102 individual cumulus cells (iCCs) from 29 patients were collected during ICSI procedure for the microfluidic-qPCR and 92 iCCs from 43 patients for the Reverse Phase Protein Array (RPPA).

Participants/materials, setting, methods: Biomarkers were identified by microfluidic-qPCR in iCCs among 95 transcripts selected from a previous microarray study and by RPPA among 7 proteins coded by the selected transcripts. The predictive model used collaborative filtering combining variants of Heterogeneous Value Difference Metric distance functions which can handle both nominal (MCE) and linear (biomarkers) attributes.

Main results and the role of chance: Seventy-four genes could be analysed by microfluidic-qPCR and 6 genes (*CUL4B*, *LGALS9*, *MERTK*, *NFYBeta*, *POLR3K*, *TRSPAP1*) were differentially expressed according the oocyte developmental competence. In a separate collection of samples, 3 proteins (RGS2, MERTK and POLR3K) were identified by RPPA as differentially expressed between CCB+ and CCB–. The predictive model only based on the MCE led to a predictive value of 0.40 (41/102 iCCs were successfully identified as CCB+ or CCB–) for microfluidic-qPCR samples and 0.36 (33/92) for RPPA samples. Interestingly, the combination of the MCE and the 74 transcripts or the MCE and 7 proteins led to a predictive value of 0.79 (81/102) and 0.81 (75/92), respectively.

Limitations, reason for caution: The antibody used for the detection of RGS2 using RPPA needs further validation. The transcriptomic biomarkers as well as the protein biomarkers identified have to be validated on a new cohort of patients. Moreover, the predictive model needs to be assessed in a randomized controlled trial.

Wider implications of the findings: This study highlighted new biomarkers of oocyte developmental competence in human cumulus cells at transcript and protein levels. Moreover, we showed the importance to combine various criteria (MCE, cumulus cells transcriptome and/or proteome) to improve accuracy of our algorithm. Furthermore, combining transcript and protein study in the same samples may further improve the predictive value of our model. Such approaches might be used in ART laboratories for oocyte and embryo selection and might limit the extended culture until blastocyst stage.

Study funding/competing interest(s): Funding by commercial/corporate company(ies), This work was supported by Merck Serono Grant for Fertility Innovation Award 2010.

Trial registration number: None.

P-081 Division behaviors and selection of high potential day 3 embryos: a time-lapse study

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Study question: Can we select day 3 (d3) embryos based on division behaviors instead of morphokinetic parameters in time-lapse study?

Summary answer: We proposed a hierarchical predictive model to select d3 embryos with high developmental potential to karyotypic normal blastocyst based on the severity and scope of abnormal division behaviors in the first three cleavages. This predictive model increased implantation rate of d3-transferred embryos in a prospective study when compared to morphological selection.

What is known already: Previous time-lapse studies suggested several morphokinetic parameters for d3 embryos selection but these parameters might vary between different laboratories and protocols and need customization. Some abnormal division behaviors (e.g. direct division from one to three or more cells) were observed and included as de-selection parameters.

Study design, size, duration: A retrospective observation study was conducted on 345 2PN embryos to establish the model and a prospective control study was conducted on 40 patients to assess the clinical efficiency of this model during October, 2012 to October 2013.

Participants/materials, setting, methods: The development of 345 fertilized 2PN embryos were monitored by PRIMO Vision system. Different abnormal division behaviors in the first three cleavages and morphokinetics parameters were analyzed. A subset of the blastocysts ($n = 73$) was biopsied for comprehensive chromosomal analysis. A hierarchical predictive model for d3 embryo selection was developed according to above observations and was further verified in a prospective controlled study.

Main results and the role of chance: Seven abnormal division patterns were observed in a high frequency during the first three cleavages: direct cleavage to more than three blastomeres (DC, 18.7%), uneven blastomeres (UB, 10.1%), fragmentation (FR, 17.2%), big fragment (BF 19.6%), irregular cytoplasm movement during cleavage (ICM, 27.0%), development arrest (DA, 4.7%) and disordered division (DD, 2.7%). We observed that DC, UB, FR and DA significantly decreased the ability to integrate into blastocyst development of the daughter cells and increased the chromosomal abnormalities of resulted blastocysts. The earlier these abnormal behaviors appeared; much less chance a normal blastocyst could develop. According to above observations, we built an embryo hierarchical predictive model solely on abnormal division. Moreover, in prospective study, compared with morphology group ($n = 20$), time-lapse group ($n = 20$) had an increased implantation rate (69.2% vs. 40.0%, $P < 0.05$) and clinical pregnancy rate (79.0% vs. 55.0%, $P > 0.05$).

Limitations, reason for caution: The effectiveness of our new model need further validation due to the small size of our clinical study and one center study design.

Wider implications of the findings: The established model in our study may provide a new approach for embryo selection in different laboratory with a relatively universal criteria based on time-lapse monitoring.

Study funding/competing interest(s): Funding by national/international organization(s), National Science foundation of China 81222007.
Trial registration number: None.

P-082 Qualitative assessment of warmed pronuclear stage zygotes after slow-rate freezing and cryotech vitrification

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Study question: To evaluate both practical and clinical relevance of frozen/warmed 2 PN Zygotes derived from two different freezing principles.
Summary answer: Warmed 2PN-Zygotes after Cryotech vitrification showed significantly higher survival rates and embryo utilization rates (derived from transferable good quality embryos) as compared to the slow-rate freezing group. On average, more embryos had to be warmed with the slow-rate freezing method in order to achieve a clinical pregnancy (3.5 vs. 2.1).
What is known already: Based on ethical restrictions, cryopreservation of 2 PN-Zygotes is the most common procedure to perform frozen embryo transfers in Switzerland. The cryopreservation of 2-PNs by slow-rate freezing is both time and LN2 consuming and leads to a limited quality of surviving 2-PN zygotes. Furthermore, non-fertilized Oocytes for emergency freezing (e.g. no sperm available) cannot successfully be cryopreserved by the slow-rate freezing method.
Study design, size, duration: 614 transfer cycles were performed from 2148 thawed 2-PNs after slow freezing between 2010 and 2012. These were compared to 252 transfer cycles derived from 525 2-PNs warmed after Cryotech vitrification in 2013.
Participants/materials, setting, methods: 2PN-Zygotes were cryopreserved 16–20 hours post insemination with either the slow-rate freezing method or the Cryotech vitrification method. Zygotes warmed with either method were cultured and compared in respect to the survival rate (SR), cleavage rate (CR) embryo utilization rate (UR), clinical pregnancy rate (CP) and the implantation rates (IR).
Main results and the role of chance: The mean age of the slow-rate freezing patients (group A) was 35.0 ± 4.6 years and the Cryotech vitrification patients (group B) 35.5 ± 4.1 years (ns). SR was 65.0% (n = 1396/2148) in group A and 99.8% (524/525; p = < 0.0001) in group B. CR was 88.6% (n = 1237/1396) in group A vs. 94.5% (n = 495/524; p = 0.4068) in group B. UR was 51.9% in group A (1115/2148) vs. 82.1% (431/525; p = < 0.0001) in group B. CP was 25.1% in group A (n = 154/614) vs. 24.6% (62/252; ns) in group B. IR was 15.2% (n = 170/1115) in group A and 16.7% (n = 72/431; ns) in group B.
Limitations, reason for caution: Neither the delivery rate, nor the baby take home rate could be assessed because of missing data. Hence, a maximum of three 2-PN zygotes were thawed per transfer due to Swiss legislation. This might limit the informative value of the post transfer data.
Wider implications of the findings: Although the comparison of the two methods does not reveal significant differences in the clinical pregnancy outcomes, a significantly higher survival rate and thus embryo utilization rate could be achieved by vitrification. Long-term follow up studies to consolidate the impact of the Cryotech vitrification method will be considered.
Study funding/competing interest(s): Funding by hospital/clinic(s). No funding to report.
Trial registration number: Not applicable.

P-083 Euploid status and single embryo transfer success is independent of Day 5 and Day 6 blastocyst development

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Study question: Are there differences in euploidy rates between Day 5 and Day 6 blastocysts? Does the day of blastocyst development predict implantation or ongoing/live birth rates in single euploid vitrified blastocyst transfers?
Summary answer: Day 5 versus Day 6 blastocysts exhibit no difference in euploidy rate. Single euploid embryo transfer pregnancy success remains high and shows no effect on implantation, clinical pregnancy, and take home baby rates between Day 5 or Day 6 vitrified blastocyst transfers.
What is known already: Early embryo aneuploidy is widely accepted as a major reason of implantation failures. The goal of many IVF programs is to offer high success without the risk of multiple gestations through single embryo transfers (SET). The use of Preimplantation Genetic Screening (PGS) is considered a valuable ART tool to help clinicians choose only top quality, genetically euploid embryos for SET.
Study design, size, duration: Euploidy was determined through PGS/vitrification-all cycles with biopsy between 1/1/2012 and 12/31/2013, resulting in 244 cycles and 1259 blastocysts. All blastocysts were biopsied on Days 5 or 6 and required a 3BB or better grade. Pregnancy results were based on 108 (average age: 35.6) vitrified-warmed single euploid embryo transfers.
Participants/materials, setting, methods: Patients autonomously chose PGS-trophectoderm biopsy/vitrification-all cycles. Embryos were laser hatched on Day 3 and herniating blastocysts were biopsied (quality grade 3BB or better) on Day 5 or 6. Euploidy results were determined on every patient enrolled and pregnancy outcomes were based on non-donor, single embryo transfers only.
Main results and the role of chance: Day 5 euploidy rate (50.4%) showed no difference (p = 0.56) compared to Day 6 (48.6%). No significance was observed with SET ongoing/live birth for Day 5 at 75.4% versus Day 6 at 72.1% (p = 1.00). Furthermore, the data revealed no differences in Day 5 and Day 6 implantation rates (83.6% versus 83.7%; p = 0.798). There was a trend toward increased spontaneous abortions, %SAB, with Day 6 blastocysts being 13.9% compared to 4.2% for Day 5 (p = 0.128). Age stratification of the data reveals no significant differences, although a majority of our Day6 SABs did occur in the 35–37 year old age group where 91.7% produced a viable sac, but only 58.3% sustained the pregnancy. Physiologic patient variation is likely a more critical factor than age in influencing pregnancy outcomes.
Limitations, reason for caution: Perceived emphasis for faster growing embryos places higher importance for transferring Day 5 blastocysts versus Day 6 when equal quality embryos are available. A prospective randomized trial could eliminate this perceived importance and correct for embryologist bias.
Wider implications of the findings: Rapid growing blastocysts have long been thought to have higher euploidy and success rates. This study indicates that when corrected for aneuploidy, day of blastocyst development does not influence success. With a demand for high SET take home baby rates, the need to better assess blastocycts beyond quality grades and developmental pace is necessary to increase IVF success.
Study funding/competing interest(s): Funding by hospital/clinic(s), SCCRM.
Trial registration number: None.

P-084 The timing of vitrification protocols and the collapse technique affects the re-expansion rates and hatching potential of expanded mouse blastocysts

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Study question: Is the effectiveness of pre-vitrification collapse using laser-pulse [LAC] or micropuncture/suction [MAC] dependent on the length of the vitrification protocol?
Summary answer: Artificially collapsing blastocysts prior to vitrification is not associated with greater survival or overall expansion rates when compared to non-collapsed controls, but higher proportions of blastocysts hatch after LAC. However, collapsing the blastocysts and vitrification protocols with long equilibration times (>10 mins) increase the time taken for blastocysts to re-expand.
What is known already: It has been reported that artificial collapse (AC) by LAC or MAC prior to vitrification improves the survival and implantation potential, particularly for expanded blastocysts. Clinical studies demonstrating improved results for the AC techniques have generally used short vitrification protocols. There is a plethora of blastocyst vitrification protocols used globally, but it remains unclear if AC is beneficial for all.

Study design, size, duration: 229 expanded mouse blastocysts were randomly assigned to 6 groups: either not collapsed (control), collapsed by laser-pulse or micro-puncture/suction and then vitrified either by short (3 min, 37ºC) or long (10 min, room temperature) protocols. Blastocysts were warmed and observed for re-expansion, survival, hatching and hatched rates.
Participants/materials, setting, methods: Blastocysts were vitrified on Fibreplugs™ (Cryologic, Mulgrave, Australia). Post-warming, blastocysts were assessed hourly to 4, 16 and 24 h then, either dual-stained with H33342 and propidium iodide to measure mean cell survival or assessed post-hatching by measuring the extent of trophectoderm and ICM cell outgrowth following *in vitro* culture for 144 h.
Main results and the role of chance: Re-expansion of AC blastocysts took longer after warming than controls (5.8 h[LAC] and 4 h[MAC] vs. 1.2 h, short protocol; 7.6 h[LAC] and 5.6 h[MAC] vs. 2.9 h, long protocol). Re-expansion was slower in all groups when using the long protocol (2-way ANOVA). Hatching rates at 16 h for LAC-short and LAC-long groups (90.6%, 91.4% respectively) was higher than in all other groups (range: 45.2%–55.9%, $p < 0.05$). Hatched rate for the LAC-short group (57%) was higher than for controls and MAC-short groups (13.8%, 17.5% respectively) but the LAC-long group (31.4%) was similar to control and MAC-long groups (15.2%, 12.9% respectively). LAC and MAC blastocyst outgrowths did not differ from controls. Overall 97.0% blastocysts survived at 24 h post-warming and of those blastocysts stained ($n = 99$), the mean cell survival/treatment group ranged between $90.7 \pm 4.4\%$ and $97 \pm 0.8\%$.
Limitations, reason for caution: Although we have demonstrated high survival and differing expansion rates with various protocols using expanded mouse blastocysts, these timings may not directly relate to human blastocysts. Also, although implantation potential was implied from the outgrowth study further studies involving embryo transfer are needed to determine true implantation and live-birth rates.
Wider implications of the findings: Vitrified blastocysts are usually warmed on the day planned for transfer and decisions to transfer, discard or warm more blastocysts are often made within 3 h by observing re-expansion. We cannot conclude the efficacy of collapse but have shown that the type of collapse and length of protocol can affect re-expansion rates, thus it would be important for clinics to consider these findings when performing their post-warm assessments.
Study funding/competing interest(s): Funding by University(ies). No direct funding or competing interests.
Trial registration number: N/A.

P-085 Correlation between blastocyst formation rate and chromosomal normality in cases without PGD - a time-lapse study

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Study question: A non-invasive model classifies embryos from A through D according to chromosomal normality. Is there a correlation between these categories and the blastocyst formation rate?
Summary answer: Yes, blastocyst formation rate is strongly correlated with the embryo categories from this model.
What is known already: The impact of chromosome normality on embryo morphology and development has been studied extensively from a static point of view. More specifically, blastocyst formation rate is among the parameters more studied. The general consensus is that chromosomally normal embryos (as determined by pre-implantation genetic diagnosis (PGD) have a higher blastocyst formation rate than abnormal ones. However PGD is not always possible and recently, time-dependent variables have been proposed to generate algorithms that may increase the probability of selecting chromosomally normal embryos. Even though we can not assure the chromosome normality of these embryos (as with PGD) it is interesting to assess if embryo categories from these algorithms are correlated, or not, with the blastocyst formation rate.
Study design, size, duration: Consecutive prospective cohort study. March 2011 and August 2012.
Participants/materials, setting, methods: University-affiliated infertility clinic. Embryo development from 77 patients undergoing pre-implantation genetic screening ($n = 508$) was analyzed with a time-lapse system (Embryoscope, Fertilitech, Denmark). Chromosomal analysis was performed through array-comparative genome hybridization (CGH)
Main results and the role of chance: Out of the 504 embryos analyzed, 54.2% (273/504) developed to the blastocyst stage on day 5 of development. Out of the 504 embryos, 28.3% were diagnosed as normal (143/504). From this last group, 79.0% (113/143) reached blastocyst stage. On the other hand, embryos were classified from A to D based on an algorithm that utilizes the variables $t_5–t_2$ ($t5$ = time to 5 cell stage, t_2 = time to 2 cell stage) and CC3 ($cc_3 = t_5–t_3$; t_3 = time to 3 cell stage) to classify them. The percentages of normal embryos in each category were: A: 36.0% (115/319), B: 25.8% (15/58), C: 10.8% (4/37), D: 10.0% (9/90). The percentages of blastocysts in each category were: A: 66.5% (212/319); B: 48.3% (28/58); C: 24.3% (9/37); and D: 26.6% (24/90); $P < 0.0001$).
Limitations, reason for caution: Blastocyst rate was analyzed only for embryos with biopsy on day 3 excluding those highly fragmented (>20%) and/or having less than 6 blastomeres. The algorithm utilized to correlate blastocyst rate only increases the probability of selecting chromosomally normal embryos but does not provide the real chromosomal content of the embryo.
Wider implications of the findings: The algorithm proposed, together with reducing the chances of transferring an aneuploid embryo, could be applied to select embryos with higher probabilities of developing to the blastocyst stage. This study is in agreement with previous ones that state that chromosomally normal embryos have higher probabilities of forming blastocysts than abnormal ones.
Study funding/competing interest(s): Funding by hospital/clinic(s), IVI.
Trial registration number: Not applicable.

P-086 Efficiency of two insemination techniques (cIVF and ICSI) on fertilization and embryo development derived from same patients

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Study question: Examine the efficiency of two different insemination techniques (cIVF and ICSI), in order to evaluate fertilization and embryo development using same condition oocytes derived from the same patient, with normal sperm sample.
Summary answer: ICSI has significantly higher fertilization rate compared to cIVF. ICSI shows to have more blastocyst formation, however there was no significant difference compared to cIVF.
What is known already: Previous studies have shown that cIVF has better embryo development, however the fertilization rate is lower, compared to ICSI. To date, there is no study comparing different techniques in the same patient, which could give more reliable results.
Study design, size, duration: Retrospective study comparing 415 mild stimulation cycles from January 2009-December 2012, including patients who had 2 MII oocytes retrieved from same size follicle to compare 2 different insemination methods.
Participants/materials, setting, methods: 415 cycles using mild stimulation protocol (Teramoto, 2007). One oocyte from each patient was inseminated by cIVF (10–20 M/ml sperm concentration), and the other using ICSI. Normal sperm samples were used in this study.
Main results and the role of chance: Normal fertilization (2PN 2PB) rate for cIVF and ICSI were 73.7% (306/415) and 89.6% (372/415) respectively, and blastocyst formation rate were 32.5% (135/415) for cIVF and 35.9% (149/415) for ICSI. Fertilization rate from ICSI was significantly higher ($p < 0.05$), but there were no significant difference in blastocyst formation rate. Choosing ICSI make more blastocyst, consequently more chances of pregnancy for patients.
Limitations, reason for caution: There are a low number of patients with two MII oocytes. Pregnancy is not able to assess because the comparison is made between embryos of the same patient.
Wider implications of the findings: There's a possibility that ICSI will become the main fertilization technique in the near future.
Study funding/competing interest(s): Funding by hospital/clinic(s), Kato ladies clinic.
Trial registration number: Not applicable.

P-087 The appearance of multinucleation at 2-cell stage does not adversely affect the implantation potential

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Study question: Does the appearance of multinucleation (MN) at 2-cell stage adversely affect the implantation potential?
Summary answer: The appearance of MN at 2-cell stage did not decrease the developmental rate to the blastocyst stage and the implantation rate after embryo transfer (ET).
What is known already: It has been controversial whether the appearance of MN at 2-cell stage causes high chromosomal abnormality and results in their pregnancy loss or not. A leading-edge technology enables us to observe morphological changes of embryo development without impairing its developmental competence.
Study design, size, duration: We intended 27 patients who underwent ET on day 3 between September and December 2013 after obtaining the informed consent. After confirmation of normal fertilization, time-lapse images of 197 embryos were taken every 10 minutes using a time-lapse cinematography system (TLC, Vitrolife). Surplus embryos were cultured until day 6.
Participants/materials, setting, methods: After day 3 ET and surplus embryo culture until day 6, the appearance of MN was confirmed using captured images. Effects of MN appearance at 2-cell stage on the development to the blastocyst stage and the implantation potential were compared retrospectively.
Main results and the role of chance: The appearance of MN at 2-cell stage was confirmed in 97 embryos (49.2%) based on the observations of TLC images. The blastulation and the morphologically good blastocyst rates in MN embryos were 38% (31/82) and 26% (21/82), respectively. These values were almost the same levels as those obtained in non-MN embryos (blastulation rate: 46% (44/95) and good blastocyst rate: 26% (24/95)). The implantation rate of MN embryos (27%) was also similar to that of non-MN embryos (20%).
Limitations, reason for caution: Further studies are required to clarify the link between the appearance of MN at 2-cell stage and its chromosome constitution.
Wider implications of the findings: This study provided new insights on the implantation potential of embryos which formed MN at 2-cell stage.
Study funding/competing interest(s): Funding by national/international organization(s), A part of this work was supported by a grant from the Japan Society for the Promotion of Science (JPS-RFTF 23580397 to S.H.). No other competing interests are declared.
Trial registration number: None.

P-088 Effect of ionomycin and calcium in the culture medium on the calcium releasing pattern of mouse oocytes and their subsequent embryonic development

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Study question: Does the concentration of ionomycin and Ca^{2+} ([Ca^{2+}]) in the culture medium affect the Ca^{2+} releasing pattern of mouse oocytes and their subsequent embryonic developmental potential when activated by ionomycin?
Summary answer: The concentration of both ionomycin and [Ca^{2+}] influence the Ca^{2+}-rise and subsequent embryonic development of mouse oocytes. From all combinations tested, 10 µM ionomycin dissolved in 1X-Ca^{2+} culture medium provoked Ca^{2+} rises with the highest amplitude and area under the curve and resulted in the highest activation and blastocyst rate.
What is known already: Ionomycin is a Ca^{2+}-selective ionophore frequently used as an agent for assisted oocyte activation (AOA) in clinical practice to overcome failed fertilization after ICSI. It has been shown recently in the bovine that ionomycin combined with a high [Ca^{2+}] in the culture medium increases the activation rate and improves embryonic development. However, neither the precise mechanism of action of ionomycin nor the possible factors influencing its efficiency have been studied yet.
Study design, size, duration: The effect of ionomycin concentration was investigated by applying 5 µM, 10 µM and 15 µM ionomycin dissolved in KSOM media to activate mouse oocytes. The effect of [Ca^{2+}] was analyzed with10 µM ionomycin dissolved in either Ca^{2+}-free KSOM, 1X-Ca^{2+} KSOM (1.71 mM), 3X-Ca^{2+} KSOM, 6X-Ca^{2+} KSOM and three common commercial IVF media.
Participants/materials, setting, methods: MII oocytes were collected from 6- to 10- week-old B6D2F1 mice. The absolute amplitude and the area under the curve (AUC, total amount of calcium released) of the ionomycin-induced Ca^{2+}-rises were measured by fluorescence time-lapse imaging. The embryo development was assessed at the two-cell, morula and blastocyst stage.
Main results and the role of chance: The amplitude, AUC and blastocyst formation rates in the 10 µM ionomycin group were significantly higher compared to the 5 µM and 15 µM groups ($P < 0.01$). In the Ca^{2+}-free KSOM group, the Ca^{2+}-rise had a similar amplitude but a significantly decreased AUC compared to the 1X-Ca^{2+} KSOM group ($P < 0.01$), and no blastocyst formation was observed. When the [Ca^{2+}] in the culture media increased 3 or 6 times, the amplitude and the AUC were significantly reduced ($P < 0.01$) compared to the 1X-Ca^{2+} KSOM group; however, no significant difference was found in the blastocyst formation rates. Similarly, different patterns of Ca^{2+} rises were observed when ionomycin was dissolved in the three different commercial IVF media, with the one medium containing the highest [Ca^{2+}], but exhibiting the lowest AUC ($P < 0.05$).
Limitations, reason for caution: The effect of ionomycin and [Ca^{2+}] can be tested reliably with mouse oocytes, yet the findings should be extended to the human oocytes with caution.
Wider implications of the findings: Both the Ca^{2+} releasing pattern and the embryonic developmental potential after ionomycin exposure were influenced by the concentration of ionomycin and [Ca^{2+}] in the culture medium. These results may explain some of the observed differences in the efficiency of AOA protocols in a clinical setting. Therefore, these parameters have to be taken into account when optimizing or applying AOA protocols. Further studies are needed to determine the optimal ionomycin and [Ca^{2+}] for human oocytes.
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.
Trial registration number: None.

P-089 Embryo assessment and selection by time-lapse evaluation using EEVA™ with transfer at the cleavage stage achieves similar clinical outcomes to blastocyst transfer

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Study question: Can EEVA™ time-lapse analyses of embryo development to the cleavage stage (EEVA™ group, EvG) be used to guide embryo transfer policy at the cleavage stage (day 3) and achieve comparable implantation rates (IR) and clinical pregnancy rates (CPR) to blastocyst transfer (day 5 group, BG)?
Summary answer: In women <37 years old, the IR and CPR were marginally higher (non-significant) in the blastocyst group. In women 37 to 42 years, the IR were identical but a marginally higher CPR (non-significant) in the EvG group. EEVA™ analyses and embryo transfer achieve similar clinical results to blastocyst culture.
What is known already: Blastocyst transfer has allowed more precise embryo selection, allowing elective single embryo transfer and minimizing multiple pregnancies. However, it is possible that extended culture may lead to altered fetal development, possibly related to epigenetic phenomena. The EEVA™ time-lapse system can predict at the cleavage stage (day 3) an embryo's potential to achieve the blastocyst stage. Hence, theoretically, transfer of cleavage stage embryos with the implantation potential of blastocysts could optimise short and longer term outcomes.
Study design, size, duration: This prospective longitudinal examination, from September 2012 to December 2013, compared clinical results in 2 age cohorts (women <37 y and 37 - 42 y) having fresh embryo transfer of day-3 embryos after EEVA™ (EvG; $N = 78$ and 110 respectively) and fresh blastocyst transfer (BG; $N = 121$ and 51 respectively).
Participants/materials, setting, methods: All patients underwent IVF/ICSI stimulation with fresh embryo transfer at a single clinic. Patients had either time-lapse analysis using EEVA™ with day-3 transfer or extended culture to blastocyst. The AMH, antral follicle count, egg and embryo yields, and the CPR and IR of the two different age groups were examined.

Main results and the role of chance: In women less than 37 years old, the EvG and the BG groups (N = 78 and 121 respectively) showed no difference in age, ovarian reserve, egg or embryo yields. The IR and CPR were similar (IR: EvG = 31%, BG group = 36%, P = 0.43; CPR: 38% and 48% respectively, P = 0.24).

In women 37–42 years old, the EvG and the BG groups (N = 110 and 51 respectively) the demographics again showed similar profiles of age, ovarian reserve, egg or embryo yields. There was no difference in IR or CPR (IR: EvG = 22%, BG group = 22%, P = 0.49; CPR: EvG group = 36%, BG = 29%, P = 0.47).

Limitations, reason for caution: This was a simultaneous cohort study and was not a randomised controlled trial. Patients elected to use EEVA™ whilst the blastocyst group was identified only after encouraging early embryo culture and development. A larger, randomised patient cohort will be more valuable.

Wider implications of the findings: These preliminary data suggest that selection of embryos by morphology, associated with computerised time-lapse analyses using EEVA™ may achieve clinical outcomes comparable to those of extended culture to the blastocyst stage. There may also be age related differences which will be worthy of further examination. If larger studies confirm these preliminary data, this would be a step towards minimizing the risks of adverse epigenetic phenomena possibly associated with extended culture.

Study funding/competing interest(s): Funding by hospital/clinic(s), GCRM Ltd.

Trial registration number: Not applicable.

P-090 Does female age matter when selecting blastocysts for transfer using a novel morphokinetic based blastocyst selection algorithm?

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Study question: Is a novel morphokinetic blastocyst selection model, which ranks embryos according to implantation potential, effective for all patients irrespective of age or, ineffective such that specific models are required which consider female age?

Summary answer: For all age categories evaluated the selection model, which ranked embryos according to implantation potential, was effective. Implantation rates reduced with decreasing rankings and relative uplifts in implantation rate, demonstrated when comparing 'high potential' with 'low potential' blastocysts, were not significantly different.

What is known already: The impact of female age on IVF outcome is well documented and this has mostly been attributed to an elevated rate of aneuploidy with increasing age. Aneuploidy has been linked with delayed development of blastocysts, and there is current debate regarding whether this may be confounded by age. Selection models based on the comparison of morphokinetic variables have been used to develop clinical evidence-based embryo selection algorithms which to date have not been age specific.

Study design, size, duration: A novel morphokinetic selection algorithm was retrospectively applied to 352 blastocysts with 'known implantation data' (KID+ or –) from May 2011 to August 2013. Blastocysts were classified low, medium or high implantation potential. KID+ rates (KID+/KID+ plus KID – ×100%) for each class were compared between four age groups.

Participants/materials, setting, methods: Morphokinetic variables were recorded using EmbryoScope™ (Fertilitech, Denmark) in a private IVF setting. A novel morphokinetic embryo selection model developed in house was retrospectively evaluated for the following female age groups, <30; 30 to <35; 35–40; >40 years in attempt to validate its use for varied age categories. Chi square tested significance.

Main results and the role of chance: For high implantation potential blastocysts, defined by the novel model, KID+ rate was 76.9%, 75.6%, 57.1%, 40% for age groups <30; 30 to <35; 35 to <40; 40+ respectively (n = 13, 41, 42, 20). For medium implantation potential blastocysts and low implantation potential blastocysts KID+ rate was 75.6%, 45.5%, 34%, 32% and 23.8%, 18.4%, 12.5% for age groups <30; 30– <35; 35-40; >40 respectively (medium n = 15, 44, 50, 25) and (low n = 21, 38, 40). There were no low implantation potential embryos in the <30 years group (n = 3). The model effectively ranked embryos for each age category according to their implantation potential demonstrated by declining KID+ rates with decreasing implantation potential. Relative increase in KID+ rate for low compared with high implantation potential blastocysts, for age groups – <35; 35-<40; 40+, were 217.6%, 210.2%, 220% respectively and were not significantly different.

Limitations, reason for caution: The outcome measure used to evaluate the potential impact of age on morphokinetics and hence to validate the effectiveness of a novel model, was blastocyst implantation. The optimal outcome measure is healthy live birth. Such models may not be transferrable between different settings and confounders may exist.

Wider implications of the findings: The evidence based development of morphokinetic models for enhanced embryo selection promises to improve outcomes for IVF patients. It is necessary to validate models prior to use and to consider potential confounding factors, such as age. This work supports the use of a single morphokinetic blastocyst selection model, irrespective of female age. Further studies will test the model prospectively and use healthy live birth as the outcome measure.

Study funding/competing interest(s): Funding by hospital/clinic(s), CARE Fertility.

Trial registration number: None.

P-091 Vimentin: a protein important for human oocyte maturation and fertilization?

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Study question: Presence of the protein vimentin has not been confirmed in mammalian oocytes until now. The aim of this study was to elucidate whether protein vimentin can be detected in human oocytes from the in vitro fertilization program and if it may be related to oocyte maturation and fertilization.

Summary answer: Our data show that vimentin was expressed in both mature and immature human oocytes and that it may be related to oocyte maturation. Moreover, vimentin was expressed in non-cleaved zygotes after in vitro fertilization but has disappeared during parthenote development thus indicating its potential role in the fertilization process.

What is known already: Vimentin is a type of intermediate filament and the major cytoskeletal component of mesenchymal cells (e.g. granulosa cells of the human ovary). The expression of vimentin is highly developmentally-regulated and related to epithelial-to-mesenchymal transition during normal development or metastatic progression. Vimentin has already been confirmed in *Xenopus laevis* oocytes and was suggested to be involved in formation and function of oocytes but has not been confirmed in mammalian oocytes until now, including human oocytes.

Study design, size, duration: We focused on the expression of vimentin in human oocytes from in vitro fertilization program by different approaches: mature (MII), non-fertilized oocytes and immature (GV or MI) oocytes without a potential of fertilization. We tried to elucidate if vimentin may be related to oocyte maturity, fertilization and parthenote development.

Participants/materials, setting, methods: Oocytes were collected after obtaining an approval from the Medical Ethics Committee and patients' written consents. Groups containing 100 oocytes each were analyzed on protein expression by proteomics and groups containing 10 oocytes each on gene expression by microarrays. The oocytes, non-cleaved zygotes and parthenotes were analyzed using immunocytochemistry.

Main results and the role of chance: The proteomic and transcriptomic analyses confirmed the expression of protein vimentin in mature and immature oocytes. Protein vimentin was accumulated in the subcortical region of oocytes, as revealed by immunocytochemistry. The pattern of vimentin expression was related to oocyte maturity; in mature oocytes it was expressed as a very thin, well-organized subcortical layer, while in immature oocytes the layer of vimentin was thicker and less organized. Moreover, in a proportion of mature oocytes vimentin was also expressed in the outer layer of zona pellucida and possibly reflects the connection with granulosa cells, while in immature oocytes this was not observed. In addition, vimentin was also expressed in the subcortical region of zygotes but disappeared in dividing parthenotes.

Limitations, reason for caution: The numbers of analyzed oocytes and replicates were limited because human oocytes are a scarce and sensitive biological material that is difficult to collect and manipulate.
Wider implications of the findings: Our data show that vimentin is expressed in human oocytes and may be involved in oocyte maturation process. This finding may be implicated in the human oocyte in vitro maturation procedure, which is still a low-success procedure in the clinical practice. The addition of vimentin to the oocyte in vitro maturation medium could possibly replace the natural function of granulosa cells and may improve the outcome of human oocyte maturation in vitro.
Study funding/competing interest(s): Funding by hospital/clinic(s), There was no special funding of this research and the authors declare that there is no financial or other conflict of interests related to this contribution.
Trial registration number: This was not a clinical trial but a basic study on human gametes.

P-092 Micro-vibration culture and group culture increase fertilization and implantation rates in human embryos

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Study question: Would micro-vibration and group culture of oocytes and embryos influence fertilization, implantation and clinical pregnancy rates?
Summary answer: Micro-vibration and group culture significantly increase the fertilization and implantation rates in IVF cycles.
What is known already: Several studies have shown that culture of human and mice embryos in groups improves embryo development, implantation and clinical pregnancy rates. Recently, it has been suggested that micro-vibration increases the cell number of blastocysts in mice and have a positive effect on pregnancy and implantation in humans.
Study design, size, duration: In this study, a retrospective analysis of a cohort of ICSI patients attending a private fertility centre between April 2012 and December 2013 was done. *In vitro* culture was performed either in a static environment with single oocyte/ embryo culture (n = 291 patients) or under micro-vibration and group culture (n = 244 patients).
Participants/materials, setting, methods: In the static group, oocytes/embryos were cultured individually, while in the micro-vibration all the oocytes were cultured together and up to 4 embryos were cultured in the drop with a three-dimensional vibration of 56 Hz for 5 s/60 min. Variables were analysed by Mann Whitney test and chi-square test.
Main results and the role of chance: There was no difference among the groups regarding patient's age, BMI, cumulative FSH dose, length of stimulation, number of total and mature oocytes, number of transferred embryos and day of transfer. We were able to observe a significant increase in fertilization rate in oocytes cultured in groups and under micro-vibration conditions compared to oocytes cultured individually in a static culture (82.13% vs. 78.24%, P = 0.0029, relative risk 0.9526), as well as in the implantation rate (41.88% vs. 35.45%, P = 0.0432, relative risk 0.8465), respectively. The clinical pregnancy rate showed a tendency to be higher in the micro-vibration group but it did not reach significance (46.72% versus 43.29%, P = 0.4279, relative risk 0.9267).
Limitations, reason for caution: Two variables were simultaneously applied (micro-vibration and group culture), so the positive effect observed could be identified and may be caused by both group culture and micro-vibration synergistically. Furthermore, this is a retrospective analysis and not all confounding factors could be considered.
Wider implications of the findings: These results emphasize the importance of culturing oocytes and embryos in groups rather than individually. Furthermore, three-dimensional vibration of oocytes and embryos would significantly improve fertilization and implantation rates, in agreement with two previous studies. Mechanical vibration of an embryo may mimic the embryo's *in vivo* environment, where oocytes and embryo are in a continuous movement in the fallopian tube and uterus which could therefore explain its beneficial effects.
Study funding/competing interest(s): Funding by hospital/clinic(s), Kinderwunsch-Zentrum Ulm.
Trial registration number: Not applied.

P-093 Retrospective assessment of second polar body alignment using time lapse imaging

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Study question: Can the relative position of the second polar body (PB2) to the first polar body (PB1) be used to predict embryo development and implantation potential?
Summary answer: Increased distance between PB1 and PB2 demonstrated a correlation with poor embryo quality morphokinetic markers, and with decreased implantation and hCG rate.
What is known already: Some fertilized oocytes have PB2 displaced from PB1, which could indicate some degree of cytoplasmic rotation, or displacement of PB1 from its original position due to manipulation to remove cumulus used prior to ICSI (Payne *et al*., 1997). Previous studies of the magnitude of the angle between PB1 and PB2 showed no significant difference for embryos of different morphological grades on day 2 after either ICSI or IVF (Garello *et al*., 1999).
Study design, size, duration: Retrospective analysis of 212 time-lapse embryos (141 ICSI cycles) with known implantation data (single UK clinic, March-November 2013). Distance between PB1 and PB2 was grouped into four: adjacent; ≤45°; 45° to 90° and ≥90°. These measurement annotations were performed at PB2 extrusion, PN appearance (PNa), and PN fading (PNf).
Participants/materials, setting, methods: The four groups were compared using morphokinetic annotations at PNf, two cell (t2), second cell cycle (CC2), four cell (t4), start of blastulation (tsB), time to blastocyst (tB), and implantation. ANOVA, Student's t-test and χ^2 test were used to determine significance. P-value < 0.05 was considered to be significant.
Main results and the role of chance: Embryos with a greater distance between PB1 and PB2 showed a significant delay at tsB (p-value = 0.001) and tB (p-value = 0.004), and a significant poorer morphokinetic embryo score on day 5 when PB2 is extruded more than 45° from PB1 (p-value < 0.001). The analysis of PB2 movement demonstrates that embryos whose PB2 has been displaced more than 45° from the extrusion position, have lower score on day 3 (p-value = 0.049). A significant difference between positive hCG rate was observed when the distance of PB2 increases from PB1 (p-value = 0.0027), but not significant difference on clinical pregnancy rate. Additionally, as distance between PB1 and PB2 increases, there is a trend to delayed tPNf, t2, t4, and shortened CC2, which may indicate reduced embryo viability.
Limitations, reason for caution: Only a two dimensional analysis is possible. As only embryos of known implantation were analysed, the number of poor quality embryos is low in this cohort. A larger cohort is needed to clarify significance.
Wider implications of the findings: The current study has demonstrated that, in a two-dimensional profile, embryos with a greater distance between PB1 and PB2 tend to have delayed morphokinetic quality markers at day 3 and day 5, and lower implantation rates opposing the findings of Garello *et al.* (1999). Further data is needed to consider whether this parameter can be incorporated into an embryo scoring systems or morphokinetic selection model.
Study funding/competing interest(s): Funding by national/international organization(s), Embryologist placement was funded by a Leonardo da Vinci grant.
Trial registration number: N/A.

P-094 Antral follicles developing from primary and preantral follicles in culture: are we getting closer to obtaining mature oocytes from in vitro grown follicles in human?

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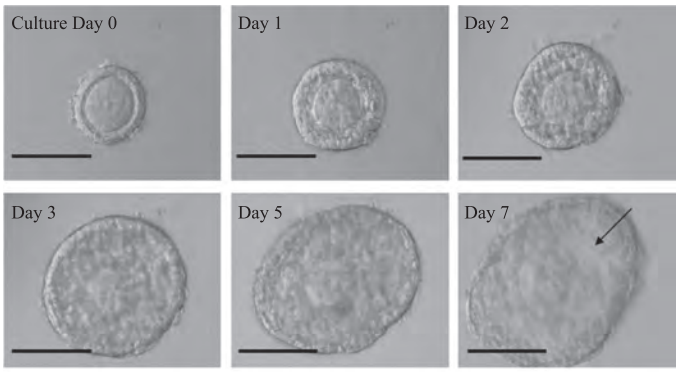
Study question: Can we grow early stage primary and small preantral follicles in vitro to antral stage in human?
Summary answer: Yes, isolated primary and preantral follicles can reach into antral stage when cultured 3-dimensionally in matrigel rich in ECM basement membrane proteins and certain growth factors.
What is known already: No data is available for human regarding the growth potentials of isolated primary and preantral follicles in culture. Early stages

of follicle growth up to antral stage is gonadotropin independent and occur via autocrine and paracrine interactions of the locally produced growth factors and extracellular matrix proteins. We therefore hypothesized that matrigel rich in ECM basement membrane proteins and certain growth factors may provide a good niche to sustain the growth and survival of isolated primary and small preantral follicles in human, which do not survive in standard culture plate.
Study design, size, duration: An in vitro human study.

Figure 1

A



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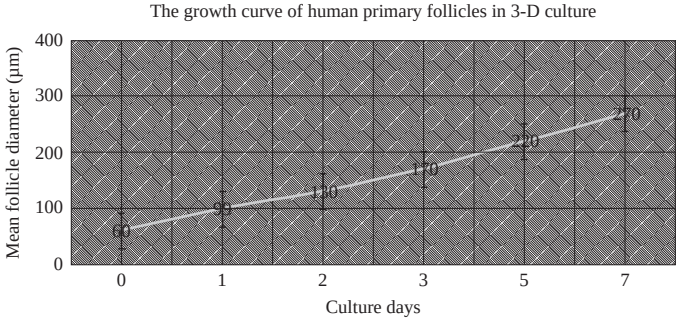

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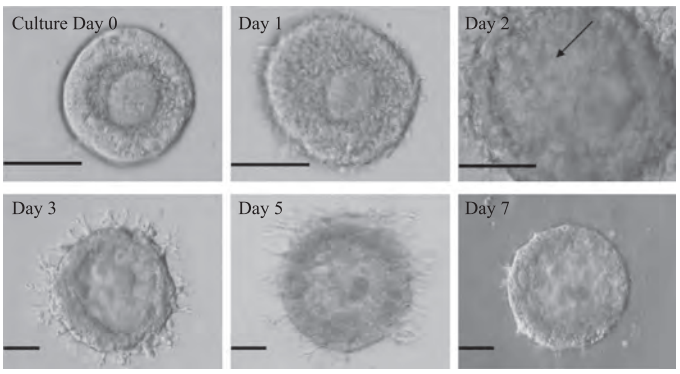

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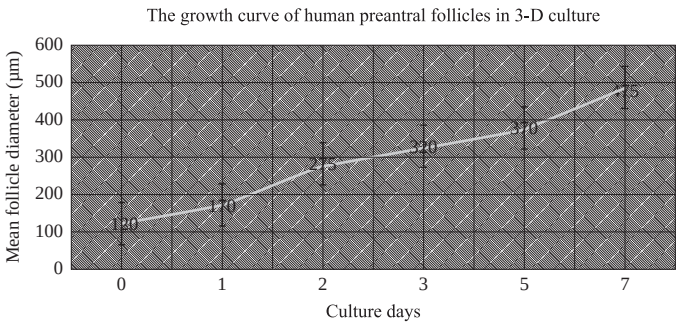

Participants/materials, setting, methods: Ovarian cortices for follicle isolations were obtained from five young patients (mean age 29.1 ± 2.4) undergoing laparoscopic excisions of benign ovarian cysts. The samples were digested with collagenase, Dnase-I in DMEM-F12 supplemented with 5% BSA for 30 minutes at 37°C. Primary (45–65 µm) and small preantral (100–150 µm) follicles were mechanically isolated using 28- to 30-gauge needles under stereomicroscope (Olympus SZX16). Matrigel was diluted with the culture medium in a 1:1 ratio (100 µL from each) and placed in 96-well format culture plates. Following transfer of isolated follicles into it, the plates were put in the incubator for 30 minutes to allow matrigel to solidify and then 100 µL of culture media was added on the top of matrigel. Half of the culture media was replaced every other day. All follicles were cultured in α-minimum essential media (α-MEM) with and without 10% fetal bovine serum (FBS) supplemented with 100 mIU/mL FSH, 3 mg/mL BSA, and 10 000 U/mL penicillin-G, 10 000 µg/mL streptomycin 25 µg/mL amphotericin-B at 37°C and 5% CO_2 in air. The images were taken every other day using Olympus IX 71 inverted microscope with digital imager under ×300 and ×150 magnifications. Follicle diameter was calculated using Olympus DPS software. A total of 10 primary and 12 preantral follicles were isolated for culture.
Main results and the role of chance
Primary follicles: Four of ten primary follicles survived at the end of 7 day culture period giving a survival rate of % 40. Single layer of granulosa cells became multilayered after 24 hr of culture as the first sign of growth and follicle diameter increased further along with expanding granulosa cell mass. On the following days of culture, the growth of follicles continued and antrum formation began to appear on culture day 5 when they reached a mean diameter of 200 µm. On day 7 antral space formation became more prominent and their oocytes were more visible within surrounding mucification areas (Fig-1A). While mean follicle diameter at baseline was 60 ± 12 µm it increased to 270 ± 22 µm, yielding a growth rate of 350% after 7 days of culture.
Preantral follicles: Survival rate was higher in preantral follicles as seven of 12 follicles survived (58.33%). In contrast to primary follicles, the growth of preantral follicles occurred in a more accelerated manner. With rapidly expanding granulosa cell layers follicle diameter increased more rapidly and antrum formation became visible even on the second day of culture. Follicle growth continued on the following days. Their initial follicle diameter of 120 ± 32 µm increased to 475 ± 45 µm, giving a growth rate of 295.8% (Fig-1B). When they reached 300–400 µm with obvious antrum formation, 4IU/mL hCG was given to induce ovulation and the oocytes were harvested 36 hrs later with a mouth pipette. 4 oocytes were retrieved of which all were at GV stage.
Limitations, reason for caution: None.
Wider implications of the findings: This study clearly illustrates that early stage follicles can be grown to antral stage in vitro. This model also herald us that combining IVM with in vitro growth (IVG) may revolutionize assisted reproduction technologies. Cancer patients seeking fertility preservation and undergoing ovarian tissue freezing as well as those with poor ovarian response to IVF may benefit in future from this approach.
Study funding/competing interest(s): Funding by University(ies), Koc University School of Medicine.
Trial registration number: None.

P-095 Time lapse monitoring: a comparison between embryos development from fresh ejaculated and cryopreserved testicular spermatozoa in ICSI cycles

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Study question: This study analyses early cellular events and embryo time lapse development relation with pregnancy (PR) and implantation rate (IR) among three male factor groups: ICSI with >1.000.000 fresh sperms/ml. (F), ≤1.000.000/ml. sperm concentration (C) and testicular cryopreserved spermatozoa (T).
Summary answer: Our data show, although second polar body extrusion, T3 (time of 3 cell division) and T4 (time of 4 cell division) are statistically different among groups, no statistically significant differences were found in the PR and IR. No differences were found in other time-lapse considered parameters.

What is known already: A relevant influence of kinetic parameters on embryo viability and implantation rate has been shown and the duration of 2-cell (T3-T2) and 3-cell stage (T4-T3) were more related factors. No data have been until now presented on the influence of male factor on timing of cellular events and their relation to PR and IR.
Study design, size, duration: We considered in a retrospective cohort study 313 cleavage stage (day 2/3) embryo-transfer ICSI cycles from April 2012 to October 2013. Timing (hours) of cellular division, pregnancy and implantation rate were evaluated. Data are expressed as median (interquartile interval). Differences among groups were explored with Kruskal-Wallis test.
Participants/materials, setting, methods: Only normal fertilised and cleaved embryos were analysed; Group F 229 cycles (216 patients) with 1277 embryos transferred; Group C 32 cycles (32 patients) with 140 embryos; Group T 52 cycles (48 patients) with 246 embryos. Female age among the 3 groups was not significantly different (37.1 ± 3.9 years).
Main results and the role of chance: Only second polar body extrusion after 3.60 (2.81–4.50), T3 at 36.755 (32.745–40.31), T4 at 39.045 (35.74–42.43) were significantly different among the 3 groups ($p < 0.05$). Pronuclear appearing 9.82 (8.39–11.9), pronuclear fading 24.71 (22.33–27.28), T2 (time of 2 cells division) at 27.375 (24.99–30.24), T3-T2 11.09 (7.02–12.26), T4-T3 0.93 (0–2.615) were all not significantly different (p = ns). The PR was 38.4% in group F, 21.9% in group C and 42.3% in group T (p = ns). The IR was 23.5% in group F, 11.4% in group C and 25.0% in group T (p = ns). Although 3 time lapse parameters were significantly different among different male populations, this difference was not related to PR and IR.
Limitations, reason for caution: A retrospective observational study has the intrinsic limits of this form of analysis and the results can be in a systematic way distorted because of the non-random distribution. The wide distribution of our data was an important limiting factor in the statistical analysis.
Wider implications of the findings: The origin of the used sperm showed significant differences in few parameters among the three studied groups, although this difference was not significantly related to the cycle prognosis. A RPT with a large sample will be needed to confirm or not this preliminary observation. Our data are the first reported analysis of male factor and time lapse imaging.
Study funding/competing interest(s): Funding by hospital/clinic(s), Humanitas Fertility Center, Department of Gynaecology, Division of Gynaecology and Reproductive Medicine, Humanitas Research Hospital, Rozzano (Milan), Italy. Yale Fertility Center, New Haven CT, USA.
Trial registration number: None.

P-096 Does embryo transfer on day 6 cause lower implantation rates than transfer on day 5

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Study question: Do implantation rates differ between embryos transferred randomly on Day 5 (D5)and Day 6 (D6)?
Summary answer: We found no difference in implantation rates between embryos transferred on D6 compared with embryos transferred on D5.
What is known already: D6 transfer has been associated with OR of 0.4 for live birth compared with D5 transfer, which has led to caution against D6 transfer in general. The conclusions are however based on studies where blastocysts were cultured till D6 only if no sufficient expansion was observed on D5 and can therefore not be applied to elective D6 transfer in general.
Study design, size, duration: Retrospective analysis of implantation rates after blastocyst transfer pseudo-randomized D5 or D6 transfer. Feb 2011-Aug 2013 patients were offered D6 transfer. Transfer was re-scheduled to D5 if aspiration was a Monday. Aug 2013- Dec 2013 D5 transfer was standard, while D6 transfer was offered to patients with aspiration on Mondays.
Participants/materials, setting, methods: Fresh embryo transfers performed on D5 or D6 at the fertility Clinic, Aarhus University Hospital were included if maternal age was <38 years and >7 oocytes retrieved. Only SET and DET were performed in the study period. Implantation rate (IR) was calculated as total number of FHB/total number of embryos transferred.
Main results and the role of chance: post hoc power calculation indicated that 91 embryos were needed in each group to detect a published reduction in IR from 40% to 20% with 80% power and a significance level of 0.05 (two-tailed test). 127 D5 cycles and 269 D6 cycles were initialised. Twenty (n = 20) D5 cycles and 25 D6 cycles were cancelled, which left 248 transferred embryos from 244 D6 cycles and 128 embryos from 107 D5 cycles for the analysis. The implantation rate was 0.41 and 0.33 for day 5 and day 6, respectively. The difference was non-significant. There was no difference in maternal age or number of oocytes retrieved between the two groups. There was a tendency for higher cancellation rates in the D5 group (p = 0.06)
Limitations, reason for caution: The analysis is retrospective and the design pseudo-randomized. The study was powered to detect a previously published decrease from 40% to 20% after D6 transfer, but smaller differences might not be detected with the present sample size.
Wider implications of the findings: In contrast to previous studies, our analysis suggests that D6 transfer does not significantly reduce implantation rates. The findings allow for a more flexible blastocyst transfer policy and lessens the concern of blastocyst transfer on D6 in relation to blastocyst biopsy on D5.
Study funding/competing interest(s): Funding by hospital/clinic(s), No external funding was provided.
Trial registration number: N/A.

P-097 Cryopreservation to synchronization endometrium and slow developing blastocysts: vitrification-warming of day 6 blastocyts

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Study question: Reproductive outcomes of blastocysts transferred on day 6 following controlled ovarian stimulation and ovulation induction have consistently been lower than for blastocysts transferred on day 5, despite having similar blastocyst scores. In this study we investigate whether the reduction in outcomes is due to blastocyst quality or endometrial receptivity.
Summary answer: In vitro cultured embryos may only reach an expanded blastocyst stage on day 6 of culture, but retardation in development rate may not be a true indication of blastocyst competence. Asynchronous transfer with regards blastocyst development day of vitrified-warmed blastocysts in hormone supplemented cycles result in highly comparative reproductive outcomes.
What is known already: Blastulation in human embryo in vitro culture should occur after 92 hours post-insemination and ideally an expanded should be reached by 116 hours. However, it is not uncommon for some developing embryos to only reach this stage by 140 hours. The transfer of expanded blastocyst have consistently be shown to have high implantation potential. However, embryos that reach an expanded blastocyst stage on day 6 do not seem to have a high implantation rate.
Study design, size, duration: A retrospective cohort observational study was performed on vitrified-warmed day 5 (N = 412) and 6 blastocyst (N = 36) transfers performed between January 2012 and December 2013.
Participants/materials, setting, methods: The study was performed in a private ART clinic. Vitrification-warming of blastocysts was performed according to the Cryotop method. Both vitrified-warmed d5 and d6 blastocysts were transferred on day 6 of progesterone supplementation in hormone timed-supplemented cycles. Blastocysts were checked for viability and re-scored 2 hours after warming.
Main results and the role of chance:

Parameter	D6 blastocyst	D5 blastocyst	*P* value
Blastocyst survival % (No)	85.4 (82/96)	90.1 (1056/1172)	0.792
Patient transfer rate % (No)	87.8 (43/49)	95.1 (500/526)	0.797
No ER (≥grade 3 blastocysts)	36	412	
Age (std)	30.12 (4.6)	30.85 (4.9)	0.390
No blastocyst transferred (std)	1.72 (0.49)	1.84 (0.37)	0.070
Pregnant % (No)	75.0 (27)	82.5 (346)	0.768
Clinical pregnancy % (No)	69.4 (25)	69.9 (288)	0.911
Ongoing pregnancy % (No)	61.1 (22)	63.8 (263)	0.989
Early pregnancy loss % (No)	18.5 (5)	22.6 (77)	0.899

t-test and Chi-square.

There were no significant differences between the rates of any of the pregnancy outcomes measured, positive, clinical, ongoing pregnancy and early pregnancy, comparing expanded day 5 or day 6 blastocysts.
Limitations, reason for caution: The power of comparison was limited by the low number of transfers in the day 6 transfer group.

Wider implications of the findings: All slow developing blastocysts with an adequate blastocysts score should be cryopreserved and replaced in a subsequent natural or hormone supplemented cycle. Warming of these slower developing blastocyst should be scheduled to occur on vitrification day minus 1 day.
Study funding/competing interest(s): Funding by hospital/clinic(s). None.
Trial registration number: None.

P-098 Evidence of differences in pregnancy rates following embryo development in an integrated time lapse incubation system compared to a standard IVF incubator

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Study question: Is there a difference in embryo development and pregnancy rates following incubation of human embryos in a classical IVF incubator compared to an integrated time lapse incubation system (iTLIS)?
Summary answer: There was evidence to support the conclusion that the use of an iTLIS resulted in improved pregnancy rates compared to a classical water -jacketed IVF incubator (SI).
What is known already: Incubation systems incorporating time lapse are relatively new in the field of IVF. The use of time lapse reduces the need to move the embryos out of the incubator to observe development, and provide more information about the progress of embryo development. There are some reports of improvements in outcomes using time lapse but there is a need to increase the knowledge base using different classical incubation systems to better assess the role of time lapse incubation in improving IVF laboratory performance and pregnancy rates.
Study design, size, duration: This was a prospective cohort study and data on 1853 IVF cycles from 2/5/2011 to 22/12/2013 were included. Oocytes were fertilized and placed into an iTLIS (EmbryoScope time lapse system) or a water jacketed incubator (Hera cell 150).
Participants/materials, setting, methods: All treatment cycles were carried out in a single IVF centre. Cycles that did not result in any viable embryos for incubation and cycles where the IVF cycle was interrupted due to the risk of OHSS (and embryos were cryo-preserved and replaced in a later cycle) were omitted from the analysis. In addition the main dataset analysed for implantation and pregnancy outcomes consisted of patients undergoing their first IVF cycle and stratified according to age (<35 and ≥35 years). *T*-test was used and 95% confidence intervals calculated.
Main results and the role of chance: In total 850 cycles were run in the iTLIS and 911 cycles were run in a SI. The average patient age was 33.9 (22.0–46.3) and 33.0 (19.8–45.3) and the average cycle number was 1.69 (1–6) and 1.4 (1–6) respectively in the 2 incubation systems. For all treatment cycles irrespective of age, the pregnancy rates were 54.3 (50.9–57.6) and 48.4 (45.1–51.6) ($P = 0.014$) respectively. For patients <35 and in their 1st IVF attempt the pregnancy rates were 64.8 (59.3–70.4) and 52.95 (48.5–57.4) ($P = 0.0012$) and clinical pregnancy rates were 51.9 (46.1–57.7) and 43.79 (39.4–48.2) ($P = 0.028$) in the iTLIS ($n = 287$) and SI ($n = 491$) groups.

For patients ≥35 and in their ≥3rd IVF attempt the pregnancy rates were 40.7 (29.8–51.7) and 26.8 (12.7–41.0) ($P = 0.133$), clinical pregnancy rate were 30.9 (20.6–41.1) and 17.1 (5.05–29.1) ($P = 0.104$) and ongoing pregnancy rate were 27.1 (17.2–37.1) and 14.6 (3.3–26.0) ($P = 0.122$) in the iTLIS ($n = 81$) and SI ($n = 41$) groups.

Implantation rates for embryos from iTLIS were higher (27.3 (24.7 – 29.8)) ($n = 1607$) in the iTLIS vs. SI group (26.4 (23.9 – 28.9) $n = 1622$) but the difference was not significant ($P = 0.648$).
Limitations, reason for caution: The data set was not randomized between the 2 interventions, however the dataset is large and slightly older patients embryos were incubated in iTLIS.
Wider implications of the findings: The dataset demonstrates that an integrated time lapse incubator can play an important role in improving pregnancy rates. More studies are warranted to further investigate the impact of embryo morphokinetics on improving the identification of viable embryos for implantation.
Study funding/competing interest(s): Funding by hospital/clinic(s), Gyn-Fiv, a.s., Záhradnícka 42, 821 08 Bratislava, Slovakia.
Trial registration number: E-03-00517.

P-099 Abnormal morphology and development of mouse embryos transfected with human oocyte retroelements preincubated with mouse spermatozoa

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Study question: To address the impact of uncontrolled retroelement expression in mouse sperm and early preimplantation embryos, in in vitro fertilization experiments using spermatozoa preincubated with plasmid vectors containing the cloned from human GV oocytes retroelements LINE-1, HERVK-10 and the mouse retroelement VL30.
Summary answer: Our results show that: 1. The spermatozoa intracellular content favors retrotransposition events 2. Spermatozoa can deliver active human retroelements to the mouse oocyte at fertilization 3. Uncontrolled human retroelement expression disrupts normal mouse embryo preimplantation development.
What is known already: Spermatozoa can be used as vectors for transfer of exogenous DNA into the oocyte. Active retroelements are present in the ooplasm and can be mobilized by reverse transcriptase present in the sperm cells. Under normal conditions retroelements are suppressed by non coding RNAs and methylation. Retroelement control failure may have an effect on the cellular proliferation. Exogenously introduced retroelements have been found to alter somatic cell phenotypes and interfere with propagation.
Study design, size, duration: In vitro preimplantation development of FVB/N mouse embryos, transfected through sperm with the retrotransposons LINE-1, HERVK-10 and VL30 was compared with control embryos. Retrotransposons were tagged with an enhanced green fluorescence (EGFP) gene-based retrotransposition cassette in order to demonstrate new retrotranspositon events when inserted into the embryonic genome.
Participants/materials, setting, methods: Spermatozoa were collected from the cauda epididymis, oocytes were retrieved from the oviducts after ovarian stimulation with gonadotropins, and processed by IVF. Retrotransposition events in spermatozoa were confirmed by PCR and in embryos by PCR and confocal microscopy. Apoptotic events, methylation, pluripotency and double strand breaks were also examined.
Main results and the role of chance: The uncontrolled presence of excessive human retroelements in the mouse fertilized oocyte fired a sequence of events characterized by accelerated asymmetrical cell division, multiple cellular fragments, cleavage arrest and embryo degeneration. Most of the embryos were arrested before the formation of the blastocyst and collapsed. These phenomena coincided with double strand DNA breaks of each particular blastomere from the first cleavage stage, apoptosis, methylation changes and insufficiency of pluripotency factors expression. We conclude that the embryo development to blastocyst depends on the presence of active retroelements transferred or expressed by spermatozoa and on the high insertion efficiency of the human retrotransposons. We have only one reservation regarding the theoretical detrimental effects of the retrotransposon cloning vector per se on the mouse embryos.
Limitations, reason for caution: The effects of human origin retroelements in mouse oocytes and embryos may limit the impact of the results to the human embryo development and the clinical relevance to human IVF.
Wider implications of the findings: Our findings show that exogenous DNA and in particular DNA from active retroelements may interrupt human embryo development and lead to morphological and genomic abnormalities. Uncontrolled human retroelements may cause the high percentages of abnormal embryos in clinical practice and the high percentages of blastomeres with genomic alterations and defects in CGH analysis of single cells.
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: Non applicable.

P-100 The time interval between blastocyst formation and freezing affects pregnancy rates in freeze-thaw embryo transfer

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Study question: We investigated the influence on pregnancy rate of both the time interval between ICSI and blastocyst formation and the time interval between the formation of blastocysts (with a diameter of at least 160 μm) and freezing.
Summary answer: The pregnancy rate was significantly higher in the groups with earlier blastocyst formation after ICSI and earlier freezing after blastocyst formation. However, the pregnancy rate was lower when the interval from blastocyst formation to freezing was long despite a shorter blastocyst formation time.
What is known already: It has been reported that pregnancy rates in freeze-thaw embryo transfer are higher when the interval between culture, blastocyst formation and freezing is short. However, embryo transfer has mostly been evaluated based on the number of culture days and the relationship between pregnancy rates and blastocyst formation period, or the time interval between blastocyst formation and freezing**,** is not well documented.
Study design, size, duration: The subjects comprised 317 cases in 458 cycles with 458 embryos that underwent ovum collection in clomiphene or letrozole stimulation cycles, followed by ICSI and freeze-thaw embryo transfer between June 2012 and October 2013.
Participants/materials, setting, methods: After ICSI, embryos were cultured with EmbryoScope™ for freeze-thaw blastocyst transfer. The period of blastocyst formation from ICSI (early/late) and time interval between blastocyst formation and freezing (short/long) were combined to compare pregnancy rates in four groups: early-short (n = 109), early-long (n = 130), late-short (n = 89), and late-long (n = 130).
Main results and the role of chance: The pregnancy rate was significantly higher in the early-short group (55.0%) than in the other groups (early-late, 30.0%; late-short, 37.1%; and late-long, 23.1%) ($P < 0.05$). There was no significant difference in the pregnancy rate between the early-short and late-short groups ($P > 0.05$).
Limitations, reason for caution: Since the time from ICSI to blastocyst formation and between blastocyst formation and freezing, the parameters under investigation in this study, may vary due to variations in culture environments and insemination methods, our study would have been improved if we had set standard times at each clinic.
Wider implications of the findings: This study demonstrates that the time from ICSI to blastocyst formation and the time interval between blastocyst formation and freezing affected the pregnancy rate thus suggesting that they could be valuable, new markers in the evaluation and selection of high quality embryos for blastocyst transfer.
Study funding/competing interest(s): Funding by hospital/clinic(s), Ochi Yume Clinic Nagoya.
Trial registration number: None.

P-101 Comparison of the clinical outcomes between Hyaluronan-enriched and autologous follicular fluid supplemented transfer medium

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Study question: The aim of this study was to evaluate the influence of hyaluronan-enriched transfer medium (HETM) on clinical outcoms compared with autologous follicular fluid (AFF) supplemented transfer medium in controlled ovarian hyperstimulation (COH) cycles.
Summary answer: The HETM did not have special effects on the clinical pregnancy and implantation rates after day 3 embryo transfers in COH cycles of unexplained infertile women compared with AFF transfer medium. AFF is likely to be an good alternative for hyaluronan.
What is known already: Hyaluronan presents in human follicular fluid, seminal plasma, oviduct and uterine. Hyaluronan is known to help the attachment of embryos on endometrium. HETM have shown to increase implatation in IVF/ET cycles and have effects on repeated implantation failure patients. Follicular fluid contains various components that are useful for oocyte maturation and embryo development. But it is still unclear whether AFF transfer medium affects on implantation in COH cycles.
Study design, size, duration: In this retrospective case control study, 165 unexplained infertile women underwent IVF-ET cycles during Aug 2012 to Dec 2013. On day 3, patients were divided by transfer media: control group was containing 20% AFF transfer medium and study group was HETM.
Participants/materials, setting, methods: All embryos have been co-cultured with autologous cumulus cell containing AFF before embryos transfer on day 3 after fertilization. We analyzed the patients' characteristics, clinical pregnancy and implantation rates between control group (AFF, N = 82) and study group (HETM, N = 85).
Main results and the role of chance: The average age (AFF, 34.9 ± 3.5 versus HETM, 35.4 ± 3.5), the average oocytes retrieval (AFF, 12.0 ± 5.0 versus HETM, 11.3 ± 4.0), fertilization rates (AFF, 77.5% versus HETM, 73.7%), cleavage rates (AFF, 97.8% versus HETM, 97.1%), and the mean of transferred embryos (AFF, 2.5 ± 0.5 versus HETM, 2.4 ± 0.6) were similar into two groups. There were no statistical differences on pregnancy rates (AFF: 50.0% versus HETM: 50.6%, odds ratio: 0.98, 95% confidence interval: 0.53–1.79) and implantation rates (AFF: 29.0% versus HETM: 29.2%, odds ratio: 0.98, 95% confidence interval: 0.64–1.50) in two groups as well ($P > 0.05$).
Limitations, reason for caution: HA is known to effective on RIF patients. This study did not divide patients with previous IVF trial attempts. With collecting more data, the effects of AFF on RIF patients need to be investigated.
Wider implications of the findings: After embryo transfer using AFF-containing medium, the clinical outcomes were comparable with HETM. Therefore, AFF could be used as transfer medium, which relieves the economic burden of patients.
Study funding/competing interest(s): Funding by hospital/clinic(s). None.
Trial registration number: None.

P-102 Clinical outcomes of vitrified- thawed blastocysts obtained from coculture with autologous cumulus cells versus sequential media

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Study question: The objective of this study was to compare the developmental competence and clinical pregnancy of vitrified-thawed blastocyst stage embryos (VTB) obtained from coculture with autologous cumulus cells (ACC) containing autologous follicular fluid (AFF) with those obtained from culture using sequential media (Cook IVF).
Summary answer: To use embryo coculture system in assisted reproductive technology (ART) seems to have an effect on developmental capability, clinical and implantation potential of VTB.
What is known already: The vitrification is now accepted as a very effective tool to cryopreserve blastocyst stage embryos in ART. However, there are no reports about clinical outcomes after VTB, in which blastocysts were cultured with ACC containing AFF.
Study design, size, duration: In this retrospective case control study, 341 patients underwent GnRH agonist or antagonist stimulation during previous cycles in 2013. The VTB were divided into two groups - Group I: VTB obtained from coculture with ACC containing AFF and Group II: those obtained from culture using Cook IVF.
Participants/materials, setting, methods: The blastocysts were vitrified using EM-grid following artificial shrinkage. Thawing was carried out by two steps, and the transfer of survived blastocysts was performed on the next day. The main outcomes were assessed the rates of survival, hatching, pregnancy and implantation into two groups.
Main results and the role of chance: In 351 VTB cycles, there were no differences in terms of the patient mean ages (34.7 ± 3.7 vs. 34.6 ± 3.4) and thawed embryo numbers (2.5 ± 0.8 vs. 2.6 ± 0.8) between Group I and II. The rates of survival and hatching in Group I and II were 90.7% (303/334), vs. 91.8% (517/563) and 71.3% (238/334) vs. 76.6% (431/563), respectively. The mean number of blastocysts per transfer was similar in Group I (2.2 ± 0.7) and II (2.4 ± 0.7). However, clinical pregnancy (50.4% vs. 36.6%) (p = 0.011, OR 0.57, 95% CI 0.37–0.88) and implantation (29.7% vs. 22.9%) (p = 0.031, OR 0.70, 95% CI 0.51–0.97) rates were significantly different between Group I and II.
Limitations, reason for caution: In this study, it is not classified the differences between stimulation methods used in controlled-ovarian-hyperstimulation

(COH) cycles. With collecting more data, it is needed that the differences between stimulation methods are clarified.
Wider implications of the findings: In this study, blastocyst stage embryos in vitrified-thawed cycles were only included. Therefore, further study will be need to investigate clinical outcomes of vitrified-thawed cleavage stage embryos obtained from various cultural environments.
Study funding/competing interest(s): Funding by hospital/clinic(s), There were no conflicts of interests to be declared.
Trial registration number: None.

P-103 Measuring the embryonic effects of vascular endothelial growth factor (VEGF) using a simple murine blastocyst outgrowth study

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Study question: Can VEGF (5, 50, and 500 ng/ml) in a simple cell culture system affect blastocyst outgrowth parameters post-hatching?
Summary answer: 50 ng/ml VEGF has an effect on blastocyst developmental progress, quality, and survival, but not on the attachment areas for both cells; trophectoderm (TE) and inner cell mass (ICM).
What is known already: Several cytokines and growth factors, including VEGF, are reported to have higher concentrations in stimulated, compared to natural, cycles. When blastocyst outgrowths were grown on fibronectin layers, 50 ng/ml gave better outgrowth outcomes than higher levels or controls (Hannan et al., 2011), but outgrowths in media alone have not been examined.
Study design, size, duration: Two-cell embryos were cultured for 78 h in SIVF-cleavage media (Cook Medical), then randomly allocated to four experimental treatments: SIVF-blastocyst media (BM, control) 5, 50 and 500 ng/ml VEGF + BM for 24 h. Hatched/hatching blastocysts were then placed individually into 100 µl DMEM + 5%v/vFBS (with equivalent VEGF concentrations) and observed to day 12.
Participants/materials, setting, methods: The TE and ICM outgrowth of 93 blastocysts were scored on Days 6, 7, 9, and 12 and, on Day 12, the outgrowth area was measured. Embryo outgrowth scores were assessed using one-way ANOVA, and areas using a Pearson chi-square test. A post-hoc test compared groups.
Main results and the role of chance: Blastocysts cultured in 50 ng/ml of VEGF had higher TE outgrowth scores on Days 9 and 12 ($p < 0.05$) in comparison with other groups, while the ICM had notable elevated outgrowth on Day 9 with VEGF 50 ng/ml ($p < 0.05$). Also, there were significantly higher proportions of D6 blastocysts with outgrowths on Day 12 in 50 ng/ml VEGF (92%, $p < 0.05$). The surface area of day 12 outgrowths was higher in the VEGF 500 ng/ml group (48.31 $\mu m^2 \pm 4.760$) compared to VEGF 5 ng/ml (34.29 $\mu m^2 \pm 4.330$, $p < 0.05$) but not different to control (46.63 $\mu m^2 \pm 3.919$) and VEGF 50ng/ml (44.81 $\mu m^2 \pm 3.769$) groups. The ICM outgrowth area of the 500 ng/ml VEGF group on Day 12 was significantly higher than those of the other groups ($p < 0.05$).
Limitations, reason for caution: While VEGF alone appears to play a part in embryo development and attachment in this in-vitro system, and that of others, it must be remembered that this is but one factor in the complex embryo-endometrial dialogue occurring in-vivo, thus further in-vivo trials are required to validate its importance.
Wider implications of the findings: Using this simple in-vitro system to test the various factors involved in driving the implantation process, and with follow up measurements of fluid in-vivo, it should be possible to create a model with the ideal factor concentrations, plus interactions, which may lead to a reduction of the implantation failure rate.
Study funding/competing interest(s): Funding by University(ies), No direct funding or competing interests.
Trial registration number: null.

P-104 Rescue of human oocytes with poor membrane stretchiness shown during ICSI, by identifying extra stretchy oolemmal regions using a piezo-micromanipulator

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Study question: The aim of our study was to evaluate the effectiveness of 'rescue' piezo-intracytoplasmic sperm injection (ICSI), in which a spermatozoon was selectively reinjected into the ooplasm through a region of the oolemma showing high resilience, among oocytes that had shown poor membrane stretchiness during the first piezo-ICSI attempt.
Summary answer: This 'rescue' piezo-ICSI into extra stretchy oolemmal regions produced markedly better survival rates and normal fertilization rates (per injected oocyte) than those derived from piezo-ICSI with a single injection. Moreover, oocytes subjected to this procedure produced viable blastocysts and fetuses, and gave rise to healthy babies.
What is known already: Poor oolemma stretchiness is a major risk factor for oocyte survival after ICSI. A piezo-micromanipulator (PMM) enabled us to avoid accidental oolemma rupture by using a blunt-end pipette and a completely separate microinjection through the zona pellucida and oolemma. The length to which a pipette could be inserted before the oolemma ruptured was defined as the degree of resilience and helped us to determine risky injection sites.
Study design, size, duration: We analyzed 673 oocytes subjected to ICSI. Oocytes with a high stretching oolemma were used for a single microinjection after application of a piezo-pulse (High/Single; $n = 571$). Those with a low stretching oolemma were penetrated suddenly by injection pipettes before application of a piezo-pulse, and used for a single injection (Low/Single; $n = 50$) or reinjection (Low/Re; $n = 52$).
Participants/materials, setting, methods: Following piezo-ICSI and *in vitro* culture, oocyte survival rates, normal fertilization rates and capacity to develop to the blastocyst stage were compared among the three categories. The outcomes of transferred oocytes originating in the Low/Single and Low/Re groups were investigated for implantation and live birth rates.
Main results and the role of chance: The survival rate of the Low/Re (94%) oocytes was significantly ($P < 0.01$) better than in the Low/Single group (66%). The fertilization rate of the Low/Re group (85%) was significantly ($P < 0.01$) better than the Low/Single group (52%), similar to the High/Single group (88.3%). The development rate to blastocyst was similar among all groups. Transfer of 20 fresh/cryopreserved embryos derived from oocytes with low stretchiness resulted in five implantations. In the Low/Re group, two healthy babies were born from nine embryos transferred. There was no difference in the *In vitro* developmental efficiency (% blastocysts/oocytes injected) between the Low/Re (34%) and High/Single (49.0%) groups, although that of the Low/Single group (22%) was significantly ($P < 0.01$) lower than in the High/Single group.
Limitations, reason for caution: Data in this study are numerically limited, and one ICSI operator performed all of the procedures.
Wider implications of the findings: We found variations in oolemma stretchiness among individual oocytes, possibly depending on membrane constituents (cholesterol, phospholipids or fatty acids) or on rearrangements of peripheral actin/microtubule networks. These oolemma characteristics and the use of a blunt-ended pipette actuated by a PMM permitted us to reinject oocytes selectively through a stretchy region of the oolemma. This novel piezo-ICSI approach might help reduce the incidence of oocyte degeneration after ICSI by selecting for oolemma stretchiness.
Study funding/competing interest(s): Funding by hospital/clinic(s). None.
Trial registration number: None.

P-105 Prediction model for blastocyst formation through multivariable analysis of morphokinetic data from an automatic time-lapse system

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Study question: Is there any combination of time range embryo cleavages that will be able to identify which embryos will become blastocyst?
Summary answer: By analyzing 7483 zygotes (the largest database ever reported), we provided a robust model by the analysis of sixteen morphokinetic parameter, three of them tM, s3 and cc3 resulted in a combination which is highly predictive (AUC = 0.849) of blastocyst formation.
What is known already: Embryo score evaluation is mainly performed under light microscope with limitations as subjectivity, prolonged exposure to light, changes in culture conditions and static perception of embryonic development. The strategy of evaluation of morphological changes using time-lapse, through Embryoscope® allows to maintain conditions and provides information useful to predict embryo implantation (Meseguer M, et al., 2011). Several attempts

has been performed to predict blastocyst formation by automatic time-lapse systems with limited numbers (Wong et al. 2010) and partially failed its prediction abilities (Conaghan et al. 2013).
Study design, size, duration: Observational, retrospective, single-center clinical study. A total of 7483 embryos were cultured and analyzed in Embryoscope® time-lapse incubator after ICSI until Day5 (D5) of development. This study was performed between May 2010 to May 2013 in IVI.
Participants/materials, setting, methods: From 990 patients timing of each cellular event was annotated along embryo development (tn) together with Morula formation (**tM**). Also we calculated cell cycle duration ($cc_n = t_{n+1}\text{-}t_n$), synchrony between blastomeres ($s_n = t_{n+1}\text{-}t_n$). We defined optimal time ranges according to consecutive quartiles with highest probability of blastocyst. A logistic regression model were performed.
Main results and the role of chance: From 7483 zygotes, 3215 (43.0%) reached blastocyts stage before 120 hours post ICSI. Logistic regression model defined the following binary variables tM (81.28–96.00 h), s3 (≤8.78 h) and cc3 (11.35–16.49 h) as predictive of blastocyst formation, ROC value = 0.844 (CI 95% 0.835–0.854). The following table shows the probabilities for an embryo to become blastocyst when the timing of cellular events are within defined ranges.

Timing of cellular event	Probability of Blastocyst (%)	
	Inside defined range	Out of range
tM (81.28–96.00 h)	81.6	69.9*
s3 (≤8.78 h)	63.7	36.3*
cc3 (11.35–16.49 h)	55.0	45.0*

*$p \leq 0.05$.

Limitations, reason for caution: These dates are from retrospective analysis, although sample size is outstanding. Results needs to be validated by a prospective study.
Wider implications of the findings: Inclusion of kinetic parameters into score evaluation improve embryo selection criteria and can predict blastocyst formation with high accuracy. By using the timing of the events here proposed we are providing an alternative algorithm to those already reported based in the largest database ever published which could be applied to forecast and select embryos for transfer and even move forward the day of transfer with comparable results.
Study funding/competing interest(s): Funding by hospital/clinic(s), This research has not funding support and may compete and question the existing commercial algorithm available for blastocyst prediction.
Trial registration number: Not applicable.

P-106 Vitrification solutions improves the immature oocyte in vitro maturation by inducing an increase of intracellular Ca2+ concentrations

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Study question: The aim of this study is to evaluate the effect of vitrification solutions (VS) on human oocyte in-vitro maturation (IVM) in order to know if oocyte quality and early embryonic development is affected by the mature stage at the vitrifying time.
Summary answer: VS improves IVM. Probably the cryoprotectants lipophilic properties used in VS have a non-specific effect on plasma/internal membranes as ER, which would lead to either Ca^{2+} influx and/or Ca^{2+} release from internal stores. However, the osmotic contraction induced by cryoprotectants might also contribute to increase the intracellular Ca^{2+} improving the IVM at Prophase-I(PI)-stage.
What is known already: The endoplasmic reticulum (ER) is the major oocyte calcium store. During oocyte maturation ER undergoes profound reorganization affecting the Ca^{2+} oscillations. Since some cryoprotectants have been shown to increase intracellular calcium in mouse oocytes, a current potential problem with oocyte freezing is the induction of a primary activation event. It has been shown that in vivo matured human oocytes exhibit a specific pattern of Ca^{2+} oscillations distinct from IVM and in vitro aged oocytes (Nikiforaki, 2013).
Study design, size, duration: PI (n = 223) from 200 IVF-ICSI cycles were randomly distributed into the following groups: G1 (control group, n = 41): IVM till MII; G2 (exposed to ionomycin, n = 41), G3 (exposed to VS, n = 43), G4 (vitrified, n = 45) and G5 (IVM till MII and then vitrified, n = 53). PI that reached MII-stage were parthenogenetically activated to assess oocyte viability.
Participants/materials, setting, methods: IVM (G1 and G5) was performed in IVF-medium during 24–48 h. G2-treatment was performed using ionomycin (10 µM, 5 min in IVF) and G3-group was exposure to VS (Irvine-Scientific) without liquid-N2 immersion. The following rates were evaluated: IVM (IVMR), activation (AR), development to cell (DRC), and development to morula (DRM) and blastocyst (DRB).
Main results and the role of chance: A significant increase ($p < 0.05$) of IVMR, AR and DRC was observed in G3 (86%, 86% and 80%); and G4 (80%, 80% and 82%) compared to G1 (63.4%, 73.1% and 68.4%), G2 (48.8%, 55% and 90.9%) and G5 (56.6%, 66.6% and 66.6%). Development to morula and blastocyst stage only was achieved in G3 (DRM: 36%, DRB: 33%) and G4 (DRM: 25%, DRB: 16%). VS improves IVM at PI-stage and early embryonic development. The highest AR obtained in G2 would be due to the ionomycin which are lipid-soluble substances that insert into the intracellular organelles membranes, allowing the release of Ca^{2+} ions. Ionophores might more effectively insert into the membranes of clustered ER in MII stage than into the membranes of more diffused ER structures in GV oocytes. That is the reason why Ionomycin does not promotes IVM at the PI-stage.
Limitations, reason for caution: Unfortunately, in the vitrification procedures, the thermal shock cannot be evaluated separately from the osmotic shock.
Wider implications of the findings: Oocyte vitrification at PI-stage and subsequent IVM can increase the number of mature oocytes. This procedure is proposed as an additional technique for fertility preservation especially in those patients that controlled ovarian stimulation is contraindicated such as pre-pubertal girls and women with hormone-dependent cancers. The immature oocytes that do not survive to the ovarian tissue cryopreservation procedures could be excised from the ovarian tissue and vitrified following this protocol, providing an additional source of gametes.
Study funding/competing interest(s): Funding by hospital/clinic(s), Hospital Universitario y Politécnico La Fe.
Trial registration number: None.

P-107 Customized morphokinetic model for blastocyst selection by time-lapse system

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Study question: Is there any correlation between morphokinetic variables (including late development events) and the resulting implantation.
Summary answer: Through the analysis of seventeen morphokinetic parameters (including those related with blastocyst formation and morphology) we were able to correlate implantation rate (IR) according to a defined morphokinetic model. The resulting categories generated were based in the synchrony of the third cell cycle (s3) and the timing of blastocyst expansion tEB.
What is known already: Using traditional incubators, inspection is limited to snapshots at a few discrete points in time, reducing the amount of information that could potentially be obtained. Time-lapse monitoring overcomes this limitation without exposing the embryos to environmental changes. Moreover, several recent studies suggest that time-lapse monitoring may introduce new dynamic markers of embryo competence, nevertheless the recent models used for embryo selection based on time-lapse, lacked sample size power and mainly used early cleavage markers.
Study design, size, duration: An observational and retrospective study on 832 transferred embryos performed in a time-lapse incubator. The majority of the embryos were transferred on Day 5 while we left some of them until day 6 when a delayed evolution was observed. This study was performed between May 2010 to December 2013.
Participants/materials, setting, methods: We studied the different cellular divisions: cleavage from 2 cells to 8 cells**,** timing of compaction (**tM**), blastocyst formation (**tB**) and expansion (**tEB**), second and third cell cycle ($\mathbf{cc_2} = t_3 – t_2$) ($\mathbf{cc_3} = t_5 – t_3$), synchrony between blastomeres ($\mathbf{s}_2 = t_4 – t_3$) and $\mathbf{s}_3 = t_8 – t_5$. A logistic regression model was performed by using defined variables to select those relevant to predict implantation.
Main results and the role of chance: Logistic regression model defined the following binary variables and optimal ranges tEB (107.9–112.9 h) and s3

(3.0–5.67 h) as predictive of blastocyst implantation, with a ROC value = 0.591 (CI 95% 0.552–0.630) with $*p < 0.001$. Based on these relevant variables we established 4 categories named from A to D from higher to lower implantation rate, finding significant differences as presented below; category A with 72.2% IR, category B with 66.2% IR, category C with 55.8% IR and D with 39.7 of IR.

$*p < 0.001$.

Limitations, reason for caution: The data are from retrospective analysis, although sample size is outstanding, we should check this result with a prospective study.

Wider implications of the findings: These data suggest the existence of a new classification algorithm based on blastocyst morphokinetic, providing a new and more objective view to the blastocyst evaluation. By using the timing of the events here proposed we are providing an alternative algorithm based on late events, being able to be applied to predict and select embryos for transfer in blastocyst stage.

Study funding/competing interest(s): Funding by hospital/clinic(s), IVI clinic.

Trial registration number: Not applicable.

P-108 Comparison of embryos morphokinetic pattern according to the sperm origin

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Study question: The aim of our study was to compare morphokinetic parameters of early embryo development in ICSI cycles according to sperm origin, i.e. fresh ejaculated (FE) or surgically retrieved (SR) spermatozoa, including testicular sperm extraction (TESE) or epididymis sperm aspiration (ESA).

Summary answer: Embryo morphokinetic parameters differ in ICSI cycles according to sperm origin.

What is known already: ICSI has been shown to be an efficient strategy in infertile couples suffering from male infertility. However, it is unclear whether sperm origin, i.e. ejaculate or surgically retrieved, has a significant impact on embryo developmental competence, with contradictory results in terms of blastulation or implantation. Time-lapse technologies have recently proved to be accurate in evaluating objectively embryo development, thus raising its interest in evaluating the eventual impact of sperm origin on early embryo development.

Study design, size, duration: This retrospective study was conducted on all ICSI cycles performed between 2011 and 2013 with the Embryoscope®. All clinical, ovarian reserve, ovarian stimulation and embryonic parameters (conventional morphology and kinetic events in hours post injection) were recorded.

Participants/materials, setting, methods: This monocentric retrospective study was conducted on 622 ICSI cycles performed with the Embryoscope®. Patients were classified according to sperm origin, i.e. fresh ejaculate ($n = 575$ cycles), epididymis (ESA, $n = 16$) or testicular biopsy (TESE, $n = 31$). Clinical, ovarian reserve, ovarian stimulation and embryo morphokinetic parameters were compared according to sperm origin.

Main results and the role of chance: Clinical, ovarian stimulation, ovarian response and number of embryo transferred were comparable in all groups. Fertilization rate was significantly lower in SR group compared to Fresh ejaculate group (58.11 vs. 67%).

Concerning morphokinetics, PN fading PN appearance, t2 (2-cell stage), t3, t4 and s2 (t4-t3) were comparable in FE and SR groups groups. Mean cc2 (t3-t2), t5 and t6 were significantly lower in SR group than in FE group. Among SR patients, only t6 and s2 were significantly different between ESA and TESE groups.

Finally, blastulation rate and pregnancy rate were comparable in all groups (FE pregnancy rate 28.70%, TESE 29.35% and ESA 31.25%).

Limitations, reason for caution: This study was retrospective and monocentric. Results should be confirmed on larger cohorts in multicentric studies.

Wider implications of the findings: Time-lapse analysis is a relevant method for the evaluation of an eventual paternal detrimental effect on embryo development in ICSI cycles. The slight modification in morphokinetic parameters observed with surgically retrieved sperm compared to fresh ejaculate sperm does not seem to lead to poorer cycle outcome.

Study funding/competing interest(s): Funding by hospital/clinic(s), CHU Nantes.

Trial registration number: Local IRB approved study.

P-109 Trends and pregnancy outcomes of assisted hatching, united states, 2000 – 2010

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Study question: To characterize trends in assisted hatching use among assisted reproductive technology (ART) cycles and to examine the relationship between assisted hatching and pregnancy outcomes (clinical pregnancy, live birth, miscarriage).

Summary answer: Use of assisted hatching increased over time in the United States. Assisted hatching was associated with poor pregnancy outcomes overall, and was not associated with improved outcomes even in 'poor prognosis' patients; prospective studies are needed to assess assisted hatching benefits.

What is known already: Assisted hatching is often performed in an effort to improve implantation rates on embryos noted to have a thick zona pellucida or among poor prognosis patients, but existing evidence of its effectiveness is limited. Meta-analyses found a higher clinical pregnancy rate of marginal statistical significance among women for whom assisted hatching was used compared to controls, but did not find significant differences in the odds of live birth or miscarriage.

Study design, size, duration: Non-cancelled ART cycles ($n = 835, 067$) initiated between 2000–2010 in the United States among patients using fresh embryos from their own eggs and reported to the National ART Surveillance System (NASS) were used in this population-based retrospective cohort study.

Participants/materials, setting, methods: We used NASS data to assess trends of assisted hatching use and associations between assisted hatching and clinical pregnancy, live birth, and miscarriage, while adjusting for patient and treatment characteristics.

Main results and the role of chance: In the United States from 2000 to 2010, assisted hatching use increased significantly in absolute number (25,724 to 35,518 cycles per year), percentages of day-3 transfers (50.7% to 56.3%) and percentages of day-5 transfers (15.9% to 22.8%), and percentage of transfers among women ≥38 years, or women with ≥2 prior ART cycles and no live birth(s) (20.1% to 25.3%); all $P < 0.05$. Cycles involving assisted hatching were associated with lower odds of clinical pregnancy and live birth, and with increased odds of miscarriage regardless of embryo stage, as compared with cycles without assisted hatching; all $P < 0.05$. In cycles for 'poor prognosis' women, the association of assisted hatching with these outcomes was not significant.

Limitations, reason for caution: Although embryo quality data were not available, we used availability of extra embryos for cryopreservation (shown to correlate well with embryo quality). We did not have data on additional factors (hypertension, diabetes, obesity, smoking, etc.) that may have influenced the decision to perform assisted hatching or affected the observed outcomes.

Wider implications of the findings: Although we observed an increasing trend of using assisted hatching among women for whom it is recommended, it is still used in a relatively large number of cycles among women <38 years of age, those with <2 prior ART cycles or history of live births, and blastocyst transfers. Well-designed prospective studies may help clinicians identify patients who may benefit from assisted hatching.

Study funding/competing interest(s): Funding by national/international organization(s), Centers for Disease Control and Prevention.

Trial registration number: Not applicable.

P-110 Factors affecting the clinical pregnancy rate of frozen-thawed blastocyst transfer cycles: a multivariate analysis of 1453 cycles

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Study question: What factors does a multivariate logistic regression analysis show that affects the clinical pregnancy rate (CPR) of frozen-thawed blastocyst stage embryo transfer cycles (TBT)?

Summary answer: There are several variables that influence the CPR of TBT including maternal age, number of blastocysts transferred, blastocyst quality, endometrial preparation method, fertilization method, and most importantly the blastocyst developmental stage.
What is known already: Maternal age, quality of the blastocyst, and number of blastocysts transferred are generally thought to affect the CPR, but the effects of developmental stage, endometrial preparation method, and some other factors are not clear. Some confounding factors were not excluded in many published studies, making the conclusions not applicable in clinical practice.
Study design, size, duration: This study retrospectively analyzed 1453 cycles of blastocyst transfer performed on 1243 couples in the past 3 years. A multivariate logistic regression analysis was performed to identify the factors that affect the CPR of TBT.
Participants/materials, setting, methods: Based on clinical experience and the literature, 26 variables were single-factor analyzed, and then were included to perform a multivariate logistic regression analysis for day 5 (D5) and day 6 (D6) blastocyst transfer. Using stepwise selection, seven variables for D5 TBT and eight variables for D6 TBT were chosen in the models ($P < 0.1$). Three factors with different effects on D5 and D6 TBT were analyzed regarding their impact on implantation rate (IR).
Main results and the role of chance: The blastocyst developmental stage was the primary factor affecting the CPR and IR of TBT cycles (CPR: 69.0% vs. 47.5%, $P < 0.001$; IR: 57.6% vs. 36.4%, $P < 0.001$), so the following analyses were performed on D5 and D6 TBT respectively. Maternal age, basal follicle stimulating hormone (bFSH) level, and number of blastocysts transferred had similar effects on D5 and D6 TBT, while the endometrial preparation method, fertilization method, and the blastocyst stage when frozen had different influences. Hormone replacement treatment (HRT) as the endometrial preparation method had a slightly higher CPR (odds ration [OR] = 1.367; 95% confidence interval [CI]: 0.986–1.895) and IR (59.5% vs. 53.8%, $P = 0.045$) than natural cycle (NC) or controlled ovarian hyperstimulation (COH) for D5 TBT, while HRT negatively affected the CPR (OR = 0.679; CI 0.477–0.967) and IR (33.2% vs. 41.4%, $P = 0.012$) of D6 TBT. D6 blastocysts derived from Intracytoplasmic sperm injection (ICSI) cycles had higher CPR (OR = 1.343; CI 1.115–1.618) and IR (45.1% vs. 35.7%, $P < 0.001$) than those from conventional in vitro fertilization (IVF) cycles. Blastocyst stage when frozen positively affected the CPR (OR = 3.441; CI 1.175–10.073) and IR (non-expanded vs. expanded, 35.9% vs. 59.7%, $P < 0.001$) of D5 TBT but not D6 TBT, most likely because there were very few cases of D6 blastocysts frozen in a non-expanded stage.
Limitations, reason for caution: These findings were only based on a retrospective analysis, and should be confirmed in a randomized controlled trial. We performed the multivariate logistic regression analysis on D5 and D6 blastocysts respectively, which is different from most of the studies in the literature, making direct comparisons difficult. Lastly, this was a single center study.
Wider implications of the findings: Blastocysts developmental stage was an important factor indicating the implantation potential. Endometrial preparation methods or the time of transfer should be carefully chosen to achieve a higher IR. D6 blastocysts derived from ICSI cycles have a higher IR than those from conventional IVF cycles. Single blastocyst transfer is highly recommended for patients with D5 or D6 blastocysts if it was derived from ICSI cycles to reduce multiple pregnancies.
Study funding/competing interest(s): Funding by national/international organization(s), National Nature Scientific Funds of China (No: 81070495) and Guangdong province (No. S2013010013404).
Trial registration number: Not applicable.

P-111 Embryo quality and clinical outcomes using Embryoscope™, Minc™ and Heracell™ 150i incubators: preliminary results from a randomized study with donor oocytes

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Study question: Are embryo quality and clinical outcomes affected by the type of incubator used for embryo culture?
Summary answer: Fertilization rates were identical among the three types of incubators included in our study. However, the percentage of good quality embryos, clinical pregnancy and implantation rates achieved using *Minc™* were significantly higher than those obtained with *Heracell™ 150i*. No differences were found between *Embryoscope™* and *Minc™* incubators for data analyzed.
What is known already: The development of designed benchtop incubators with controlled temperature and gas, has been performed in the last few years to provide more stable conditions for the culture of human embryos. In addition, the incorporation of time-lapse monitoring systems to these new incubators provides supplementary information about morphokinetics of embryo development. The advantages of using this new generation of incubators have led to improved embryo quality rates and better clinical outcomes. The aim of our study was to compare the performance of different types of incubators under our laboratory conditions.
Study design, size, duration: This prospective randomized study, included 72 recipient cycles performed between October and December 2013. Fertilization, good quality embryos, embryo replacement, clinical pregnancy and implantation rates were compared among the different incubators.
Participants/materials, setting, methods: Embryo culture was performed using *Embryoscope™* (24 cycles; 231 embryos), *Minc™* (24 cycles; 235 embryos) or *Heracell™ 150i* (24 cycles, 256 embryos) incubators.

Injected oocytes were cultured in Global Total® medium (*LifeGlobal*) in low oxygen tension. Embryo replacement was performed on Day 3. Results were compared by X^2 or student's *t-test.*
Main results and the role of chance: Fertilization rates were identical between *Embryoscope™*, *Minc™* and *Heracell™ 150i* incubators (88.2%, 84.7% and 81.9%, respectively). The percentage of good quality embryos on day-3 obtained using *Minc™* (72%) was identical to that obtained with *Embryoscope™* (68.4%), but statistically higher than with *Heracell™ 150i* (61.1%, $p = 0.0037$). Statistically significant differences were also found on clinical pregnancy (65.2% vs. 30.4%, $p = 0.0039$) and implantation (55.6 vs. 24.4, $p = 0.0052$) rates between *Minc™* and *Heracell™ 150i*. No differences were found on miscarriage rates among the three different types of incubators.

All groups were comparable in terms of oocyte donors and recipients ages, number of donated oocytes and number of replaced embryos.
Limitations, reason for caution: These are preliminary results of an ongoing study and a larger sample size is required to increase statistical power. Results are based on observations using embryos from oocyte donors and need to be confirmed using embryos from infertile patients of different ages and with embryo replacements performed on Day 5.
Wider implications of the findings: Our preliminary data suggest that the usage of benchtop incubators in human IVF improves embryo quality and clinical outcomes. These results could be attributed to more stable culture conditions in terms of both temperature and pH offered by the new benchtop incubators compared to standard *Heracell™ 150i*. Further studies should be carried out to confirm the beneficial effects of using benchtop incubators in IVF laboratories.
Study funding/competing interest(s): Funding by hospital/clinic(s), This study was funded by IVI-Barcelona. The authors do not have any conflict of interests with the commercial identities mentioned in this abstract.
Trial registration number: None.

P-112 New method of visualization of native sperm intracellular organelles for sperm selection and diagnostics of male infertility

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Study question: The aim of this study was developing a new microscopy method of sperm morphology evaluation, allowing us to visualize the intracellular organelles of spermatozoa.
Summary answer: The new mycroscopy method for Native Assessment of Sperm Ultramorphology (NASUM) was developed. NASUM gives possibility to visualize chromatin, acrosome and its granularity, vacuoles, mitochondria, nuclear membrane pores, microfibrils of the tail, and chromocenter, as well as evaluate the degree of chromatin condensation in native spermatozoa.

What is known already: Various authors have demonstrated that selection of vacuole-free spermatozoa for intracytoplasmic sperm injection (ICSI) leads to higher pregnancy rates and lower rates of pregnancy loss. Bartoov et al. (2001) developed the intracytoplasmic morphologically selected sperm injection (IMSI). It was shown that selection of vacuole-free spermatozoa for ICSI leads to higher pregnancy rates and lower rates of pregnancy loss. However, IMSI does not provide us with the possibility of visualization of the intracellular organelles.
Study design, size, duration: We obtained 12348 NASUM microphotographs of native immobilized spermatozoa from in vitro fertilization (IVF) program patients with male factor of infertility as well as from sperm sperm donors.
Participants/materials, setting, methods: The developed microscope system employed both Hoffman and Nomarski contrast techniques. Resolution increase was attained with light interference suppression via using circularly polarized light. The introduction of additional lenses into the optical system allowed us to reach 20000X total magnification. We used green 500 mW laser lighting (wavelength 532 ± 10 nm) with grain suppression.
Main results and the role of chance: The developed method of observation and the constructed microscope system allows for studying sperm morphological features at subcellular level and detect structural anomalies invisible during the usual light optical microscopy (see picture). The subcellular organelles can be morphologically classified on the basis of the presence of specific malformations defined according to the descriptive approach reported by Bartoov et al. (2002): acrosome: absent, partial or vesiculated; post-acrosomal lamina: absent or vesiculated; neck: abaxial, disordered or showing cytoplasmic droplet; tail: absent, coiled, broken, multi or short. For the nucleus, the morphological normal state can be defined by the shape and content of the chromatin. It is proposed to name the new method as native assessment of sperm ultramorphology (NASUM). Estimated NASUM technique resolution is 0.05 µm.
Limitations, reason for caution: The developed method NASUM is time consuming and requires about 2 hours to assess the ultramorphology of 100 spermatozoa. Sperms must be firmly immobilized to collect multiple images of the same cell for digital processing.
Wider implications of the findings: Together with electron microscopy of fixed spermatozoa, NASUM might be used efficiently in male infertility diagnostics. Further study of human sperm nucleus chromatin architecture using NASUM will fill the gaps existing at present in the scientific understanding of the non-random arrangement of chromosomes. We expect that NASUM would assist in improving fertilization rates, embryo quality, blastocyst development rates, rates of implantation and pregnancy, as well as decreasing the incidence of pregnancy loss.
Study funding/competing interest(s): Funding by hospital/clinic(s), Altravita IVF clinic, Moscow, Russia.
Trial registration number: Not clinical trial.

P-113 Elective vitrify-all: a good choice when embryo transfer conditions are not ideal

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Study question: The aim of the study is to analyse pregnancy rates (PR) in the first transference cycle of couples whose embryo transfer was performed with fresh embryos compared to those whose first transfer was done after vitrification of every available embryos due to any clinical or personal circumstance.
Summary answer: Vitrify--all technique will not be detrimental for pregnancy rates and it is a good option when the conditions for the transference of the embryos in the fresh cycle are not ideal.
What is known already: Some IVF or ICSI cycles are accompanied by high progesterone levels in the late follicular phase, risk of ovarian hyperestimulation syndrome, need for the accumulation of embryos for a preimplantational genetic diagnosis cycle, bad quality endometrium, etc. In such cases, vitrification of all embryos can be a good option to improve the efficiency of the cycle in terms of pregnancy rate by deferring the transference to a later natural or medically assisted cycle.
Study design, size, duration: A retrospective observational study including all the consecutive 73 couples with elective 'vitrify-all' and the 185 couples with a fresh transference cycle performed between may 2011 and December 2013, was done.
Participants/materials, setting, methods: Only patients under forty years old with at least one good quality embryo were included. A Fisher's test ($p < 0.05$) was used for statistic analysis.
Main results and the role of chance: PR in the elective 'vitrify-all' group was 54.5%, with an average age of 36.4 ± 4.1 years and an average of 1,97 embryos transferred. Those patients with fresh embryo transfer had a PR of 56.4% with an average age of 35 ± 4.4 years and an average number of 2,06 embryos transferred. There were not statistically significant differences between the two groups analysed ($p > 0.05$)
Limitations, reason for caution: The small size of the population analysed and the retrospective origin of data suggest the need of a larger and prospective study to confirm these findings.
Wider implications of the findings: Embryo vitrification yields a high rate of embryo survival in good quality conditions and produce comparable results in terms of PR in subsequent cycles, permitting the correction or avoidance of clinical and personal circumstances that could compromise the results if a fresh embryo transfer is performed.
Study funding/competing interest(s): Funding by hospital/clinic(s), no funding.
Trial registration number: No.

P-114 Chromosomal integrity of human preimplantation embryos at cleavage and blastocyst stage in recurrent pregnancy loss patients

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Study question: Investigate the dynamics of genomic alterations that occur at different developmental stages in embryos from recurrent pregnancy loss (RPL) patients.
Summary answer: In a population of RPL patients different embryo chromosomal abnormalities were detected depending on D3 or D5 of in vitro embryo culture.
What is known already: The high incidence of chromosome abnormalities in human gametes and early preimplantation embryos provides an explanation for the low success of IVF treatment cycles. The development of comprehensive chromosome screening (CCS) has offered valuable insight into the chromosomal status of human gametes and preimplantation embryos. And applied as a therapeutic tool it could improve implantation and live birth rates from in-vitro fertilization and provide a means of attenuating pregnancy loss in recurrent pregnancy loss patients.
Study design, size, duration: A retrospective study was performed. We included the array-CGH results of 198 embryos (133 on D3 and 55 on D5) from 45 RPL patients performing CCS cycles in 2013 from January to December at Instituto Bernabeu. The main outcome measures were aneuploidy, chromosome alteration and chromosome monosomy and trisomy rates.
Participants/materials, setting, methods: RPL was defined as two or more miscarriages. We examined the chromosome content of human preimplantation embryos by array-CGH in single-cell blastomeres from D3 embryos and trofoectoderm cells from D5 embryos. For the array-CGH analyses we used the Agilent technology platform (SurePrintG3) according to manufacturer recommendations applied to single-cell.
Main results and the role of chance: Significant differences were reported in the aneuploidy rate between D3 (62.6%) and D5 (25.5%) embryos ($p < 0.001$). According to multiple aberrations we observed that the 39.5% of aneuploidy embryos on D3 has more than 3 chromosome alterations in contrast to 14.3% of D5. As for each chromosome, alteration on chromosome 22 (22%) and 16 (22%) were the most frequently involved in aneuploidy on D3 embryos, followed by chromosomes 19, 20 and 5. All the chromosomes were involved in aneuploidy. Alteration on chromosome 19 (28%) was the most frequently involved in aneuploidy on D5 embryos, followed by chromosomes 16 and 21. Chromosomes 8, 9, 11 were not involved in aneuploidy in D5 embryos. The most chromosomal aberration on D3 was monosomy 18 versus trisomy 19 on D5.
Limitations, reason for caution: CCS uses to increase the IVF-results mainly in patients suffering RPL is a promising tool. The present work shows that embryo culture could select the euploid embryos suggesting key chromosomes

could be involved in embryo arrest. More data are needed to conclude what chromosomes are important in each stage.
Wider implications of the findings: This investigation reveals that in a population of RPL different chromosomes are involved in aneuploidy at different stage. These dynamic changes that occur at early developmental stages and the natural selection produced by the embryo culture to blastocyst stage suggest that CCS from trofoectoderm cells may be more appropriate.
Study funding/competing interest(s): Funding by hospital/clinic(s), Instituto Bernabeu.
Trial registration number: None.

P-115 Automated time-lapse analysis in adjunctive use with morphology is highly informative in allowing diverse embryologists to select embryos with high developmental potential

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Study question: Embryo assessment by traditional morphology is subject to inter- and intra-observer variation (Paternot et al., 2009). We assessed whether computer-derived time-lapse analysis used in adjunct with traditional morphology would allow embryologists to consistently benefit in selecting embryos with higher development potential.
Summary answer: Quantitative and objective results from automated time-lapse analysis are informative for embryologists to select good/fair morphology embryos on day3 with higher potential to form blastocysts. Five embryologists from 5 clinics with a diverse range of training and experience demonstrated consistent embryo assessment after using time-lapse information adjunctively with morphology.
What is known already: Since many transferred good morphology embryos fail to implant, technologies to identify embryos with high developmental potential would be beneficial. ***Eeva™*** (Early Embryo Viability Assessment), an integrated time-lapse and automated analysis system has been shown to benefit embryo selection for a panel of three very experienced embryologists (Conaghan et al., 2013). Here we examined if Eeva, when used adjunctively with morphology, is informative for a new panel of embryologists with diverse clinical experience.
Study design, size, duration: Prospective multi-center study (Jun.2011-Apr.2012) with 54 patients undergoing blastocyst transfer cycles consented to have embryos imaged using Eeva, which automatically measures cell division timings P2 (time between first and second mitosis) and P3 (time between second and third mitosis), and categorizes embryos into groups with different development potential.
Participants/materials, setting, methods: Five embryologists representing a diverse range of clinical practices, laboratory training, and geographical areas predicted blastocyst formation using day3 morphology alone vs. morphology + Eeva. To assess the effect of using Eeva with morphology, odds ratio (OR) was calculated by comparing prediction results to true blastocyst outcomes (OR > 1.0 indicates morphology + Eeva is informative in blastocyst-prediction).
Main results and the role of chance: When time-lapse results of Eeva were used adjunctively with traditional morphology for embryos that were graded good or fair on day3, the odds of an embryo forming a blastocyst was 2.56-fold (95% CI = 1.75–3.75) higher in the group predicted to develop into blastocysts than in other embryos. In contrast, the OR using morphology alone was 1.65 (95% CI = 0.78–3.51) and not statistically significantly >1.0. When individual embryologist's adjunctive prediction performances were compared to morphology alone, every embryologist's OR was improved to >2.0. Furthermore, the variability in prediction performance across all five embryologists was reduced when time-lapse information was used: the range of embryologists' adjunctive prediction ORs narrowed (OR 2.33–2.82), compared to the range of ORs from morphology alone (1.14–2.20).
Limitations, reason for caution: The panel of five embryologists in this study represented a diverse range of clinical practices, laboratory training, and geographical areas; however, the embryo morphology data used for analysis were collected from only three IVF clinics.
Wider implications of the findings: Our data demonstrate that computer-derived time-lapse analysis used adjunctively with morphology is highly informative in helping embryologists select which embryos have developmental potential. In contrast, traditional morphology was not informative in predicting embryo development for good morphology embryos. Our results also demonstrate that, irrespective of the clinic practice, experience level, and training, embryologists were able to consistently improve their ability to select embryos with higher developmental potential using Eeva adjunctively with traditional morphology.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Auxogyn, Inc.
Trial registration number: NCT01369446

P-116 Frozen embryo transfer outcomes with the Rapid i closed vitrification carrier: impact of delayed blastulation and blastocyst morphology on clinical pregnancy and implantation rates

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Study question: Does blastocyst morphology or day of blastulation affect clinical success rates with vitrified blastocysts?
Summary answer: The pregnancy potential of day 5 versus day 6 vitrified blastocysts was significantly different. Delayed blastulation appears to negatively impact embryo competence to implant. Even in transfers with high grade expanded day 6 blastocysts, clinical pregnancy and implantation rates were lower than with Day 5 vitrified blastocysts of equivalents grade.
What is known already: Pregnancy rates appear higher with fresh day 5 versus day 6 transfers. Not clear if this is due to blastocyst quality or endometrial receptivity. Time lapse data suggest that kinetics of blastocyst formation correlate to implantation potential and possible risk of aneuploidy. Frozen embryo transfer results with blastocysts vitrified on day 5 or 6 and replaced in a more synchronized endometrium may provide a better understanding of the impact of delayed blastulation on embryo competence.
Study design, size, duration: Data was retrospectively analyzed from frozen embryo transfers carried out between April 2011 and December 2013. The study group consisted of 209 consecutive transfer cycles with vitrified-warmed blastocysts. Data was stratified according to day of vitrification (D5 VBL vs. D6 VBL) and blastocyst grade. Survival, clinical pregnancy rate (CPR) and implantation rate (IR) were compared. Post-warming morphology was also examined.
Participants/materials, setting, methods: Blastocysts (n = 394) were cryopreserved using the Rapid i and a two step vitrification protocol: 7.5% ethylene glycol (EG)/DMSO for 5 minutes followed by 15% EG/DMSO for 45 seconds. Blastocysts were collapsed before vitrification. estrace and progesterone were used for endometrial priming. Warmed blastocysts were transferred after six days of progesterone.
Main results and the role of chance: The overall CPR and IR were 54% and 42%, respectively with a 96% post-warming survival rate. Percent survival was similar between blastocyst grades. Mean embryos transferred were 1.78 ± 0.48. We had 105 FET cycles with D5 VBL, 85 with D6 VBL and 19 with both D5 and D6 VBL. Frozen outcomes were significantly higher with D5 VBL (CPR 64%, IR 50%; $p < 0.05$) as compared to D6 VBL (CPR 38%, IR 30. Even comparing pregnancy potential of only the high grade D6 VBL to D5 VBL, we observed a decrease in CPR and IR (CPR 71% IR 55% vs. CPR 45%, IR 38%, respectively; $p < 0.05$). Patient age was higher in the D6 VBL group (36.7 ± 4.3) versus D5 VBL (34.9 ± 4.1; $p < 0.05$).
Limitations, reason for caution: This was a retrospective analysis. Confounding factors such as patient age, diagnosis and cycle characteristics were not controlled for. Further investigation with a larger data set is warranted to determine if late developing blastocysts have limited potential. This may ultimately affect the laboratory's cryopreservation program and criteria for freezing.
Wider implications of the findings: Delayed blastulation on day 6 may be indicative of impaired embryonic potential. The cause of delayed blastulation could be intrinsic to the embryo (chromosomal content) or alternatively, the result of a less than optimal in vitro culture environment that is negatively influencing embryo development. Currently, limited published studies are available

on the Rapid i vitrification system. Our present work demonstrates the effectiveness of the Rapid i for vitrification of blastocysts of all grades.
Study funding/competing interest(s): Funding by hospital/clinic(s), Cleveland Clinic.
Trial registration number: NA.

P-117 Randomized controlled comparison of single embryo culture versus group culture using a micro-well group culture dish in humans

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Study question: Does group culture using the micro-well group culture dish improve human blastocyst development compared to single embryo culture?
Summary answer: A midpoint intermediate analysis does not yet reach the estimated difference, but reveals interesting results favoring group culture. No differences in embryo development are observed until day 3.
What is known already: Animal studies have shown improved embryo development after group culture. A micro-well dish improved bovine embryo development. In humans, a number of clinics routinely perform group culture. However, few prospective studies investigating the possible beneficial effects of group culture have been performed.
Study design, size, duration: Patient allocation was at the time of fertilization check according a randomization list. For a difference of 15% blastocyst formation and a power of 80%, 167 embryos were required per group. An intermediate analysis was planned after inclusion of 50% of the required number.
Participants/materials, setting, methods: Fertilized oocytes were cultured using a primo vision embryo culture dish either individually in 30 µl droplets or in a group with up to 9 embryos in 80 µl under oil. On day 3, embryos were transferred to fresh medium. Embryos were cultured until day 5 or day 6.
Main results and the role of chance: Patient characteristics, oocyte number, oocyte maturity and fertilization rate were similar. Until day 3, developmental speed and embryo quality were similar. Overall blastocyst development up to day 6 was not different. However, significantly more embryos reached the full or expanded blastocyst stage after group compared to individual culture (type ≥3 according to the Gardner criteria) (37.4% versus 25.2%, respectively; $p = 0.0025$). On day 5 group culture also supported significantly higher grade inner cell mass and trophectoderm morphology than individual culture (ICM type A: 25.4%versus 12.6%, respectively; $p < 0:0001$) (TE type A: 21.3% versus 11.4%, respectively; $p = 0.0007$). This difference resulted in significantly more embryos available for replacement and cryopreservation after group compared to individual culture (52.% versus 39.1%, respectively; $p = 0.017$).
Limitations, reason for caution: Although the trend is clear, this intermediate analysis did not reach the assumed difference in blastocyst formation.
Wider implications of the findings: Our findings confirm the observations of improved blastocyst development after group culture by Ebner et al. (2010). Improved blastocyst formation possibly results in improved overall cycle efficiency leading to more pregnancies or births per oocyte collection cycle. At the same time, the micro-well culture dish allows individual monitoring of the embryos which is useful during time-lapse monitoring. Current findings suggest that routine implementation of group culture should be considered for extended embryo culture.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), media and dishes used in this study were provided by Vitrolife.
Trial registration number: NCT02050516

P-118 Spindle presence in metaphase-II oocytes and its relationship with blastocyst formation

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Study question: Is there a relationship between the presence of the spindle in metaphase-II (MII) oocytes and their potential to development into blastocyst in assisted reproductive technology (ART)?
Summary answer: There is no significant difference in fertilization and cleavage rates between the oocytes that had spindle visualisation compared to oocytes without. However, blastocyst rate is significantly higher in oocytes with visible spindle.
What is known already: The presence of meiotic spindle birefringence in human oocytes viewed by Polscope can predict a higher fertilization rate. However, the relative position of the spindle within the oocyte does not appear to influence the embryo quality on cleavage stage and the pregnancy rate.
Study design, size, duration: This is a retrospective study of 58 patients who had mature oocytes at retrieval time were selected at the MUHC reproductive center between January - December 2013.
Participants/materials, setting, methods: Oocytes were collected from stimulated cycles 35 hours after human chorionic gonadotropin (hCG) injection. Immediately before sperm injection, the MII oocytes were screened using Polscope to visualize the meiotic spindle and were grouped according to the presence or absence of spindle.
Main results and the role of chance: A total of 830 eggs were collected of which 641 were MII on retrieval day. 574 out of 641 (89%) had visible spindles seen with Polscope (Group 1) and 67 out of 641 (11%) had no visible spindle (Group 2). There was no significant difference in fertilization rate between the two groups (73% and 67%, $p = 0.31$). Additionally, similar results were found in regards to cleavage rates. A total of 219 (52%) out of 419 zygotes developed to the blastocyst stage in MII oocytes with visible spindle. However, MII with non-visible spindle had a significantly lower blastocyst rate (24.4%) when compared to those with visible spindle ($p < 0.001$).
Limitations, reason for caution: Results are based on a retrospective analysis. Clinical pregnancy and live birth rates are needed to validate this initial finding.
Wider implications of the findings: *In vivo* MII with visible spindle can be an important marker of selecting which oocyte has more potential to develop to blastocyst compared with those without visible spindle. Further studies should be conducted to compare different spindle positions and their effects on blastocyst formation rate.
Study funding/competing interest(s): Funding by hospital/clinic(s), MUHC Reproductive Center. McGill University Health Center.
Trial registration number: None.

P-119 Applicability of a microfluidic device to produce blastocysts in vitro: how close is embryo quality in the device to quality in vivo

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Study question: How do mouse blastocysts, individually cultured in a microfluidic device, compare to in vivo-developed blastocysts in the allocation of blastomeres to trophectoderm (TE), primitive endoderm (pEnd) and primitive ectoderm (pEct)?
Summary answer: Blastocysts cultured in microfluidic devices have a larger proportion of blastomeres that coexpress TE, pEnd, and pEct markers and thus are less advanced in differentiation, compared to in vivo-developed blastocysts.
What is known already: Mouse zygotes subjected to individual in vitro culture (IVC) are a model to mimic the clinical situation in human assisted reproduction (ART). Evidence that mouse blastocysts developing individually in microfluidic platforms are competent for full development has been obtained only recently in a single study. The cellular characterization of these blastocysts is limited and necessary, because fetal rates are known to correlate more with blastocysts' cell numbers than with blastocyst rates.
Study design, size, duration: In vivo-fertilized oocytes developed individually in static microfluidic system ($n = 152$) or in vivo ($n = 26$) or in two conventional IVC systems (microdrops 1X under oil, $n = 168$; group 20X culture, $n = 280$). Endpoints were rate of blastocyst formation, total cell number, and cell allocation to TE, pEnd and pEct on day 4.
Participants/materials, setting, methods: Embryos (B6C3F1xCD1) were scored for blastocyst formation. For these blastocysts the number of total, TE, pEnd, and pEct cells was determined by confocal immunofluorescence microscopy using antibodies targeting cell lineage specific markers (CDX2, SOX17, and NANOG). Image and statistical analysis were performed using ImageJ and chi square test, respectively.
Main results and the role of chance: Blastocysts cultured individually in nanoliter volumes attain developmental rates and cell numbers (70.4%; 36.7 ± 11.6 cells)

lower than blastocysts developed in vivo (91.2%; 59.7 ± 8.3). In-depth analysis within the same interval of cell number (30–60 cells) shows that cell allocation was 58.9% to TE, 9.7% to pEnd and 18.1% to pEct for nanoliter volumes, as compared to 71.4%, 11.0% and 12.9% for development in vivo (chi square test, p = 3.8E-04). A proportion of blastocyst's cells (microfluidic device, 12.3%; in vivo, 4.7%; chi square test, p = 7.3E-06) coexpressed TE, pEnd and pEct markers. These multi-marker cells are also observed in blastocysts produced in conventional IVC. Thus, the differentiation of the blastocyst may be slower in a microfluidic system than in vivo, as observed also in conventional IVC.
Limitations, reason for caution: This is an animal model study. The size and physiology of a naturally fertilized mouse embryo in a nanoliter chamber may differ from that of a human embryo, which is produced by IVF or ICSI.
Wider implications of the findings: While mouse blastocysts produced in a microfluidic device have been shown to be competent for full development after embryo transfer, details of their metabolic and functional features may differ from the in vivo situation. Pathways underlying cell lineage formation in mice and humans are also in part different. Delayed differentiation of the blastocyst's cells cultured in microfluidic devices may allow better adaptation after embryo transfer.
Study funding/competing interest(s): Funding by national/international organization(s), Deutsche Forschungsgemeinschaft (BO 2540/4-1), Nederlandse Organisatie voor Wetenschappelijk Onderzoek (63-258).
Trial registration number: Not applicable.

P-120 Ignorance is bliss - the importance of the cleavage stage morphology evaluation for blastocyst transfer in good prognosis patients

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Study question: (i) Is the embryo morphology evaluation at the cleavage-stage still needed for the selection of the embryo with the best implantation potential in extended embryo culture programmes? (ii) Is the transfer of low quality embryos on day-three a better approach than extended embryo culture and transfer in the blastocyst stage?
Summary answer: The embryo morphology evaluation at the cleavage stage is still needed for the selection of the embryo with the best implantation potential in extended embryo culture programmes. In addition, the transfer of low quality embryos on day-three is a better approach than transfer at the blastocyst stage.
What is known already: Serial observation of embryo morphology is a common technique to evaluate embryos and has been considered a key predictor of implantation and pregnancy. However, morphological assessments have limitations. This is a highly subjective method and to perform the assessment, embryos are removed briefly from the incubator, but due to concerns for the safety and stability of culture conditions, the observation of embryos outside the incubator should be avoided as much as possible.
Study design, size, duration: This retrospective observational study performed between January/2010 and July/2013 evaluated the influence of the embryo quality on days two and three on successful blastocyst formation and implantation. Moreover, a comparison was made between the implantation potential of low-quality embryos at the cleavage-stage that were transferred on day-three versus day-five.
Participants/materials, setting, methods: The study included 8444 embryos obtained from 1125 good prognosis patients evaluated at 16–18 h post-ICSI and on days two, three and, for extended culture, on day five of development. From the 8444 embryos evaluated, 2280 embryos were transferred on day-three and 6164 were cultured until day-five.
Main results and the role of chance: Low-quality embryos on day-two had an approximate 20% decreased chance of achieving the blastocyst-stage, and blastocysts derived from low-quality embryos on day-two had a nearly 40% decrease in the implantation chance. Low-quality embryos on day-three had a 30% decreased chance of achieving the blastocyst-stage, and blastocysts derived from low-quality embryos on day-three had an almost 40% decreased implantation chance. The implantation rate didn't differ when low-quality embryos on cleavage-stage were left in culture and transferred at the blastocyst or at the cleavage-stage (25.06% vs.20.39%, p = 0.320, for day-two; 26.25% vs.23.08%, p = 0.432, for day-three). Conversely, high-quality embryos on days-two or -three resulted in an increased implantation rate if left in culture and transferred at the blastocyst-stage rather than at the cleavage-stage. (22.54% vs.38.16%, p = 0.036, for day-two; 22.97% vs.41.18%, p = 0.035, for day-three).
Limitations, reason for caution: This is a retrospective observational study and therefore subjected to bias. A prospective randomized trial is needed to confirm our findings.
Wider implications of the findings: Here, we challenged the predictive value of the cleavage-stage embryo morphology in the blastocyst formation and implantation. It was hypothesized that embryo morphology evaluation on the cleavage-stage would be dismissed in extended embryo culture programs, for good prognosis patient. However, we demonstrated that the embryo morphology on cleavage-stage is an important indicator to predict blastocyst development and implantation. When the morphology is compromised at the cleavage-stage, the odds of blastocyst implantation may significantly decrease.
Study funding/competing interest(s): Funding by hospital/clinic(s), Fertility - Centro de Fertilização Assistida.
Trial registration number: Not applicable, due to the retrospective design of the study.

P-121 Closed oocyte vitrification in an egg donation program: from oocyte to live birth

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Study question: What is the efficiency of closed oocyte vitrification in terms of oocyte survival, embryo developmental capacity and clinical outcome in an egg donation program?
Summary answer: Our results demonstrate that closed oocyte vitrification is an efficient, safe and valuable alternative to open vitrification, with reassuring results from survival up to neonatal outcome.
What is known already: Most literature data regarding successful survival, embryological and clinical outcome of vitrified-warmed oocytes have been obtained using open vitrification devices. They report outcomes comparable to those using fresh oocytes. However, little information is available on the efficiency and safety of closed oocyte vitrification which avoids the use of sterilized liquid nitrogen and completely prevents cross-contamination.
Study design, size, duration: We performed a retrospective analysis on all oocyte warming cycles performed between March 2010 and May 2013. A total of 286 patients received 2602 vitrified-warmed oocytes in 372 recipient cycles. Outcome parameters were survival, developmental capacity, pregnancy outcome and neonatal outcome. The outcome of subsequent frozen-embryo transfers was also analyzed.
Participants/materials, setting, methods: All donated oocytes were vitrified using the CBS High Security Straws (CryoBioSystem). On average, 7.0 donor oocytes were warmed per recipient cycle. Surviving oocytes were injected and cultured in Sage cleavage media (Origio). Eighty-three single and 259 double embryo transfers were performed on day 3. Supernumerary embryos were vitrified.
Main results and the role of chance: Survival rate was 76.2% (1983/2602) and fertilization rate was 70.9% (1406/1983). On day 3, 43.5% top-quality and 23.8% good-quality embryos were obtained and an average of 1.8 embryos were transferred in 342 transfer cycles. Overall, 127 out of 601 transferred embryos implanted (21.1%), resulting in 90 live born children. The outcome of 10 pregnancies (with 14 implantations) is still unknown. Mean birth weight (±SD) of singletons and twins was 3143 g (±710) and 2437 g (±535), respectively. One congenital major malformation was observed. Out of 319 vitrified surplus embryos, 195 were warmed in 137 subsequent cycles. Thirty-four out of 169 transferred embryos implanted (20.1%) and 21 children are born. With still 124 vitrified embryos remaining, the current overall implantation per warmed oocyte is 6.2% (161/2602).
Limitations, reason for caution: Although our retrospective data demonstrate reassuring outcomes after closed oocyte vitrification, prospective

randomized trials are needed to compare the outcome of open and closed oocyte vitrification.
Wider implications of the findings: This present retrospective study demonstrates that closed oocyte vitrification is a valid alternative to the use of open devices, providing safe vitrification and banking of oocytes.
Study funding/competing interest(s): Funding by hospital/clinic(s), UZ Brussel, laarbeeklaan 101, 1090 Brussel, Belgium.
Trial registration number: NA.

P-122 Predictive factors of embryo implantation, either at cleavage or blastocyst stage, according to time-lapse analysis

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Study question: The aim of our study was to determine if morphokinetic parameters could predict embryo implantation, both at the cleavage stage or at the blastocyst stage.
Summary answer: Early morphokinetic parameters are predictive of cleavage-stage embryo implantation, whereas only few late parameters are predictive of blastocyst implantation.
What is known already: Before the onset of time-lapse technology, embryo selection for transfer was only based on conventional static morphological parameters, which have demonstrated relatively poor prediction interest for implantation. Some very recent studies reported the potential interest of time-lapse as a relevant tool for embryo selection for transfer, based on few early kinetic parameters, such as timing of 5cell stage, or duration of the 3cell stage.
Study design, size, duration: This monocentric retrospective study was conducted on all ICSI cycles performed between 2011 and 2013 with the Embryoscope®. All clinical, ovarian reserve, ovarian stimulation and embryonic parameters (conventional morphology and kinetic events in hours post-ICSI until day 6) were recorded. Only embryos with known implantation were considered in the analysis.
Participants/materials, setting, methods: This study was conducted in a public setting, in an unselected population. All ICSI cycles performed with the Embryoscope® were included, all oocytes/embryos being incubated in the Embryoscope® until transfer, either on day 3 or on day 5/6. Embryo transfer strategy was always established on day 1.
Main results and the role of chance: A total of 317 non implanted and 115 implanted cleavage-stage embryos were analysed. Univariate analysis demonstrated that t2, t3, t4, t5, t8 and s2 (t4-t3) were significantly lower in implanted embryos than in non implanted. Logistic regression showed that t4-t2 (OR = 0.79 [0.67–0.93]) and t5 (OR = 1.14 [1.04–1.33]), as well as smoking status, were independent and significant predictors of implantation.

115 non implanted and 54 implanted blastocysts were also analysed. Univariate analysis showed that trophectoderm morphology only tB were significantly different between the 2 groups. In implanted blastocysts, the frequency of blastocyst contractions was higher, whereas the proportion of blastocysts with major contractions was lower. Logistic regression demonstrated that only major contraction of the blastocyst (OR = 0.4 [0.13–0.9]) could independently predict implantation.
Limitations, reason for caution: This study was monocentric and retrospective.
Wider implications of the findings: The predictive interest of time-lapse analysis appear to depend on embryo transfer strategy, with apparent lower accuracy in blastocyst tranfers than in cleavage-stage transfers.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), University Hospital of Nantes, France.
Trial registration number: None.

P-123 Follicular fluid levels of anti-Mullerian hormone (AMH) as a predictor of oocyte maturation and embryonic development in modified natural cycle-IVF (MNC-IVF)

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Study question: To investigate follicular fluid (FF) concentrations of anti-Mullerian hormone (AMH) are associated with oocyte maturation and blastocyst rate in (MNC-IVF) patients.
Summary answer: This study suggests that there is an association between FF AMH levels and the blastocysts potential formation.
What is known already: AMH is a glycoprotein of the transforming growth factor-beta (TGF-β) super family and is a biomarker of ovarian reserve. AMH is produced by granulosa cells of preantral and early-antral follicles and is released in FF, where folliculogenesis and oocyte growth and development take place. Studying poor explored fluid environment, we could find a marker of oocyte maturation and quality, which correlates to obtain a good quality embryos.
Study design, size, duration: From May 2010 to August 2011, 145 patients undergoing the MNC-IVF were recruited to this prospective study. Number of MII and blastocyst were analyzed in the tree group according to FF AMH level - group A: <1.6, B: 1.7 - 5 and C: >5 ng/ml.
Participants/materials, setting, methods: FF was obtained at oocyte retrieval for intracytoplasmic sperm injection. AMH levels were tested using Gen II ELISA assay.
Main results and the role of chance: In the group A was analyzed 48 cycles (FF AMH level mean 0.9 ± 0.5 ng/ml), in which achieved 41 oocyte-corona-cumulus complex (OCCC), 31 MII (75.6%) and 9 blastocysts (29%). In the group B was analyzed 47 cycles (AMH level mean 2.02 ± 0.85 ng/ml) – 61 OCCC, 43 MII (70.5%) and 15 blastocysts (34.9%) and in the group C respectively 50 cycles (AMH level mean 11.55 ± 6.35 ng/ml), 97 OCCC, 62 MII (63.9%) and 25 blastocysts (40.3%). Significant differences was found between group C and A in respect to blastocyst number ($p < 0.05$).
Limitations, reason for caution: Because of differences in culture conditions between laboratories number of blastocysts obtained may be different.
Wider implications of the findings: This study suggests that there is an association between FF AMH levels and the blastocyst potential formation. This investigation requires more trial and further analysis to clearly determine the prognostic value of AMH in FF in oocyte maturation.
Study funding/competing interest(s): Funding by hospital/clinic(s), INVICTA Fertility and Reproductive Center, Gdansk, Poland.
Trial registration number: Not applicable.

P-124 Comparison of blastocyst culture outcome obtained in 180 ICSI cycles with standard or time-lapse incubation and with three different media systems

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Study question: We compared blastocyst formation rates (BFR) obtained with standard incubators, where only individual culture per patient were performed, or time-lapse incubators where maximum 6 patients were cultured. We also compared the outcome obtained with three different culture media (one single-step and two sequential media). Culture were performed at 37°C, 5% 02,6% CO_2.
Summary answer: There are no statistical differences in blastocyst formation performing individual cultures in standard incubators or cultures with time-lapse technology. The single step media allows to achieve a higher blastocyst formation rate than the sequential media. Anyway, clinical pregnancy and implantation rates are higher when a sequential medium is used.
What is known already: The time-lapse technology allows evaluating embryo development without interrupting the incubation. It is also possible to use two different culture strategies: sequential culture, where two media with different composition are used, or single-step culture, where the same medium is used for the whole culture until the blastocyst stage. There are few studies comparing standard with time-lapse incubators. Most of them use standard incubators with atmospheric O_2 as control.
Study design, size, duration: In this retrospective observational study, the outcomes of 180 performed from July to December 2013 were analyzed. Particularly, the BFR obtained with the two types of incubators and by culturing with different media systems were compared.
Participants/materials, setting, methods: Firstly, the BFR achieved with the time-lapse (106 cycles) or standard incubators (74 cycles) were compared. Then, the use of different culture media were compared (39 cycles in single-step medium, 56 and 85 cycles respectively in two different sequential media).
Main results and the role of chance: In standard incubators, 444 oocytes were injected, 302 fertilized (68.0%), 302 embryos and 128 blastocysts (42.4%) were obtained. In time-lapse incubators, 993 oocytes were injected, 744 fertilized

(74.9%, $p = 0.0071$), 744 embryos and 356 blastocysts (47.8%, NS) were obtained. Using single-step medium, a higher percentage ($p < 0.05$) of blastocysts was achieved (54.7%, 152 blastocysts out of 278 embryos) then using the two sequential media (134/323 = 41.5% and 198/431 = 45.9% respectively). Clinical pregnancy rates were 23.1% (3/14 transfers), 38.3% (23/60 transfers) and 60.0% (12/20 transfers) and implantation rates were 21.4% (3 sacs/14 blastocysts transferred), 34.6% (25 sacs/72 blastocysts transferred) and 57.1% (12 sacs/21 blastocysts transferred, $p < 0.05$ vs. single-step medium) respectively using single-step medium and the two sequential media.

Limitations, reason for caution: Preimplantation embryos are able to adapt to different culture conditions. Other factors, in addition to incubators and/or culture media, can influence embryo development. For example, some studies report better results performing group culture. Others important parameters, are the pH of the media and the percentages of gas.

Wider implications of the findings: In the present study, standard incubators with 5%O_2 were used for culturing embryos of one single patient up to the blastocyst stage. The ideal combination of culture media and incubation systems has to be developed. Although no difference appeared between time-lapse and standard incubators, time-lapse has the additional relevant advantage of allowing complete embryo observation and proper selection for transfer. For this reason, every laboratory has to develop its own set-up.

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 declared.

Trial registration number: Not applicable.

P-125 Antimetabolite cancer drug gemcitabine selectively impacts preantral and antral follicle cohorts in the ovary as evidenced by quantitatively histomorphometric, hormonal and cell viability markers

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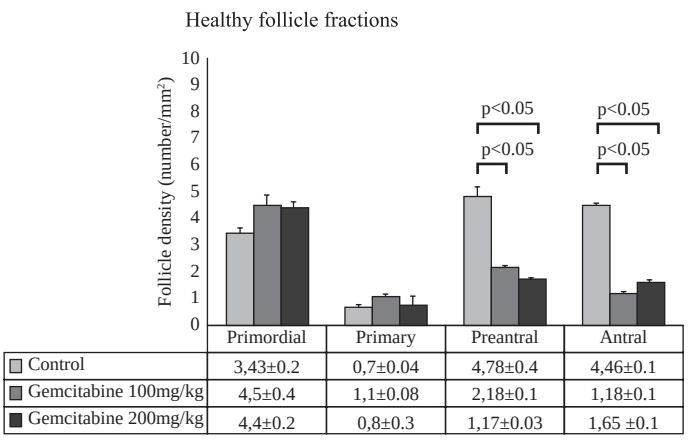
Study question: Does antimetabolite cancer drug gemcitabine (a pyrimidine analog) exert the same magnitude of cytotoxicity on dormant primordials and growing follicle fractions in the ovary?

Summary answer: No, gemcitabine selectively impacts preantral and antral follicle cohorts in the ovary and decreases serum AMH levels in the rat model. The drug also causes dose-dependent growth arrest and apoptosis of non-luteinizing mitotic granulosa cells in vitro, which resemble the growth characteristics of proliferating granulosa cells of preantral and early antral follicle cohorts, providing another evidence for its impact on growing follicle fraction. By contrast human luteal granulosa cells as an example of non-dividing granulosa cells are resistant to the cytotoxic effects of gemcitabine.

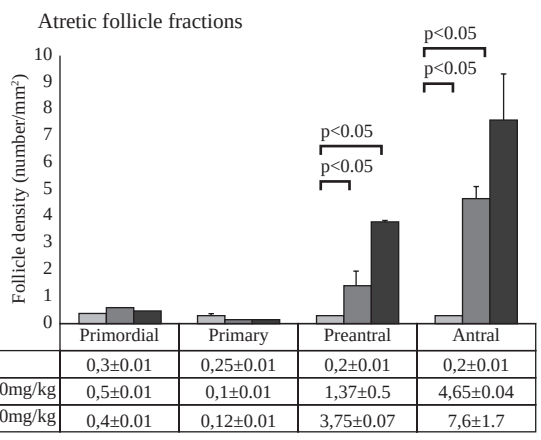
What is known already: It was already known that antimetabolite cancer drugs are less detrimental to the ovary than alkylating drugs. But no data is available either for gemcitabine or other antimetabolite drugs regarding the detailed analysis of their gonadotoxicity on the ovary. The question is unanswered as to whether gemcitabine induced ovarian toxicity is limited to follicles at a particular stage of development, or is equally toxic on entire subclasses of follicles.

P-125 – Figure 1

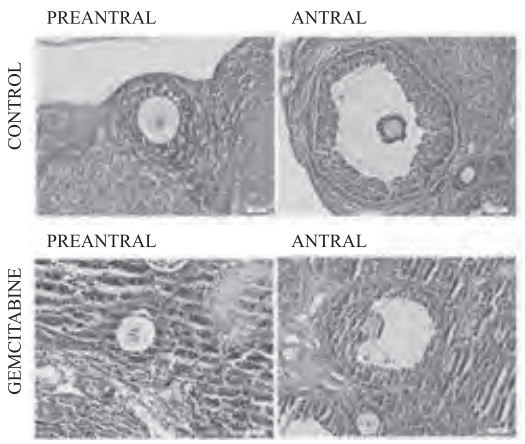
A

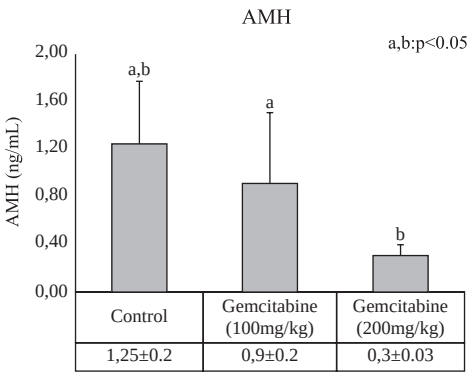


	Primordial	Primary	Preantral	Antral
Control	3,43±0.2	0,7±0.04	4,78±0.4	4,46±0.1
Gemcitabine 100mg/kg	4,5±0.4	1,1±0.08	2,18±0.1	1,18±0.1
Gemcitabine 200mg/kg	4,4±0.2	0,8±0.3	1,17±0.03	1,65 ±0.1



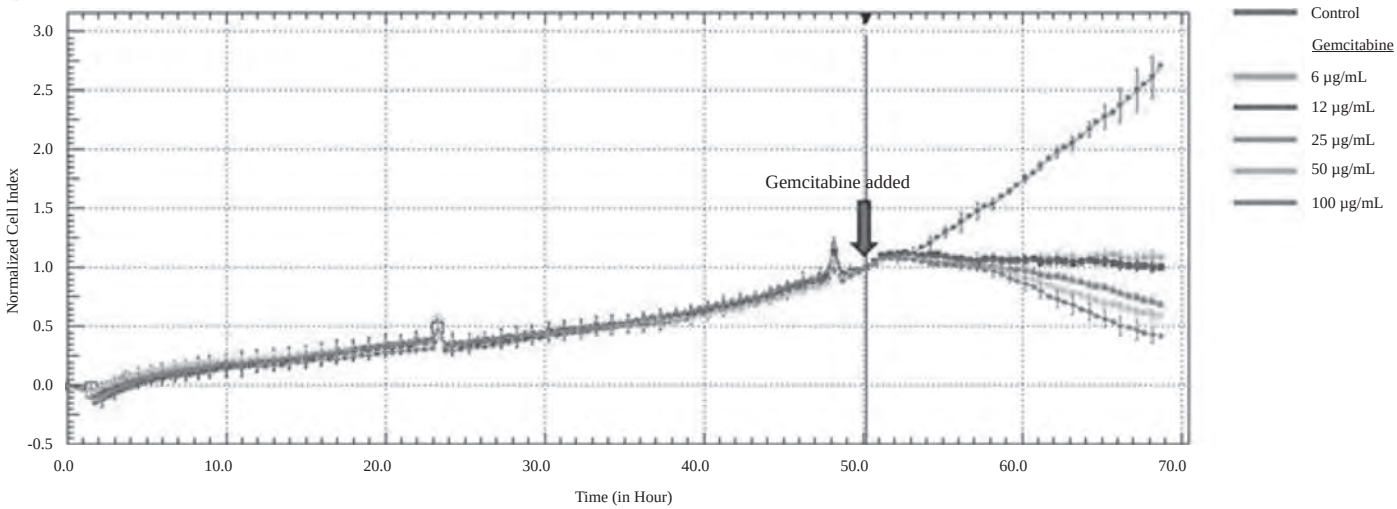
	Primordial	Primary	Preantral	Antral
Control	0,3±0.01	0,25±0.01	0,2±0.01	0,2±0.01
Gemcitabine 100mg/kg	0,5±0.01	0,1±0.01	1,37±0.5	4,65±0.04
Gemcitabine 200mg/kg	0,4±0.01	0,12±0.01	3,75±0.07	7,6±1.7

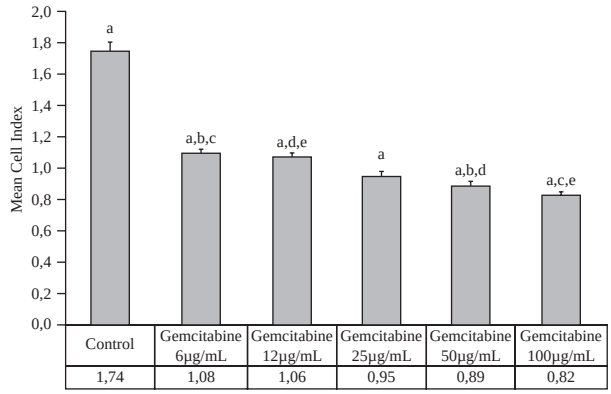




Control	Gemcitabine (100mg/kg)	Gemcitabine (200mg/kg)
1,25±0.2	0,9±0.2	0,3±0.03

B





Control	Gemcitabine 6µg/mL	Gemcitabine 12µg/mL	Gemcitabine 25µg/mL	Gemcitabine 50µg/mL	Gemcitabine 100µg/mL
1,74	1,08	1,06	0,95	0,89	0,82

a:p<0.0001
Control vs. Gemcitabine subgroups

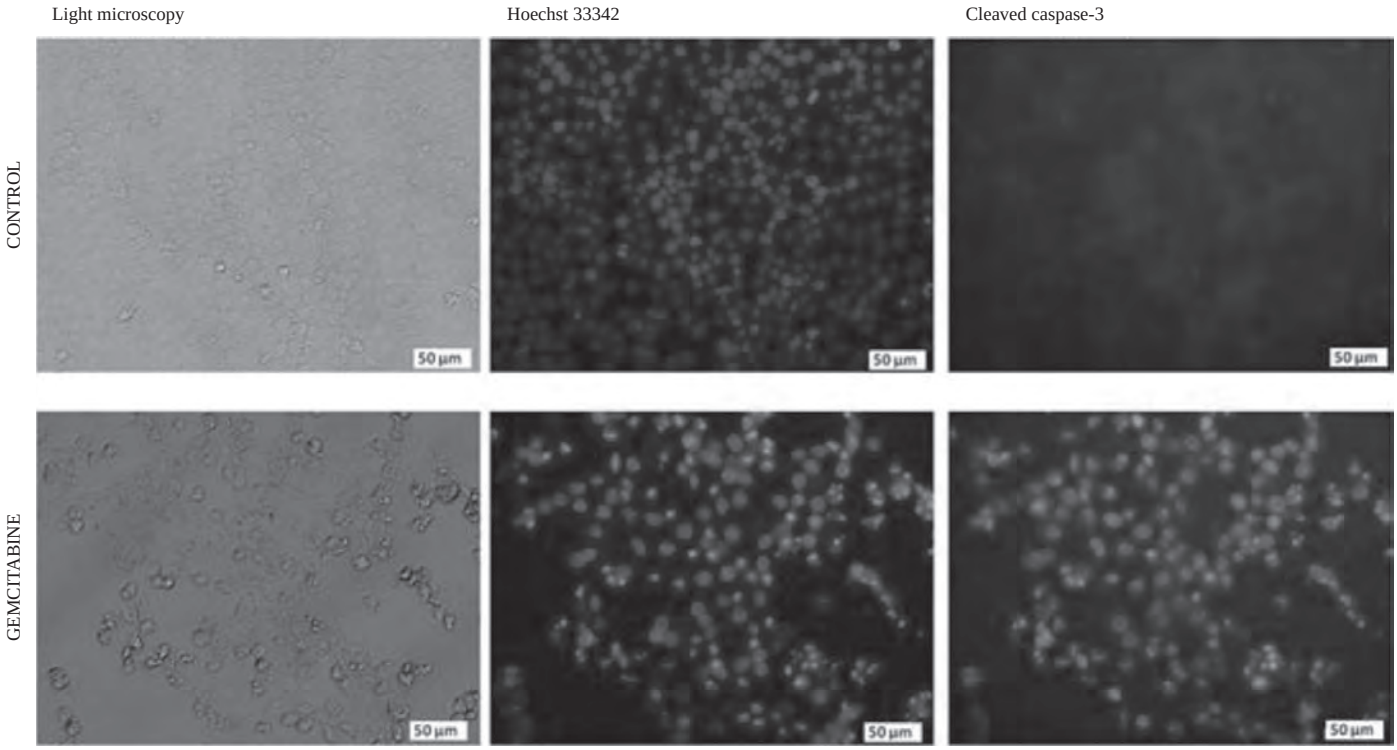
b:p<0.0001
Gemcitabine (6µg/mL vs. 50µg/mL)

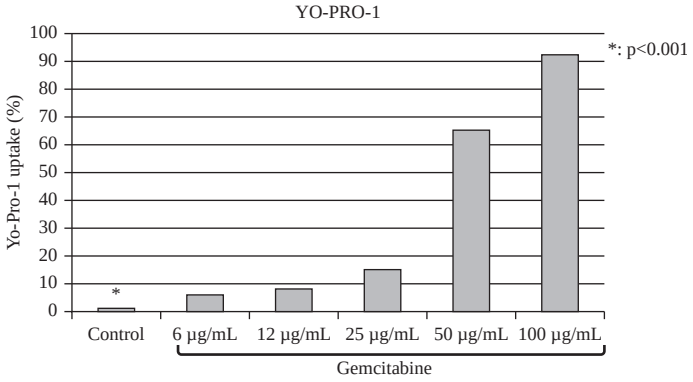
c:p<0.0001
Gemcitabine (6µg/mL vs. 100µg/mL)

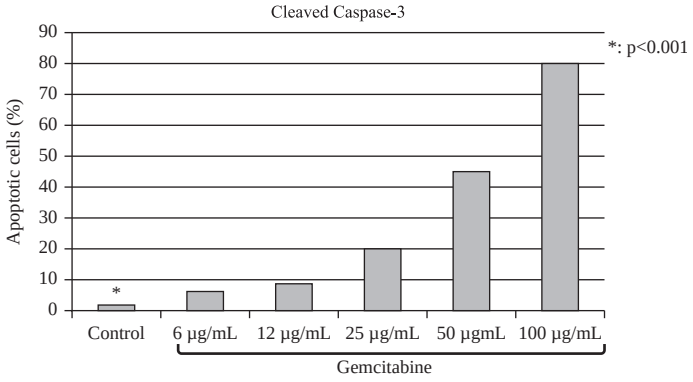
d:p<0.05
Gemcitabine (12µg/mL vs. 50µg/mL)

e:p<0.0001
Gemcitabine (12µg/mL vs. 100µg/mL)

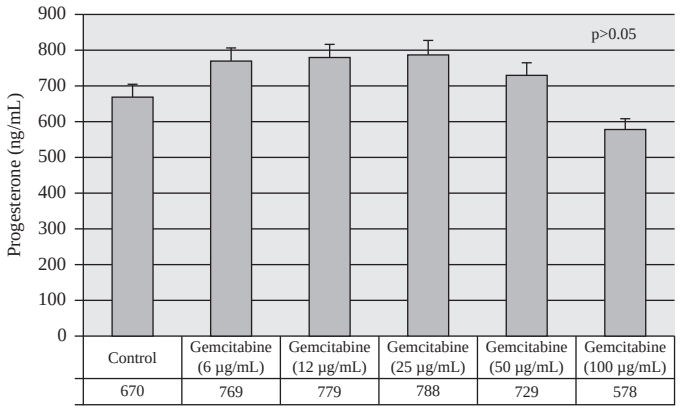
C

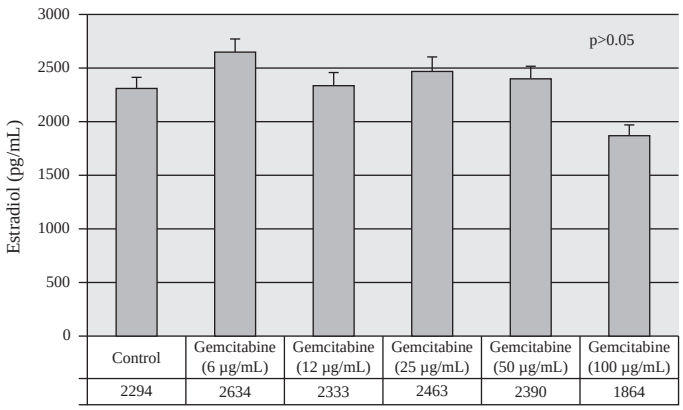


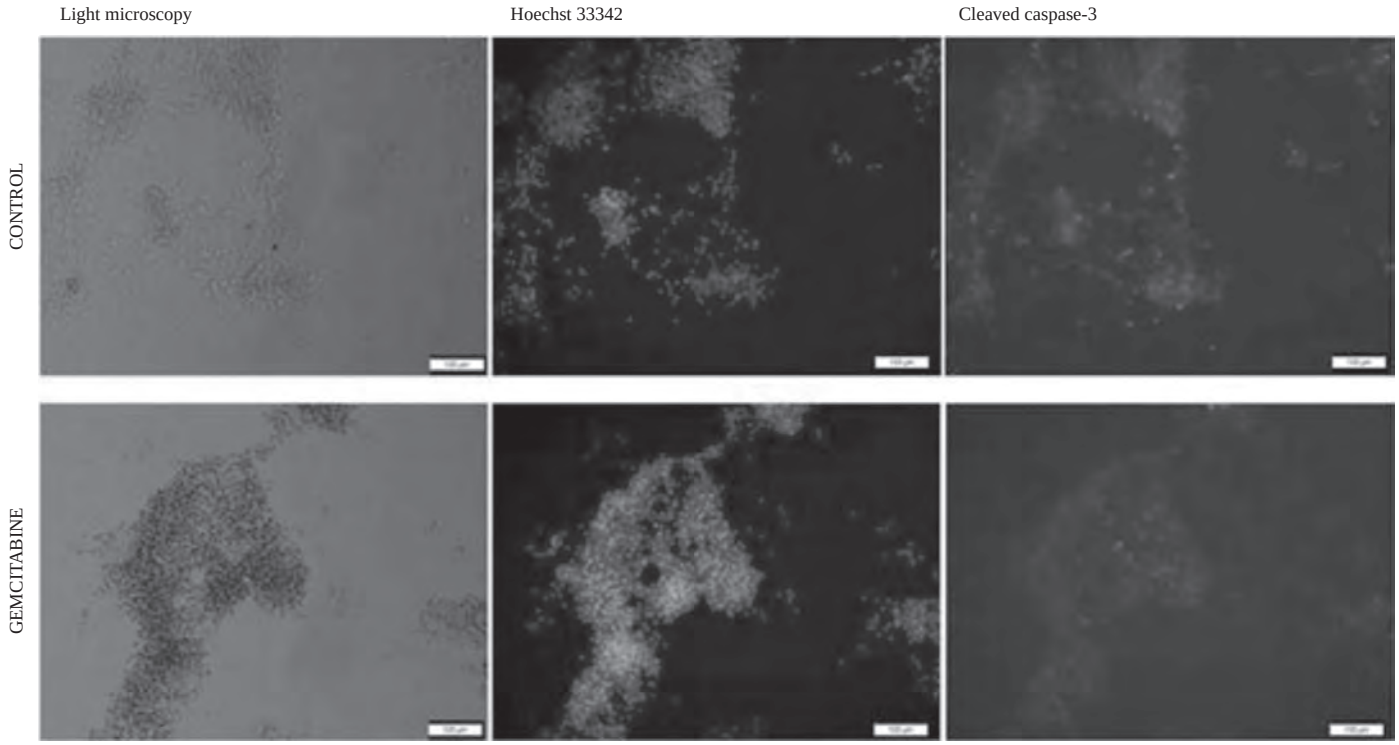


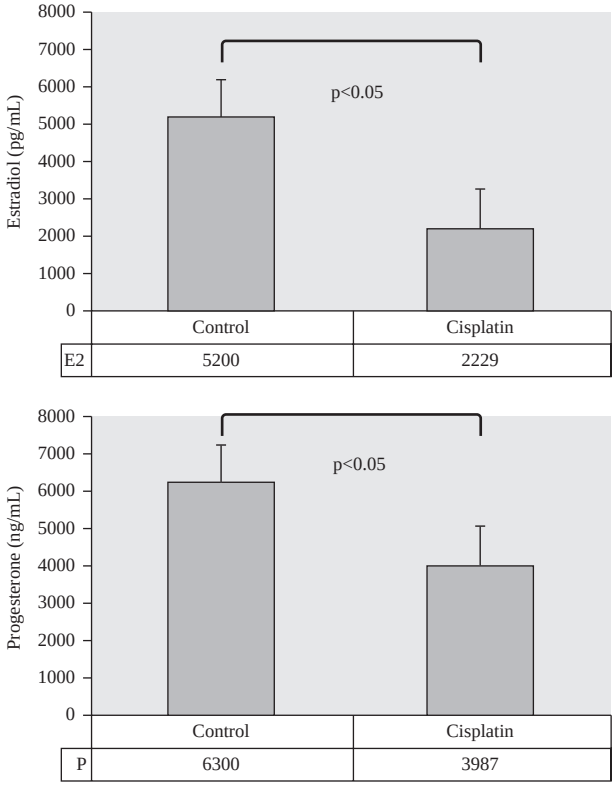


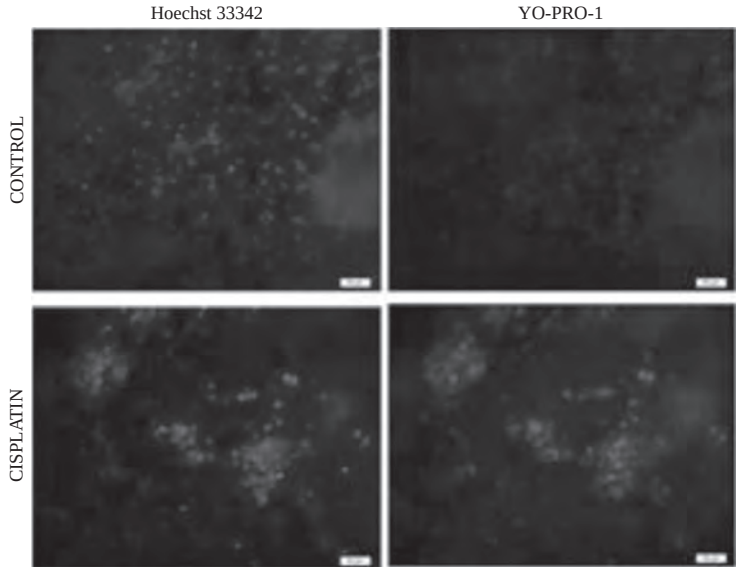
P-125 – Figure 2











To address these questions we designed a joint study involving both in vivo and in vitro ovary and granulosa cell models of human and rat origin to analyze the impact of gemcitabine on the ovary using quantitative histomorphometric, hormonal and cell death/viability markers.

Study design, size, duration: A cross-sectional joint study involving in vivo and in vitro models of human and rat origin.

Participants/materials, setting, methods: Fifty-four 4–6 week old Sprague-Dawley young female rats were either given saline injections only (control) or single dose of gemcitabine at 100 and 200mg/kg doses intraperitoneally. Healthy and atretic fractions of primordial, primary, preantral and antral follicles were determined and antimullerian hormone (AMH) levels were measured 72 hrs post-exposure. Immortalized rat granulosa cells (SIGC) and human luteal granulosa cells (HLGC) were used to analyze the cytotoxicity of gemcitabine in vitro. Gemcitabine-induced cytotoxicity in cultured granulosa cells was monitored in a real-time and quantitative manner using xCelligence system for up to 70 hrs. Live/dead cell and apoptosis assays were also carried out using intravital Yo-Pro-1 staining and cleaved caspase-3 expression, respectively. HLGCs exposed to gemcitabine in vitro were also assayed for their estrogen and progesterone production.

Main results and the role of chance: Primordial and primary follicle counts were comparable in control animals and those treated with gemcitabine. However, the number of healthy preantral and antral follicles were significantly lower, and the atretic preantral and antral follicles were significantly higher in the ovaries compared to control animals. Accordingly, AMH levels in the animals treated with gemcitabine were significantly lower than controls (Fig-1A). Mitotic granulosa cells (SIGC) exposed to gemcitabine in-vitro first exhibited growth arrest and then underwent apoptosis in a dose-dependent fashion as evidenced by their growth curves, increased Yo-Pro-1 uptake and higher cleaved caspase-3 expression (Fig-1B and 1C). But non-dividing granulosa cells (HLGCs) treated with and without gemcitabine were similar in terms of live/dead cell index, apoptosis and in vitro hormone production, indicative of resistance to cytotoxic effects of gemcitabine. When HLGCs were treated with a more toxic chemotherapy drug such as cisplatin, increased apoptosis and decreased hormone production was observed in these cells (Fig-2). These results strongly suggest that antimetabolite cancer drug gemcitabine preferentially targets growing follicles and mitotic granulosa cells and that non-dividing granulosa cells are resistant to its cytotoxic effects. The absence of such findings in controls and dose-dependent toxicity of gemcitabine practically rule out the possibility that these observations occurred by chance.

Limitations, reason for caution: None

Wider implications of the findings: This data can be useful when counseling female cancer patients who will receive gemcitabine-based chemotherapy regimens and have concern about their fertility. Developed methodology can be extended to other drugs.

Study funding/competing interest(s): Funding by University(ies), Koc University School of Medicine.

Trial registration number: None.

P-126 Assessment of oocyte activation capability of sperm using live-cell imaging and the rescue of non-activated embryos after ICSI by artificial activation

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Study question: What is happening in gametes which are not activated after insemination by ICSI?

Summary answer: The female chromosomes were arrested at meiosis II. However, the male chromatin was dispersed in the cytoplasm. Live cell imaging

of mouse oocytes injected with husband's sperm showed that many of them were also not activated after ICSI with dispersal of the male chromatin being observed from 4 h after ICSI.
What is known already: ICSI is a powerful technique in the field of human-assisted reproduction and improves the outcomes for patients who cannot achieve fertilization by routine IVF. However, there are reports of cases where fertilization as evidenced by the appearance of two pronuclei (2PN) is not seen after ICSI (nonfertilized, NF). Failure of pronuclear formation can be caused by defective oocytes or from a deficiency in the spermatozoons' ability to activate the oocyte.
Study design, size, duration: In this study, we examined the oocyte-activating ability and dynamics of parental chromosomes among couples with a tendency to show NF after ICSI and who had never obtained 2PN embryos.
Participants/materials, setting, methods: NF oocytes were obtained from couples with a history of NF after obtaining their informed consent. The oocytes were stained with an anti-dimethylated lysine 9 on histone H3 antibody to detect the female chromosomes. To assess the oocyte-activating ability of the husband's spermatozoa, they were microinjected into mouse oocytes and chromosomal dynamics during the first mitosis were tracked using a live cell imaging system. KSOM with 5 mM $SrCl_2$ and 2 mM EGTA were used for artificial activation of the mouse oocytes. Ethics Committee approval was obtained for the study.
Main results and the role of chance: Immunofluorescence staining of human NF oocytes after ICSI showed that the female chromosomes were arrested at meiosis II. However, the male chromatin was dispersed in the cytoplasm. Live cell imaging of mouse oocytes injected with the husband's spermatozoa showed that many of them were also not activated after ICSI and with dispersal of the male chromatin observed from 4 h after ICSI. However, when the mouse oocytes subjected to ICSI were artificially activated within 4 h of ICSI, the rate of 2PN formation increased compared with the non-activated group.
Limitations, reason for caution: The number of samples for this study was relatively small. Nevertheless, this is the first study to assess the dynamics of chromosome in non-activation embryos after ICSI using live-cell imaging system.
Wider implications of the findings: These results suggest that the ability of the husband's spermatozoa to activate oocytes is low in cases with a history of producing NF oocytes. In addition, it appears necessary to activate oocytes after ICSI to prevent dispersal of the male chromatin. Artificial activation of the oocytes at an appropriate time after ICSI might be an effective technique for initiating embryo development in such cases.
Study funding/competing interest(s): Funding by hospital/clinic(s), Asada Ladies Clinic.
Trial registration number: None.

P-127 Utility of elective single frozen embryo-transfer (eSFET)

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Study question: Does elective single frozen embryo transfer (eSFET) decrease the risk of multiple pregnancy without reducing the accumulative live birth rate?
Summary answer: Our results show that eSFET contributes to reducing multiple pregnancies without affecting the cumulative live birth rate in a selected population.
What is known already: FET cycles have increased significantly due to the increased effectiveness of embryo vitrification, and there has been a corresponding increase in multiple pregnancies following cryotransfer. The reduced rate of multiple pregnancies achieved by fresh eSET programmes for good-prognosis patients is partially offset after cryotransfer when two embryo are transferred (DFET)
Study design, size, duration: Retrospective study carried out from January 2010 to February 2013 at the Virgen de las Nieves University Hospital (Granada, Spain) of 158 cryotransfers performed on good-prognosis couples (women aged under 38 years, first cycle, BMI 18.5–29 kg/m², no repeat miscarriages, no previous surgery, no uterine malformations).
Participants/materials, setting, methods: We analysed the FET results for women who had not become pregnant in their fresh embryo transfer and who had at least two class A/B vitrified embryos. The following study groups were established: DFET (n = 75), non-eSFET (n = 48), eSFET (n = 35). The latter group was comprised of 35 single-embryo cryotransfers belonging to 21 patients with individually vitrified embryos.
Main results and the role of chance: No significant differences were observed in epidemiological variables, protocol, response to stimulation or laboratory parameters. The implantation rate was similar in the three groups (26.0%, 21.3% and 25.7% for DFET, non-eSFET and eSFET, respectively). There were no significant differences in the cumulative clinical pregnancy rate (43.0% eSFET vs. 41.3% DFET). The rate of multiple pregnancies was dramatically lower in the eSFET group than in the DFET group (0% vs. 25.8%). There was no statistically significant difference in the cumulative live birth rate between eSFET and DFET (33.3% vs. 32.0%). The rate of embryonic survival was similar in the three groups (96.0% DFET, 90.6% non-eSFET, 92.1% eSFET). With respect to the number of cleaved embryos, there were no significant differences among the three groups.
Limitations, reason for caution: No culture to blastocyst stage was performed. We believe the incorporation of these protocols would significantly improve the performance of eSFET programmes.
Wider implications of the findings: The increased utility of eSFET suggests that eSET policies should be generalized to FET cycles. To introduce eSET programmes it is essential to plan previously the number of embryos in each device, specially in good prognosis women. This study may be generalised to IVF units with experience in eSET and vitrification.
Study funding/competing interest(s): Funding by hospital/clinic(s), Hospital Universitario Virgen de las Nieves de Granada.
Trial registration number: Does not apply.

P-128 Oocytes affected by smooth endoplasmic reticulum aggregates can produce healthy babies

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Study question: Does the presence of Smooth Endoplasmic Reticulum aggregates (SERa) in oocytes affect clinical and neonatal outcomes?
Summary answer: Six healthy babies were born from SERa+ oocytes and eleven from SERa+ cycles.
What is known already: Several reports, focusing either on SERa+ cycles or SERa+ oocytes, have shown negative results in terms of fertilization, embryo development and pregnancy rates as well as compromised obstetrical and neonatal outcomes. A recent publication showed however, that healthy babies can be born from embryos originating from SERa+ oocytes.
Study design, size, duration: A prospective observational study including 402 ICSI cycles and 72 SERa+ ICSI cycles was performed during 2012.
Participants/materials, setting, methods: Comparisons of fertilization rates, embryo quality, pregnancy and neonatal outcomes were performed between SERa+/SERa− cycles and oocytes.
Main results and the role of chance: 70 out of 472 ICSI cycles (14.8%) showed at least one SERa+ oocyte. Amongst these positive cycles, 108 out of 420 oocytes (25.7%) were affected. No significant differences in embryological, clinical or neonatal outcomes were found when comparing SERa+ and SERa− cycles. Moreover, 11 healthy babies originating from these SERa+ cycles were born. For SERa+ oocytes, fertilization and embryo quality were not affected by the presence of the aggregates. We avoided the transfer of SERa+ embryos when possible. For ten fresh transfers, with mainly good quality embryos, only one pregnancy was obtained and resulted in a healthy new-born. An additional two healthy births occurred from cryopreserved cycles involving SERa+ embryos. Altogether, 6 healthy babies have been born in our center from SERa+ oocytes.
Limitations, reason for caution: Our study is small and a bias was introduced since SERa+ embryos were only transferred when no other embryo or embryo of good quality was available.
Wider implications of the findings: The birth of healthy babies from SERa+ oocytes is encouraging. Data in the literature is scarce and conflicting. The current consensus established by ESHRE and Alpha on embryo assessment might be revised in the future if larger studies confirm these findings. In the meanwhile transfers of SERa+ embryos should be done with caution.

Study funding/competing interest(s): Funding by University(ies), CHU St Pierre.
Trial registration number: None.

P-129 Predictive factors of good quality blastocyst formation according to time-lapse analysis

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Study question: The aim of the study was to assess whether early morphokinetic parameters obtained with a time-lapse device could provide relevant information for the prediction of good quality blastocyst formation.
Summary answer: We confirm that the early morphokinetic parameters are predictive of good quality blastocyst formation, even in an unselected population undergoing ICSI cycles.
What is known already: Several recent studies have addressed the relevance of time-lapse technology in the selection of embryos with good developmental competence. Significantly different cleavage patterns and morphokinetic parameters have been reported between embryos that reached the blastocyst stage and those who arrested their development before the blastocyst stage or who lead to a poor quality blastocyst. In summary, embryos cleaving earlier have a significantly higher chance of achieving optimal blastocyst stage (Meseguer et al., 2013).
Study design, size, duration: This retrospective observational study was conducted on all ICSI cycles performed between 2011 and 2013 with the EmbryoScope® in an unselected population. All clinical, ovarian reserve, ovarian stimulation and embryonic parameters (conventional morphology and kinetic events) post injection were recorded until day 6.
Participants/materials, setting, methods: This monocentic retrospective study was conducted on 162 couples undergoing ICSI cycles who were scheduled for blastocyst transfer. All oocytes/embryos were cultured in the EmbryoScope®. There were no exclusion criteria. A total of 1740 cleaved embryos were recorded. Only blastocysts with ≥B2 stage and A/B trophectoderm were transferred/frozen.
Main results and the role of chance: Among the 1740 cleaved embryos analyzed, 355 reached Good Quality Blastocyst (GQB) stage on day 5 or 6 and were transferred or frozen, whereas 1385 were discarded (D). Basal and ovarian response characteristics did not differ between women with at least 1 good quality blastocyst and those with none. Almost all kinetic parameters were significantly different between GQB and D embryos, with t2, t3, t4 and t8 occurring earlier in GQB than in D embryos. Duration of second cell cycle (Cc2 = t3–t2), synchrony of second cell division (s2 = t4–t3) and cell evenness at the 2 and 4 cell stages were also significantly different between the 2 groups. Only t5 was not significantly different between the 2 groups.
Limitations, reason for caution: This study was retrospective and monocentric. Blastocyst transfer policy was advised to almost any couple undergoing ICSI cycle, thus resulting in a heterogeneous population, with patients with various prognoses.
Wider implications of the findings: We report that early morphokinetic parameters are predictive of good quality blastocyst formation, even in an unselected population. Time lapse analysis seems to be a useful tool for the selection of embryos with higher developmental potential, and might be help in implementing blastocyst transfer policy.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), University Hospital of Nantes, Nantes, France.
Trial registration number: None.

P-130 Temperature stability is improved by introducing a novel air warming system during extended micromanipulation procedures

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Study question: Can gamete and embryo temperature stability be improved during extended micromanipulation procedures by the introduction of a novel air warming system (AWS)?
Summary answer: An AWS provides improved temperature stability both over time and when changing microscope objectives during micromanipulation procedures. These improvements were shown when compared to a range of commercially available alternatives.
What is known already: Micromanipulation workstations are routinely used to perform extended laboratory procedures such as intra-cytoplasmic sperm injection (ICSI), intra-cytoplasmic morphologically selected sperm injection (IMSI) and embryo biopsy. Temperature fluctuations during such procedures have been demonstrated to have deleterious effects on meiotic spindle integrity which in turn impacts on fertilisation and subsequent clinical pregnancy rates.
Study design, size, duration: Standard ICSI dishes were prepared and temperature readings performed in 5 µl culture droplets directly above the microscope objective immediately and every 5 min thereafter for 1 h on 3 different micromanipulation workstations. Tests were carried out on each objective lens and were repeated 5 times for each relevant objective.
Participants/materials, setting, methods: Temperature stability comparison of 3 micromanipulation workstations: Workstation 1 (WS1): Metal heated stage with aperture. Workstation 2 (WS2): Glass heated stage without aperture. Workstation 3 (WS3): Metal heated stage with aperture and AWS. On relevant procedural objective lenses; ×20, ×40, ×60 and ×40 laser.
Main results and the role of chance: *Comparison of temperature stability over time*. The temperature stability over the 1 h test period was lowest on WS1 for all objectives (×20 = 33.2 ± **0.9**; ×40 = 33.3 ± **1.1**; ×60 = 33.1 ± **1.0**). Both WS2 and WS3 demonstrated improved temperature stability for all 4 objectives (WS2: ×20 = 36.7 ± **0.4**; ×40 = 35.4 ± **0.2**; ×60 = 36.2 ± **0.4**; laser = 36.4 ± **0.3** and WS3: ×20 = 36.0 ± **0.2**; ×40 = 36.1 ± **0.3**; ×60 = 36.3 ± **0.4**; laser = 36.0 ± **0.2**). *Comparison of mean objective temperatures on the same workstation.* Mean droplet temperature did not differ significantly between the objective lenses on WS1 and WS3. A significant difference was observed on WS2 ($p < 0.0001$) where the ×40 read 1.3°C lower than the ×20, 0.8°C lower than the ×60 and 1.0°C lower than the laser objective; further to this the ×60 objective read 0.5°C lower than the ×20 objective.
Limitations, reason for caution: The study suggests that culture drops are susceptible to significant changes in temperature caused by different working objectives when using a glass heated stage (WS2). The changes in temperature appear to be related to variables such as objective mass and the working distance between the objective and the dish.
Wider implications of the findings: An AWS provides improved culture dish temperature stability over extended periods of time and is not significantly affected by the choice of working objective. When compared to alternative methods of temperature control currently available, the AWS provides a consistent and optimised temperature during micromanipulation procedures; this may help maintain gamete and embryo integrity and result in increased clinical pregnancy rates.
Study funding/competing interest(s): Funding by hospital/clinic(s), Cambridge IVF, Cambridge University Hospitals NHS Foundation Trust.
Trial registration number: Not applicable.

P-131 Single versus double blastocyst embryo transfer

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Study question: Does the transfer of one or two blastocysts affect pregnancy rate?
Summary answer: The pregnancy rate of single blastocyst transfer is similar to double blastocyst embryo transfer.
What is known already: With the development of assisted reproduction techniques, the pregnancy and implantation rates have increased. However with this higher implantation rate; there is also an important increase in multiple births rate. However, multiple pregnancy is considered a high-risk for the woman and for the babies. The most effective way to avoid twins is to transfer only one embryo.
Study design, size, duration: Retrospective cohort study with 416 transfers during 2011–2013.
Participants/materials, setting, methods: The patients were matched to two different groups. In group 1, 86 patients had only one blastocyst transferred (SET), while group 2 with 330 patients had 2 blastocysts transferred (DET). In all cycles the best embryos were transferred and the remaining blastocysts embryos were frozen. The averages age were 33.6 and 33.9 years for groups 1 and 2, respectively. The pregnancy and children born were compared between the groups. The results were compared using t test ($P < 0.05$).

Main results and the role of chance: In group 1 (SET) it was observed a pregnancy rate of 50% (43/86), with 43% (37/86) clinical pregnancies. Out of the clinical pregnancy there were 6 miscarriages, 20 ongoing pregnancy and 11 newborn. In the group 2 (DET) there were a pregnancy rate of 53.6% (177/330) from these 45.4% (150/330) were clinical pregnancies. Out of the clinical pregnancies there were 25 miscarriages, 1 ectopic pregnancy, 41 ongoing and 83 newborn. There were no statistical differences between the groups ($p > 0.05$).
Limitations, reason for caution: The study was conducted with a small number of patients.
Wider implications of the findings: Our data showed that there was no difference in pregnancy rate when transferred one or two blastocyst in women with the average age of 33 years old. Therefore when there is one good quality blastocyst and the patients are young, it is better to transfer only one blastocyst, in order to decrease the chance of twins.
Study funding/competing interest(s): Funding by hospital/clinic(s), Fertilitat – Centro de Medicina Reprodutiva.
Trial registration number: Not available.

P-132 Proteome, survival rate and developmental potential of control and vitrified mouse oocytes matured with or without a glutathione donor

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Study question: Does supplementation with glutathione ethyl ester (GEE), a cell permeable glutathione donor, during *in vitro* maturation (IVM) affect the oocyte proteome, the survival rate after CryoTop vitrification at metaphase II stage, and the developmental capacity?
Summary answer: GEE supplementation during IVM alters the mitochondrial redox-potential but the proteome is neither affected by GEE nor by vitrification as shown by high resolution 2D-DIGE-saturation-labeling. However, GEE supplementation increases significantly the activation rate and the developmental capacity to the blastocyst stage of embryos cultured in low (5%) oxygen.
What is known already: Glutathione (GSH), the major non-enzymatic antioxidant involved in maintaining the intracellular redox status, is decreased in IVM oocytes. Oocytes with low GSH have reduced developmental competence. We showed recently that supplementation of culture medium with 1 mM GEE during IVM supports spindle recovery and chromosome alignment after Cryotop vitrification, potentially by accelerating the recovery of intrinsic redox-homeostasis after vitrification.
Study design, size, duration: Denuded GV oocytes from F1 C57/Bl6xCBA/Ca mice were matured with or without 1 mM GEE. MII oocytes were CryoTop vitrified. Proteome patterns of MII oocytes were analyzed by 2D-DIGE-saturation-labeling. ATP content, survival and developmental rate to the blastocyst stage were assessed prior to and after vitrification and parthenogenetic activation, respectively.
Participants/materials, setting, methods: Oocytes were matured in M2 without or with 1 mM GEE. Proteome patterns were analyzed by 2D-DIGE-saturation-labeling. ATP was determined with bioluminescent-somatic-cell-assay-kit (Sigma-Aldrich). Parthenogenetic activation was performed with 5 mM SrCl2, 2 mM EGTA and 4 µg/ml cytochalasin B. Embryos were cultured in KSOM (5% CO_2, 5% O_2) until the blastocyst stage.
Main results and the role of chance: IVM with 1 mM GEE increased slightly the MII rate. ATP content was not significantly affected by GEE. Gel matching by DeCyder 7.0 software algorithm detected consistently 1492 spots after 2D-DIGE-saturation-analysis. The quantitative evaluation using a stringent false discovery rate revealed no significant protein abundance differences between the IVM groups. There was also no significant difference between the vitrified and control groups underlining the efficiency of CryoTop vitrification. GEE during IVM improved significantly the 2-cell embryo rate. There was a slight increase in the blastocyst rate as well as in the blastocyst diameter in the GEE supplemented groups, suggesting an improved development by GEE.
Limitations, reason for caution: Sample size must be increased to confirm the improved development by GEE. Concerning the proteome after ZGA and redox-homeostasis in embryos of vitrified and GEE supplemented groups, methods to study protein expression and intra-mitochondrial-redox-potential expressing a redox-sensitive reporter protein (Mito-Grx1-roGFP2) are established and extended to all groups.
Wider implications of the findings: GEE improves the redox capacity and protects mouse oocytes from spindle aberrations and chromosome misalignment after vitrification. This study indicates that GEE improves also the activation rate and the developmental competence. Supplementation with GEE prior to vitrification may therefore also boost redox capacity as well as prevent spindle and chromosome alterations by cryo-preservation in human oocytes, especially those of lower quality possessing low stress resistance e.g. from aged patients or IVM, thus improving outcomes.
Study funding/competing interest(s): Funding by national/international organization(s), The study was supported by the German Research Foundation (DFG; FOR 1041; 'Germ cell potential'). Authors declare no conflict of interest.
Trial registration number: N/A.

P-133 Low oxygen tension may increase implantation potential by enhanced expression of LIFR and antioxidant related genes MnSOD and PRDX5 in mouse blastocyst cultured *in vitro*

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Study question: Does low O_2 tension affect the embryo development, quality and gene expression profile cultured *in vitro*?
Summary answer: 3% O_2 tension culture system provides a more conducive environment by increased antioxidant effect (MnSOD and PRDX5), enhanced embryonic glycolysis (GLUT-3), increased angiogenesis (VEGF) and induced adhesion (LIFR) by up regulation of related genes during preimplantation period.
What is known already: In human IVF, the embryos cultured in lower O_2 tension (5%) can give rise to the higher success rates, when compared with nomoxic condition (20%). However the beneficial effects of reduced oxygen tension in embryogenesis remain unclear.
Study design, size, duration: A retrospective experimental animal study in a university hospital.
Participants/materials, setting, methods: The 2-cell embryos were cultured to blastocyst stage under 3% O_2 ($n = 330$) or 20% O_2 ($n = 317$) tension. Real-time PCR were performed to investigate the expression of oxygen-related genes (HIF-1α, HIF-2α, GLUT-3 and VEGF) and antioxidant related genes (MnSOD and PRDX5) in blastocysts from each group. Protein expression levels were validated by Immunofluorescence analysis.
Main results and the role of chance: The blastocyst formation rate was not affected by oxygen tension. However the hatching rate was significantly increased in 3% O_2 group compared to 20% O_2 group ($P < 0.05$). Expression of GLUT-3 and VEGF was increased 4.14 and 7.99-fold, respectively in 3% O_2 group ($P < 0.05$). although HIF-1α and HIF-2α mRNA level was similar in two groups, immunofluorescence staining showed the intensity of MnSOD, LIFR was higher in 3% O_2 than 20% O_2 group, respectively. Apoptotic index was significant increase in 20% O_2, compared with 3% O_2 group ($P < 0.05$).
Limitations, reason for caution: Our study was based in mouse model and further studies on implantation or birth rate are required to confirm our findings.
Wider implications of the findings: This study provided new evidence of a beneficial effect of 3% oxygen tension in embryonic hatching; it may assist in developing best culture condition for IVF.
Study funding/competing interest(s): Funding by University(ies), Taipei Medical University.
Trial registration number: NA.

P-134 Are late morphokinetic parameters useful in identifying the blastocysts within a cohort that are most likely to implant?

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Study question: Early embryo development kinetics can help to predict blastocyst formation as well as pregnancy. Can late morphokinetic parameters also be useful in identifying the blastocysts within a cohort most likely to implant?
Summary answer: Late kinetic parameters, specifically start of blastulation and formation of the blastocyst are significantly different between implanting and non-implanting blastocysts. Use of kinetic criteria to refine blastocyst

selection for day 5 transfer may potentially increase implantation rates and be a useful tool in single embryo transfer (SET) cycles.
What is known already: Continuous time lapse imaging provides new criteria for embryo evaluation and selection for transfer. Studies have focused on comparing early cleavage kinetics. Recent data analyzing PGS results after trophectoderm biopsy and embryo morphokinetics suggest that initiation of blastocoel formation and blastulation are significantly different between euploid and aneuploid embryos.
Study design, size, duration: This retrospective study conducted between April 2012 and October 2013 analyzed morphokinetic data collected after time lapse imaging of embryos from 185 patients having a day 5 transfer. Known implantation data (KID) from cycles where all transferred blastocysts implanted (KID+) were compared to those where none of the transferred blastocysts implanted (KID–).
Participants/materials, setting, methods: Zygotes (n = 1522) were cultured in an EmbryoScope. Kinetic markers assessed were: time to syngamy (tPnf), t2, t3, t4, t5, t8, t-Mor (morula), tSB (start of blastulation) tBL (blastocyst formation), tEBL (expanded blastocyst). Timings for the kinetic parameters were divided in to quartiles. Data for KID+ and KID− embryos were compared using the Mann-Whitney-Wilcoxon test.
Main results and the role of chance: Clinical pregnancy and implantation rates were 70.8% and 57.9%, respectively. A mean of 1.92 ± 0.47 blastocysts were transferred. The mean patient age was 32.97 ± 4.42. KID data was available on embryos from 144 patients, of which 96 were KID+ and 48 were KID–. Early kinetic markers tPnf, t2, t3, t4 and t8 were significantly different between the two groups. All of the late kinetic parameters (tMor, tSB and tBL) were also significantly different between implanting and non-implanting blastocysts.

	Median of KID+	Quartiles (25–75%)	Median of KID–	Quartiles (25–75%)	*p*-Value
tPnf	23.38	21.92–25.14	24.55	23.23–26.60	0.001
t2	25.62	24.10–27.49	26.92	25.28–28.93	0.001
t3	36.87	34.80–39.46	38.23	36.02–40.33	0.005
t4	38.17	36.43–40.81	39.29	37.51–42.35	0.017
t8	56.77	52.63–64.62	58.85	55.18–66.17	0.04
t-Mor	89.58	84.79–94.94	92.22	87.38–97.64	0.009
tSB	97.19	94.07–102.8	100.5	95.34–104.8	0.043
tBL	102.1	98.36–106.7	104.9	101.7–109.1	0.001

Limitations, reason for caution: A prospective study design is needed to determine if the interquartile ranges associated with KID+ embryos is useful in refining blastocyst selection. Confounding factors such as diagnosis, patient age, stimulation and lab protocols may also affect embryo morphokinetics and should be analyzed using a larger patient series with known implantation data.
Wider implications of the findings: This study once again supports the use of time lapse and kinetic endpoints to assess embryo competence. Implanting and non-implanting blastocysts differed in many kinetic parameters, both early and late. Our data indicate that late kinetic parameters such as tSB and tBL may be especially helpful in identifying specific blastocysts within a cohort with increased likelihood of implanting. Non-invasive methodology such as time lapse imaging for embryo selection requires further validation by individual laboratories.
Study funding/competing interest(s): Funding by hospital/clinic(s), Cleveland Clinic Fertility Center: Internal Funding.
Trial registration number: N/A.

P-135 Improvement of survival rate in euploid blastocysts coming from day 3 biopsied embryos and collapsed with a laser shot before vitrification

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Study question: Is possible to improve the results of the vitrification program referred to the euploid blastocysts using the laser for collapsing them?
Summary answer: Collapsed euploid blastocysts have better prognosis in terms of survival rate than those which are not. To be collapsed makes them more resistant and the laser is a safe tool easy to use.
What is known already: Since the introduction of vitrification, the results in terms of survival rate reach levels close to 95%, a figure unthinkable not so long time with slow freezing. However, the group of euploid blastocysts coming from the Preimplantation Genetic Diagnosis (PGD) program, did not met the standards expected in the global levels of vitrification, with survival values of about 70%. The hole in the pellucida for removing the cell during biopsy, seems to impair the vitrification procedure due to the entry of cryoprotectant.
Study design, size, duration: Retrospective study from January 2012 to April 2013 in 43 couples of our PGD program. A total of 71 euploid blastocysts were collapsed and vitrified. All the blastocysts collapsed were hatching or hatched (BHi, BH).
Participants/materials, setting, methods: Day 3 biopsies were performed by laser (OCTAX). Comprehensive chromosome screening was performed by array-CGH (BlueGnome). The euploid blastocysts were collapsed by laser shot on day 5 or day 6. Vitrification was carried out using Cryotop®.
Main results and the role of chance: Up to now, 30 blastocysts were devitrified with a survival rate of 96% (29) and were completely intact. They were warmed and incubated for 2 h and checked again to ensure viability; A total of 17 transfers were done with a pregnancy rate of 70%, ongoing pregnancy rate of 53% and an implantation rate of 48%.
Limitations, reason for caution: This is a retrospective study with the intrinsic limitations to draw stronger conclusions. The low number of blastocysts is a big limitation. Not all the patients of the PGD program have supernumerary embryos for vitirification that makes the study long and difficult.
Wider implications of the findings: Despite this is a retrospective study, our results are reassuring in two aspects: on one side, the laser shot is a useful tool for collapsing blastocysts; on the other side, survival rates improve significantly in this group of embryos. Further studies with more embryos included are necessary to confirm this trend.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Instituto Valenciano de Infertilidad, Instituto Universitario, IVI Valencia.
Trial registration number: NO.

P-136 Categorisation of embryo developmental potential using computerised time-lapse evaluation of early stage development reveals differences in morphology and viability between three defined categories

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Study question: Computerised time-lapse analyses of early embryo morphokinetic events can indicate the potential viability of an embryo, by categorising embryos into those with high potential (HP), moderate (MP) or low potential (LP). Do these viability categories reflect morphology at cleavage stage, and ability to attain blastocyst stage in a routine setting?
Summary answer: Evidence from embryo selection for cleavage stage transfer combined with subsequent ability to achieve blastocyst stage indicate that the three categories of embryo potential show distinct viability performance. HP and MP embryos show significantly higher utilisation rates than LP embryos.
What is known already: Time-lapse analyses of early cell divisions can predict an embryo's potential to achieve the blastocyst stage of development *in vitro*. The Early Embryo Viability Assessment (EevaÔ) system (version 2.2) records timings of the second and third cell divisions to diagnose embryo viability as: high (HP), medium (MP) or low (LP) potential. HP embryos show higher potential to achieve blastocyst stage than LP embryos. The role of the MP categorisation has not been reported.
Study design, size, duration: This retrospective examination compared the viability of embryos in women (<43 years) undergoing IVF/ICSI whose embryos were graded into 3 categories by the computerised EevaÔ system. The study period was from September 2012 to December 2013.
Participants/materials, setting, methods: Patients underwent stimulated cycles with embryo transfer on day 3 at a single clinic, after embryo viability assessed by computerised time-lapse imaging. Embryos were categorised into HP, MP or LP prior to transfer or subsequent culture to blastocyst stage for vitrification. Embryos were 'utilised' when transferred or vitrified.
Main results and the role of chance: The cohort of embryos (n = 1217, in 213 cycles) showed a distribution of categorisation with LP embryos as a majority: HP (29%), MP (19%) and LP (47%). There was no difference of distributions of embryo categories between the age-groups (HP = 31% in the younger (<37 years), and 28% in the older (37–42 years) groups. Utilisation of the HP

group (81.3%) was markedly superior to the remaining embryos ($P < 0.001$), and utilisation of the MP embryos (58.3%) was superior ($p < 0.001$) to LP embryos (34.8%). The utilisation rates of the HP and MP embryos showed no difference in the 2 age-group cohorts, while more LP embryos were utilised in the older age-group. When MP embryos were transferred in isolation (13 cases), the implantation rate was 32%.

Limitations, reason for caution: Embryo selection for transfer by morphological assessment has an intrinsic degree of subjectivity, and there was no blinding to the time-lapse evidence.

Wider implications of the findings: It is important that computerised time lapse imaging of early embryo development can reliably identify embryos with high and low developmental potential. These data provide strong evidence that LP embryos, which constituted the majority proportion in both age-groups, showed significantly poorer morphology at both the cleavage and blastocyst stages than did the HP and MP embryos. MP embryos showed encouraging viability.

Study funding/competing interest(s): Funding by hospital/clinic(s), Internal study at GCRM Ltd.

Trial registration number: Not applicable.

P-137 Software based analysis of Local Zona Pellucida Thickness Variation: Bending Energy as a predictive window on Day 2 embryo fate

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Study question: Objective of the present retrospective observational study is to evaluate the clinical effectiveness of 'Embryon', a new proposed software program based on the semiautomatic Local Zona Pellucida Thickness Variation (LZPTV) computation of Day 2 Intracytoplasmatic Sperm Injection (ICSI) embryo images to provide a reliable additional information regarding embryo fate

Summary answer: Preliminary results from software based analysis of LZPTV on Day 2 ICSI digitalized embryos images appear to provide, by a single numerical value, a possible integrative approach to Assisted Reproduction Technologies (ART) practice and research

What is known already: Zona Pellucida Thickness (ZPT) and Zona Pellucida Thickness Variability (ZPTV) have been related to clinical outcome of ART. A number of morphological, morphokinetics, automated and semi-automated non-invasive methodologies for the assessment of various human embryo stages have been developed to evaluate its potential to implant and proceed to term. Up to date, none has been proven superior to the others.

Study design, size, duration: 342 digitalized Day 2 embryo images from 147 ICSI patients treated at ART Unit 'Sapienza' University of Rome, Italy, during October 2011–October 2012 were blindly retrospectively analyzed using 'Embryon' software program (#ITRM2012A00025, ACS, Rome, Italy), which characterized the LZPTV of each embryo by a single numerical value

Participants/materials, setting, methods: The a-dimensional fractal-based descriptor akin to the Bending Energy (BE) value obtained by 'Embryon' captures the high frequency variability of embryo ZPT digitalized images. Embryos with high LZPTV value (Top) were evaluated for capacity to proceed to Live Birth (LB) compared to embryos with lower score (Poor)

Main results and the role of chance: According to LB, 147 patients were split in two clusters: Group A (38 Live Birth patients) and Group B (109 patients where the previous condition was not verified for implantation failure or abortion). The two Groups presented similar clinical and biological parameters, including age, etiologies, number and quality of transferred embryos classified according to Veeck Morphological Classification (VMC). Quantitative data of LZPTV values are presented as the mean value ± standard deviation (SD). Student t test was carried out to evaluate the significance of differences. Group A: $21.19 \times 10^3 \pm 8.33 \times 10^3$; Group B: $15.92 \times 10^3 \pm 5.18 \times 10^3$ (P value <0.001). In addition patients having transferred embryos with LZPTV greater than threshold value of 18.5×10^3 presented 63.6% of LB, significantly above the overall patients term delivery rate of 25.9%

Limitations, reason for caution: Our observations present underestimation of prediction accuracy as consequence of Italian Law that explicitly bans embryo selection. LZPTV analysis whether confirmed in a larger prospective study with single or homogeneous embryo transfers, for robustness and reproducibility, may represent an integrative tool to distinguish embryos able to proceed to term

Wider implications of the findings: LZPTV measurement could be used to select embryos for transfer. This appears to be even more important when embryos of suboptimal morphology are available. It might provide an addition criterion that may be of benefit in a policy of selecting single embryo transfer. A crucial point to be addressed is the relationship between LZPTV and VMC. At a preliminary evaluation both parameters appear to share the same biological basis.

Study funding/competing interest(s): Funding by University(ies), Department of Obstetrics and Gynecology, 'Sapienza' University of Rome, Italy. The authors can identify no competing interests whatsoever in writing or publishing this abstract.

Trial registration number: This is a purely observational digitalized study in which medical intervention is not at the discretion of the investigator.

P-138 Promising transfer and pregnancy rates utilizing consecutive "freeze all"-cycles and trophectoderm aneuploidy screening

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Study question: We report on our experience with a novel preimplantation genetic screening (PGS) protocol including cryopreservation that was followed for couples with extensive histories of assisted reproductive treatment (ART) demonstrating repetitive failure to develop high grade blastocysts.

Summary answer: Iterative PGS with cryopreservation allows to accumulate an appropriate number of blastocysts needed for the transfer of an euploid embryo in a reasonable time frame.

What is known already: With most technical drawbacks having been overcome during the last years, vitrification capabilities for blastocysts have been optimized and successfully introduced to ART routines. With regard to the manifold clinical indications for ART, there is an ongoing discussion on which couples would benefit most from these new techniques.

Study design, size, duration: We included 11 couples displaying normal karyotypes. Maternal age was 35–43 years. They had experienced at least one loss of pregnancy following ART and multiple previous unsuccessful IVF-cycles. The novel approach included repetitive stimulation according to standard protocols, ICSI, embryo culturing to blastocyst stage and trophectoderm biopsy by laser dissection.

Participants/materials, setting, methods: Embryos were vitrified following a closed vitrification protocol. Biopsies were whole genome amplified and hybridized to 24 sureV3 microarrays utilizing the SurePlex DNA amplification system and the 24 sure protocol (BlueGnome, Cambridge, United Kingdom). After evaluation, embryos were thawed, recultured for 2 h and transferred 5 days post ovulation in natural cycles.

Main results and the role of chance: After two to three completed stimulation cycles, each couple accumulated an adequate number of blastocysts for PGS. Seven couples received recommendations for the transfer of at least one euploid tested embryo (range: 1 to 4). Vitrification did not adversely affect the vitality of the six embryos that were thawed for single transfer. Up to today, one couple is awaiting transfer, another is a waiting pregnancy testing. One did not achieve a pregnancy, two pregnancies were confirmed by ultrasound and two healthy babies were born. We point out that for five couples with extremely low numbers of mature oocytes, additional cryopreservation at pronuclear stage was performed (slow freezing protocol) and that we did not see any adverse affect of this procedure.

Limitations, reason for caution: We evaluated the efficiency of a vitrification approach utilizing this highly selected cohort and saw good benefit for couples with distinct blastocyst development failure due to presumed aneusomy.

Wider implications of the findings: Cryopreservation gives reproduction professionals the flexibility to organize analytical workflows without time pressure and counselees the possibility to actively participate in the stepwise decision process.

Study funding/competing interest(s): Funding by hospital/clinic(s), No external funding was received.
Trial registration number: The results were gathered through diagnostic routine.

P-139 The use of live birth as an outcome measure for a new morphokinetic based blastocyst selection algorithm

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Study question: Using morphokinetic data from transferred blastocysts with known implantation and live birth outcome (KID), can a predictive embryo selection model be devised for prospective clinical use?
Summary answer: Two morphokinetic variables were found to be significantly different between viable and non viable transferred blastocysts, defined by foetal heart beat (FHB) and live birth (LB). These were used to build a model for prospective clinical use.
What is known already: Time lapse monitoring (TLM) allows the detailed comparison of morphokinetic variables between embryos, according to their fate following transfer. These data can be used to develop clinical evidence-based embryo selection algorithms. To date only a few embryo selection models have been published and it has yet to be established whether models are transferrable between centres. The only published morphokinetic model for blastocyst selection was based on aneuploidy risk classification. This model introduces live birth outcome.
Study design, size, duration: Morphokinetic variables and outcome to FHB (n = 250) or LB (n = 73), for ICSI embryos following blastocyst transfer, were measured and annotated, prior to outcome being known, using EmbryoScope™ (Fertilitech, Denmark). Data were collected between May 2011 and August 2013 and the morphokinetic profiles compared according to fate.
Participants/materials, setting, methods: Time, in hours from pronuclear fading (pnf) to – all cleavage stages (relt2 to relt9); morula formation (reltM); start of blastulation (reltSB) and full blastocyst (reltB) were annotated using the EmbryoViewer software. We prefixed the variable with 'rel' to distinguish it as time from PNf devising a model applicable to both IVF and ICSI. Where the number of transferred embryo(s) at least equaled the number of FHB(s) or LB(s), KID of all embryos per treatment was positive, otherwise negative. KID rates were compared between model classes.
Main results and the role of chance: ReltM and reltSB were found to differ significantly between KID positive and negative embryos. An algorithm was derived using recursive partitioning which ranked embryos according to their implantation potential calculated using KID rate (KID positive/KID negative +KID positive × 100%).

The overall KID rate was 38% FHB and 30% LB. We grouped them A to C, embryo numbers in brackets. Class C (where reltSB ≥75.8 h) – KID Rates 11% FHB (97) and 8% LB (97) we considered an exclusion event. This corresponds to earlier published models where tSB was a strong predictor for implantation potential (Campbell et al., 2013). Class A (where rtM >52.6 h and ≤61.4 h and rtSB <75.8 h) – KID rate 64% FHB (89) and 71% LB (17) and Class B (<52.6 h or >61.4 h and rtSB <75.8 h).KID rate 39% FHB (128) and 38% LB KID (24). The area under the ROC curve (AUC) was 0.74 FHB and 0.80 LB indicating good predictive power.
Limitations, reason for caution: This model was developed using ICSI only so requires further validation for IVF. The size of the data assessed may limit the predictive value and different models may be applicable in different settings or patient groups.
Wider implications of the findings: This finding may encourage centres to analyse their own time-lapse data – utilizing IVF and ICSI data by using the relative time from pronuclear fading, comparing positive and negative outcomes, per embryo, in order to develop selective algorithms. Regular review of in house data, allows fine-tuning of selection models as data increases and promises to be a powerful tool for wider use in embryo selection.
Study funding/competing interest(s): Funding by hospital/clinic(s), CARE Fertility.
Trial registration number: None.

P-140 Human ART culture media have no effect on apoptosis in mouse pre-implantation embryos

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Study question: Is apoptosis the primary mechanism accountable for the reduction of the relative number of cells in murine pre-implantation embryos during in-vitro-culture in human culture media?
Summary answer: In-vitro-culture (IVC) of murine pre-implantation embryos using the human sequential media ISM1 and Blast Assist (Origio, Berlin, Germany) leads to a decline in cell numbers in contrast to controls. However cell loss was not due to a higher rate of apoptosis since caspase activity was not significantly elevated from controls.
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 development of pre-implantation embryos in mice. Apoptosis, playing a major role in peri- and postimplantation processes of mammalian development, might also mediate the effects of IVC on pre-implantation embryos.
Study design, size, duration: For caspase analysis we used 441 zygotes, cultured until 4.5 days post coitum (dpc) either in human sequential media (ISM1/Blast Assist) or in KSOM(aa) (*in vitro* control). Blastocysts obtained from the uterus on dpc 3.5 served as *in vivo* control. The potent apoptosis inducer Actinomycin D served as positive control.
Participants/materials, setting, methods: Per group 6 to 10 superovulated female mice (B6C3F1, 6–8 weeks) were mated with C57Bl/6 males. *In vivo* or *in vitro* developed blastocysts were fixed and immunohistochemically stained for cleaved caspase-3, -8, and -9. Total cell numbers and cells positive for any of the three cleaved caspases were counted.
Main results and the role of chance: Analysing a total of 441 embryos showed that blastocysts of the ISM1/BlastAssist group and the *in vivo* control had significantly lower total cell numbers compared to the *in vitro* group (ISM1/BlastAssist: 47.79 ± 1.36 N = 146; *in vitro*: 68.09 ± 1.7 N = 144; *in vivo*: 41.01 ± 0.9 N = 151; $p \leq 0.0001$). In both groups we detected cells, which were positive for cleaved caspase-3 (ISM1/BA: 1.35 ± 0.32 N = 48; *in vitro*: 1.09 ± 0.25 N = 47; *in vivo*: 1.24 ± 0.32 N = 46) and cleaved caspase-9 (ISM1/BlastAssist: 1.6 ± 0.32 N = 48; *in vitro*: 0.84 ± 0.20 N = 51; *in vivo*: 1.43 ± 0.3 N = 52), although results were not significantly different. For cleaved caspase-8 nearly no positive cells were found (ISM1/BlastAssist: 0.08 ± 0.06 N = 50; *in vitro*: 0.18 ± 0.08 N = 46; *in vivo*: 0.53 ± 0.26 N = 53). Actinomycin D effectively induced apoptosis in cultured embryos (Caspase 3: 6.1 ± 1.2 N = 19; Caspase 8: 0.4 ± 0.27 N = 4; Caspase 9: 0.16 ± 0.1 N = 4).
Limitations, reason for caution: Due to the fact that the mouse was used as an animal model, the results cannot be directly transferred to the human situation, but 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: We found low caspase activity in murine blastocysts cultured in KSOM(aa), which is consistent with previous findings. We observed a restriction in development, but no increased incidence of apoptosis when blastocysts were cultured in ISM1/Blast Assist. Other mechanisms, e.g. changes in epigenetic profile or expression pattern of metabolic genes during IVC, might be responsible for the restriction in development and need to be explored in future studies.
Study funding/competing interest(s): Funding by national/international organization(s), IMF NO 11 12 12.
Trial registration number: Not applicable.

P-141 Microfluidics device applied to embryo culture improves blastocyts morphology

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Study question: It is microfluidic system device, presented as IVF-Lab6, advantageous compared to conventional microdrops culture platforms for *in vitro* embryo development?
Summary answer: Both systems are able to provide optimal culture conditions in human embryos, although development to blastocyst is significantly improved in the microfluidic device; however implantation rates are comparable in both systems.
What is known already: Until now it is known that microchannels provide an environment that seems to mimic *in vivo* situation than traditional static microdrop methods. Microfluidics cell culture devices permits gradual change of the culture medium, which might result in better embryo development, and also reduces the embryo manipulation, which is potentially damaging to the cell
Study design, size, duration: 58 patients undergoing egg donation were included prospectively in this study between January and July 2013 at IVI Valencia (Spain). A total of 641 metaphase II oocytes were inseminated using ICSI, obtaining 457 embryos that were randomly cultured in one of the described systems. Embryos were transferred in blastocyst stage.
Participants/materials, setting, methods: After ICSI, oocytes were divided into two groups: a) conventional culture, making a different Petri dish containing the new medium with manual embryo manipulation; and b) inside microflow dish where the medium change and flow was generated manually until transfer day. Both were incubated at same incubator on equal conditions
Main results and the role of chance: Analyzing all embryos, 223 from microdrops group and 234 from microfluidics dish, we have observed that those who had an optimal development on day 2 and 3 according embryo culture dish, were statistically comparable (Day-2: 56.9% vs 52.56%; Day-3: 57.14% vs 55.45% respectively). Optimal blastocyst quality according to embryo culture dish were significantly improved on Day-5: 33.5% vs 42.31% ($p < 0.05$) and similar in Day-6: 29.03% Vs 31.51% respectively. However, implantation rates (IR) were not significantly different between both systems in both groups (64.7% vs 44.4%; $p = 0.199$)
Limitations, reason for caution: The number of embryos transferred in both groups were still limited lacking statistical power, only morphology comparison between both culture systems is consistent and reliable
Wider implications of the findings: Microfluidics and single drop culture systems present optimal conditions for embryo culture, since embryo development and IR were comparable on both devices. However, the efficiency of these systems to offer a pathway toward future still need to be investigated, because the real advance of microfluidic devices lay in their ability to automate flow and replenishment of nutrients without manipulating the embryos outside the incubator. Microfluidics is a change of paradigm as embryos stay in the same place during all process. It is plausible that future microfluidics devices can replace current IVF platforms
Study funding/competing interest(s): Funding by hospital/clinic(s), IVI-Valencia.
Trial registration number: Not applicable.

P-142 Does blastocyst pre-freeze grade, extent of survival and expansion have an effect on pregnancy outcome?

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Study question: Does pre-freeze blastocyst quality, percentage cell survival and the degree of re-expansion of vitrified embryos after warming affect clinical pregnancy rate (CPR)?
Summary answer: The pre-freeze grade of the blastocysts and the degree of re-expansion after 1 h were found to be statistically significant factors in relation to CPR following frozen embryo replacement (FER). Degree of blastomere survival and re-expansion at time of embryo transfer may also have a positive correlation with CPR.
What is known already: The pre-freeze blastocyst quality is known to influence the outcome of a FER cycle. Less is known about the effect of percentage survival and the degree of re-expansion after warming on CPR and implantation potential.
Study design, size, duration: This is a retrospective study of clinical outcome from 208 cycles where a single warmed day 5 blastocyst was transferred between January 2009 to July 2012 inclusive. Only suitable quality blastocysts with expansion between 3–6, as described by Gardner and Schoolcraft, were selected for freezing.
Participants/materials, setting, methods: Suitable supernumerary blastocysts were frozen using the Cook Blastocyst Vitrification method and warmed using the Cook Blastocyst Warming procedure. Pre-freeze day 5 blastocysts were graded according to quality of both inner cell mass (ICM) and trophectoderm morphology. Post-warming, percentage cell survival and re-expansion were assessed. Statistical analysis used Chi-squared.
Main results and the role of chance: There was a statistically significant difference between the different pre-freeze grade blastocysts and CPR ($p < 0.05$), with blastocysts of 3–6 A/B ICM and a/b trophectoderm resulting in a higher pregnancy rate (40%; 57/142) than those with grade c trophectoderm (20%; 11/55). Although numbers were small, no pregnancies resulted from 3–6Cc blastocysts (0/6). There was a trend between percentage blastomere survival and CPR, with the highest CPR resulting from those blastocysts where survival was 100% (40%; 29/73), compared to 26% (12/47) where survival was 50–75%. No pregnancies resulted from those with 10–40% survival. There was a statistical difference in CPR between embryos with no re-expansion after 1 h (14%; 3/22) compared to those with between 10–100% re-expansion (36%; 52/146).
Limitations, reason for caution: The study is on-going as some data sets are small. Additionally, assessments of this nature are open to a degree of subjectivity. Whilst there may be several other variables involved in FER cycles, these factors are nonetheless important in contributing to overall CPR.
Wider implications of the findings: The results indicate that pre-freeze grade significantly affects the CPR, supporting other papers in the literature documenting higher quality blastocysts have a greater implantation rate. Degree of blastomere survival and re-expansion at the time of embryo transfer had a positive correlation with CPR. Additionally, re-expansion after 1 h showed a significant correlation with CPR when comparing blastocysts with no sign of re-expansion to those with 10–100%, supporting previous studies.
Study funding/competing interest(s): Funding by hospital/clinic(s), Oxford Fertility Unit.
Trial registration number: N/A.

P-143 Use of GM-CSF supplemented IVF medium in patients with recurrent implantation failure: a controlled trial

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Study question: May the use of GM-CSF supplemented IVF medium in women with Recurrent Implantation Failure improve their chance to became pregnant?
Summary answer: GM-CSF supplemented IVF treatment may be useful in women with implantation failure
What is known already: In the recent years it has been recognized the role of growth factors as well as of cytokines in promote and regulate embryo development from the earlier stage through the implantation and placentation. With the development of Assisted Reproductive Technology has became crucial the conditions of embryo culture to allow fertilized eggs to growth in a most physiological way. Recently it has been recognized the role of GM-CSF in early embryo development. Embryo culture medium supplemented with this growth factor may be useful in recurrent implantation failure patients.
Study design, size, duration: This randomized controlled study was conducted from the March 2012 to January 2014 on 100 women with recurrent implantation failure, at least three failed previous IVF attempts with at least 8 good embryos transferred no more than 40 years old. Patients were randomized by a computer generated number sequence. All patients before allocation signed an informed consents form.
Participants/materials, setting, methods: Women were randomly divided in two groups of 50 women each. All patients underwent the same controlled ovarian hyperstimulation with gonadotropins from the first day of menstrual cycle plus Cetrorelix when the leading follicle exceeding 14 mm in mean diameter and/or estradiol levels were higher than 400 pg/ml. The fertilized oocytes by ICSI were cultured in the study group with GM-CSF supplemented IVF medium (Embryogen, Origio, Denmark), whereas in the other group the embryos obtained were cultured with a standard IVF medium. A maximum of three embryos were transferred. Primary outcome was: pregnancy rate and implantation rate.
Main results and the role of chance: The epidemiological data of patient groups did not show statistically significant differences. The pregnancy rate in the group treated with GM-CSF was of 38.0% (19/50) whereas in the control group 20.0% (10/50), this difference was statistically significant $P = 0.0385$.

The implantation rate in the study group was 24.2% (22/91) whereas in the control group it was 11.2% (11/98): this difference was statistically significant $P = 0.0154$. However the number of patients included in the study was enough big to reach the right power (Power of the study 0.635).
Limitations, reason for caution: These data need to be confirmed in larger group of patients in order to reach the power needed.
Wider implications of the findings: The use of GM-CSF supplemented IVF culture medium may be useful in women experiencing implantation failure.
Study funding/competing interest(s): Funding by hospital/clinic(s), none.
Trial registration number: NCT01718210.

P-144 Randomized study assessing the impact of primo vision time-lapse embryo monitoring system (tlm) on embryo culture and selection

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Study question: (1) Could the use of a time-lapse monitoring system including embryo culture in group result in a better culture environment for embryos? (2) Could the system help to determine embryo selection criteria resulting in increased implantation and clinical pregnancy?
Summary answer: (1) The stress-free environment provided by Primo Vision System including the micro-well group culture dish resulted in increased number of embryos available for transfer. (2) The morphokinetic data provided by the system can be used to improve the embryo selection criteria, possibly resulting in increased implantation and clinical pregnancy.
What is known already: (1) By using time-lapse technology, the complete process of embryo development can be monitored continuously without the need to remove embryos from the incubator. (2) Continuous monitoring allows exploring whether dynamics of embryo development correlate with embryo implantation potential.
Study design, size, duration: Patients (female age ≤35; Body Mass Index: 18–25; 5–15 oocytes; tubal factor infertility) were randomly assigned to the TLM group or control group between April 22 and September 19, 2013.
Participants/materials, setting, methods: Totally, 129 patients (TLM group, 64 patients; control group, 65 patients) were included. Embryos in TLM group were cultured in group using the primo vision embryo culture dish. Embryos in control group were cultured singly in conventional culture dishes. Embryos were transferred on day 3.
Main results and the role of chance: (1) More Embryos were available for transfer or cryopreservation in the TLM group compared to control (4.5 ± 2.23 vs. 3.68 ± 1.76, $p = 0.011$). After transfer of 264 embryos in 129 patients, pregnancy and implantation rate showed a trend towards increased rates in the TLM group compared to control (70.31% vs. 58.46%, $p = 0.160$; 49.24% vs. 40.91%, $p = 0.174$). (2) The duration of the first cytokinesis was shorter for implanted embryos compared to the ones that failed to implant ($0.34\ h \pm 0.26$ vs. $0.60\ h \pm 0.92$, $p = 0.0468$). Similarly, the duration of the second cytokinesis differed between these two groups ($0.56\ h \pm 0.42$ vs. $1.39\ h \pm 2.61$, $p = 0.0268$).
Limitations, reason for caution: This is an intermediate data analysis. Final conclusion is possible when a sufficiently large sample size is obtained.
Wider implications of the findings: Significant higher quality of embryos from the TLM group may be attributed to the stable culturing environment, the group culture condition, avoiding mechanical movement during time-lapse monitoring, minimizing electromagnetic strength in culture environment and the overall undisturbed culture environment with minimal handling throughout the culture period. The morphokinetic data collected by the time-lapse system provide information that can be used to improve the embryo selection resulting in increased implantation and clinical pregnancy.
Study funding/competing interest(s): Funding by hospital/clinic(s), No study funding for this research. The authors declare no competing financial interests.
Trial registration number: No.

P-145 IntegraTI versus Integra3 (I3): a prospective randomised sibling study assessing fertilisation outcome, embryo quality and morphokinetic parameters

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Study question: To establish if the use of two different micromanipulator systems (I3 versus IntegraTI, Research Instruments™) had an effect on fertilisation outcome, embryo quality and morphokinetic parameters.
Summary answer: The performance indicators (PIs) assessed were not affected when using the I3 compared to the IntegraTI. Embryos derived from oocytes injected using the I3 had a longer second cell cycle (CC2), shorter second cell cycle synchrony (S2) and were twice as likely to be transferred compared to IntegraTI.
What is known already: With the availability of different micromanipulation systems, practitioners must validate any new equipment. The I3 advances over the IntegraTI with the emission of warm air towards the petri dish and additional manipulation functions. Previous research found that intracytoplasmic sperm injection (ICSI) practitioners affect morphokinetic parameters including the time to pronuclei fading (PNf), two cells (t2) and three cells (t3). To date there is no research project assessing the effect of ICSI equipment on morphokinetics.
Study design, size, duration: In total 160 mature oocytes derived from 22 patients were injected by two practitioners. Oocytes from each patient were randomly split to be injected either using the I3 ($n = 82$) or the IntegraTI ($n = 78$) systems. Injected oocytes were cultured in an EmbryoScope® to assess PIs and morphokinetics.
Participants/materials, setting, methods: Continuous data (morphokinetics) were analysed using a generalised linear model (assessed parameter = ICSI rig*practitioner), with the patient as the random factor. Categorical data (PIs) were assessed using Chi square test and considered significant where $p < 0.05$. All embryos were blindly selected for transfer.
Main results and the role of chance: PIs [fertilisation (72%vs67%), degeneration (7%vs6%), 3PN (2%vs1%), embryos with even first cell division (78%vs74%) and overall day 5 embryo ultilsation rate (63%vs52%)] were not significantly affected by treatment (I3 versus IntegraTI respectively). The proportion of embryos selected for transfer was two times higher for I3 compared to IntegraTI (27%vs12%, $p < 0.05$).The morphokinetic data indicates that the time to reach cell stages from two cells to hatching blastocyst (t2, t3, t4, t5, t6, t7, t8, tsC, tsB, tB, teB and tHB) were not significantly different between the two systems. I3 embryos had longer CC2 compared to IntegraTI embryos (mean ± standard deviation: 10.7 ± 5.2 h versus 7.2 ± 6.1 h respectively, $p = 0.004$), and the difference in S2 was not significant (1.7 ± 2.9 versus 3.5 ± 5.5 h, $p = 0.06$). None of the assessed parameters were affected by the practitioner.
Limitations, reason for caution: This study has a power of 0.8 for detecting 12% difference in fertilisation rate. To detect a 5% difference, the analysis of 253 patients is required. Extending the study to assess effects at the clinical outcome level would be beneficial.
Wider implications of the findings: This study not only validates the I3 as performing at least equal to the established IntegraTI system, but also demonstrates that ICSI equipment can have an effect on embryo morphokinetic development.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Research Instruments Limited.
Trial registration number: N/A.

P-146 The effects of maternal hyperhomocysteinemia on embryo quality in mice

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Study question: Does maternal hyperhomocysteinemia (HHcy) affect embryo quality and injury on gene expression in mice?
Summary answer: Maternal HHcy reduces embryo mitochondrion membrane potential ($\Delta\psi m$), induces embryo endoplasmic reticulum (ER) stress condition and alters expressions of stress and development genes, finally causing less cell number and higher apoptosis rate till blastocyst stage.
What is known already: Elevated maternal homocysteine is a well-established risk factor for embryonic toxicity. A lower rate of transsulfuration in early gestation was seen in women with pregnancy wastage. Although HHcy has been identified as a potential disturbance in developmental processes and is capable of upsetting the proper growth of the cells, little is known about the etiopathology through which homocysteine alters embryo quality.

Study design, size, duration: This was a randomized study based on a mouse model for hyperhomocysteinemia. Thirty-eight BALB/c strain mice were used and HHcy was induced by dissolving homocysteine in drinking water at a concentration of 53 mg/dL for 5 weeks prior to super-ovulation.
Participants/materials, setting, methods: Either control (n = 18) or a diet high in homocysteine (n = 20) were fed for 5 weeks. Zygotes were recovered and cultured to D3; six-eight cell embryos were cultured continuously to blastocyst. Δψm and relative mRNA abundance of 11 candidate genes were determined in D3 and blastocysts by RT-PCR.
Main results and the role of chance: Mice maternal HHcy significantly reduced embryo mitochondrion Δψm, elevated ER stress (Atf4 and Hsp70), apoptosis (p53 and caspase3) along with other stress genes (MnSOD and GADD45) in eight-cell embryos. Expressions of development (E-Cadherin, ZO-I and IGF-II) related genes decreased, although not significant enough as compared to the fertilization related genes (ZP2, ZP3) in blastocysts developed from induced hyperhomocysteinemic females. Simultaneously, a lower cell number and higher apoptosis rate was also observed in the hyperhomocysteinemic group. To limit the role of chance, the experiments were conducted in a defined laboratory setting with the proper controls, and the animals were randomly assigned to each experimental group. Moreover, a P-value of <0.05 was chosen to determine whether the differences observed between the groups were statistically significant.
Limitations, reason for caution: The results obtained may not fully extrapolate to humans. Also, as we did not perform embryo transfer on the pseudopregnant mice, the effect on hormone secretion cannot be correctly adjudged; hence, effect of mice maternal HHcy on gene expression level is only demonstrated.
Wider implications of the findings: Mice maternal HHcy reduced organelles function of embryonic cell, changed embryo genes expression and lowered embryo *in vitro* development rate. Homocysteine causes ER stress by inducing the expression of GADD45, HSP70, ATF-4, and MnSOD. Furthermore, growth arrest, development and fertilization impairment will provide additional important information on the mechanism by which homocysteine promotes poor embryo quality simultaneously increasing the understanding of the genetic pathways involved in the pathogenesis of HHcy.
Study funding/competing interest(s): Funding by hospital/clinic(s), This study was supported by Institute of Reproductive Medicine. There were no competing interests.
Trial registration number: Not applicable.

P-147 *In-silico* analysis of follicular fluid proteome for the identification of molecular biomarkers related to oocyte quality in IVF programs

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Study question: Considering all the so far unambiguously detected proteins of human follicular fluid (HFF), we performed an *in-silico* functional processing to delineate protein patterns with reasonable potentialities for oocyte quality estimation in IVF programs.
Summary answer: Our study proved the occurrence in HFF of a highly dynamic functional network which is space- and time-controlled by specific effectors. The net mainly converges on some very specific key factors, i.e. metalloproteinases (MMP), MMP inhibitors, α-1-anti-trypsin, whose combined presence and functionality may be applied as biomarkers for oocyte quality.
What is known already: The HFF contains molecules that may affect follicle growth, oocyte maturation and competence acquiring. Nevertheless, HFF protein composition as well as its role in follicular growth and oocyte maturation still remain to be clarified since even if a number of proteomics attempts have been performed, no comprehensive study on the whole HFF protein composition has been carried out.
Study design, size, duration: An extensive review of the literature from January 2000 was performed and all the proteins registered in order to depict the functional microenvironment of the human follicle.
Participants/materials, setting, methods: We included in our study more than 600 proteins detected in HFF; which were then processed *in-silico* to highlight their functional correlations by using the DAVID functional enrichment analysis tool and the MetaCore pathway analysis bioinformatics resource.
Main results and the role of chance: According to the performed functional analysis, we highlighted two main biochemical processes that characterize the pre-ovulatory follicle physiology: 1) protease cross-reactions in inflammation, healing, and coagulation events and their balancing by a fine regulatory interfacing between protease activators and inhibitors; 2) activity of dietary-related factors on metabolism, health and fertility. As regard to the nutritional status, it may profoundly impact on follicle development, oocyte quality and embryo viability. Indeed, vitamins (i.e. A and D) interfere with lipid and glucose metabolism by binding specific receptors and these activate transductional signals which regulate gene expression of key factors active in such metabolic paths, such as apolipoprotein A1.
Limitations, reason for caution: Our analysis is based on data from literature analysed *in silico* by bioinformatic resources. The results have to be applied in specific clinical trials in order to validate the detected key factors.
Wider implications of the findings: This is the first time that all the proteins described in HFF are functionally processed at once. This led to a complex and wide overview on the follicular biochemical and biomolecular dynamics and allows to identify some key factors whose combined presence and functionality may be useful for oocyte quality estimation in IVF programs in order to improve pregnancy rate.
Study funding/competing interest(s): Funding by University(ies), University of Siena, Siena, Italy.
Trial registration number: None.

P-148 Successful treatment with Ca2+ -ionophore in cases of previous developmental problems: a multicentre study

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Study question: Does an ionophore treatment (A23187, calcimycin) improve embryo development and outcome in patients with a history of developmental problems/arrest?
Summary answer: Application of A23187 compensates for a calcium deficiency in the embryo which leads to increased rates of cleavage, blastocyst formation, and clinical pregnancy/live birth.
What is known already: Studies on lower animals indicate that changes in intracellular free calcium initiate and regulate the events of cell division. In human, calcium fluctuations were detected with a maximum peak shortly before cell division. Interestingly, these calcium oscillations disappeared in arrested embryos. Mitotic division blocked with a Ca^{2+} chelator could be restored by means of ionophores in an animal model.
Study design, size, duration: This prospective multicenter (5 Austrian centres) 1-year study includes 38 patients (41 cycles) who gave informed consent.
Participants/materials, setting, methods: Inclusion criteria were complete developmental arrest in a previous cycle (no transfer), complete developmental delay (no morula/blastocyst on day 5), or reduced blastocyst formation (<15%). Severe male factor patients and patients with <30% fertilization rate after ICSI were excluded because these would be routine indications for ionophore usage. A total of 44 previous cycles of the same patients were used as a control group (matched for age and stimulation details). In the present treatment cycles all MII-oocytes were exposed to a commercially available ready-to-use ionophore (CultActive) for 15 min immediately after ICSI. After a 3-step washing procedure *in vitro* culture was performed as in the control cycles, preferably up to blastocyst stage.
Main results and the role of chance: Fertilization rate did not differ (72.8% vs. 70.4%), however, further cleavage on day 2 was significantly higher (P = 0.009) in the ionophore group (97.3%) as compared to the control cycles (92.1%) In addition, significantly more (P < 0.0001) blastocysts formed on day 5 in the

study group (54.0% vs. 13.2%). This was associated with an significant increase ($P < 0.0001$) in the rates of implantation (50.0% vs. 8.6%) and clinical pregnancy (51.2% vs. 6.8%), and live birth (48.8% vs. 6.8%). All babies born so far (17/20) were healthy.

Limitations, reason for caution: Due to the expected low frequency of patients showing developmental problems a multicenter approach was chosen in order to increase sample number. It should be noted that in one third of the cycles the stimulation protocol was changed, however, this switch did not improve/influence developmental rate.

Wider implications of the findings: This is first evidence that developmental incompetence of embryos is an additional indication for ionophore treatment. Apart from IMSI (one published study) the present approach is exclusive in order to overcome cleavage arrest.

Study funding/competing interest(s): Funding by hospital/clinic(s), Kinderwunsch Zentrum Linz provided the ionophore for free.

Trial registration number: D-17-13 (Ethical Committee Upper Austria).

P-149 Morphokinetic assessment using time-lapse imaging allows more accurate identification of embryos with multiple rather than single aneuploidies up to the 8-cell stage

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Study question: Does non-invasive, morphokinetic assessment allow aneuploid embryos to be classified according to their with chromosome copy number.

Summary answer: Multivariable analysis of time-lapse morphokinetic parameters in early stages of development can be used as a predictive factor for selecting aneuploid embryos. Predicting aneuploidy in embryos at cleavage stage is enhanced as the number of chromosomal abnormalities identified per embryo increases.

What is known already: Embryo selection based on standard morphological criteria at the time of transfer only poorly correlates with the aneuploid status of the embryos. Non-invasive, morphokinetic analysis using time lapse imaging of the embryo has demonstrated that delayed first and second cleavage divisions combined with prolonged transition between 2- to 4-cells significantly correlates with chromosome aneuploidy. Also, recently the timing of cavitation of some aneuploid embryos has been shown to be significantly delayed.

Study design, size, duration: Blind retrospective analysis of time-lapse imaging, to measure timing and synchronicity of the first cleavage divisions. These parameters were correlated with aneuploid status of the embryos. 94 couples had 102 IVF cycles for aneuploidy testing, between 2011 and 2013. All procedures and protocols were carried out in Embryogenesis, Athens, Greece.

Participants/materials, setting, methods: 456 normally fertilized embryos were monitored using the Embryoviewer™. The frequency of direct cleavage events was introduced as a dynamic score. Embryos were categorized based on single cell 24 chromosome aneuploidy testing to a) (euploid) normal chromosome copy number, b) single aneuploidy, c) multiple aneuploidies (complex) or d) (chaotic).

Main results and the role of chance: Statistically significant differences were observed across all study groups for t2, t3, t5 and t6. The time intervals between t5–t2; t5–t3; t5–t4; t3–t2 ($p < 0.001$) and synchrony from 2–4 cells (t4-t3, $p = 0.03$) were also significantly different across the groups. The occurrence of dynamic score was 10%, 16%, 21% & 62% for Groups A, B, C & D, respectively.

Multivariable logistic regression analysis identified the time interval t4-t3 (Odds Ratio = 0.82; 95% Confidence Interval, 0.72–0.94) as the most relevant variable in selecting normal embryos. This value in combination with direct cleavage frequency (OR = 0.33; 95%CI, 0.18–0.61) provides the best model for selecting normal embryos.

Time interval t3–t2 along with direct cleavage frequency, has the highest predictive value when comparing Group A versus Group C. Correspondingly, the combination of t6 and t5–t4 has the highest predictive value when comparing Group A versus Group D.

Limitations, reason for caution: Mosaicism is the main factor that could lead to misdiagnosis when biopsy and aneuploidy testing are performed at cleavage stage embryos. The origin of aneuploidy is complex and multifactorial, therefore, careful assessment of the time-dependant variables and models utilized should be validated prior to clinical use.

Wider implications of the findings: Time-lapse technology could potentially distinguish embryos with multiple chromosome aneuploidies and may allow a more targeted approach to aneuploidy testing, reducing the overall cost. The embryo selection models could be powerful tools in the diagnosis of embryo viability and may prove extremely important when culture/society prohibits embryo biopsy. However, these findings should be treated with caution in clinical practice.

Study funding/competing interest(s): Funding by hospital/clinic(s), Study funded by Embryogenesis, Athens, Greece.

Trial registration number: This is not a clinical trial.

P-150 Consensus guidelines on the terminology and definitions for human pre-implantation embryo time-lapse monitoring by an expert panel

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Study question: How can the approach to, and terminology for, dynamic monitoring of pre-implantation embryo development be uniformly defined in order to improve the utilization and impact of this novel technology?

Summary answer: The need for consensus, and best practice, in the rapidly developing field of IVF time-lapse imaging has been highlighted by the varied scope and approaches to collection and interpretation of data. Minimization of variation in time-lapse imaging practice will allow validation of published embryo selection algorithms and facilitation of progress.

What is known already: An increasing quantity of researches relating to time lapse imaging of *in vitro* embryo development have demonstrated the added clinical value of morphokinetic data for embryo selection. Several articles have identified similar embryo selection or de-selection variables but have termed them differently. An evidence based consensus document exists for static embryo grading and selection but, to date, no such evidence based reference document is available relating to time-lapse methodology or dynamic embryo grading and selection.

Study design, size, duration: A series of consensus meetings were held between September 2011 and June 2013 involving expert time-lapse users from seven different European centres. The group reached to a consensus on commonly identified time-lapse variables.

Participants/materials, setting, methods: Guidelines on uniform annotation of pre implantation morphokinetic parameters are presented. The ALPHA and ESHRE assessment consensus criteria (2011) were used as reference and time-lapse monitoring (TLM) literature, as a resource.

Main results and the role of chance: Guidelines for the definitions and annotation of the following variables are presented; 'When & How' to annotate the first cleavage and subsequent cleavage events to the hatched blastocyst; cytoplasmic waves; second polar body extrusion; pronuclear appearance and fading; pronuclear scoring; duration and synchronization of cell cycles; compaction, blastulation and collapse; incidence of multinucleation; degree of fragmentation; direct and reverse cleavage events; cell evenness; presence of vacuolation, anomalies in the embryo development and cytoplasmic strings. Each generable calculation from these timings was explained.

Limitations, reason for caution: Variables here considered may not be exhaustive. Identification of new variables may require revised consensus. Different experts may have proposed different definitions for some of the variables discussed. Technical processed involved in TLM imply a not absolute continuous monitoring, so aspects of embryo development may not all be visualized.

Wider implications of the findings: This is the first consensus of TLM proposed by experienced clinical scientists. A systematic evaluation of current evidence and theory was undertaken to reach consensus on a uniform methodology and terminology for the use and study of TLM and its clinical application in IVF. Adoption of uniform approaches to the terminology and definitions of morphokinetics time lapse variables, would allow improved interpretation and sharing of data which could impact positively on patient treatment outcome.
Study funding/competing interest(s): Funding by hospital/clinic(s), IVI, Care Fertility, Centro Hera, Klinikk Hausen, Fertility Clinic.
Trial registration number: Not applicable.

P-151 Aneuploidy and implantation potential of euploid blastocysts cannot be predicted by non-invasive morphokinetic analysis during *in vitro* culture

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Study question: Is morphokinetic assessment related to aneuploidy and/or implantation potential of euploid blastocysts?
Summary answer: Morphokinetic parameters commonly assessed by time-lapse investigation up to blastocyst stage are not related to aneuploidy. Moreover, euploid implanted and not implanted embryos have similar timings of cell division and blastocyst formation.
What is known already: Recent studies have suggested a correlation between aneuploidy and/or implantation and morphokinetic parameters. However, there is not a general consensus on which parameter is the predictive one. Timing of singamy, to reach 5-cell-stage, and cell-cycle synchrony and duration have been suggested from some groups, timing to reach 8-cell stage from other, finally only correlations at blastocyst stage have been reported. Moreover, these studies were often underpowered and the results not adjusted for female age.
Study design, size, duration: A longitudinal cohort study was performed from September 2012 and December 2013. A total of 455 blastocysts, derived from 136 patients, cultured in timelapse incubator and subjected to trophectoderm biopsy and comprehensive chromosomes screening for aneuploidies, have been included.
Participants/materials, setting, methods: ICSI/PGS cycles due to advanced maternal age were analysed. 13 morphokinetic features were registered. Transfers were performed with single-vitrified-warmed-euploid blastocysts, and implantation (>12 weeks of gestation) was recorded. Logistic mixed effects models analysis adjusted for female age, with a subject-specific intercept was performed.
Main results and the role of chance: 186/455 (40.9%) blastocysts resulted euploid. Logistic regression analysis found no statistical correlation between the 13 morphokinetic characteristics analysed (syngamy, completion of cleavage to 2, 3, 4, 5 and 8-cells, length of first and second cell cycle and synchrony in the 2 divisions; initiation of compaction, initiation of blastulation, completion of blastulation) and aneuploidy (24.5 ± 3.3, 27.1 ± 3.3, 37.9 ± 4.9, 39.8 ± 4.9, 51.7 ± 7.3, 61.7 ± 10.7, 2.6 ± 0.9, 10.8 ± 3.3, 1.9 ± 3.0, 10.1 ± 8.8, 91.7, 104.8 ± 9.5, 119.7 ± 11.6 and 24.6 ± 3.3, 27.2 ± 3.3, 38.2 ± 4.7, 40.1 ± 5.1, 52.1 ± 8.3, 61.9 ± 11.6, 2.5 ± 0.9, 11.1 ± 2.9, 1.9 ± 3.1, 9.7 ± 8.2, 92.0 ± 9.5, 104.0 ± 9.7, 120.7 ± 11.9 for euploid and aneuploid blastocysts, respectively). As expected advancing female age was significantly predictive of aneuploidy ($P < 0.001$). Out of 74 transferred blastocysts 37 implanted (50%). Implanted and not implanted blastocysts also had similar timings of development.
Limitations, reason for caution: The study was restricted to a selected group of patients with increased risk of aneuploidy due to advanced maternal. A larger study would be required to prove if the findings are applicable for the whole cohort or other selected groups of patients. Furthermore, this study design did not allowed to distinguish between meiotic and mitotic derived aneuploidies. Future studies can be performed to specifically investigate the role of pre-compaction morphokinetic parameters in predicting mosaic aneuploidies.
Wider implications of the findings: In contrast to several recent publications, our results suggest that morphokinetic analysis cannot be considered as a predictive indicator of aneuploidy and/or implantation potential of euploid blastocysts. The lack of correlation found in this selected group of patients, prone to aneuploidy, indicates at this day no clinical value of morphokinetic investigation to improve embryo selection in PGS cycles.
Study funding/competing interest(s): Funding by hospital/clinic(s), none.
Trial registration number: None.

P-152 The effect of group embryo culture strategy based on pronuclear pattern on blastocyst development: a two-centre analysis

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Study question: to compare two different strategies of group culture from the zygote to the blastocyst stage. Does separate culture of grouped zygotes with symmetrical pronuclear pattern from grouped zygotes with non-symmetrical pronuclear pattern improve blastulation rate compared to random allocation of zygotes to group culture?
Summary answer: the two strategies of group culture gave similar results in terms of blastulation rate and top quality blastocyst rate. Regarding clinical pregnancy rates, the "random" group strategy showed statistically significant higher results compared to grouping according to the pronuclear pattern, after logistic regression analysis.
What is known already: in several animal models, increased embryo density has been suggested to improve development, potentially through secretion of autocrine/paracrine factors. In humans, group culture has been shown to be superior in terms of compaction and blastulation rates and blastocyst quality as compared to individual culture, albeit not consistently. Whether human embryos may take advantage from group culture is still controversial.
Study design, size, duration: Retrospective time-course study. IVF-ICSI patients with at least 6 fertilized oocytes recruited in two Centres. Culture strategies and time period: "Random" group strategy: September 2012–November 2012, zygotes were randomly grouped. "Definite" group strategy: December 2012–March 2013, zygotes were grouped based on the pronuclear pattern.
Participants/materials, setting, methods: "random" strategy: zygotes were randomly grouped under a binocular microscope not allowing for identification of pronuclear pattern. "Definite" strategy: zygotes were grouped based on the pronuclear pattern, namely Type-1 (symmetrical) separately from Type-2 (other arrangements) (ref. Istanbul Consensus). Zygotes were group cultured up to 4/20-ml-drop till blastocyst stage.
Main results and the role of chance: In 80 patients belonging to "random" group and 89 patients belonging to "definite" group, a mean number of 11.9 ± 3.9 and 12.7 ± 3.8 oocytes were obtained, respectively. Baseline clinical characteristics and cycle parameters for the two groups were not significantly different. A total of 594 and 649 zygotes were grouped in the "random" and in the "definite" arm, respectively. The blastulation rate was similar: 42% and 41% day (5 + 6) blastocysts, 32% and 31% top quality blastocysts were obtained in the two groups, respectively ($p > 0.05$). Cumulative pregnancy rate showed a marked tendency in favour of the "random" strategy compared to "definite" strategy, being 58% and 44%, respectively ($p = 0.08$). After adjusting for baseline and cycle variables with a stepwise forward regression analysis, this difference reached statistically significant difference ($p < 0.03$).
Limitations, reason for caution: Blastulation rate was the primary outcome in good prognosis patients. Secondary outcomes such as pregnancy rate should therefore be interpreted with caution. The study was retrospective but the setting, staff and procedures that characterized the entire study period did not change over time.
Wider implications of the findings: A similar blastulation rate was observed using the two strategies of group culture. However, the developmental potential of blastocysts was shown to be better in the "random" group. In fact, a stepwise forward logistic regression analysis confirmed results in terms of blastulation rates and highlighted a significant increase in pregnancy rates in favour of "random" group when adjusting for baseline and cycle variables.
Study funding/competing interest(s): Funding by hospital/clinic(s), Fondazione Ca' Granda Policlinico Milan.
Trial registration number: Not applicable.

P-153 Clinical benefit of zona free embryo transfer examined with blastocyst grade

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Study question: Blastocyst transfer is generally able to obtain high pregnancy rate by selecting good embryos, however, some cases are failed to pregnancy

despite several times of transfer. To lead to pregnancy with less frequency of transfers, we examined the utility of zona free embryo transfer (ZFET).
Summary answer: Regardless of the frequency of transfers, ZFET could maintain high pregnancy rate and had no influence on abortion rate, and also it was very higher effective in Inner Cell Mass (ICM) grade than Trophectoderm (TE) grade.
What is known already: By improvement of culture and cryopreservation technique, blastocyst transfer is known as the method which results high pregnancy rate and low multiplets rate with fewer blastocyst.
Study design, size, duration: We classified 264 cases (216 patients) in zona free method and 478 cases (397 patients) in normal PZD (Partial Zona Dissection) method, which underwent single blastocyst transfer from September 2011 through October 2013 in our clinic.
Participants/materials, setting, methods: We compared the clinical pregnancy and abortion rate in zona free method and normal PZD method with Gardner's classification which were ICM and TE cell numbers. We also compared in frequency of transfers.
Main results and the role of chance: The patient mean age was 36.5 ± 4.3 years old in zona free method, 35.7 ± 3.8 years old in normal PZD method, so there was a significant difference ($P < 0.05$). Whereas there was no difference in pregnancy or abortion rate between ICM and TE grade, the pregnancy rate in ZFET tended to increase at any grade when we classified by frequency of transfers. Especially, the pregnancy rate in ICM grade A ($P < 0.05$) and TE grade B ($P < 0.05$) is significantly high pregnancy rate in ZFET. In the case with multiple failures of conception, only zona free method could lead ICM poor embryo to pregnancy.
Limitations, reason for caution: None.
Wider implications of the findings: ZFET is very effective method not just for the case with multiple failures but to improve pregnancy rate with less frequency of transfers. As classified by blastocyst grades, the method is more effective in ICM grade than TE grade. It is also useful for lower quality embryo.
Study funding/competing interest(s): Funding by hospital/clinic(s), No competing interest is declared.
Trial registration number: None.

P-154 Assessing the impact of factors derived from morphokinetic analysis in decreasing implantation potential of morphologically good embryos

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Study question: The aim of the study is to evaluate the extent of reduction of the implantation potential of embryos characterised as groups A–D with the hierarchical model of classification (Meseguer et al., 2011) when they also have associated multinucleation or direct cleavage from 1–3 cells
Summary answer: Morphologically good embryos with a direct cleavage at any stage during early development up to the 8 cell stage have a significantly reduced potential for implantation when compared with embryos classified similarly A-D on hierarchical models. The impact of micronucleation on implantation potential was not significant.
What is known already: The hierarchical model of classification has a gradation of embryo viability which is greatest at grade A and lowest at grade D. The presence of micronucleation in cells at early cleavage stages has often been a characteristic associated with a reduced viability. Similarly, the advent of time lapse has defined direct cleavage events as associated with low viability and increased aneuploidy.
Study design, size, duration: A retrospective study with a total of 182 patients, <40 years of age undergoing IVF between Jun 2011–Dec 2013 and embryos evaluated by non-invasive time-lapse imaging system. Patients were allocated into 3 different groups and the outcome of clinical pregnancy was the completion point of study.
Participants/materials, setting, methods: All groups were matched for ages, number of embryos transferred (2–3) and morphological grade of embryos day 3 (grade 1–2). All embryos were categorised as A–D on hierarchical model, then subdivided into; Group 1 (A–D), Group 2 (A–D with micronucleation), & Group 3 (A–D with direct 1–3 cleave at any stage).
Main results and the role of chance: The number of embryo transfers for Group1, 2 & 3 embryos were 102, 38 & 42 respectively. The clinical pregnancy rate were: Group 1 – 61/102 (59.8%), Group 2 – 2138 (55.3%) & Group 3, with a significantly lower pregnancy rate of– 2/42 (5%) ($p < 0.0001$). The implantation rates for Group 3 embryos also significantly lower than in the other groups: Group 1 – 109/275 (39.6%), Group 2 – 33/100 (33%), & Group 3 2/119 (1.7%) $p < 0.0001$.
Limitations, reason for caution: The number of cases is relatively low because it is rare for patients to have only multinucleated embryos or only direct cleave embryos for transfer. If only one type is available for ET, there may be an underlying aetiology creating these types of embryos which we have not examined here.
Wider implications of the findings: In all models of embryo viability we need to heavily weight the incidence of direct cleavage as a negative impact factor, even when the abnormal cleave occurs in the second or third cell cycle which becomes more difficult to detect and has virtually no impact on embryo morphology and grading. The presence of micronucleation for early implantation does not have significant impact.
Study funding/competing interest(s): Funding by hospital/clinic(s), Embryogenesis Assisted Conception Unit Athens – Greece.
Trial registration number: None.

P-155 A novel aspect of cytoplasmic vacuole formation in human zygotes and its clinical impacts on embryonic development revealed by high-resolution time-lapse cinematography

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Study question: We recently found a novel aspect of cytoplasmic vacuole (CV) formation by using high-resolution time-lapse cinematography (hR-TLC). Based on this finding, we further investigated the dynamic morphology of CVs and their clinical impacts on embryonic development in human conventional IVF (c-IVF) and intracellular sperm injection (ICSI) programs.
Summary answer: We confirmed by hR-TLC that the CV first appears in the zygote periphery simultaneously with formation of the male and female pronucleus (mPN, fPN), and then migrates toward the center of the zygote surrounding the mPN and fPN. The appearance of CVs in zygotes was closely associated with multinucleation.
What is known already: Oocyte dysmorphisms, including vacuoles, smooth endoplasmic reticulum clusters, and cytoplasmic granularity, are strongly associated with a decreased fertilization rate and/or poor embryonic development. It is also widely accepted that formation of the CV during embryonic development is closely related to poor outcome with assisted reproductive technologies (ART). However, the dynamic morphology of CV formation and evolution during embryonic development was still unknown.
Study design, size, duration: Since 2003, we collected 199 oocytes donated from 186 patients (c-IVF: 95 and ICSI: 104) and 144 fertilized normally (c-IVF: 55, ICSI: 89) for hR-TLC observation. Of the 144 normally fertilized oocytes, 13 (c-IVF: 2, ICSI: 11) showed CV formation, and these were prospectively analyzed for 2 days by hR-TLC.
Participants/materials, setting, methods: The hR-TLC was commenced at 1.5 h after insemination in c-IVF oocytes, and immediately after the ICSI procedure. Digital images were acquired for 2 days with an exposure time of 1/20 s. After hR-TLC observation, good-quality embryos developed to the 4- to 8-cell stage were cryopreserved for future clinical use.
Main results and the role of chance: There was no significant difference in the frequency of CV appearance between c-IVF and ICSI oocytes (3.6% vs. 12.4%, respectively). The CV appeared in the peripheral cytoplasm after extrusion of the second polar body (PB) and moved synchronously, simultaneously with the mPN and fPN migrating toward the central cytoplasm. The CV size gradually enlarged in the cytoplasm and was retained within the blastomere after the first cleavage. Although there was no difference in the developmental velocity between the embryos with and without CV, the rate of multinucleated blastomeres was significantly higher in embryos with CV than in those without CV (61.5% vs. 22.1%; $P < 0.05$).
Limitations, reason for caution: In this study, we excluded oocytes with vacuoles, because we could not evaluate morphology before insemination in c-IVF oocytes. Therefore, we focused on the CV appearance only during the zygotic stage. We could not determine future clinical outcomes for zygotes with and without CV due to the limited sample size.
Wider implications of the findings: This hR-TLC study demonstrated CVs forming in the periphery of the zygote cytoplasm in accordance with the timing

of PN appearance after c-IVF and ICSI, and then migrating toward the center of the cytoplasm surrounding mPN and fPN. The rate of blastomere multinucleation was significantly higher in zygotes with a CV than in those without ($P < 0.05$), suggesting that CV appearance at the zygote stage could predict aberrant embryonic development.
Study funding/competing interest(s): Funding by hospital/clinic(s), None.
Trial registration number: None.

P-156 Analysis of cleavage patterns and centrosome formation during the first mitotic division in abnormal human zygotes using an immunofluorescence staining and live-imaging system

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Study question: We used immunofluorescence staining and a live-imaging system to investigate cleavage patterns and centrosome formation in abnormal human zygotes with monopronucleus (1PN) and tripronucleus (3PN) obtained from conventional *in vitro* fertilization (c-IVF).
Summary answer: We found that half of the zygotes with 1PN were generated by parthenogenic activation and the other half failed to form the male pronucleus after fertilization. Most of the zygotes with 3PN demonstrated dispermic fertilization followed by tripolar cleavage.
What is known already: In humans, maternal centrosomes are reduced and inactivated during oogenesis and the centrosomes are contributed by the sperm during fertilization. The known mechanisms involved in formation of 1PN zygotes in c-IVF are as follows; 1) asynchrony of the pronuclear appearance, 2) male and female genome fusion, and 3) parthenogenesis. 3PN zygotes from c-IVF arise from either dispermy or digyny caused by retention of the second polar body.
Study design, size, duration: Frozen/thawed zygotes with 1PN ($n = 10$), 3PN ($n = 13$) and 2PN ($n = 3$; control) donated from infertile copules were used in this study. Three 1PN, five 3PN and three 2PN zygotes were used for immunofluorescent staining, and the remaining 1PN and 3PN zygotes were used to visualize nuclear segregation by live-imaging.
Participants/materials, setting, methods: Centrosomes were identified using a rabbit polyclonal anti-pericentrin antibody (1:500 dilution, abcam). Alexa Fluor 568 goat anti-rabbit IgG antibody was purchased from Life Technologies. We synthesized H2B-mCherry mRNA for live-imaging from pGEM-H2B-mCherry from Dr. Takashi Hiiragi (European Molecular Biology Laboratory). Images were obtained using FV1000 (Olympus) and CV1000 (Yokogawa).
Main results and the role of chance: We confirmed the presence of two centrosomes in all 2PN control zygotes. One of three 1PN zygotes retained the centrosome in the cytoplasm and the others had no centrosomes, suggesting parthenogenesis. Live-imaging followed by immunostaining of centrosomes showed that three of seven 1PN zygotes had no centrosome suggesting parthenogenesis, and two of the 1PN zygotes with centrosomes had not cleaved. The remaining two 1PN zygotes had one or two centrosomes in each blastomere after the first cleavage.

Three of five zygotes with 3PN had four centrosomes, suggesting dispermic fertilization. The remaining two zygotes showed five centrosomes, suggesting dispermy with duplication of one centrosome. Of eight zygotes with 3PN, five demonstrated tripolar cleavage and the remaining three were poorly cleaved.
Limitations, reason for caution: We were unable to obtain fluorescent signals from zygotic stage to first cleavage stage embryos using H2B-mCherry mRNA due to temporal differences between mRNA injection and translation. Though this is a preliminary study, it is important in clarifying the physiology of fertilization and early stages of embryonic development.
Wider implications of the findings: This study demonstrated that half of the zygotes with 1PN had paternally derived centrosomes and half showed parthenogenetic development. However, our identification of 1PN zygotes with both male and female genomes in one nuclear envelope, suggested the involvement of several aberrant mechanisms. In addition, as most 3PN zygotes showed dispermic fertilization and demonstrated tripolar cleavage, investigation of the function of centrosomes in zygotes with 3PN due to dispermic fertilization is also important.
Study funding/competing interest(s): Funding by hospital/clinic(s), Mio Fertility Clinic.
Trial registration number: None.

P-157 Vitrification using hydroxypropyl cellulose (HPC) as a novel cryoprotectant demonstrates significant improvement of survival rate of zona pellucid (ZP) free human blastocysts

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Study question: Can HPC-supplemented vitrification media improve survival rate of human ZP free blastocysts after vitrification and warming?
Summary answer: HPC-supplemented vitrification media showed significant improvement of survival rate of ZP-free blastocysts in human.
What is known already: HPC is plant-derived non-ionic water soluble cellulose which is already listed as pharmacopeia and is widely used in drug manufacturing.

Our previous studies indicated that the use of HPC as a substitute for serum substitute solution (SSS) in Cryotop® vitrification media improved the survival rate of mouse blastocysts that normally show poor cryoresistance such as ZP-free blastocysts and blastocysts from inbred strain. Yet, the effectiveness of HPC in human blastocysts is still unknown.
Study design, size, duration: Cryopreserved human blastocysts ($n = 97$) donated from patients in our center were warmed and used for the present study. Some of blastocysts ($n = 33$) were treated in acid Tyrode's solution to dissolve ZP to serve as ZP-free blastocysts. They were vitrified and warmed in either HPC or SSS-supplemented vitrification/warming media using Cryotop® devices. The survival rates of blastocysts and cell viability were assessed and compared among experimental groups.
Participants/materials, setting, methods: HPC solution was made by dissolving 60 mg/ml of HPC in Milli-Q water. Human blastocysts with or without ZP were re-vitrified and warmed using either 5% (v/v) HPC or 1% (v/v) SSS-supplemented media. The survival rate of blastocysts were assessed based on the re-expansion of the blastocoele 2 h after warming. Cell viability of survived blastocysts was examined by Hoechst and PI staining.
Main results and the role of chance: For ZP intact blastocysts, the survival rate of after re-vitrification/warming was 100% in both HPC and SSS groups (32/32, 33/33, respectively). The cell viability in HPC and SSS were 93.2 ± 0.02% and 89.2 ± 0.03% respectively and no significant differences were observed between 5% HPC and 1% SSS for non-re-vitrified blastocysts (89.6 ± 0.03%). For ZP-free blastocysts, the survival rate was significantly higher in HPC compared with SSS (90.0%, 9/10 and 20.0%, 2/10, respectively, $P < 0.05$). The cell viability was significantly higher in HPC (85.9 ± 3.0%) than SSS (49.5 ± 7.6%, $P < 0.05$). The HPC group showed comparable cell viability for non-re-vitrified blastocysts (76.1 ± 8.7%).
Limitations, reason for caution: Although human donated blastocysts were used to confirm the effectiveness of HPC-supplemented vitrification/warming media, randomized clinical trials are necessary to evaluate the long term effects of HPC before it can be used in medical practice.
Wider implications of the findings: The present study revealed that HPC is an acceptable substitute for SSS in vitrification media because it improved the survival rate of human blastocysts, especially ZP-free blastocysts. Also our results speculate that HPC would advantageous for human hatched blastocysts which frequently exhibit poor survival rate after vitrification/warming. Our findings suggest the possibility of adopting HPC as a novel cryoprotectant in ART.
Study funding/competing interest(s): Funding by hospital/clinic(s), Kato Ladies Clinic.
Trial registration number: N/A.

P-158 Abnormal spindles in human blastocyst increase with maternal age

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Study question: Does the incidence of morphologically abnormal spindle in human blastocysts increase with maternal age?
Summary answer: The incidence of morphologically abnormal spindle increased and the implantation competence decreased with an increase of maternal age.

The appearance of abnormal spindle adversely affected the cell cycle. The increase in morphologically abnormal spindles would be one of the critical causes of age-related infertility.

What is known already: An increase of chromosomal aneuploidy in oocytes with maternal age is the dominant contributor of age-related infertility. However, the infertility could not be explained simply by the increased aneuploidy.

Study design, size, duration: We performed a retrospective cohort study including 727 single vitrified–warmed blastocyst transfers that developed to the blastocyst stage on day 5 after insemination between 2010 and 2012, and 2 experimental studies using 154 day-5 blastocysts and 36 pronuclear embryos donated for research. The local IRB approved this study.

Participants/materials, setting, methods: We analyzed the relationship of the pregnancy rate of vitrified–warmed day 5 blastocysts with maternal age. In experimental studies, vitrified blastocysts were immunostained with anti-α-tubulin antibody, anti-γ-tubulin antibody and DAPI. Pronuclear embryos were injected with cRNA encoding mRFP1 fused with histone H2B.

Main results and the role of chance: The clinical study revealed that the pregnancy rate of day 5 blastocysts on 9 weeks of gestation decreased with maternal age ($R^2 = 0.7982$, $P < 0.0001$). We analyzed spindles of totally 392 specimens. The incidence of morphologically abnormal spindles increased with maternal age ($R^2 = 0.59$, $P = 0.0038$). Dynamic changes of chromosomes of the cRNA-injected embryos were monitored using a confocal microscope inside an incubator. Seventeen embryos developed to blastocysts after imaging (47.2%). More than half embryos appeared to form abnormal spindles and multi-nuclei. The cell cycles of such blastomeres were found to delay or arrest. Comparative genomic hybridization (CGH) analysis showed that 12 blastocysts were euploid (70.6%) though morphologically abnormal spindles were observed during their development.

Limitations, reason for caution: Further studies are required to clarify the relationship between an increase in morphologically abnormal spindle and a decrease in embryonic implantation potential.

Wider implications of the findings: This study provides new insights into the implications of a decrease of fertility with maternal age.

Study funding/competing interest(s): Funding by national/international organization(s), The Japan Society for the Promotion of Science (JPS-RFTF 23580397 to S.H.).

Trial registration number: None.

P-159 Air bubble position after embryo transfer affects pregnancy outcome differently in cleavage- and blastocyst-stage embryos

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Study question: To evaluate whether embryo deposited site assessed by air bubble location after embryo transfer (ET) is associated with clinical outcome.

Summary answer: Favorable clinical outcome was associated with further ET distance from the fundus for cleavage-stage embryos, while closer for blastocyst-stage embryos.

What is known already: Embryo transfer is the seemingly simplest but the most crucial step that guarantee a reliable clinical pregnancy rate while the best embryo depositing site remained to be discussed. The existing literature consisting of small sample size has yielded contradictory results

Study design, size, duration: Retrospective clinical study of 5082 cycles that enrolled between Jan 2011 and Jan 2013.

Participants/materials, setting, methods: 3504 patients that met follow-up endpoint (negative blood/urine test, early spontaneous abortion, ectopic/heterotopic pregnancy, late term abortion or live birth) enrolled in Reproductive Medical Center of Sun Yat-Sen Memorial Hospital. Distance from the air bubble to the fundus was measured after embryo transfer under the guide of abdominal ultrasound.

Main results and the role of chance: In fresh ET cycles ($N = 3214$) of cleavage-stage embryos, the clinical pregnancy rate (CPR), implantation rate (IR) and taking-home-baby rate of (THR) the ≤10 mm distance group ($N = 1619$) were reduced compared to the >10 mm group ($N = 1595$) (46.16% vs 50.28%, $P = .016$; 30.30% vs 32.64%, $P = .035$;and 37.00% vs 43.82%, $p = .004$; respectively) while ectopic/heterotopic pregnancy rate was significantly higher (4.30% vs 2.24%, $P = .023$). In fresh blastocyst transfer cycles ($N = 156$), on the contrary, PR, IR and THR were significantly higher in the ≤10 mm group (54.32% vs 36.00%, $P = .045$; 43.40% vs 23.08%, $P = .000$;and 49.38% vs 29.33%, $p = .011$; respectively). Similar trend was observed in FET cycles, but only IR of cleavage-stage embryos ($N = 1604$) was found of significant difference (≤10 mm vs >10 mm, 18.91% vs 21.94%, $P = .024$).

Limitations, reason for caution: Due to the retrospective nature of the study, the parallel groups were not controlled for all factors. As the length of endometrial plate was not provided, the precise relative position was unknown and the results should be interpreted with care.

Wider implications of the findings: The implantation mechanism may be associated with the spatiotemporal embryo-stage specific interaction between the embryos and the endometria during the window of implantation.

Study funding/competing interest(s): Funding by University(ies), Sun Yat-Sen Memorial Hospital.

Trial registration number: None.

P-160 Pregnancy and childbirth resulting from transfer of frozen-thawed blastocysts generated by culture of zona-free ICSI embryos

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Study question: Zona-free oocytes are occasionally encountered during collection of human oocytes for ART. Here, we investigated whether zona-free oocytes could be fertilized by ICSI and if the resulting embryos could develop to the blastocyst stage. We also investigated whether transfer of such blastocysts resulted in successful pregnancies and childbirth.

Summary answer: Fifty-six zona-free oocytes were collected and 37 (66.1%) were successfully fertilized by conventional or piezo-ICSI. Seven (18.9%) of these zygotes developed into good blastocysts. Four frozen-thawed blastocysts were used for transfers and three (75.0%) produced pregnancies; two healthy babies were delivered.

What is known already: It has been reported that zona-free oocytes can be fertilized by ICSI and that pregnancies and healthy babies can be produced after embryo transfer. However, it is not easy to obtain undamaged embryos, and the pregnancy rate is lower than that for zona-intact oocytes.

Study design, size, duration: During the collection of human oocytes from 2009 to 2013, 56 zona-free oocytes were identified and used for this study.

Participants/materials, setting, methods: Zona-free oocytes were fertilized by conventional or piezo-ICSI. The embryos were cultured for 5 days until the blastocyst stage; blastocysts ≥160 μm in diameter were frozen using a vitrification method. Frozen-thawed blastocysts were transferred into patients undergoing hormone replacement cycles.

Main results and the role of chance: Thirty-seven (66.1%) of the 56 zona-free oocytes were successfully fertilized by ICSI. Seven (18.9%) of these embryos developed into good quality blastocysts; four frozen-thawed blastocysts were used for transfers and three (75.0%) resulted in a pregnancy. Two healthy babies were delivered from these women. Over the same period, the rate of successfully fertilized embryos after ICSI using zona-intact oocytes was 77.9% (8996/11545), and the rate of development of good blastocysts was 33.0% (2542/7694). Thus, the fertilization rate for zona-free oocytes after ICSI was significantly lower than that for zona-intact oocytes. However, there was no significant difference in the rates of formation of good blastocysts between the two groups.

Limitations, reason for caution: None.

Wider implications of the findings: Zona-free oocytes are not always used for fertilization because of the risk of physical damage to the oocytes by ICSI. Here, we found that about 70% of zona-free oocytes could be fertilized by ICSI and that good blastocysts could be obtained for successful transfers to produce live born children. Our study indicates that ICSI of zona-free oocytes may be a viable strategy for infertile women from whom a limited number of oocytes is available.

Study funding/competing interest(s): Funding by hospital/clinic(s), Ochi Yume Clinic Nagoya.

Trial registration number: None.

P-161 Blastocyst culture using continuous culture medium and time-lapse monitoring

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Study question: Can the outcome of *in vitro* embryo culture be improved if embryos are incubated in conditions in which they are maintained in the same medium throughout the culture period, are not exposed to the external environment, and are monitored using a time-lapse system?
Summary answer: Compared to conventional culture methods in which the medium is regularly renewed, we found an acceleration of blastocyst development and an improved rate of generating good quality blastocysts for cryopreservation; the rate of successful implantation was similar to that obtained in conventional culture.
What is known already: It was previously reported that embryos cultured in Continuous Single Culture medium (Irvine Scientific), which does not need to be renewed during culture unlike conventional media, showed an increase in the rate of developing to the blastocyst stage.
Study design, size, duration: Eggs were retrieved in 203 cycles from 190 women in October to December 2013; the eggs were fertilized *in vitro*, cultured and 402 embryos were obtained. Control cultures (430 embryos) were performed using QA: SAGE medium, with two changes of medium, from August to October 2013.
Participants/materials, setting, methods: Each embryo was cultured in 28 µl Continuous Single Culture medium (Irvine Scientific), which obviates the need for medium renewal, to day 6 of embryonic development. Cultures were placed in an EmbryoScope incubator (Unisense FertiliTech), which has a time-lapse monitoring system, and not exposed further to the external environment.
Main results and the role of chance: Blastocysts with an inner diameter of 160 µm or more and in which an identifiable inner cell mass (ICM) was present, were deemed to be good quality blastocysts and were cryopreserved. The rate of good quality blastocysts selected for cryopreservation in the single medium culture group (167/402; 41.5%) was significantly higher ($P < 0.05$) than in the control group (147/430; 34.2%). We also found that the development of the embryos was significantly accelerated as 91/402 (22.6%) blastocysts were cryopreserved on day 5 compared to 67/430 (15.6%) in the conventional culture ($P < 0.05$). The respective rates of implantation/pregnancy for the two groups of blastocysts were 61.1% (11/18) and 57.3% (47/82); the difference was not significant.
Limitations, reason for caution: None.
Wider implications of the findings: The experimental culture system used here produced a higher rate of good quality blastocysts for cryopreservation; moreover, good quality blastocysts developed more rapidly. Thus, an embryo culture system that does not require renewal of the medium, and which avoids exposure of the embryos to the external environment, provides good conditions for embryo growth and greatly reduces external environmental stresses on the embryos. These factors appeared to improve the outcome of the culture.
Study funding/competing interest(s): Funding by hospital/clinic(s), Ochi Yume Clinic Nagoya.
Trial registration number: None.

P-162 The process of blastocyst development in frozen-thawed single transfer and effectiveness of the grade of ICM and TE

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Study question: How do the process of blastocyst development and ICM and TE grade affect clinical outcome?
Summary answer: Both in ICM and TE grade, the clinical outcome of Blasto 3 whether in grade A or B are equal. When we developed into Blasto 4, alphabetically the better grade, the higher pregnancy rate and the lower abortion rate both in ICM and TE grade.
What is known already: Progress in embryo culture and cryopreservation techniques permit to get multiple blastocyst at the oocyte pick up. Selecting the best embryo for transfer is essential to lead to pregnancy with less frequency of transfer. Recently, Gardner's classification is widely used as morphological evaluation of blastocyst in generally.
Study design, size, duration: We examined 1216 cycles (808 cases) which underwent frozen-thawed blastocyst single transfer from January 2009 through October 2013 in our clinic.
Participants/materials, setting, methods: After we evaluated blastocyst with Gardner's classification at cryopreservation, we retrospectively examined by comparing the process of development and the rate of pregnancy and abortion both in ICM and TE grade. In addition, to exclude the case with multiple failure, we targeted only the patient who have had ET 2 times or less.
Main results and the role of chance: The patients mean age ± SD was 35.6 ± 3.9 years old. The pregnancy rate of Blasto 3 ICM at grade A and B were significantly high compared with that at grade C ($P < 0.005$). As for Blasto 4, the pregnancy rate at grade A was significantly higher than that either at grade B or C ($P < 0.005$).The pregnancy rate of Blasto 3 TE at grade A and B were significantly high compared with that at grade C ($P < 0.05$). As for Blasto 4 at grade A and B, the rate was significantly higher than that at grade C ($P < 0.005$). Meanwhile, there was no significant difference in the abortion rate of either Blasto 3 or 4 between each grade both in ICM and TE. In the case of Blasto 4, the better grade is, the lower the abortion rate.
Limitations, reason for caution: None.
Wider implications of the findings: In Gardner's classification, pregnancy and abortion rate of Blasto 3 were almost equal at grade A and B either in ICM or TE. As for Blasto 4, the pregnancy rate became high and the abortion rate became low with alphabetical order. Therefore, we conclude that grade A and B of Blasto 3 are equal; on the other hand, the grade alphabetically affects the clinical outcome in the case the blastocyst develops into Blasto 4.
Study funding/competing interest(s): Funding by hospital/clinic(s), No competing interest is declared.
Trial registration number: None.

P-163 Cleavage synchronicity thresholds derived from aneuploid embryos predict embryo implantation and live birth in an infertile cohort without PGS

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Study question: Can the implantation potential of an embryo and the live birth be predicted from its ploidy status calculated using morphokinetic parameters obtained until day 3?
Summary answer: Two kinetic ratios based on selected cleavage cycles defining mitotic synchronicity were used to construct a ploidy model, which provided high predictivity of implantation and of live birth in an infertile cohort without PGS.
What is known already: Embryo implantation 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). 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).
Study design, size, duration: This retrospective cohort study included 3176 embryos having a (t8). A total of 359 embryos biopsied on day 5 and diagnosed using arrayCGH were used to derive the ploidy model, which was further retrospectively applied to 997 embryos with known implantation data (KID). Live birth was available for 669 KID embryos belonging to 387 patients.
Participants/materials, setting, methods: Incubation was performed in time-lapse incubators (EmbryoScope™). Four categories were defined in the non-PGS group using cut-off values obtained from biopsied embryos. The mean female age was 33.18 and 32.35 in the PGS and non-PGS group, respectively.

Implantation was defined by the presence of a gestational sac observed by ultrasound.
Main results and the role of chance: Cleavage synchronicity from 2–8 cells CS2–8 = ((t3–t2) + (t5–t4))/(t8–t2) reflects the ratio of time the embryo spends at 2 and 4 cells over the time from 2–8 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. Similarly, the cleavage synchronicity from 4 to 8 cells CS4–8 = ((t8–t5)/(t8–t4)) was defined. Cut-off values were calculated as 0.8315 for CS2–8 and as 0.2734 for CS4–8 in PGS embryos. The KID rate increased from 22.48% to 42.86% (two-fold increase, chi-square $p < 0.0001$). Likewise, the live birth rate was multiplied by three in the high euploidy category when compared to the high aneuploidy category (from 14.44% to 42.10%, chi-square $p < 0.0001$).
Limitations, reason for caution: Embryos having a (t8) were exclusively included in the ploidy model. The non-PGS cohort studied involved infertile patients with different etiologies (female, male or combined) and is in this respect a heterogeneous population. The mean female age of the four categories defined in the ploidy model are statistically non-significant.
Wider implications of the findings: Time points defining precise embryo cleavage events may not be generalized to infertile patients with different etiologies. However, using ratios based on selected cleavage cycles defining synchronicity of embryos, allowed in this study an individualized analysis giving high predictivity of implantation and live birth. Time-lapse cannot be substituted to arrayCGH, but can provide additional valuable data in order to choose the most suitable embryo(s) for a cost-effective trophectoderm biopsy strategy and for single embryo transfer.
Study funding/competing interest(s): Funding by hospital/clinic(s), Sisli Memorial Hospital.
Trial registration number: Not required.

P-164 Disproportion of sex ratio within aneuploid embryos after aCGH

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Study question: Does sex ratio of embryos undergoing array comprehensive genome hybridization (aCGH) reflect pattern of human population?
Summary answer: Although the ratio of males and females embryos within euploid group is almost 50:50, the group of aneuploid embryos has explicit disproportion to males. Besides, the strict correlation was not found between embryo morphology and sex.
What is known already: Male embryos are prone to achieve expanded blastocyst stage faster and thus are more preferable to be chosen for transfer. Also embryo morphology is connected with its chromosomal status.
Study design, size, duration: Retrospective randomized study. Data was collected from May 2012 to December 2013 in private IVF centre.
Participants/materials, setting, methods: Embryo culture was performed in EmbryoScope (Unisense Fertilitech A/S, Denmark). Cleavage embryos were scored using manufacturer software and blastocysts were scored according to Istanbul consensus. 438 blastocysts from 79 patients underwent trophectoderm biopsy on day 5 or day 6 or day 7 and aCGH. The average patients age was 35.5 years.
Main results and the role of chance: 233 embryos were found to be euploid and 205 – aneuploid. In the euploid group the proportion of females to males was 121/112 respectively, whereas in the aneuploid group the proportion of females to males was 68/98 respectively, $p < 0.05$. Thus aneuploid embryo is more likely to be male than female. The morphology within groups of euploid and aneuploid embryos was comparable with a slightly deterioration in the aneuploid group. Interestingly, that biopsy of aneuploid embryos was performed mostly on day 6 or 7 whereas for euploid embryos it was day 5. The difference was statistically significant when compared (trophectoderm biopsy on day 5 to day 6 (7) proportion was: 67/105 for aneuploid and 131/101 for euploid embryos respectively, $p < 0.05$).
Limitations, reason for caution: Despite of relatively young patients age they all had aggravated anamnesis and thus formed specific group that probably did not represent the whole population. Besides, donor cycles were not excluded from the study.
Wider implications of the findings: Gender disproportion of aneuploid embryos could be possibly caused by the same factors that force male embryos to develop faster than female embryos ignoring chromosomal pathologies, but still this question is opened for debate.
Study funding/competing interest(s): Funding by hospital/clinic(s), Clinic of Reproductive Medicine 'Nadiya'.
Trial registration number: Not applicable, due to the retrospective design of the study.

P-165 Simple steps to identify viable embryos using time lapse monitoring. Can it replace classical "Pronuclear-scoring" under the auspice of the German Embryo Protection Law?

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Study question: The aim of this attempt is the use of time lapse system EmbryoScope™ immediately after the ICSI insemination process to replace static pronuclear scoring under the auspice of the German Embryo Protection Law (GEPL). Does appearance and fading time of pronuclei work as a sufficient tool to avoid static observations?
Summary answer: The preliminary data show clearly the useful application of a time lapse monitoring system. It allows watching the dynamic process of oocytes shortly after the insemination process and judging the possible embryo quality without any decrease of the pregnancy and implantation rates.
What is known already: Recent studies have shown the usefulness of EmbryoScope™. But most countries do not face such strict GEPL like Germany does. Some attempts describe the innovation of models to identify viable embryos. This is done by cultivating all of the oocytes. Static pronuclear scoring is a good and also proven tool to identify possible viable embryos to induce a pregnancy after embryo replacement. EmbryoScope™ allows minimizing time of physiological stress for oocytes outside the incubator.
Study design, size, duration: This is a prospective observational attempt at a private IVF-centre. 366 patients are included, undergoing the IVF or ICSI program after pituitary down-regulation or with following GnRH-antagonist treatment, irrespective of their indications of subfertility. It is a continuing study to gather more data for a substantial based data base.
Participants/materials, setting, methods: Ovarian stimulation of patients aged 22–46 years was performed with gonadotrophins. Time of appearance and fading of pronuclei, 2 pronuclei at 18 h post inseminationem, the development at 42 h and at 62 h as well of oocytes and embryos was calculated. Embryo replacement was performed 3 days after retrieval.
Main results and the role of chance: Overall pregnancy rate was 34.8% per transfer. After replacing 447 embryos, 88 embryos implanted, leading to a implantation rate of 19.7%. The time of pronuclei appearance in non-pregnant was a mean of 9.7 h vs. 5.9 h in pregnant women ($p \leq 0.05$); the time of fading of pronuclei in non-pregnant was a mean of 26.9 h vs. 24.7 in pregnant women. All embryos of women who got pregnant showed 4 cell stage embryos after 42 h and 8 cell stage embryos after 62 h. All embryo replacements were done on day three after ovum pick up. There seems to be a relation between the appearance and the fading of pronuclei, and the development of 8 cell stage embryos on day 3 as well.
Limitations, reason for caution: The study at the moment lack a greater database for consistent calculation. Presenting only preliminary data, efforts are needed to strengthen the statistical power. The attempt shows unselected couples with variation of age and indications, which should be splitted in subgroups.
Wider implications of the findings: More detailed investigation of surplus parameters may enhance the power of information, especially in finding reproducible cut off parameters. But the preliminary results shown above allow the idea of implementing a calculation model. With this model it should be possible to identify most viable oocytes and embryos respectively. With the help of the EmbryoScope™ it seems to be possible to convince static observation even under restrictive conditions like the German Embryo Protection Law.

Study funding/competing interest(s): Funding by hospital/clinic(s), IVF-centre Dr. Krieg.
Trial registration number: None.

P-166 The rate of blastocyst development affects cycle outcome in both fresh and frozen embryo replacement cycles

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Study question: The aim of this study was compare the pregnancy rate (PR), clinical pregnancy rate (CPR) and implantation rate (IR), of embryos forming blastocysts on day 5 or day 6 of development in fresh and frozen embryo replacement (FER) blastocyst transfers.
Summary answer: PR, CPR and IR following transfer of blastocysts that form on day 5 of *in vitro* culture are significantly higher than those forming on day 6 in both fresh and vitrified/warmed cycles. This suggests that the rate of blastocyst development is significantly associated with cycle outcome and cannot be associated to endometrial asynchrony. Early miscarriage rates are also similar in both normally and slow developing blastocysts. This suggests that developmental retardation affects implantation potential.
What is known already: Studies on IVF cycles with fresh blastocyst transfers have demonstrated that the rate of blastocyst development affects the treatment cycle outcome, with blastocysts forming on day 5 reported to have higher implantation and pregnancy rates than those forming on day 6. Results reported for FER cycles, however, are conflicting as to whether the rate of blastocyst formation prior to cryopreservation affects the pregnancy outcome. Other studies have shown that slower developing embryos reaching the blastocyst stage on day 6 have similar clinical pregnancy and live birth rates to those forming blastocysts on day 5, provided that the morphology is of a similar grade.
Study design, size, duration: Retrospective analysis of the outcome of 3154 fresh and frozen blastocysts forming on day 5 or day 6 of *in vitro* embryo culture, during the period 01/01/2011 to 18/09/2013.
Participants/materials, setting, methods: A total of 2077 patients had blastocyst transfer in a fresh or frozen cycle in a private IVF Unit. Patients were a day 5 and day 6 bastocysts were transferred in a single cycle were excluded from the study.
Main results and the role of chance: The PR and CPR following transfer of day 5 blastocysts were shown to be significantly higher than those forming on day 6 in fresh and frozen cycles. The IR following transfer of blastocysts that formed on day 5 in fresh cycles was shown to be significantly greater (49.8% compared to 35.8%; $P < 0.001$) than those forming on day 6. Similarly in FER cycles, the IR following transfer of warmed day 5 blastocysts was significantly higher than transfers of warmed day 6 blastocyst (51.3% as compared to 36.6%; $P < 0.001$). There was no significant difference between survival rates of warmed day 5 blastocysts as compared to warmed day 6 blastocysts (95.7% compared to 94.0%; $P = 0.07$). No significant difference was observed in IR between fresh and vitrified/warmed embryo transfers using day 5 blastocyst (49.8% vs 51.3%; $P = 0.593$). Similar results were also observed in IR for fresh embryo transfer cycles using blastocysts forming on day 6 of *in vitro* embryo culture when compared to FER cycles were day 6 blastocysts was used for transfer (35.8% vs 36.6%;$P = 0.821$).
Limitations, reason for caution: Further studies are needed in order to confirm that implantation potential is affected by developmental retardation rather than endometrial asynchrony.
Wider implications of the findings: Data collected in this report demonstrate that the PR, CPR and IR following transfer of blastocysts that form by day 5 are significantly higher than those forming on day 6 in both fresh and FER cycles. This suggests that the rate of blastocyst development is associated with cycle outcome, rather than embryo-endometrium asynchrony.
Study funding/competing interest(s): Funding by hospital/clinic(s), Center for Reproduction and Genetic Health, London.
Trial registration number: The present study did not require a trial registration number.

P-167 Thyrotropin and thyroid hormones in *in vitro* follicle growth of mouse primary preantral follicles, in *in vitro* fertilization and early embryo development

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Study question: What is questioned in this study is whether Thyrotropin (TSH) and Thyroid hormones (T_3 and T_4) have an impact in *in vitro* follicle growth of mouse primary preantral follicles, in *in vitro* fertilization and early embryo development to the morula/blastocyst stage
Summary answer: In line with the negative effect of thyroid hormones in folliculogenesis and fertilization, a negative impact of T_3 was found in the early stages of embryo development. Contrary, a positive effect of T_4 was shown in specific embryo stages. TSH had no impact on folliculogenesis, fertilization and early embryo development.
What is known already: Thyroid disorders, namely hyper- of hypo thyroidism have been long related to menstrual disturbances and impaired fertility. Given that Thyrotropin receptor (TSHR) and Thyroid hormone receptors (TRα and TRβ) are expressed in ovarian follicles, a direct role of these hormones in the regulation of ovarian function is considered indisputable.
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 though an 8-month period.
Participants/materials, setting, methods: Preantral follicles were mechanically dissected from ovaries and cultured *in vitro*. Mature oocytes were fertilized using sperm from adult male hybrids. The effect of TSH (2 mIU/ml, 1 mIU/ml, 0.2 mIU/ml) and the effect of T3 and T4 (10^{-9} M, 10^{-8} M, 10^{-7} M each), were studied.
Main results and the role of chance: T_3 was found to negatively affect follicular growth and maturation in the lower 10^{-9} M and intermediate 10^{-8} M concentrations. Similarly, T_4 was found to negatively affect follicular growth and maturation of mouse preantral follicles in the intermediate concentration 10^{-8} M. Contrary to thyroid hormones, no impact of TSH was found in the process of folliculogenesis. T_3 had a negative effect in fertilization and early embryo development to the 2-cell stage at a concentration of 10^{-8} M and T_4 negatively affected fertilization at a concentration of 10^{-9} M and 10^{-7} M. Interestingly, higher 2-cell, 4-cell and morula/blastocyst stage rates were shown at a concentration of 10^{-8} M. TSH had no effect on fertilization and early embryo development.
Limitations, reason for caution: Hormones were added in the culture medium throughout follicular growth, thus only an indirect late hormone effect in early embryo development could this study reveal. Furthermore, the hormone effect was evaluated at a single timepoint, at the end of follicular culture, thus transient effects during folliculogenesis could not be determined.
Wider implications of the findings: Findings of this study may encourage researchers to work on the effect of TSH, T_3 and T_4 in various combinations during folliculogenesis, fertilization and early embryo development, that is in conditions that mimic follicular physiology, given that *in vivo* these hormones co-exist in the follicular milieu.
Study funding/competing interest(s): Funding by hospital/clinic(s), Alexandra Hospital.
Trial registration number: The study was registered to Local University Hospital Ethics Committee (registration number: 5/07-09-2007).

P-168 The synchronicity of mitotic divisions predicts embryo implantation and live birth

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Study question: Can the implantation potential of an embryo and the live birth be predicted using morphokinetic parameters obtained until day 3?

Summary answer: Three kinetic ratios based on selected cleavage cycles defining mitotic synchronicity and two morphologic penalty criteria were used to construct an additive scoring model, which provided high predictivity of implantation (AUC = 0.639; 95% CI: 0.604–0.673) and of live birth (AUC = 0.704; %95 CI: 0.661–0.744).
What is known already: Embryo implantation 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). 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).
Study design, size, duration: This retrospective cohort study was conducted from October 2011 to October 2013. It included 776 embryos having a (t8) and with known implantation data (KID) from 457 infertile patients. Live birth data was available for 489 KID embryos belonging to 293 patients.
Participants/materials, setting, methods: Incubation was performed in time-lapse incubators (EmbryoScope™). For the prediction of implantation and live birth, a 10% approach was used; dividing the study group in 77 and 49 embryos, respectively. Clinical pregnancy was defined as the presence of a gestational sac with fetal heartbeat detected on ultrasound on week 7.
Main results and the role of chance: Cleavage synchronicity from 2–8 cells ((t3–t2) + (t5–t4))/(t8–t2) reflects the ratio of time the embryo spends from 2–4 cells over the time from 2–8 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. Similarly, the cleavage synchronicity from 4 to 8 cells ((t8–t5)/(t8–t4)) and the DNA replication time ratio ((t3–t2)/(t5–t3)) were calculated. The unevenness of blastomeres at 2 and 4 cells and the presence of direct cleavage were set as morphologic penalty criteria. KID and known live birth rates were 26.03% and 19.3%, respectively. Prediction with the additive model gave an AUC of 0.639 (95%CI: 0.604–0.673) for implantation and an AUC of 0.704 (95%CI: 0.661–0.744) for live birth.
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 the implantation potential and of live birth in this retrospective cohort study.
Study funding/competing interest(s): Funding by hospital/clinic(s), Sisli Memorial Hospital.
Trial registration number: Not required.

P-169 Soybean phosphatidylcholine modifies the lipid profile of bovine oocytes after short-term exposure during *in vitro* maturation

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Study question: Thus, the aim of this work was to evaluate the lipid profile of oocytes supplemented with soybean phosphatidylcholine (PC) during IVM, using bovine oocytes as an experimental model. Preimplantational development of oocytes and interfacial properties of the PC with both culture medium and mineral oil were also assessed.
Summary answer: Assessment of lipid chemical composition seems therefore to provide an attractive approach to develop *in vitro* maturation systems for best human oocytes and post cryopreservation survival.
What is known already: Limited success of cryopreservation technology appears to be associated with high intracellular lipid contents and phospholipid (PL) constitution of the oocyte cell membrane. The knowledge of PL profiles and protocols for developing suitable *in vitro* maturation (IVM) able to modify membrane's composition may result in significant improvements of the resistance of embryos and oocytes to cryopreservation.
Study design, size, duration: Were used 1916 bovine oocytes matured in TCM supplemented with 50 or 100 µM PC (PC *n* = 994) or without it (control *n* = 922).
Participants/materials, setting, methods: Oocytes were matured in TCM supplemented with 50 or 100 µM PC (PC *n* = 994) or without it (control *n* = 922). The maturation media and mineral oil prior and after IVM incubation, and oocytes were submitted to matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), and the lipid profiles were compared via principal component analysis.
Main results and the role of chance: The present MALDI-MS results demonstrate that the culture medium properly solubilize the phospholipids of the PC-supplement and that lipid profile of mineral oil incubated during IVM period was unaltered either by PC supplement and metabolism of the control oocytes nor by PC-oocytes. Regarding oocytes, we observed that when the oocytes are supplemented with only 50 µM PC mixture during IVM, slight modifications occur in their lipid profile. However, the PC supplement at 100 µM dose mainly resulted in substantially higher relative abundances of phospholipid species PC (32:1), PC (32:0), PC (34:2), PC (36:6), PC (36:4), and PC (38:6). Interestingly, the ions that most affect this separation, observed running the PCA, are those of *m/z* 758.6, 782.6 and 806.6 and belonging to the PC-supplement. Oocytes exposed to PC supplement during IVM did not display significant differences in terms of embryo cleavage rate (78.8% vs. 74.9%), blastocyst development at day 8 (42.1% vs. 40.4%) and hatching rates (73.9% vs. 69.2%) compared to untreated control, respectively.
Limitations, reason for caution: Nothing to declare.
Wider implications of the findings: These results show that indeed short-term exposure to PC supplement is able to modify lipid profiles of IVM-oocytes without affecting preimplantational embryo development.
Study funding/competing interest(s): Funding by national/international organization(s), We thank the Brazilian science foundations FAPESP (2011/06191-7) and CNPq for assistance.
Trial registration number: Nothing to declare.

P-170 Comparison of embryo culture with a single medium, global®, lifeglobal or with sequential media from cook laboratories in a prospective study

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Study question: Two types of culture media are available for IVF. In this study, we compared pregnancy rate, kinetic of embryo development, embryo freezing obtained either with a single medium, Global®, or with sequential media from Cook laboratories, whose composition changes according to the stage of embryo development.
Summary answer: Between the two types of media, the composition of glucose and amino acids can explain the differences observed in the kinetic of embryo development. The better imitation of the physiological environment sequential media could explain the more physiologic embryo development kinetics and higher rates of blastocyst.
What is known already: Two studies compared the use of Global® and sequential media Cook®. For Mellon (2005): pregnancy rate as well as proportion of good quality embryos at day 3 are significantly higher in the Global study group. For Ruttanajit (2012), the comparison of Blastocyst culture either with Global® or with sequential media Cook® resulted in identical clinical pregnancy rates in the two groups.
Study design, size, duration: This is a preliminary prospective randomized study for 6 months in CMCO IVF center comparing embryo culture either with a unique medium: Global® (Lifeglobal) or with sequential media from COOK laboratories: Gamete Buffer®, Fertilization Medium®, Cleavage Medium® and Blastocyst Medium® for embryo culture.
Participants/materials, setting, methods: Were included first and second attempts of IVF or ICSI with women less than 37 years old. Elective single

embryo transfer was the rule at day 3 or at blastocyst stage. Were excluded attempts of Preimplantation Genetic Diagnosis (PGD) and Oocyte Donation program. 173 patients were included in this study.

Main results and the role of chance: Pregnancy rates per oocytes retrieval and per embryo transfer were similar in the two groups: 40% per oocyte retrieval and 51% per transfer in the Global® group and 44% per oocyte retrieval and 48% per transfer in the sequential group. The cinetics of early embryo development was significantly accelerated with the Global® medium: at day 3, 27% of embryos presented more than 8 cells, versus 16% with the sequential media. At day 3, embryos present more frequently a compaction in the Global® group (25% versus 15%). We obtained significantly more blastocysts in the group of sequential media: 80 blastocysts versus 50 in the Global®.

Limitations, reason for caution: This study is a comparison of embryo culture media already marketed and CE certified and used by other ART laboratories. For this reason, this study did not need a request for approval by a CCPRB.

Wider implications of the findings: In the context of PGD, early embryo compaction requires a longer incubation of embryos in a medium lacking Ca^{2+} and Mg^{2+} ions and containing EDTA as a chelating agent. The comparison of birthrates and children birth weight according to the culture media used is crucial, because recent publications rapport the impact of embryo culture media on the later development and on children birth weight. These preliminary results have to be confirmed in a total of 300 attempts.

Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by commercial/corporate company(ies), This study has been partially supported by the COOK® Company and the University Hospital of Strasbourg. No competing interests.

Trial registration number: This study has not been registered in the EU Clinical Trials Register.

P-171 The impact of sperm-free DNA (f-spDNA) in combination with follicular fluid oocyte/cumulus-free DNA (ff o/c-free DNA) on embryological results in couples undergoing IVF/ICSI-ET treatments

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Study question: To investigate the possible role of f-spDNA in combination with ff o/c-free DNA on embryological data, such as fertilization and cleavage rate, embryo quality and IVF outcome of couples undergoing IVF/ICSI-ET treatments.

Summary answer: The sum of f-spDNA and ff o/c-free DNA, as a combined gamete apoptotic marker, seems to impact on embryo quality and on pregnancy outcome.

What is known already: The fragments of DNA which are released from the cell nucleus due to apoptotic processes are called cell-free DNA. Apoptosis has been considered as an essential component of various processes including proper early human embryonic development and functioning of the reproductive system. The increased serum cell free DNA in men has been associated with impaired sperm parameters, while increased serum cell free DNA in women has been related to impact on the IVF outcome. Data regarding the correlation between the couple's free DNA and the IVF outcome do not exist.

Study design, size, duration: Seventy eight couples undergoing 78 consecutive IVF/ICSI-ET cycles were randomly investigated during 2013. All couples were allocated to two groups. Group 1 (n = 39), where the sum of f-spDNA and ff o/c-free DNA is less or equal to 1800 ng/ml and group 2 (n = 39) where the sum is more than 1800 ng/ml. This value was the median of our study population and was arbitrarily chosen as a cut-off level to discriminate the two groups with low and high free DNA concentrations respectively.

Participants/materials, setting, methods: 78 semen and follicular fluid samples were collected and aspirated, respectively, on the day of ovum pick up (OPU). A volume of 0.5 ml of each sperm specimen and 1 ml portion of the total collected follicular fluid were centrifuged and 0.4 ml cell free supernatant from each sample was carefully transferred to a new eppendorf tube and frozen at –20 C for future analysis. F-spDNA and ff o/c-free DNA were determined by conventional quantitative real time PCR–Sybr green detection approach.

Main results and the role of chance: Overall, the sum of both free DNAs, as a combined gamete apoptotic marker, was found to correlate positively with cumulative embryo score (CES) (r = 0.24, $p < 0.05$) and inversely with mean score of embryo quality (MSEQ) (r = –0.27, $p < 0.05$). The comparison of the embryological data between the two groups revealed that the embryo quality of group 1 was significantly higher compared to group 2. Treatment outcome, in terms of positive hCG, was comparable between the two groups (p = 0.08), but the clinical pregnancy rate was higher in group 1 than in group 2 (28.2% vs 7.7%, $p < 0.05$).

Limitations, reason for caution: The sample size of the present study is relatively small, therefore a future larger study is needed.

Wider implications of the findings: The combined gamete apoptotic marker appears to play a significant role in embryo quality and IVF outcome, suggesting a possible clinical application.

Study funding/competing interest(s): Funding by University(ies), University of Thessaly.

Trial registration number: N/A.

P-172 Developmental competence of human mature oocytes derived from preovulatory and antral follicles: reflections on follicle and oocyte physiology

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Study question: Is the competence of human mature oocytes derived from antral follicles comparable with that of oocytes obtained from preovulatory follicles?

Summary answer: Mature oocytes derived from antral and preovulatory follicles appear have comparable developmental competence.

What is known already: Previous studies compared the competence of oocytes recovered from follicles of different sizes in controlled ovarian stimulation (COS) cycles. Collectively, these experiences indicated that oocytes from smaller follicles are less developmentally competent. Nevertheless, these findings should be considered with caution. In fact, in COS cycles it is plausible that oocytes derived from small follicles have an intrinsically reduced competence, considered the reduced growth response of such follicles to gonadotropin stimulation.

Study design, size, duration: From June 2011 to December 2012, in an observational retrospective study, mature oocytes from preovulatory follicles of women undergoing COS/IVF treatment (Group A) were compared with mature oocytes recovered from antral follicles sized 4–12 mm of women selected for hCG-primed *in vitro* maturation (IVM) treatment (Group B).

Participants/materials, setting, methods: Patients were matched for age, BMI, FSH, AMH and AFC. ICSI was used to achieve fertilization. In Group B, only transfers involving embryos derived exclusively from oocytes found mature at recovery were included. Rates of fertilization, implantation and babies born per transferred embryo were compared between the two groups.

Main results and the role of chance: Oocyte pick-ups (OPU) were 315 and 204 in Groups A and B, respectively. Fertilization rates were comparable (72.8% and 75.9%, respectively; P = 0.137). In Group A, in which the average number of embryos transferred was higher, clinical pregnancy rates per OPU (37.5%) and embryo transfer (38.4%) were superior in comparison to Group B (27.0%, P = 0.013; 29.4%, P = 0.041; respectively). On the contrary, implantation rates (Group A, 23.7%; Group B, 20.8%) and proportions of babies born per transferred embryo (Group A, 19.5%; Group B, 16.9%) were similar (P = 0.528 and 0.332, respectively).

A multivariate logistic model indicated that the higher average number of embryos transferred was the only independent factor associated with a higher probability to achieve a pregnancy in Group A.

Limitations, reason for caution: Being generated by a retrospective analysis, our data cannot be considered conclusive. Additional studies are needed

to test more extensively, also at the molecular and cellular level, similarities and difference between oocytes and follicles of different developmental stage.

Wider implications of the findings: This study suggests that mature oocytes recovered from antral (4–12 mm) and preovulatory follicles retain a comparable ability to give rise to a viable pregnancy. The data are consistent with the hypothesis that full acquisition of oocyte developmental ability is completed at the antral stage, preceding final preovulatory growth. As a consequence, this would suggest that late follicle growth play a modest role in the process of oogenesis.

Study funding/competing interest(s): Funding by hospital/clinic(s), Biogenesi Reproductive Medicine Centre.

Trial registration number: N.A.

P-173 The effect of ovarian reserve parameters on biomarkers identified with time-lapse imaging

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Study question: Is there an association between ovarian reserve parameters [i.e.: antral follicle count (AFC), serum day-3 follicle stimulating hormone (FSH)] and early embryonic time-lapse biomarkers?

Summary answer: AFC showed no association with either the duration of the 2- to 3-cell (P2) or the 3- to 4-cell stage (P3). The latter lasted longer in patients with a FSH >8 U/L and a strong positive correlation was noted between FSH and P3-duration.

What is known already: Recent studies suggest that certain time-lapse markers might predict embryo development, embryo quality and implantation potential. However, studies investigating the biology underlying these biomarkers are limited. It is well established that ovarian reserve parameters correlate with ovarian response, embryonic development and pregnancy rates. Whether there is a visible effect of these biomarkers during the very early stages embryonic development (as studied by time-lapse image technologies) is unknown.

Study design, size, duration: *Design*: Retrospective cohort study.

Size: 553 embryos derived from 44 women undergoing IVF and cultured in a time-lapse incubator till day-6.

Duration: 09/2013 to 12/2013.

Participants/materials, setting, methods: *Setting*: Academic Fertility Center (Boston, MA, USA).

Materials/Methods: Serum day-3 FSH, and ultrasound-determined AFC were recorded prior to treatment initiation.

Outcome Measures: Duration of 2- to 3-, and 3- to 4-cell stage.

Statistics: Pearson correlation and *t*-test, used as appropriate.

Main results and the role of chance: AFC was modeled in tertiles (mean ± SD; T1: 8.5 ± 1.7, T2: 13.8 ± 1.3, T3: 26.9 ± 8.9 follicles). T1 women were older (T1: 36.9 ± 3.6, T2: 35.7 ± 3.8, *T3: 35.0 ± 4.1* years, $P < 0.05$), with a higher day-3 FSH (T1: 7.9 ± 2.3, T2: 7.6 ± 1.9, T3: 6.4 ± 1.6 U/L, $^{*}P < 0.001$).

Duration (hours) of phases P2 and P3 (mean ± SD) in increasing AFC tertiles were: 10.7 ± 5.2, 8.8 ± 4.3*, and 10.1 ± 4.7 (*P: 0.03 for T1 vs. T2, P-trend >0.05), 2.5 ± 3.3, 3.3 ± 3.3, and 2.5 ± 2.8 ($P > 0.05$), respectively.

Duration (hours) of P2 from women with an FSH ≤8 U/L did not differ from that of women with an FSH >8 U/L (10.1 ± 3.6 vs. 9.5 ± 6.2, P: 0.4). However, P3 lasted longer in the latter group (2.4 ± 2.8 vs. 3.5 ± 3.7, FSH ≤8 vs. >8 U/L, P: 0.03) and day-3 FSH correlated positively with P3-duration (r: 0.44, $P < 0.01$; r: 0.75, $P < 0.001$ in the FSH >8 U/L subgroup).

Limitations, reason for caution: Anti-Mullerian hormone levels were not available, thus not included in the analysis. However, AFC is a robust ovarian reserve marker. Our analysis was limited to the time-lapse parameters that may predict high-quality blastocyst development and evaluated all embryos not only the high-quality ones leading to either transfer or cryopreservation.

Wider implications of the findings: Biomarkers identified by time-lapse imaging have been under investigation for use in clinical embryo selection. Since ovarian reserve is associated with both ovarian response, oocyte and embryonic yield, as well as embryonic developmental potential, identifying the time-lapse markers that correlate best with ovarian reserve might aid in selecting those that best predict embryonic and treatment outcomes.

Study funding/competing interest(s): Funding by hospital/clinic(s), Massachusetts General Hospital.

Trial registration number: None.

P-174 Laser assisted hatching of cleavage stage mouse embryos impairs developmental potential and increases genetic instability in blastocysts

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Study question: What is the influence of 2 cell (day 2) and 6–8 cell (day 3) stage laser assisted hatching on the developmental potential and genetic integrity of the embryos?

Summary answer: Laser manipulation of mouse embryos induces genetic instability at blastocyst stage.

What is known already: Although assisted hatching is routinely performed either on day 2 (2–4 cell stage) or day 3 (6–8 cell stage) of development, its benefits in enhancing pregnancy rate are still debated.

Study design, size, duration: In this prospective experimental study, 2-cell and 6–8 cell stage mouse embryos were subjected to laser hatching and then assessed for the developmental potential and DNA integrity in blastocysts.

Participants/materials, setting, methods: *In vivo* fertilized embryos were collected from 6 to 8 week old *Swiss albino* mice. The embryos (2-cell and 6–8 cell stage) were randomly divided into control and laser hatched groups and then subjected to laser hatching. Embryos were cultured for 72 h and 48 h followed by morphology based evaluation and TUNEL test was performed.

Main results and the role of chance: Laser assisted hatching in mouse embryos significantly enhanced the blastocyst hatching potential on day 4.5 ($P < 0.0001$). However, a significant decline in blastocyst total cell number (TCN) was observed in 6–8 cell stage laser hatched embryos ($P < 0.001$). Attempt to understand the genetic integrity in laser hatched mouse blastocysts revealed significantly higher labelling index when hatching was done at 2 cell ($P < 0.01$) and 6–8 cell stage ($P < 0.05$).

Limitations, reason for caution: Findings in the mouse embryo may not be fully extrapolated to humans. In addition, it is important to look into *in vitro* derived embryos to mimic clinical situation.

Wider implications of the findings: Genetic instability induced by the laser manipulation may affect implantation and postimplantation developmental potential of the embryos. However, further studies are required to elucidate the impact of laser induced genetic instability on the reproductive outcome.

Study funding/competing interest(s): Funding by national/international organization(s), This work was supported by Indian Council of Medical Research (ICMR) Grant # 2010-00020.

Trial registration number: Nil.

P-175 Expression of oocyte secreted factors in cumulus cells as predictor of oocyte developmental potential

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Study question: Can the expression level of growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) mRNA in cumulus cells be used as molecular markers for predicting oocyte developmental potential?

Summary answer: The expression levels of *GDF9* and *BMP15* mRNA were closely associated with oocyte maturation, fertilization, embryo quality, and pregnancy outcome; therefore, GDF9 and BMP15 mRNA in cumulus cells may be considered as new molecular markers for predicting ooctye developmental potential.
What is known already: GDF9 and BMP15 play an important role in the process of follicular development. Previous studies have confirmed that both GDF9 and BMP15 are expressed both in oocyte and cumulus cells with consistent expression levels.
Study design, size, duration: Cumulus cells were collected from one hundred and ninety-nine female subjects who underwent *intracytoplasmic sperm injection* (ICSI) assisted reproduction in a retrospective study from October 2012 to January 2013.
Participants/materials, setting, methods: The cumulus cells were stripped off for detecting the expression levels of *GDF9* and *BMP15* mRNA. Pearson analysis was used to analyze the correlation between *GDF9* and *BMP15* expression and oocyte developmental potential. The *t*-test was used to compare *GDF9* and *BMP15* expression between the different groups.
Main results and the role of chance: The expression levels of *GDF9* and *BMP15* mRNA were significantly associated with ooctye maturation, normal fertilization, and cleavage rate ($P < 0.001$). The expression levels of *GDF9* and *BMP15* mRNA in the high-quality embryo group were significantly greater than those in the low-quality embryo group ($P < 0.05$). The expression levels of *GDF9* and *BMP15* mRNA in the pregnant group were significantly greater than those in the non-pregnant group ($P < 0.05$).
Limitations, reason for caution: The limitation of this study was that the cumulus cells were not harvested from individual oocyte, so the expression of *GDF9* and *BMP15* mRNA did not reflect individual oocyte and embryo quality.
Wider implications of the findings: *GDF9* and *BMP15* mRNA in cumulus cells may be considered as new molecular markers for predicting ooctye developmental potential. As a quick diagnostic method, this transcriptomic approach may pave a new way for enhancing the accuracy of oocyte developmental potential.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Funding by national/international organization(s), This study was supported by the National Science Fund of China (No. 81200417 and 81200476), the Doctoral Fund of the Ministry of Education of China (No. 20120171120093 and 20120171120122), the Science Fund of Guangdong Province (S2012040007621 and S2012040007770), the Medical Research Fund of Guangdong Province (B2012104 and B2012150), the Young Teacher Fund of Sun Yat-Sen University (11ykpy24), the Project of Science and Technology New Star in Zhu Jiang of Guangzhou City, and the Project of Excellent Medical Talents in Sun Yat-Sen Memorial Hospital.
Trial registration number: None.

P-176 Reduced and delayed expression of GDF9 and BMP15 in unstimulated ovarian tissues from women with PCOS

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Study question: What are the expression features of the proteins growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) in unstimulated ovarian tissues from PCOS patients and normal ovulatory women?
Summary answer: The expression of GDF9 and BMP15 demonstrates a stage-dependent characteristic both in oocytes and granulosa cells in unstimulated ovarian tissues. The expression of two factors was reduced and delayed in the early follicular phase in PCOS tissues, suggesting that might be associated with aberrant follicular development in women with PCOS.
What is known already: As members of the transforming growth factor-β (TGF-β) superfamily, both GDF9 and BMP15 play crucial roles in the regulation of follicular development and oocyte maturation.
Study design, size, duration: From May 2010 to December 2012, unstimulated ovarian tissues were collected from 28 patients with PCOS and 26 women with normal ovulatory cycles, and were examined in a retrospective study.
Participants/materials, setting, methods: Immunohistochemical staining was performed in a lab of university affiliated hospital to examine the expression levels of GDF9 and BMP15 proteins in oocytes and granulosa cells of the unstimulated ovarian tissues.
Main results and the role of chance: The expression of both GDF9 and BMP15 was stage-dependent in oocytes and granulosa cells of ovarian tissues. The expression of GDF9 began from primordial follicles while the expression of BMP15 began from primary follicles, their expression increased with follicular development gradually, reaching the highest level in Graafian follicles. However, the expression of GDF9 and BMP15 was reduced and delayed in oocytes and granulosa cells of ovarian tissues from PCOS patients compared with the control group ($P < 0.05$).
Limitations, reason for caution: The study was limited by the low availability of ovarian tissues from PCOS patients and controls.
Wider implications of the findings: The study reveals a mechanism that may be responsible for aberrant follicular development in PCOS.
Study funding/competing interest(s): Funding by national/international organization(s), the present study was supported by grants from the National Natural Science Foundation of China (Grant No.81200476), Natural Science Foundation of Guangdong Province (Grant No. S2012040007770), National Doctoral Foundation of China (Grant No. 20120171120122), and Medical Science and Technology Research Foundation of Guangdong Province (Grant No. B2012150).
Trial registration number: None.

P-177 The effect of sperm source and artificial oocyte activation (AOA) on early embryo morphokinetics can diminish before implantation

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Study question: Embryos that are obtained after AOA can create successful implantation and pregnancies, albeit to a lower extend. This study questions whether differences in early morphokinetic parameters may have an impact on such inferior outcome.
Summary answer: Our study show that, starting from the pronuclear stage, there exist early embryo developmental disturbances or variations which may affect the development until blastocyst stage in cases with AOA.
What is known already: Fertilization failure can be due to a variety of reasons. Rescue of failed or low fertilization by AOA different methods has previously been shown to improve ART outcome in especially sperm-related failure. However, clinical success rate in such cases is somewhat lower than standard IVF/ICSI cycles.
Study design, size, duration: Morphokinetic parameters of 57 embryos with known implantation value (KID) from 39 cycles (24 with ejaculated and 15 with testicular sperm) were retrospectively analysed using time-lapse monitoring system. One hundred and seventy seven embryos (without AOA) from standard ICSI cycles with known implantation value were included as controls. Study included the data from cycles performed between January 2012–August 2013 in Bahceci Fulya and Umut Assisted Reproductive Technology Centers.
Participants/materials, setting, methods: Embryos derived from ejaculated (Group I), testicular spermatozoa (Group II) and control group were analysed for parameters of extrusion of 2nd polar body, pronuclei appearance, pronuclear fading, early cleavage time points and duration in each cleavage from two cells – until hatching blastocyst stages (t2 to tHB), time interval between cleavages (cc2, cc3, S2, t4–t2 and t8–t4) and compared between groups.
Main results and the role of chance: Fertilization rates were higher in the standard ICSI group (75.1%) compared to Group I (64%) and group II (54.6%) respectively. When the KID data of both groups were compared with the KID data of standard ICSI cycles, significant developmental delays before blastulation were observed. This may imply that there exist developmental disturbances

in such embryos but this negative effect can be overcome before blastulation in order to create a successful implantation.
Limitations, reason for caution: The number of embryos and cycles analyzed are the main limitation of our study. Further research and larger sample sizes with the current parameters and technical setting are required to confirm our findings.
Wider implications of the findings: Our preliminary study indicates that AOA, either alone or in combination with the use of spermatozoa from different sources may create abnormal development pattern and significant yet unknown developmental effects on early embryo morphokinetics. Such studies are very scarce and should be increased to analyze and document such effects with regard to the different source of spermatoza used for insemination.
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: This study was not an RCT and therefore there is no registration number.

P-178 Higher pregnancy rate using low oxygen incubators in comparison with standard high oxygen incubators for day 3 embryotransfer: a prospective randomised clinical trial

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Study question: Are low oxygen incubators (6% CO_2, 5% O_2, 89% N2) performing better than standard incubators (6% CO_2 in air (high oxygen-20%)) for in vitro fertilisation (IVF) culture systems, resulting in higher clinical pregnancy rate (CPr), using day 3 embryotransfer?
Summary answer: The clinical pregnancy rate for embryos transferred after 3 days of culture in low oxygen incubator was higher than for embryos cultured in high oxygen incubator.
What is known already: In vivo, blastocyst and implantation stage embryos are exposed to lower O_2 concentration than cleavage stage embryos. In tubes and uterus of most mammalian species, the embryos are exposed to 2%–8% O_2. An increased O_2 concentration create an excess of free oxygen radicals, which could affect embryos in preimplantation period. The low oxygen incubators perform better than standard incubators with day 5 embryotransfer, the benefits of reduced oxygen being critical between 8 cells to blastocyst stage.
Study design, size, duration: This is a prospective randomised study on 264 patients evaluated between 2011–2014, first IVF attempt. Patients were randomely divided in two groups based on the incubator used for embryos culture: group A (124 patients) with high oxygen atmosphere and group B (140 patients) with low oxygen atmosphere.
Participants/materials, setting, methods: Using same long agonist protocol, egg collection was done 36–37 h after hCG trigger. All patients undergone an IVF procedure and the same media were used for culture system. Two embryos were transferred in day 3. The exclusion criteria were: severe male factor (ICSI cases), endometriosis, PCOS, hydrosalpynx, uterine pathology, trombophilia.
Main results and the role of chance: Patients were between 25–43 years old, and the body mass index (BMI) were between 18–32 kg/m^2. The two groups were similar in age, BMI, days of stimulation, total dose of gonadotropins, the number of oocyte retrieved, the number of 2PN obtained embryos, the number of embryos that achieved 6 or 8 cells on day 3. Patients in group B had significantly higher CPr (39%) and biochemical pregnancy rate (42%) in comparison with group A (28.5%, $p < 0.05$ and 30.7%, $p < 0.05$ respectively). We also found significantly higher implantation rate ($p < 0.05$), fertilisation rate (68% vs. 52%, $p < 0.05$) and embryoscores in group B patients vs. group A. Although in poor responder CPr (18% vs. 14%) and in older women (>40 years age) (CPr15% vs. 12%), the results were better.
Limitations, reason for caution: This is a small study, it should be confirmed by larger prospective, randomized studies. Other factors that might be consider seems to be the indication for IVF, and the composition of the media used for cultured.
Wider implications of the findings: Our findings show that it is worth to consider using low O_2 incubators, even in day 3 embryotransfer. A lower O_2 environment, mimicking physiological conditions, seems to determine better embryos quality, higher implantation rate and higher pregnancy rate. Also it appears that low oxygen level influence positive the IVF cycle with low embryos number (poor responders and older patients).
Study funding/competing interest(s): Funding by hospital/clinic(s), Life Memorial Hospital, Bucharest.
Trial registration number: Not applicable.

P-179 Trophectoderm gradation and time of transfer are robust factors governing selection of best single blastocyst for transfer to predict implantation and live births in IVF

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Study question: To evaluate trophectoderm (TE) and inner cell mass (ICM) morphology during blastocyst scoring in order to assess their individual impact as well as consequence of time of transfer on implantation and pregnancy outcome so as to facilitate selection of single best blastocyst for transfer in IVF cycles.
Summary answer: Morphologic gradation of trophectoderm and timing of transfer are robust factors for predicting implantation and live births thus aiding embryologists to select appropriate embryo, counsel the patient and facilitate single best blastocyst transfer in IVF to avoid multiple gestation hence obviating the inconclusive conventional selection method based on ICM gradation.
What is known already: Current routine practice of blastocyst assessment in cycles involving multiple blastocyst transfers has a limitation that TE and ICM grades are grouped together, making it difficult to interpret the impact of individual grading of TE and ICM on individual embryo implantation and live birth potential. However, three recent studies have demonstrated that the TE grade but not the ICM grade, correlate with ART outcomes in both fresh and frozen embryo transfers.
Study design, size, duration: In this retrospective cohort study, 412 women (mean age: 31.0 ± 4.0 years, BMI: 23.8 ± 2.8) underwent conventional IVF with fresh autologous single blastocyst transfer during January 2011 to December 2012 at our private IVF centre.
Participants/materials, setting, methods: Blastocyst Gradation: TE grades: A: many cells organized in epithelium; B: several cells organized in loose epithelium; C: few large cells. ICM grades: A: numerous tightly packed cells; B: several loosely packed cells; C: very few cells. Blastocelic cavity grades: early, expanded, hatched. Total time in hours was noted from time of insemination till time of embryo transfer.
Main results and the role of chance: Overall IR & LBR was 45.1% & 36.1% respectively. ICM (A) group [n = 256], ICM (B) group [n = 124] and ICM (C) group [n = 32] did not differ significantly in IR and LBR among them (IR = 50.8%, LBR = 41%; IR = 47.8%, LBR = 41.9%, and IR = 9.3%, LBR 0% respectively; P: 0.1679). ICM also did not correlate with IR (P: 0.18) or LBR (P: 0.47). However, TE grades were significantly higher in the pregnant patients of each of the ICM (A), (B) and (C) groups compared to their non-pregnant counterparts in the same groups (P:0. 0003; P: 0.0364 & 0.0313 respectively). The time in hours from insemination to embryo transfer for implantation in pregnant women was found to be significantly lower compared to non-pregnant women ($P < 0.0001$ & $P = 0.0364$) in ICM (A) & ICM (B) groups respectively.
Limitations, reason for caution: Study is limited by a small sample size. Although TE is relatively a more static structure, morphologic assessment of the dynamic ICM structure may tend to get subjective and vary with timing of observation. Frequency of opening incubator door may also have its impact on the morphokinetic assessment of embryo.
Wider implications of the findings: Single blastocyst transfer in this study made assessment of each morphologic parameter with ART outcome possible.

Use of live birth as an end point and inclusion of multiple parameters strengthened our findings. Time in hours for embryo transfer has been another strong point of this study. This data supports recent reports that TE grade is the strongest morphologic predictor for ART outcomes. This data also challenges the practice of embryo selection based on ICM grade.

Study funding/competing interest(s): Funding by hospital/clinic(s), Funded by our own private infertility clinic, Vaunshdhara Clinic and Assisted Conception Centre.

Trial registration number: Not applicable.

P-180 An increase in the contraction of human blastocysts reduces the recovery rate of frozen-thawed embryos and the pregnancy rate after blastocyst transfer

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Study question: The contraction of human blastocysts was often observed by time-lapse monitoring system. Here we examined whether the number of contraction in blastocysts affected the recovery rate of frozen-thawed blastocysts, and influenced the rate of pregnancy after blastocyst transfer.

Summary answer: An increase in the contraction of human blastocysts reduced the recovery rate of frozen-thawed blastocysts, and the subsequent rate of pregnancy was also diminished.

What is known already: The contraction of blastocysts during culture is the results of the collapse of blastocoel by the bankrupt of trophoblastic cells. It has been reported that the contraction of blastocysts *in vitro* has the negative effect of blastocyst hatching.

Study design, size, duration: Between October 2012 and December 2013, human oocytes were collected, cultured *in vitro* to the blastocyst stage and cryopreserved. In total, 1619 were assessed as good blastocysts and frozen. The motion picture of the blastocysts by time-lapse monitoring system was analyzed.

Participants/materials, setting, methods: Oocytes were cultured in an EmbryoScope incubator that has a time-lapse monitoring system (Unisense FertiliTech). The number of blastocyst contraction was counted as one time when the blastocoel shrank. Single frozen-thawed blastocysts were transferred. The proportions of blastocysts recovered for transfer after thawing, and subsequent pregnancy rates were determined.

Main results and the role of chance: In total 1619 blastocysts, the proportion of the group of zero, one, two, three and more than four times of contraction was 29.6%, 29.5%, 21.7%, 11.4% and 7.8% individually. The recovery rate of frozen-thawed blastocysts was 99.6%, 95.6%, 98.1%, 97.6% and 85.0%. The recovery rate in the group of less than three times was significantly higher than that in the group of more than four times (97.7% (719/736) vs. 85.0% (51/60)). Pregnancy rate in each group was 57.3%, 49.6%, 34.6%, 31.4% and 21.6%. Pregnancy rate in the group of less than one time was significantly higher than that in the group of more than two times (53.5% (286/520) vs. 31.4% (116/314)).

Limitations, reason for caution: None.

Wider implications of the findings: The results showed that an increase in contraction number of blastocysts gave the negative effect, and decreased the recovery capability of freezing and thawing and the pregnancy rate. Therefore, the contraction number in blastocysts is a useful marker for evaluating blastocyst quality.

Study funding/competing interest(s): Funding by hospital/clinic(s), Ochi Yume Clinic Nagoya.

Trial registration number: None.

P-181 Evaluation of developmental rate and expression of Bax, Bcl-2 and ErbB4 in pre-implantation mouse embryos after re-vitrification

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Study question: Does re-vitrification affect on developmental rate and expression of Bax, Bcl-2 and ErbB4 genes in mouse pre-implantation embryos?

Summary answer: Re-vitrification could change the developmental rate and expression of Bax, Bcl-2 and ErbB4 genes in mouse pre-implantation embryos.

What is known already: many studies have been shown that there are relationship between vitrification and gene expression. Necessary for re-vitrification of embryos sometimes is Unavoidable. Many researchers try to find out which developmental stage is more suitable to do re-vitrification.

Study design, size, duration: Control versus treatment. Embryos at cleavage stage were collected from hyper stimulated mice and divided to five groups. 1: Fresh embryos, 2: Vitrified embryos at 5–8 cells 3: Vitrified embryos at blastocyst stage. Groups 4 and 5, first vitrified at 5–8 cells, then re-vitrified at compaction or blastocyst stage respectively.

Participants/materials, setting, methods: 400 embryos were used. Vitrification was done using cryolock method according Kuwayama report. The relative quantification of Bax, Bcl-2 and ErbB4 genes was carried out by real time polymerase chain reaction. Total RNA was isolated from blastocyst embryos using RNX-plus. RAN was transcribed to cDNA. Then real time PCR was performed.

Main results and the role of chance: Survival rate after re-vitrification in groups 4 and 5 was 87.5% and 84.5% respectively and these rates didn't show any significant difference with vitrification groups 2 and 3 (88.8% 1 and 92.2%) respectively ($p > 0.05$).

Blastocyst formation rates showed no significant differences between vitrified and re-vitrified groups ($p > 0.05$). Although there was significant difference between re-vitrified and fresh embryos ($p < 0.05$).

Re-vitrification up regulated expression of bax (pro-apoptotic gene) and down regulated of Bcl-2 (anti-apoptotic gene) in re-vitrified embryos. There were strong relationship between reduced developmental rate and altered apoptotic genes. Re-vitrification down regulated the expression of ErbB4 (implanting gene). Re-vitrification could reduce implanting rate.

Limitations, reason for caution: This study was done on mouse embryos. To generalize the results to Invitro fertilization (IVF) clinics, it is necessary to do research on human pre-implantation embryos.

Wider implications of the findings: The results are in agreement with literature regarding gene expression changes after vitrification and re-vitrification comparing to fresh embryos. Our results showed significant decrease in developmental rate between fresh and re-vitrified embryos. But some studies concluded that re-vitrification up to three time didn't effect developmental ability of embryos (Ito, 2010).

Study funding/competing interest(s): Funding by University(ies), Tarbiyat Moderes University.

Trial registration number: N/A.

P-182 Can a prolonged warmed blastocysts culture improve the selection of embryo for transfer? The value of blastocyst re-expansion assessed by time-lapse monitoring

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Study question: Can a prolonged post-thaw culture promotes blastocyst re-expansion and improves the selection of embryos for transfer?

Summary answer: Re-expansion is a direct morphological response after thawing; prolonged embryo culture does not improve embryo selection and might deteriorate blastocyst viability.

What is known already: Currently, post-thaw degree of blastocoele re-expansion is considered a good indicator of cryosurvival and a significant predictor of reproductive potential. The time required to warmed-blastocysts evaluation hasn't been never defined: some embryologist focus on immediate survival while others suggest an additional waiting period of 24 h to monitor survival and growth.

Study design, size, duration: Retrospective study of all the patients who received a frozen-thawed single blastocyst transfer ($n = 136$) performed at our clinic between January and November 2013. A total of 143 blastocysts were warmed and were cultured in Embryoscope.

Participants/materials, setting, methods: According to post-thawed culture period blastocysts were divided in two groups: group I ≤ 3 h culture ($n = 62$) and group II > 3 h culture ($n = 81$). Outcome measures evaluated were: re-expansion rate, positive hCG, implantation, ongoing pregnancy. Chi-square test was used for percentages and Student's *t*-test for mean.
Main results and the role of chance: Prolonged culture significantly increased blastocyst re-expansion (group I 42.0 ± 40.3% vs. group II 60.9 ± 57.5%; $p = 0.0368$); however a culture > 3 h impacted only on expansion of blastocysts with negative outcome (group I 27.06 ± 31.7% vs. group II 64.2 ± 58.2%; $p = 0.0017$). In culture ≤ 3 h we observed a different expansion between implanted and not implanted blastocysts (59.9 ± 42.8% vs. 27.06 ± 31.7%; $p = 0.0019$); this diversity was not evident in culture > 3 h (61.53 ± 51.7% vs. 64.2 ± 58.16%). Positive hCG (45.5% vs. 32.0%) and implantation (29.1% vs. 22.7%) were not different; however, higher rate of ongoing pregnancy was obtained in short culture ≤3 h (25.5% vs. 13.3%; $p = 0.04$). Blastocysts that implanted have a rapid expansion and a delay in this process may indicate alterations of osmotic and/or metabolic conditions.
Limitations, reason for caution: Blastocyst expansion was the only post-thaw morphological parameters considered.
Wider implications of the findings: Blastocysts that fail re-expansion process should not be transferred. Re-expansion is a rapid response after thawing; culture time of 3 h is adequate for to assess blastocysts viability.
Study funding/competing interest(s): Funding by hospital/clinic(s), Tecnobios Procreazione. No external funding was obtained for this study. There was no competing interest.
Trial registration number: Not applicable.

P-183 The duration of the first cell cycle in fertilized human oocytes is related to ploidy: observations on abnormally fertilized oocytes after IVF and ICSI

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Study question: The aim of this study is to elucidate the relationship in human embryos between ploidy, the timing of early cleavage and the cleavage rate by comparing the development of monopronuclear (1PN) and tripronuclear oocytes (3PN) after IVF and ICSI with matched normally fertilised oocytes (2PN).
Summary answer: The first cell cycle of monopronucleate and tripronucleate oocytes is significantly delayed compared to normally fertilized oocytes, confirming the association between early cleavage and ploidy. The bipolar spindle present in digynic 3PN embryos after ICSI results in a more normal distribution of cleavage stages on day 2 compared to those developing from 3PNs after IVF.
What is known already: While early embryo cleavage after normal fertilisation predicts an embryo's developmental potential, the relationship between early cleavage and ploidy is unclear. Observations on haploid and polyploid mouse embryos suggest that the duration of the first cell cycle is correlated with ploidy, while in humans, the cell cycle of triploid embryos is delayed compared to normal zygotes. Nevertheless, the timing of first cell cycle events in abnormally fertilized oocytes after ICSI and IVF remains unknown.
Study design, size, duration: A retrospective study performed on data collected at the Queensland Fertility Group's Toowoomba Clinic from October 2008 to December 2013. A total of 4032 oocytes showing normal and abnormal fertilisation was included in the study.
Participants/materials, setting, methods: 413 couples undergoing 503 IVF/ICSI treatment cycles in which one or more embryos developed from 1PN or 3PN oocytes within a cohort showing normal fertilisation. All oocytes were assessed for early cleavage on day 1 (23.9 ± 0.9 h post insemination) and their cleavage rate on day 2 (41.9 ± 1.3 h post insemination).
Main results and the role of chance: In both IVF and ICSI groups, significantly more 3PNs showed pronuclei at the early cleavage check compared to controls ($P < 0.0001$) and significantly fewer were at syngamy ($P < 0.01$) or 2-cells ($P < 0.001$). Similarly, significantly more monopronuclear oocytes were still 1PNs ($P = 0.02$) after IVF with lower, but not significantly so, numbers at syngamy and 2-cells. By contrast, there was no difference in the proportion of 1PNs at 1PN or syngamy compared to controls after ICSI, although significantly fewer were 2-cells ($P = 0.003$). On day 2, significantly fewer 3PNs were at 4-cells after IVF and ICSI compared to controls (19% and 38% respectively versus 53%; $P < 0.005$) but after IVF, significantly more 3PNs had reached the 5 to 8-cell stage than controls or 3PNs obtained after ICSI ($P < 0.0001$). The development of 1PNs after IVF and ICSI was similar, with both groups showing significantly lower percentages of 4-cell embryos and higher numbers of 2-cells than 2PN oocytes.
Limitations, reason for caution: The significance of this study is limited by the relatively small numbers of 1PN and 3PN oocytes obtained after IVF and ICSI. Nevertheless, the finding that abnormally fertilised oocytes show significant delays in the timing of early cleavage confirms that the duration of the first cell cycle is correlated with ploidy.
Wider implications of the findings: Normally fertilised oocytes that undergo early cleavage 24 to 26 h after insemination are associated with improved embryo quality and higher pregnancy and implantation rates. The reasons for the known variation in the timing of the first cleavage division are unclear but are in part related to chromosomal abnormalities and DNA repair processes. Studies on abnormally fertilised oocytes, which must follow the same basic mechanisms as normal zygotes, provide insights into the biology of fertilisation and early cleavage.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), The study was a retrospective analysis of existing data and was supported by the Queensland Fertility Group as part of Virtus Health. There are no competing interests.
Trial registration number: Not applicable.

P-184 Comparison of the clinical outcomes between fresh blastocysts transfer and vitrified-thawed blastocysts transfer

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Study question: Fresh vs. vitrified-thawed day 5 blastocyst transfer cycle: to compare the clinical outcome of blastocyst vitrification with EM-grid.
Summary answer: The results showed that, the implantation and ongoing rates were not significantly different between fresh and vitrified-thawed blastocysts transfer. The vitrified-thawed surplus blastocysts would be transferred in subsequent cycles to improve the cumulative pregnancy rate.
What is known already: Blastocyst has various advantages than cleavage stage embryo. The survival rates and developmental competency of frozen-thawed blastocyst has been improved because of vitrification, successful cryopreservation techniques. The Vitrification compromises the viability of embryos and consequently results in higher cumulative pregnancy rates than those obtained from embryo transfer of fresh embryos.
Study design, size, duration: A total of 568 cycles (fresh group: 284 and vitrified-thawed group: 284) transferring blastocysts assessed retrospectively from January 2009 to December 2011.
Participants/materials, setting, methods: All the embryos were derived from IVF or ICSI and had undergone COH. The surplus day 5 embryos were vitrified by EM-grid following artificial shrinkage. The equilibrium (EG20), vitrification (EFS40) solutions were prepared (Son et al., 2003) and embryo warming was performed by a two-step dilution method.
Main results and the role of chance: Of the 284 cycles in the fresh group, the chemical pregnancy and implantation rates were 54.2% (154/284), 33.2% (197/594). Of the 284 cycles in vitrified-thawed group, the chemical pregnancy and implantation rates were 46.8% (133/284), 28.4% (162/571). The ongoing pregnancy rate for fresh and vitrified-thawed groups was 58.9% and 62.9%, retrospectively. The number of embryos transferred with an average of 2.09 in Fresh group and 2.01 in vitrified-thawed group. The percentages of singleton, twin and triplet pregnancies were 67.1%, 24.2% and 1.1% for fresh group and 68.4, 31.6% and 0.0% for vitrified-thawed group, retrospectively. The chemical pregnancy, implantation, ongoing and multiple rates did not differ significantly between the two groups.
Limitations, reason for caution: The number of cycles was relatively low. Thus, the results are required large study population.

Wider implications of the findings: The implantation and ongoing rates were not significantly different between fresh and vitrified-thawed groups. All available blastocysts would be vitrified for frozen embryo transfer in patients who are unsuitable in fresh embryo transfer cycle to improve the cumulative pregnancy rate.
Study funding/competing interest(s): Funding by hospital/clinic(s), Maria hospital.
Trial registration number: None.

POSTER VIEWING

ENDOMETRIOSIS/ENDOMETRIUM

P-185 Dual NFKB and mTOR inhibition required to significantly reduce endometrial cell viability

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Study question: Can the inhibition of signal transduction kinases reduce the cellular proliferation of endometrial epithelial and stromal cells that results from exposure to an inflammatory micro-environment and thus serve as potential targets for non-hormonal treatment of endometriosis.
Summary answer: A significant reduction in the viability of primary endometrial stromal cells and immortalized endometriotic epithelial cells exposed to tumor necrosis factor alpha (TNFa) occurred only when both NFkB and mammalian target of rapamycin (mTOR) were concomitantly inhibited. No significant effect was observed when each pathway was inhibited alone.
What is known already: Ectopic endometrial cell attachment stimulates the infiltration of macrophages and the production of inflammatory cytokines in the peritoneal fluid. This inflammatory microenvironment further stimulates the lesion progression. How this extracellular inflammatory environment regulates lesions progression however is not yet clear. Both the NFkB and mTOR signaling pathways play key roles in transmitting extracellular signals into a cellular response. Whether the inhibition of these pathways is effective in reducing endometriotic lesions is not yet clear.
Study design, size, duration: Endometrial epithelial and stromal cell cultures were treated with TNFa to simulate an inflammatory microenvironment and either NFkB (Pyrrolidine dithiocarbamate (PDTC)), mTOR (Rapamycin or Temsirolimus), or both (PDTC and Rapamycin) were inhibited by pharmaceutical intervention. The effect on kinase activation, production of inflammatory mediators and cellular proliferation were assessed.
Participants/materials, setting, methods: Primary endometrial stromal cells and an immortalized endometriotic epithelial cell line (12Z) were used for the *in vitro* analysis. Kinase activation was determined by Western blot, the inflammatory reaction by an ELISA measurement of interleukin (IL)-6, IL-8 and monocyte chemotactic protein (MCP)-1 and cellular proliferation by an MTS assay.
Main results and the role of chance: The activation of the downstream kinases S6K and 4EBP1 was significantly reduced by PDTC, Rapamycin and Temsirolimus confirming the targeted activity of the small molecule kinase inhibitors. In addition PDTC significantly upregulated phosphorylated AKT in both cell types. PDTC also showed strong inhibition of all cytokines examined in both cell lines, where as both rapamycin and temsirolimus resulted in significant inhibition of IL-8 and MCP-1 in the 12Z epithelial cells. Neither PDTC, or rapamycin and temsirolimus significantly inhibited cellular proliferation alone. A dual incubation with both PDTC and rapamycin however significantly reduced cellular proliferation in both epithelial stromal and epithelial cells.
Limitations, reason for caution: To stimulate an inflammatory micro-environment we used concentrations of TNFa that may not be present in the peritoneal fluid and thus the signaling pathways may behave different under different stimulation. In addition small kinase inhibitors may have additional unknown targets that may have affected the cell viability.
Wider implications of the findings: The inhibition of signal transduction kinases has been suggested for the non-hormonal treatment of endometriosis. The results of this study indicate that while neither PDTC or rapamycin alone did not reduce cell viability there was a significant reduction when used in combination. It therefore suggests multiple pathways are utilized to transmit the extracellular inflammatory signal into a cellular response, which should be taken into account when testing kinase inhibitors for endometriosis.
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by national/international organization(s), This study was supported by grants from the Stiftung fur klinische experimentelle tumor forschung, Bernische Krebsliga and Swiss National Science Foundation.
Trial registration number: 149/03.

P-186 Witch pharmacological scheme is better to luteal phase support after different IVF-protocols in order to improve clinical and ongoing pregnancy rate – randomized controlled trial

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Study question: Which is the best pharmacological luteal-phase-support after IVF cycles according to the ovarian stimulation protocol in order to increase clinical and ongoing pregnancy rate? Could endometrial thickness at pick-up <10 mm, E2 max at pick up <5 nmol/L and age >35 years have a role in decision making?
Summary answer: Long-agonist-protocol benefits from high-dose-progesterone supplementation particularly if endometrial-thickness <10 mm, E2 <5 nmol/L and age >35 years; short-agonist-protocol benefits from both high-dose-progesterone and estradiol-supplementation in clinical and ongoing pregnancy rate (strongly if E2 max <5 nmol/L and endometrial thickness <10 mm); short-antagonist-protocol benefits from high-dose-progesterone and estradiol-supplementation in almost all of cases.
What is known already: There's a lot of studies about luteal-phase-support after IVF-cycle but poor data are available in terms of ongoing-pregnancy. Poor data are available about additional effects of estradiol-supplementation to the progesterone-administration. It is universally demonstrated the benefit of progesterone administration but poor data are available regarding the best daily/dose. There is no agreement between the results for short or long protocol progesterone despite the effect of progesterone after pituitary desensitation with GnRH-agonists seems to be bigger.
Study design, size, duration: Perspective-randomized-superiority trial (January-2011/June-2013) on 360-patients undergoing IVF-cycle by long-agonist-protocol (Group_A: 180-patients), short-agonist-protocol (Group_B: 90-patients) and short-antagonist-protocol (Group_C: 90-patients). Patients were 1:1 randomly assigned to three different luteal-phase-support schemes: low-dose vaginal progesterone (200 mg/daily; subGropus1); high-dose combined vaginal/intramuscular progesterone (400 + 50 mg/daily; subGroups2); high-dose combined vaginal/intramuscular progesterone plus oral valerate estradiol (4 mg/daily; subGroups3).
Participants/materials, setting, methods: IVF-Cycles were performed according to our Unit-protocols, patient's history and ovarian reserve test. We included cases with almost one embryos obtained by FIVET/ICSI technique, registering the embryos quality. Starting from pick-up day, we support luteal-phase using the scheme obtained by randomization until a negative pregnancy test/miscarriage or until 12th gestational-week.
Main results and the role of chance: In Group_A no differences were found between the subGroups in terms of clinical-pregnancy (31.7%-vs.-35%-vs.-33.3%) and ongoing-pregnancy (36.8%-vs.-47.6%-vs.-45%).

In Group_B significant differences were found between subGroups_B1 vs. subGroups_B2 and subGroups_B3 in term of clinical-pregnancy (26.7% vs. 40.0% vs. 36.7%). Interestingly, data about ongoing-pregnancy showed that no differences were found between subGroups_B1 vs. subGroups_B2 (50%-vs.-58.3%) but both differed from the ongoing-pregnancy rate in subGroups_B3 (72.7%).

In Group_C differences were found between subGroups_B1 and subGroups_B2 vs. subGroups_B3 in term of clinical-pregnancy (20%-vs.-33.3%-vs.-53.3%). Similar trend was observed in term of ongoing-pregnancy (33.3%-vs.-50%-vs.-75%).

High-dose-progesterone improved ongoing-pregnancy in case of endometrial-thickness <10 mm and age >35 years (Group_A) and E2 max <5 nmol/L (Group_A and Group_C) while estradiol supplementation in case of endometrial-thickness <10 mm (Group_A and Group_B) and age >35 years (Group_B and Group_C).

Limitations, reason for caution: Weakness point of our study are: not-standardized number of transferred embryos, inability to blind patients or clinicians, large range of patients' age, different number of previous IVF cycle, no investigations about different administration way of drugs.

Wider implications of the findings: Low-dose progesterone seem to be an insufficient luteal-phase support for a large part of IVF-patients and it should be not recommended particularly in case of endometrial-thickness <10 mm, E2 max <5 nmol/L and age >35. High-dose of progesterone alone seems to improve clinical pregnancy rate in short-agonist-protocol and short-antagonist-protocol while in long-agonist-protocol benefits seem to be obtain only in a selected cases. Estradiol-supplementation should be administered only in a selected cases with intent to improve ongoing-pregnancy rate.

Study funding/competing interest(s): Funding by University(ies), Authors declare no funding. Authors declare no competing of interest.

Trial registration number: LUTEAL-IVF-11.

P-187 The endometrial stem cell marker Musashi 2 is associated with severity of endometriosis and cycle phase

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Study question: Is the adult stem cell marker Musashi-2 (Msi-2) of the eutopic endometrium associated with endometriosis ?

Summary answer: Msi-2 of the eutopic endometrium is associated with severity of endometriosis as assessed by ASRM stage. In addition Msi-2 is associated with cycle phase.

What is known already: Endometrial stem cells have been hypothesized to contribute to the etiology of endometriosis. Adult stem cells expressing markers of the Musashi (Msi) gene family contribute to the determination of cell fate. While we have demonstrated an association of Msi1 with endometrial cancer and endometriosis, data on Msi2 are limited. We studied the expression of Msi2 in endometrial samples obtained by transcervical biopsy in subjects with endometriosis and in healthy controls.

Study design, size, duration: In this case control study, prospectively collected data of 94 female subjects over a 3 years interval were included. Thirty-seven patients with endometriosis were compared with 57 disease free controls.

Participants/materials, setting, methods: Subjects underwent standardized diagnostic procedures including endometrial biopsy and laparoscopy in the IVF unit and the tertiary endometriosis referral centre of the university hospital. Endometrial biopsies were immunostained for Msi-2. Stem cell marker expression was correlated with clinical data of endometriosis patients and controls.

Main results and the role of chance: When comparing Msi2 expression between all endometriosis patients and healthy controls, no significant difference was detected. However, endometriosis patients with ASRM stages 1 and 2 displayed a significantly higher luminal Msi2 expression compared with ASRM 3 and 4 ($P = 0.032$). Additionally stromal Msi2 expression was higher in proliferative endometrial samples compared with secretory samples in all patients ($P = 0.032$) and the same was the case for luminal Msi2 expression ($P = 0.041$).

Limitations, reason for caution: Although the number of cases and controls appears adequate in this pilot study, a possible limitation derives from the fact that endometriosis was present in different clinical manifestations with partial overlap. Future studies with a high number of well characterized patients are needed to confirm and extent these results.

Wider implications of the findings: This pilot study contributes novel evidence to the hypothesis that endometrial stem cells contribute to endometriosis. Our findings could help to develop future diagnostic or therapeutic strategies involving stem cell markers, with Msi2 potentially serving as an early marker of endometriosis. The association of Msi2 with cycle phase could convey progesterone resistance a mechanism suspected to contribute to endometriosis. It also suggests to control for cycle phase in future studies on endometrial stem cells.

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

Trial registration number: None.

P-188 Expression patterns of VEGF and Flk-1 in human endometrium at the various phases of the natural menstrual cycle

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Study question: To investigate the expression and regulation of VEGF and its receptors in the human endometrium during the menstrual cycle is limited.

Summary answer: The expression of Flk-1 was higher in the luminal and glandular epithelium as compared to the stroma during the periovulatory, early-secretory and late-secretory phases ($P < 0.05$), as well as differentially expressed among the phases in the glandular epithelium.

What is known already: Angiogenesis is fundamental for human endometrial development and differentiation, which are necessary for implantation. These vascular changes are thought to be mediated by the vascular endothelial growth factor (VEGF) and its specific receptors.

Study design, size, duration: In vitro experimental study involving 60 tissue samples.

Participants/materials, setting, methods: Archived paraffin-embedded endometrial samples from 60 normally cycling women (age 23–39) were obtained from the Department of Pathology of the Johns Hopkins Hospital. The samples were divided into five groups according to the day of sampling: proliferative (day 7–12, $n = 14$), periovulatory (day 13–15, $n = 9$), early secretory (day 16–18, $n = 12$), mid-secretory (day 19–21, $n = 11$) and late secretory (day 24–26, $n = 14$) phases. Tissue microarrays (TMAs) were assembled from formalin-fixed, paraffin-embedded endometrial samples at cores of 1.5 mm in diameter for each core and three representative punches from each specimen. Immuno-histochemical staining was performed using monoclonal antibodies of anti mouse VEGF (sc-7269) and anti-human Flk-1(sc-6251). The intensity of staining of the antibody was then analyzed by a semi-quantitative method, the HSCORE. The intensity of the staining was evaluated in the anatomical compartments of the endometrium (luminal epithelium, glandular epithelium and stroma) through out the menstrual cycle.

Main results and the role of chance: VEGF and Flk-1 were immunolocalized in the luminal epithelium, glandular epithelium and stroma throughout the phases of the menstrual cycle. No immunoreactivity was detected when a monoclonal antibody MiTF was used as the primary antibody in the negative control staining.

The expression of VEGF was similar in the luminal, glandular epithelium and stroma at the same phase of the menstrual cycle. In the luminal epithelium, the expression of VEGF showed a trend of down regulation from the proliferative to late-secretory phase but it did not reach statistical significance. In the glandular epithelium, VEGF reached a peak expression during the early-secretory phase, and then trended down during the late-secretory phase. Significant differences in VEGF expression were observed in the stroma among the phases ($P < 0.05$), as tested by one-way ANOVA.

Flk-1 expression was similar between the luminal and glandular epitheliums in all phases of the menstrual cycle. In contrast, Flk-1 expression was significantly reduced in the stroma as compared to the luminal and glandular epithelium during the periovulatory, early-secretory and late-secretory phases ($P < 0.05$).

Limitations, reason for caution: Due to small sample numbers, the study could be underpowered to document additional significant changes in VEGF and Flk-1 expression in the different components of the endometrium.

Wider implications of the findings: Further investigation of the expression of VEGF and its receptor FLK-1 as well as of additional factors that are implicated in endometrial angionennesis and remodeling could provide valuable information on the process of normal and abnormal implantation.

Study funding/competing interest(s): Funding by hospital/clinic(s), Johns Hopkins Hospital.

Trial registration number: N/A.

P-189 Endometriosis related infertility: painful symptoms are associated with deep infiltrating endometriosis

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Study question: To evaluate the significance of severe painful symptoms for endometriotic women presenting with infertility.
Summary answer: In case of infertility, severe pelvic pain is significantly associated with previous history of surgery for endometriosis, higher rAFS adhesions scores and associated deeply infiltrating lesions. In this situation, the practitioner should address the patient to a referral centre.
What is known already: The association between endometriosis and infertility is quite common. Surgery and ART are both efficiency in enhancing pregnancy rates in infertile endometriotic women. Diagnosing deep infiltrating endometriosis is an important criteria in the management of endometriotic women wishing to conceive.
Study design, size, duration: We conducted a cross sectional study in a tertiary-care university hospital between January 2004 and March 2013. This study enrolled a cohort of 870 patients with histologically proven endometriosis who underwent surgery for pain and/or infertility. Complete surgical excision of all recognizable endometriotic lesions was achieved in each patient.
Participants/materials, setting, methods: For each patient data were collected preoperatively using a structured questionnaire. The pain was considered severe when the VAS ≥7. Infertile endometriotic women were compared according to the existence of severe pain or not. Disease severity was evaluated using both rAFS and surgical classifications.
Main results and the role of chance: In the whole endometriotic population women with infertility were more likely to have a previous history of surgery for endometriosis (PHSE) than women without infertility (137/307 (44.6%) vs. 195/562 (34.7%); $p = 0.004$). In addition women with infertility showed a more aggressive disease with higher rAFS adhesion scores than women without infertility (24.2 ± 26.2 vs. 18.1 ± 22.8; $p = 0.001$) and more severe deep infiltrating endometriosis (DIE) multifocal and with intestinal involvement ($p = 0.009$). Among endometriotic women with infertility, severe painful symptoms (n = 223) were associated with PHSE (14/84 (16.7%) vs. 123/223 (55.2%); $p < 0.001$) as compared to women with moderate or no painful symptoms ($n = 84$). In addition women with infertility and severe pain showed a more aggressive disease with higher rAFS adhesion scores (29.2 ± 26.8 vs. 10.9 ± 19.2, $p < 0.001$) and associated DIE (153/223 (68.6%) vs. 19/84 (22.6%), $p < 0.001$).
Limitations, reason for caution: There was a possible selection bias due to inclusion of only surgical patients. Specific biases may have occurred because the prevalence of DIE may be overestimated in our specific study population. Actually, the recruitment at our center, where we specialize in the care of severe endometriosis, may contribute to the elevated rate of patients affected by DIE.
Wider implications of the findings: We demonstrate in a large cross sectional study that infertile painful endometriotic women display a more severe disease with higher rate of previous history of surgery for endometriosis, higher rAFS adhesions scores and associated deeply infiltrating lesions. However, even if an association does not constitute proof of cause and effect, investigating the effects of painful symptoms in endometriotic women wishing to conceive is a step towards a more appropriate multidisciplinary approach.
Study funding/competing interest(s): Funding by University(ies), none.
Trial registration number: None.

P-190 The endometrial gene expression signature of recurrent implantation failure after IVF

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Study question: The aims of this study were to elucidate how the endometrial gene expression profile differs between women with recurrent implantation failure (RIF) and controls, and to investigate whether a predictor set of genes could be identified able to distinguish between these women.
Summary answer: An endometrial mRNA expression profile characteristic of RIF developed in two independent cohorts of both women with RIF and controls predicts the RIF phenotype with 88% precision.
What is known already: Transcriptomic studies have identified gene expression profiles characteristic of the window of implantation, and preliminary studies have indicated that these may be disrupted in some women with RIF.
Study design, size, duration: Between 2006 and 2013, mid-luteal phase endometrial biopsies were taken in the natural cycle from two serial cohorts, each consisting of women with RIF, defined as ≥3 failed IVF/ICSI treatments or replacement ≥10 embryos without the occurrence of a pregnancy ($n = 23$ and $n = 20$), and controls ($n = 23$ and $n = 50$).
Participants/materials, setting, methods: All subjects had undergone IVF/ICSI treatment in two tertiary hospitals. The control group had all conceived within the first two cycles of ICSI ($n = 50$) or IVF treatment ($n = 23$), and were therefore considered unlikely to have an endometrial factor. Microarray profiling and subsequent bioinformatic analysis sought a gene expression signature predictive of RIF.
Main results and the role of chance: Samples from 43 women with RIF and 73 controls were included in the analysis. A predictive gene signature was determined in a training set, which was validated in 34 samples. The RIF endometrial gene expression signature was shown to predict the RIF phenotype with 88% precision (95% CI: 53–99%). Sixteen percent of RIF patients showed a control-like expression profile, indicating that in these patients RIF may have an embryonic rather than endometrial aetiology.
Limitations, reason for caution: Endometrial sampling was performed after retrospective data analysis and although women were recruited from two hospitals, further validation of the identified profile in other hospital populations is desirable.
Wider implications of the findings: This study represents the largest validated gene expression cohort study in RIF to date. The expression profile of a predictor set of 374 genes provides the basis for a novel diagnostic approach to identify the endometrial factor in infertility, and can be of value in guiding further treatment in women who fail to conceive from IVF. Further secretomic and histologic studies are now required to identify specific putative therapeutic targets.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), this study was funded by a GFI grant from Merck Serono.
Trial registration number: NCT00351481.

P-191 A safety and efficacy study on long term treatment with Letrozole after GnRH A down-regulation in premenopausal patients with moderate and severe endometriosis

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Study question: The use of aromatase inhibitors (AI) is recommended for the treatment of refractory endometriosis in combination with i.e. GnRHa. However, the effect of long term AI monotherapy in premenopausal women is unknown. This study evaluates the safety, tolerability and efficacy of letrozole after down regulation with the GnRHa leuprorelin acetate.
Summary answer: Long-term letrozole monotherapy is insufficient to achieve a sustainable pain relieve and a frequent development of large functional cysts and SAEs has led to a frequent premature discontinuation of treatment. This suggests that long-term letrozole monotherapy is unsafe in premenopausal women and ineffective for the treatment of endometriosis.

What is known already: One possible explanation for the failure standard medical treatment in endometriosis may extraovarian estradiol synthesis by the aromatase pathway and aromatase expression has been described within endometriotic lesions. In the literature less than 20 cases have been described of premenopausal women suffering from endometriosis treated with AI monotherapy. However, due to the fear that AI may induce follicular growth there is no widespread use of AI as monotherapy in premenopausal women.
Study design, size, duration: This was a multicenter, open-label, exploratory phase II randomized controlled trial (RCT). 40 patients were recruited and monitored in gynaecological departments of five German hospitals from October 2002 (first enrolment) to June 2006 (last completion).
Participants/materials, setting, methods: 40 premenopausal women with moderate/severe endometriosis received 2 months GnRHa pretreatment before randomization (N = 36) to 4 months treatment of letrozole 2.5 mg/day (N = 19) or GnRHa 3.75 mg/month (N = 17). Safety variables included adverse event profile, gynaecological and ultrasound examination and laboratory parameters. Efficacy was assessed by pain reduction using the visual analog scale (VAS).
Main results and the role of chance: Five SAEs in the letrozole group – arthralgia, large ovarian cysts, vaginal bleeding, low abdominal pain – led to premature discontinuation and were considered to be related to the study medication. Under GnRHa treatment a significant decrease of LH (p = 0.018), estradiol (p = 0.028), estrone (p = 0.036), progesterone (p = 0.046) and cortisol (p = 0.043) and Ca125 (p = 0.012) levels were observed. However, in the letrozole group only estrone levels were significantly reduced (p = 0.047), whereas a significant increase of LH (p = 0.001), FSH (p = 0.033), estradiol (p = 0.006), progesterone (p = 0.015) and Ca125 (p = 0.036) was observed. In the letrozole group 13 patients developed new functional cysts >3 cm, but none in the GnRHa group. The VAS pain score decreased significantly under GnRHa treatment (p = 0.005) but showed an increase under letrozole treatment.
Limitations, reason for caution: The number of participants is rather limited. Furthermore, the endocrine profile under therapy was only tested once monthly, insufficient to permit a conclusion on the course of the endocrine profile in detail in premenopausal women with long term letrozole treament.
Wider implications of the findings: Due to the large number of SAEs under letrozole monotherapy in premenopausal women suffering from endometriosis it seems unethical to us to consider a repetition of a study with long-term monotherapy of any AI in premopausal women suffering from endometriosis. Hence, it seems difficult to overcome the limitations of this study due its relatively small study size. Indeed, this study gives the evidence that the treatment with AI can only be recommended in combination with hormonal treatment suppressing gonadotrophin secretion, i.e. combined hormonal contraceptives, progestagens or GnRha, Futhermore, the treatment with AI should be restricted to a limited time due to its severe side effects with possible long term implications for the women's health.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Novartis Pharma Nuremberg.
Trial registration number: ClinicalTrials.gov identifier NCT00240942.

P-192 G-CSF instillation in bad prognosis patients may improve embryo implantation in an oocyte donation program

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Study question: Can endometrial perfusion of granulocyte colony-stimulating factor (G-CSF) improve endometrial thickness and pregnancy rates in recipients with a low chance of pregnancy due to suboptimal endometrium (<7 mm thickness and/or low volume or VFI) after administration of high dosage of oestrogens in an oocyte donation program?
Summary answer: This study supports the benefits of endometrial perfusion with G-CSF 2 or 3 days before embryo transfer in thin endometria resistant to oestrogen stimulation, achieving a better endometrial thickness ensured by ultrasound scan the same day of embyo-transfer, and improving success rates in an oocyte donation program.
What is known already: A small number of IVF patients fail to achieve normal endometrial thickness with routine hormone treatments. Even when vasodilators are given, many women fail to reach minimal endometrial thickness/volume associated with low pregnancy chances.

Recent reports have suggested that intrauterine perfusion with (G-CSF) may be effective in women who are otherwise resistant to treatment.
Study design, size, duration: Retrospective study over 12 months including 47 egg-donation patients with endometrial thickness less than 7 mm after oestrogens therapy for at least 20 days. Chronic endometritis and Asherman were excluded from the study population. All patients failed to conceive in previous embryo-transfers.
Participants/materials, setting, methods: In a private fertility clinic, the study group (A) included 29 patients who received uterine instillation with 1 ml Granocyte© 34 IU using a intrauterine transfer catheter under sonographic control, 2–3 days before embryo transfer. The control group (B) included 18 patients who did not receive perfusion. In both groups endometrium was evaluated with power Doppler before and at the day of embryo-transfer.
Main results and the role of chance: There were no differences in both groups regarding mean age (Group A: 40.3 ± 5.68 years; Group B: 40.6 ± 5.57 years), cause of infertility or seminal parameters. With 3.2 ± 1.1 days between perfusion and embryo-transfer, endometrial thickness increased from 6.0 ± 1.2 to 7.3 ± 1.1 mm, while in the control group the increase was 6.3 ± 1.1 to 6.5 ± 1.7 mm (p > 0.05). In the study group, a 34.4% clinical pregnancy rate (PR) was observed while in the control group only 11.1% (p = 0.08). There seems to be a benefit of G-CSF instillation in terms of pregnancy rates in these bad prognosis patients, although the endometrial thickness didn't improve significantly, probably due to the small sample size.
Limitations, reason for caution: Small sample size (but a highly selected patient population) in a retrospective observational study. A prospective study comparing instillation with GCSF and placebo is ongoing to determine if it is the growth factor or the instillation procedure (with possibly pro-inflammatory effects) responsible for the clinical differences.
Wider implications of the findings: This study may support the utility of G-CSF in the treatment of chronically thin endometrium and suggests that such treatment will, in very adversely affected patients, still result in low, but reasonably improved clinical pregnancy rates. Increase in the study population will allow us to confirm the present data.
Study funding/competing interest(s): Funding by hospital/clinic(s), IVF Spain.
Trial registration number: Not registered.

P-193 Down-regulation of milk fat globule EGF factor 8 (MFG-E8) impairs endometrial receptivity

Abstract withdrawn by the author

P-194 Gene expression profiling of cumulus oophorus cells reveals significant altered pathways in patients with endometriosis

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Study question: Is there a difference in the global gene expression profile of cumulus cells (CC) associated with mature oocytes in patients affected by severe endometriosis compared to control patients referred to in-vitro fertilization for a severe male factor?
Summary answer: In patients with severe endometriosis, a down-regulation of a large number of genes involved in key regulatory pathways, such as extracellular matrix assembling and inflammation-like processes, is shown. The impairment of these mechanisms could be responsible for poor oocyte quality, with low fertilization and implantation rates, observed in endometriosis patients.
What is known already: Patients with severe endometriosis undergoing IVF show a decrease in the number of retrieved oocytes and lower fertilization,

implantation and pregnancy rates in comparison with patients affected by tubal factor infertility. Different studies demonstrated a major impairment in oocyte quality rather than in endometrial receptivity in endometriosis patients. A non-invasive method for evaluating the oocyte competence is the gene expression analysis of cumulus cells which establish a functional cross-talk with the oocyte.

Study design, size, duration: Experimental study in which the global gene expression profile of CC associated with mature oocytes in 18 patients affected by severe endometriosis was compared to control CC obtained from 18 patients affected by a severe male factor.

Participants/materials, setting, methods: The patients were recruited at ANDROS Day Surgery Clinic, Palermo, Italy and underwent a long-protocol. rFSH was used and ICSI was performed.

For each group, CC were pooled, RNA was extracted and microarray performed. Subsequently, quantitative real-time PCR was performed in CC of other endometriosis patients ($n = 5$) and controls ($n = 7$).

Main results and the role of chance: Considering as significant only the genes with fold change ≥1.5 and $p < 0.05$, a list of 595 differentially expressed genes was identified. 320 genes resulted down-regulated and 275 up-regulated. The most significant changes were observed in key genes involved in chemokine signaling pathway and cell-cell or cell-extracellular matrix adhesion. Several genes of these pathways were down-regulated in endometriosis patients. Individual RT-PCR assays confirmed for ten genes (*RUNX1, ARGHAP10, NLRP2, CYP11A1, TNFAIP6, CXCL2, CD44, TENASCIN C, IL-8, INTEGRIN b2*) the results of microarray analysis.

Limitations, reason for caution: In both groups, the CC associated with mature oocytes of all patients were pooled together, with no noticeable individual differences. This procedure was mandatory due to the very low quantity of obtained RNA.

The results of this paper should be validated in further studies.

Wider implications of the findings: The results of this paper show that several genes involved in chemokine mediated-signaling pathway and in the functional cross-talk between CC and the oocyte are down-regulated in endometriosis CC, indicating an impairment of these processes which could explain the reduction of oocyte competence in endometriosis patients. This preliminary knowledge may represent the starting point for a complete, future elucidation of the relationship between endometriosis and oocyte competence.

Study funding/competing interest(s): Funding by hospital/clinic(s), ANDROS Day Surgery Clinic, Palermo, Italy.

Trial registration number: None.

P-195 The in-vitro effect of the peritoneal fluid and TGFβ1 on fibroblast and endometriotic epithelial cell proliferation

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Study question: Can TGFβ1 affect the in-vitro proliferation rate of the mouse fibroblast (MF) and endometriotic epithelial cell (EEC) in a different way than that by the peritoneal fluid?

Summary answer: It was clear that although the TGFβ1 had no significant effect on the proliferation rate of MF and EEC in-vitro, the peritoneal fluid (PF) from women with endometriosis (EM) could stimulate the MF proliferation (but not EEC) which could be partially attributed to the estradiol (E2) in PF.

What is known already: TGFβ1 is known to mediate Epithelial-Mesenchymal Transition (EMT) during embryogenesis, fibrosis and cancer metastasis. EMT is important in the development of both EM and adenomyosis (AM). The endometrial epithelial cells are transformed into stromal cells then into fibroblasts laying down collagen. Endometriotic lesions are usually associated with varying degrees of fibrosis which contribute to chronic pelvic pain in these patients. EMT is thought to occur in the basal endometrium (BE) in AM as well.

Study design, size, duration: It is an experimental study that included patients with peritoneal endometriosis ($n = 20$) and control ($n = 20$). PF from endometriotic patients ($n = 19$) and healthy ($n = 13$) was collected.

Basal endometrium from AM patients ($n = 10$) and control ($n = 10$) were collected. It was carried out at Charite Universitätsmedizin – Berlin, Germany (2012–2013).

Participants/materials, setting, methods: The MF(L-929) and EECline(12Z) were incubated till 72 h with TGFβ1(0.1–10 ng/ml), 10% EM and control PF and E2(0.1 nM–10 nM) separately. TGFβ1 in PF in EM and control was measured by ELISA. Immunoexpression of TGFβ receptors (1.2 and 3) in healthy, EM peritoneum and in AM was done.

Main results and the role of chance: The PF from EM patients had a stimulatory effect on the proliferation of MF at 24–48 h incubation which could be reproduced with 1 nM E2 at 48 h incubation. TGFβ1 could not affect the proliferation rate of either MF or the EEC lines up to 72 h. The immune-expression of the three TGFβ receptors could be shown in both cell lines by immunofluoresence.

TGFβ1 level in PF was insignificantly different between EM and control, although TGFβ receptor 2 was significantly more immune-expressed in healthy peritoneum than EM. The BE showed significantly higher immune-expression of TGFβ receptor 3 in AM than control and higher TGFβ receptor 2 than receptor 1 and 3.

Limitations, reason for caution: The lack of in-vivo model of EM or AM could weaken any in-vitro experiments. Testing other growth factors in PF from EM which could partially stimulate the MF proliferation is essential. Testing TGFβ2 and 3 level in PF should follow.

Wider implications of the findings: According to our results we could suggest that targeting E2 level in the PF in EM might control the fibroblastic proliferation and hence the fibrosis. The TGFβ receptor 3 might be a future target for controlling AM progression.

Study funding/competing interest(s): Funding by national/international organization(s), Funding by commercial/corporate company(ies), the study is funded by Bayer pharmaceuticals, Germany and a doctoral scholarship from Ernst Schering foundation, Germany and Nachwuchsförderung des Landes Berlin, Germany.

Trial registration number: Basic science.

P-196 Dienogest for pain symptoms caused by rectovaginal endometriosis resistant to norethisterone acetate: prospective cohort study

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Study question: Is dienogest (DNG) efficacious in treating pain symptoms caused by rectovaginal endometriosis resistant to norethisterone acetate (NETA)?

Summary answer: DNG improves patient satisfaction compared with NETA. It ameliorates endometriosis-related pain symptoms and quality of life without causing significant changes in the volume of rectovaginal nodules and adverse effects.

What is known already: Progestins are valuable options for the treatment of endometriosis-related pain symptoms; however, no study compared the efficacy and safety of different progestins in the treatment of endometriosis.

Study design, size, duration: 24-week open-label prospective cohort study.

Participants/materials, setting, methods: The study included 17 women with rectovaginal endometriosis who had persistence of pain symptoms after treatment with NETA. Patients received DNG (2 mg/day) for 6 months. Patient satisfaction was the primary endpoint. Secondary endpoints were: pain symptoms, EHP-30, FSFI and adverse effects.

Main results and the role of chance: Patient satisfaction improved at 3- ($p = 0.020$) and 6-month ($p < 0.001$) treatment with DNG. DNG decreased the intensity of deep dyspareunia and non-menstrual pelvic pain at 3- ($p < 0.001$) and 6-month ($p < 0.001$). By week 24, the absolute reduction in visual analogue scale was 31.3 mm for deep dyspareunia and 18.3 mm for non-menstrual pelvic pain. There was an improvement in three modular

dimensions of the EHP-30: emotional well-being (p = 0.023), self-image (p = 0.014) and sexual intercourse (p = 0.037). There was a tendency towards better total FSFI score (p = 0.055). Nodule volumes did not change during treatment (p = 0.372). The number of analgesics used significantly decreased by week 24 ($p < 0.001$). There was no significant difference in the incidence of adverse effects between NETA and DNG (p = 0.295).

Limitations, reason for caution: The study was open label and treatments were not randomly allocated; the sample size was small.

Wider implications of the findings: This study confirms the efficacy of DNG in treating endometriosis-related pain symptoms and suggests for the first time that it may be more efficacious than NETA. If these findings will be confirmed by randomised controlled trials with larger sample size, DNG may become the first choice progestin for the treatment of pain caused by endometriosis.

Study funding/competing interest(s): Funding by University(ies), University of Genoa, Italy, PRA 2012.

Trial registration number: None.

P-197 Association of SLC18A1 gene polymorphisms with embryo implantation and early pregnancy loss in recipients undergoing IVF with donated oocytes

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Study question: Genotyping of Thr136Ile (rs1390938) and Thr4Pro (rs2270641) polymorphisms in SLC18A1 gene were used to assess their possible influence in success of IVF treatments between recipients using oocyte donors in an endometrial receptivity model.

Summary answer: Thr136Ile polymorphism of SLC18A1 gene was associated different clinical outcomes between recipients grouped according to their genotype. Recipients homozygous for Thr136 allele presented significantly lower implantation rates (IR) and suffered higher biochemical pregnancy loss events. Allele distribution for Thr4Pro polymorphism was differentially distributed when compared with pregnancy outcome.

What is known already: The protein product of SLC18A1, the vesicular monoamine transporter 1 (VMAT1), has been detected at glandular cells in the uterus. VMAT1 is responsible of monoamine storage inside vesicles and release of them to the extracellular space, being the serotonin (5-HT) one of the substrates more efficiently transported. Serotonergic system has been proposed to influence reproductive processes and 5-HT in the embryo maternal interface has been proposed to be determinant during embryo development and implantation success.

Study design, size, duration: A nested case-control study was used involving recipients undergoing IVF treatments with donated eggs. A total of 227 patients were recruited between July 2009 and February 2011 at the Instituto de Fertilidad Clínica Rincón facilities. Groups were established according to the final results obtained after the treatments.

Participants/materials, setting, methods: Genomic DNA isolation was performed from buccal swaps. TaqMan® OpenArray® Genotyping System (Applied-Biosystems) was used for genotyping selecting probes included for rs1390938 and rs2270641. Chi-squared or Fisher exact test and logistic regression model were used to assess possible differences between genotype frequencies and groups of recipients according to the outcome.

Main results and the role of chance: Assessment of genotype distribution between groups of recipients was in Hardy-Weinberg equilibrium ($p > 0.05$).

Different IR were obtained for different genotypes of the Thr136Ile polymorphism Thr/Thr (22.86%), Thr/Ile (51.03%) and Ile/Ile (35.86%) (p = 0.001). Thr4Pro allele frequencies varied between recipients successfully pregnant (Thr = 0.59) and those that did not (Thr = 0.47) (p value = 0.002). Thr136 allele was higher in recipients that suffered a biochemical pregnancy loss compared to those that get pregnant 22% vs. 57%, respectively (p = 0.0056). In addition higher frequencies of homozygous Thr136 were associated with biochemical pregnancy loss under a recessive inheritance model [13.82 (2.58-74.10) OR (95% CI)] (p value = 0.0053).

Haplotype analysis revealed that haplotype Ile136-Thr4 was associated with increased pregnancy rates [0.61 (0.39–0.97) OR (95% CI)] (p value = 0.039).

Limitations, reason for caution: The present study is based on a possible influence of gene polymorphism that could affect implantation and pregnancy maintenance. Still are needed further studies that could provide new insights about functional effects of Thr136Ile and Thr4Pro polymorphism at SLC18A1.

Wider implications of the findings: Thr136Ile and Thr4Pro polymorphisms in SLC18A1 gene are associated with success of pregnancy in the present endometrial receptivity model. Homozygous Thr136 carriers showed reduced implantation and pregnancies rates might related to reduced VMAT1 activity associated to this genotype. Decreased monoamine flux release at the extracellular location in the glandular region of the uterus, could compromise 5-HT availability that is needed for the maternal-embryo dialogue during the implantation and the onset of pregnancy.

Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Institutional Fundings Univeristy of Malaga (SAF2008-03314). Instituto de Fertilidad Clínica Rincón (PTQ 09-01-00496).

Trial registration number: None.

P-198 Dyspareunia in women with endometriosis

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Study question: What characterizes women with dyspareunia and endometriosis? Is there an association with specific anatomical lesions?

Summary answer: 18.5% of the 470 women with endometriosis investigated in our German multicentre study, reported to suffer from dyspareunia. Contrary to current literature the presence of dyspareunia was not related to specific anatomical lesions. As to expect no association with the ASRM stages could be demonstrated.

What is known already: Endometriosis i.e. endometriotic tissue implants outside the uterine cavity is a chronic, often progressive disease occurring in up to 10% of women during reproductive age. Classical symptoms are chronic pelvic pain, disturbed bleeding patterns and reduced fertility. Dyspareunia is one of the characteristic pain complaints in women with endometriosis. Current research gives emphasis to the hypothesis, that specific location of endometriosis, i.e. those of the uterosacral ligaments are associated with a higher prevalence of dyspareunia.

Study design, size, duration: Retrospective data analysis of 470 women with surgically/histologically confirmed endometriosis. Women were recruited in different Swiss and German hospitals. Data on dyspareunia were collected using a self-administered questionnaire designed to investigate quality of life quality (qol) of life in endometriosis.

Participants/materials, setting, methods: Data acquisition was made with a questionnaire in gynecologic units throughout Switzerland and Germany, on average 37.5 months after the first surgery. Socio-demographic data, medical, gynaecologic and obstetric history, and different aspects of the *qol* were collected in women suffering from endometriosis and controls matched for age and nationality.

Main results and the role of chance: A total of 90.8% (427) women answered the question about dyspareunia. Out of these 20.4% (87) women who reported to suffer from pain in every or almost every intercourse were considered presenting dyspareunia. In addition, 45.7% (195) women rarely or sometimes experienced dyspareunia and 33.9% (145) described to never or almost never experience dyspareunia. Dyspareunia was experienced independently from the ASRM stages (i.e. 20% in ASRM I, 16.9% in ASRM II, 21.9% in ASRM III and 21.4% in ASRM IV). The occurrence of dyspareunia after surgical treatment of an obliterated Douglas (21.2%), resection of implants on the uterosacral ligament (20.6%) or a resection in the vaginal fornix (21.2%) did not differ significantly.

Limitations, reason for caution: Time between diagnostic surgery and data acquisition varied between less than a year and 21 years, so that further analysis will clarify the effect of time since surgery on the development of dyspareunia.

Future analysis will also investigate the occurrence of dyspareunia on the background of medical treatments.
Wider implications of the findings: Not only women with lesions of the uterosacral ligament suffer from dyspareunia. In addition, Douglas pouch obliteration and resected lesions in the vagina were associated with dyspareunia. Consequently, any women suffering from endometriotic lesions close to the vagina should systematically be counselled to reduce potential consequences of endometriosis on sexual activity.
Study funding/competing interest(s): Funding by hospital/clinic(s), University Hospital of Zurich, Switzerland.
Trial registration number: None.

P-199 Human endometrial stroma cells express CXCL1 in vitro through different signalling pathways

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Study question: Are the two signalling pathways – MAPK and JNK – involved in the expression of the chemokine C-X-C-motif ligand 1 (CXCL1) in the human endometrial stroma cell line St-T1 either decidualized or not decidualized and is there a role for Syndecan-1 (Sdc-1) in this process?
Summary answer: Both pathways are involved in CXCL1 expression, but only the MAPK signalling pathway is influenced by Sdc-1 investigated by sdc-1 knock-down. JNK pathway regulates CXCL1 independent of Sdc-1. Decidualized cells had a higher sensitivity towards used signalling pathway inhibitors.
What is known already: CXCL1 is one of the most important chemokines during the implantation and mediates its function through a G-protein activated second messenger cascade by the CXC-receptor 2 and the co-receptor Sdc-1. Previous studies of Sdc-1 by use of the knock-down cell line KdS1 offered changes in cytokine and angiogenic factor expression of decidualized KdS1 as well after incubation with embryonic stimuli (IL-1β).
Study design, size, duration: This study was performed in vitro with the human endometrial stroma cell line St-T1 (Brosens, 2009) and the inducible Sdc-1 knock-down cell line KdS1 based on St-T1. Treated vs. untreated controls were evaluated with $n \geq 4$.
Participants/materials, setting, methods: St-T1 and KdS1 were both treated with decidulization stimuli, embryo surrogate IL-1β (10 ng/ml for non- and 0.1 ng/ml for decidualized cells) and MEK1/2 and c-Jun inhibitors. Culture media supernatant were used in a CXCL1-ELISA and total protein in western blot.
Main results and the role of chance: After treatment with decidualization stimuli, IL-1β and inhibitors the ELISA evaluation showed a significant decrease of CXCL1 in c-Jun inhibitor treated St-T1, dSt-T1, KdS1 and dKdS1 (decidualization was proven by prolactin PCR). Here the decidualization sensitized the cells for the inhibitor effect. In St-T1 and KdS1 the significant decrease started from 10 µM or 25 µM c-Jun inhibitor and in dSt-T1 and dKdS1 from 5 µM already. In St-T1 and dSt-T1 the MEK1/2 inhibitor caused no significant alteration in CXCL1 expression. However KdS1 and dKdS1 showed a significant decrease of CXCL1 at 50 µM or 25 µM inhibitor. The decidualization produced no change in CXCL1 expression in St-T1 vs. dSt-T1, nevertheless untreated KdS1 showed a lower sensitivity towards MEK1/2 inhibitor in contrast to dKdS1.
Limitations, reason for caution: This study was performed in vitro. The knock-down cell line KdS1 was generated by a double transfection of the initially endometrial stroma cell line St-T1.
Wider implications of the findings: This study showed that MAPK signalling pathway and JNK signalling pathway are both involved in the expression of CXCL1, an important chemokine during human embryonic implantation. Dysregulations in these pathways could cause interference during implantation process and threaten the establishment of a healthy pregnancy. Embryo transfer medium containing additions maintaining these two pathways could assist the implanting embryo during IVF treatment.
Study funding/competing interest(s): Funding by national/international organization(s), German research foundation (DFG) He 3544/2-2 and He 3544/2-3.
Trial registration number: Basic science.

P-200 Syndecan-1 knock down alters apoptotic susceptibility to embryonic stimuli in the human endometrial epithelial cell line RL95-2

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Study question: Is the expression of apoptosis related proteins in endometrial epithelial cell line RL95-2 altered by a knock down (kd) of the co-receptor Syndecan-1 (Sdc-1) and if so, does the alteration influence the apoptotic susceptibility to embryonic stimuli like cytokines and anti-Fas antibody (ab)?
Summary answer: A lower Sdc-1 expression in RL95-2 influences the expression of apoptosis related proteins compared to control which is correlated with higher apoptotic susceptibility of these cells displayed by higher levels of active Caspase-3 after treatment with embryonic stimuli.
What is known already: Trophoblast invasion in the decidua is accompanied by the apoptotic cell death of cells of the maternal epithelium and a suggested mechanism is via the extrinsic Fas/ FasL pathway but intracellular signalling pathways remain unknown. Sdc-1 as a growth-factor and chemokine co-receptor fulfils many functions regarding binding, storage and signalling of its ligands. Recent publications reveal contrary influences of Sdc-1 to the apoptotic inducibility of human carcinoma cells depending on the origin of tissues.
Study design, size, duration: RL95-2 vs. RL95-2 with Sdc-1 kd; 4-5 independent experiments.
Participants/materials, setting, methods: RL95-2 human endometrial epithelial cells were chosen to generate a stable Sdc-1 kd. Induction of apoptosis was determined after treatment with embryonic stimuli (anti-Fas ab, IL-1β, IFN-γ, TGF-β1 and TNF-α). Furthermore, the expression of apoptosis related proteins before and after induction was investigated via Antibody Arrays and active Caspase-3 ELISA.
Main results and the role of chance: The kd of Sdc-1 was generated successfully in RL95-2. Different proteins with an anti-apoptotic impact were significantly decreased in RL95-2 with Sdc-1 kd (IAPs, heme oxygenase-2, heat shock proteins-27 and -70). Incubation with an anti-Fas ab induced apoptosis in both cell types with a peak after 7 h. Treatment with the cytokine combination sensitized the cells to Fas-mediated apoptosis. RL95-2 with Sdc-1 kd revealed higher apoptotic inducibility displayed by higher levels of active Caspase-3. After induction of cell death the expression of apoptosis related proteins revealed an increase of death receptors Fas and TRAIL in RL95-2 with Sdc-1 kd.
Limitations, reason for caution: The study was limited to *in vitro* experiments with the human cell line RL95-2. Embryo contact was mimicked with cytokines known to be secreted by the trophoblast during implantation and an anti-Fas ab as an alternative for the Fas-Ligand bearing trophoblast.
Wider implications of the findings: Sdc-1 is supposed to influence endometrial apoptosis during embryo implantation and further experiments will reveal the signaling pathways behind these interaction.

The role of Sdc-1 *in vivo* might be observed during IVF-treatment by endometrial scratching, quantification of Scd-1 and afterwards be correlated with IVF-results for the individual patient.
Study funding/competing interest(s): Funding by national/international organization(s), German research foundation (DFG) He 3544/2-2 and -3.
Trial registration number: None.

P-201 Disruption of progesterone signaling by microRNA-200a precludes embryo implantation in the cervix

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Study question: The embryo does not normally implant in the cervix but in the uterus. Are there any local molecular machineries to prevent embryo implantation in the cervix?

Summary answer: Progesterone (P_4) signaling in the uterus makes epithelial differentiation and stromal proliferation in the receptive phase and further provides successful implantation, while such P_4 signaling with the appropriate proliferation/differentiation switching is disrupted in the cervix by microRNA-200a (miR-200a). These findings indicate that P_4 signaling deficiency confers cervical non-responsiveness to implantation.

What is known already: In mice and humans, P_4 regulates epithelial differentiation and stromal proliferation in the receptive endometrium, and this differentiation/proliferation switching is one of indicators for endometrial receptivity. P_4 acts via progesterone receptor (PR), and its cochaperone FKBP52 optimizes PR activity. FKBP52 null mice show implantation failure due to the reduction of P_4-PR signaling, and P_4 supplementation restores its signaling and implantation. P_4 metabolizing enzyme 20α-HSD is induced by miR-200a through the reduction of Stat5 in the pregnant myometrium.

Study design, size, duration: Human uterine and cervical tissues were obtained from 30 women with normal menstrual cycles undergoing hysterectomy due to gynecological disorders. Wild-type (WT) and FKBP52 null mice were used for the evaluation of P_4 signaling and cell proliferation. Cell lines MCF-7 and COS-7 were used for functional analyses of miR-200a.

Participants/materials, setting, methods: Proliferation/differentiation status in the cervix and uterus was evaluated by Ki-67 immunostaining using human and mouse tissues. The expression of PR signaling-related molecules in mice was assessed by immunostaining, Western blotting, qPCR and in situ hybridization. Cellular functions of miR-200a were assessed by Western blotting and reporter assay.

Main results and the role of chance: The proliferation/differentiation switching observed in the uterus did not occur in the cervix during the mouse and human receptive phase. Impaired P_4-PR signaling in FKBP52 null mice or WT ones with the injection of PR antagonist abolished the uterine proliferation/differentiation switching in the receptive phase, while such inhibition of PR activity never affected the proliferation/differentiation status in the cervix during this period. Interestingly, the protein levels of PR were significantly low in the cervix compared to the uterus in WT mice; conversely the cervical miR-200a levels were higher than the uterine ones. The in-vitro analyses showed that miR-200a directly represses PR translation. In addition, 20α-HSD was upregulated and its transcriptional repressor Stat5 was inversely downregulated in the cervix compared to the uterus.

Limitations, reason for caution: We performed most of the analyses using mouse tissues and cell lines, and found that miRNA-PR-P_4 signaling is a critical regulator of cervical non-responsiveness to embryo implantation. However, further investigations using human tissues and/or cells are needed to confirm the presence of this molecular pathway in the human cervix.

Wider implications of the findings: These findings provide for the first time molecular evidence for the non-responsiveness of the cervix to P_4-PR signaling, which may contribute its inability to allow implantation, and may also help us better understand not only the endometrial receptivity in mice and humans but also the pathological basis of cervical pregnancy and placenta previa in humans.

Study funding/competing interest(s): Funding by national/international organization(s), this work was supported by the Precursory Research for Embryonic Science and Technology and the Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science.

Trial registration number: Not applicable.

P-202 Spatio-temporal pattern of endometrial vascular extracellular matrix expression is dysregulated in women with menorrhagia

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Study question: How does the pattern of expression of vascular extracellular matrix proteins change during the course of the normal menstrual cycle in the stratum functionalis, stratum basalis and myometrium and is this pattern of variation altered in women with menorrhagia?

Summary answer: The pattern of expression of vascular extracellular matrix (ECM) components: osteopontin, laminin, fibronectin and collagen IV ditters between the normal myometrium, stratum functionalis and stratum basalis. In addition, this pattern of expression is altered in menorrhagia. The most notable alterations occurred in the stratum basalis and functionalis followed by myometrium.

What is known already: Menorrhagia affects 30% of women of reproductive age and accounts for ~60% of all hysterectomies. Previous studies suggested important structural roles for endometrial blood vessels, including early breakdown. We have previously shown alteration in vascular calponin expression in menorrhagia. ECM provides structural framework for endometrial stroma, maintaining vascular structure. Vascular ECM includes osteopontin, laminin, fibronectin and collagen IV. Although important to normal functioning of endometrium, ECM has not been studied in depth, especially in menorrhagia.

Study design, size, duration: Endometrial biopsies were taken from hysterectomy specimens from control women, without endometrial pathology (proliferative (PP), early secretory (ESP), mid secretory (MSP), late secretory (LSP); n = 5 each group) and women with menorrhagia (PP, ESP, MSP, LSP; n = 5 each group). Biopsies were formalin fixed and paraffin embedded (FFPE).

Participants/materials, setting, methods: FFPE sections were immunostained for osteopontin, laminin, fibronectin and collagen IV. Vascular ECM within the myometrium, endometrial stratum basalis and stratum functionalis were scored for the intensity of immunostaining using a modified quickscore, taking into account the proportion of positive cells and the intensity of immunostaining.

Main results and the role of chance: In menorrhagia osteopontin expression was higher in LSP (P = 0.008) stratum functionalis, while in stratum basalis it was lower in ESP (P = 0.008), compared to control samples. In control stratum functionalis laminin expression remained stable between PP and MSP endometrium, before increasing in LSP. In menorrhagia samples this stability was disturbed, and expression was increased (P = 0.05) in LSP stratum basalis. In menorrhagia fibronectin expression was altered especially in stratum basalis, where it was lowered in MSP (P = 0.006), and increased in LSP (P = 0.02). Collagen IV expression was lower in menorrhagia than control samples in ESP (P = 0.008) and LSP (P = 0.04) stratum functionalis, ESP (P = 0.007) and MSP (P = 0.002) stratum basalis as well as LSP (P = 0.03) myometrium.

Limitations, reason for caution: This study included vascular ECM expression for both straight and spiral arterioles. A semi-quantitative approach was utilised to analyse ECM component expression. Repeated measures two-way ANOVA compared the three layers within control and menorrhagia groups for each phase of the menstrual cycle, followed by Bonferroni correction for multiple comparisons.

Wider implications of the findings: Vascular osteopontin expression was increased in menorrhagia. We previously showed reduced calponin expression in these vessels. Evidence suggests regulation of vascular calponin expression by osteopontin; our data supports this. Collagen IV has been shown to restrict VSMC growth, promote contractile phenotype; decreased collagen IV expression in menorrhagia LSP may reflect weaker vascular structure, definition and altered differentiation status. Our results indicate potential misregulation of vascular ECM and consequent functional alterations in endometrial vessels in menorrhagia.

Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), Newcastle University, Wellbeing of Women (RG1342), There are no competing interests to declare.

Trial registration number: Not applicable.

P-203 Andullation therapy promotes implantation and increases pregnancy rates in IVF-cycles

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Study question: Besides all technical innovations which were implemented in the clinical course of reproductive medicine within the last decade, the implantation

rates (IR) are still unsatisfactory low. We evaluated whether andullation, a known relaxation massage therapy for pain relief is a beneficial complementary treatment for ART to support the implantation process.

Summary answer: We found that andullation therapy, a specific massage method, significantly increased pregnancy (PR) – and ongoing pregnancy rates (oPR) when administered prior to embryo transfer. No adverse effects of andullation were observed.

What is known already: Several studies suggest that stress and anxiety impede the implantation process. Complementary and adjuvant therapies such as acupuncture or yoga are supposed to alleviate psychological stress and to be beneficial within the scope of ART. The whole body exposure to biomechanical oscillations is widely used by physiotherapy experts as it is known to positively affect the endocrine-, the cardiovascular- and sensory system. However, its benefit has not been tested within the field of ART.

Study design, size, duration: A randomized study including 267 IVF-patients between January and December 2012 was conducted. Patients received a transfer with vitrified/warmed blastocysts during this period. One group of the patients received treatment on a commercial massage mattress including thermal infrared deep heat for 30 min for deep relaxation prior to embryo transfer.

Participants/materials, setting, methods: Patients were prepared for embryo transfer (ET) with a hormonal substitute protocol with increasing dosages of estradiol. One week prior to ET endometrial thickness had to be >6 mm. Blastocysts were aseptically vitrified and warmed using the VitriSafe protocol. The quality of ET was categorized into three groups, easy and difficult.

Main results and the role of chance: No statistically significant differences between the group of patients with or without andullation therapy regarding type and reason for infertility, number of previous cycles and patients' age (35.2 vs. 35.2 years) were found. Endometrial thickness was similar in both groups as well as the expected gametes performance (EGP: e.g. amount and quality of blastocysts transferred). Interestingly, the andullation treatment did not increase the percentage of "easy" ETs as could be expected as it is thought to be a relaxation therapy. However, a significantly higher PR as well as oPR was observed (PR: 58.9% vs. 41.7%, $p < 0.05$; oPR: (53.6% vs. 33.2%, $p < 0.01$). A patient-tailored complementary andullation therapy prior ET might be simple and efficient tool for supporting the implantation.

Limitations, reason for caution: Additional studies will be necessary to prove these findings and to investigate the mode of therapeutic effect. Further, our observation has to be confirmed in fresh ETs.

Wider implications of the findings: The application of complementary medicines during ART is increasing. IVF-patients are often under massive emotional pressure during a fertility treatment. The role of psychological impact on ART outcome is – to date-completely underestimated. We demonstrated for the first time, that andullation therapy in combination with infrared deep heat prior cryo-transfer positively impacts on pregnancy rates. Applying this technique complementary to IVF might improve implantation rates.

Study funding/competing interest(s): Funding by hospital/clinic(s), IVF Centers Prof. Zech.

Trial registration number: Informed consent was signed by all patients. All works performed were in concordance with the principles for medical research according to the WMA declaration of Helsinki.

P-204 Endometriosis in primary care practice: general practitioners' medical strategies and knowledge

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Study question: What is a General Practitioner's (GPs) medical strategy when encountering a patient who may have endometriosis, and on which knowledge is this stategy based?

Summary answer: 50% of GPs starts an empirical medical treatment when suspecting endometriosis. When answering questions concerning knowledge about endometriosis, an average of 61% of questions is answered correctly. All GPs in this study express the need of and motivation for further education about endometriosis.

What is known already: In The Netherlands, patient delay in endometriosis is 5.3 years, whereas doctors delay is 7.8 years. The current ESHRE guideline on diagnosis and treatment of women with endometriosis identifies awareness and early diagnosis of endometriosis among primary care specialists as one of the important topics for further research.

Study design, size, duration: Ten Dutch GPs participated in a quantitative postal survey about endometriosis management and knowledge. The GPs answered a 41-item questionnaire concerning (1) their medical strategies when encountering a patient with complaints suspect for endometriosis, (2) their actual knowledge on endometriosis and (3) whether they feel a need to improve this.

Participants/materials, setting, methods: Of the participating GPs, 50% was male and 50% female. Average years of working experience was 13.9 (range 1–39 years). 60% had predominantly higher social class patients, 20% predominantly lower social class, and 20% had a mixture of all classes in their practice.

Main results and the role of chance: When encountering a patient with complaints suspect for endometriosis, 20% of GPs observes the natural course for a certain period, 50% of participants starts empirical medical treatment, and 30% refers to a gynaecologist or refers for diagnostic tests.

The average score on the questions about knowledge is 61% (15.1 out of 25) correct answers (range 32%–92%). GPs with ten or more years of working experience score higher (73%) than GPs with working experience of less than 10 years (48%) ($p < 0.05$).

All GPs think that their knowledge on endometriosis could be improved, and they all express the need for education. 70% prefers frontal education whereas 30% prefers individual education using an (online) course. 30% of GPs uses the ESHRE guideline on endometriosis.

Limitations, reason for caution: The results are based on a pilot study of ten participants. In order to get a representative pilot group, GPs were selected on sex, number of years of working experience and demographic characteristics of their practice.

At the moment, this study is undertaken among a larger group of GPs.

Wider implications of the findings: When eventually suspecting endometriosis, a majority of GPs expresses an adequate strategy. However, the level of knowledge is only sufficient in GPs having over 10 years of working experience. All GPs feel a need for education. Because doctors delay to the diagnosis of endometriosis in The Netherlands is 7.8 years an education program about recognition of signs and symptoms is under development in conjunction with GPs, in order to enhance early diagnosis and treatment of endometriosis by GPs.

Study funding/competing interest(s): Funding by hospital/clinic(s), there was no funding.

Trial registration number: N/A.

P-205 Induction of IGFBP-1, PRL and Mn-SOD by cAMP is associated with histone acetylation status of their promoters in human endometrial stromal cells

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Study question: How are gene expressions of insulin-like growth factor binding protein-1 (IGFBP-1), prolactin (PRL) and manganese superoxide dismutase (Mn-SOD) regulated by C/EBP β, a transcriptional factor, in human endometrial stromal cells (ESC)?

Summary answer: H3 lysine 27 acetylation (H3K27ac) status, which activates transcription, of the C/EBP β binding site of the promoter is critical for the expression of IGFBP-1, PRL and Mn-SOD.

What is known already: IGFBP-1 and PRL are preferentially expressed in human ESC undergoing decidualization, and are recognized as specific markers of decidualization. Mn-SOD is expressed in ESC without decidualization stimulus, and its expression increases by decidualization stimulus and contribute to cell survival. C/EBP β is a transcriptional factor that regulates a number of genes related with decidualization including IGFBP-1, PRL and Mn-SOD.

Study design, size, duration: None.

Participants/materials, setting, methods: ESC isolated from the proliferative endometrial tissue were incubated with or without dibutyryl-cAMP (cAMP, 0.5 mM) for 4 days to induce decidualization. IGFBP-1, PRL and Mn-SOD mRNA expression were examined by real time RT-PCR. The DNA binding of C/EBP β and H3K27ac levels were examined by ChIP assay. Knockdown of C/EBP b was done by using siRNA method.
Main results and the role of chance: cAMP increased mRNA expressions of IGFBP-1, PRL and Mn-SOD with the increased binding of C/EBP β to their promoters, and the stimulatory effects of cAMP were inhibited by C/EBP β knockdown. In non-decidualized-ESC, C/EBP β-binding activity and H3K27ac levels of Mn-SOD-promoter were high whereas those of IGFBP-1- and PRL-promoters were low. cAMP induced H3K27ac of C/EBP β-binding site of IGFBP-1-and PRL-promoter while H3K27ac of Mn-SOD-promoter remained high. In conclusion, cAMP up-regulates IGFBP-1, PRL and Mn-SOD expression through the recruitment of C/EBP β to their promoters. The C/EBP β-binding site of Mn-SOD-promoter is high H3K27ac status in the absence of cAMP, which allows C/EBP β-binding to keep basal Mn-SOD expression. On the other hand, cAMP induces high H3K27ac at C/EBP β-binding site of IGFBP-1-and PRL-promoters, which allows C/EBP β recruitment to induce IGFBP-1 and PRL expression.
Limitations, reason for caution: This work is an in vitro study.
Wider implications of the findings: Our results show the molecular mechanism for decidualization-specific IGFBP-1 and PRL expression. This study provides new insights to understand the mechanism for gene regulation by decidualization in human ESC.
Study funding/competing interest(s): Funding by University(ies), this work was supported in part by Grants-in-Aid 21592099 for Scientific Research from the Ministry of Education, Science, and Culture, Japan. The authors declare no competing interests.
Trial registration number: None.

P-206 Effects of S100 protein knockdown in primary epithelial and stromal endometrial transcriptome of fertile patients

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Study question: Using omics, we previously identified biomarkers of endometrial receptivity. We investigated the function(s) of one S100 family member, expressed in both epithelial and stromal cells, using shRNAs. We analyzed the impact of the S100 protein extinction on gene expression profiles of primary epithelial and stromal endometrial cells of fertile patients.
Summary answer: S100 expression protein extinction affected the genes expression profile of both epithelial and stromal endometrial cells particularly on genes involved in the extracellular matrix. Alteration of signaling pathways which are crucial for the implantation process, the TGFb signaling and the leukocyte transendothelial migration were deregulated by this S100 protein extinction.
What is known already: Many biomarkers of human endometrial receptivity have been previously reported. However, few studies have been performed to identify their role(s) and function(s) during the implantation window of fertile patients.
Study design, size, duration: The stromal and epithelial endometrial cells were isolated using anti-EpCam coated magnetic dynabeads. Then, an approach by loss of function (pLKO.1-puro-CMV-tGFP, 3 shRNAs) was used in each endometrial cellular type for stable gene silencing of the candidate. Transcriptomes of infected cells were analyzed by microarrays and compared with non-infected cells.
Participants/materials, setting, methods: Primary endometrial cells were obtained from biopsies performed during the implantation window of fertile patients. After epithelial cells purification and S100 gene silencing in each cell type, RNAs were extracted and analyzed using DNA microarray chips. Gene expression profiles and biological pathways impacted by S100 gene silencing were analyzed.
Main results and the role of chance: In epithelial cells, S100 extinction induced the deregulation of 34 genes that are all up-regulated in the S100 shRNA cells compared with normal cells. A majority of these genes were components of the extracellular matrix and intercellular connections [such as COL4A5 (×2.6), COL4A6 (×6.1) and GJA5 (×3)]. In stromal cells, S100A10 extinction induced a more severe impact with 256 genes (174 up- and 82 down-regulated genes) differentially expressed compared with non-infected stromal cells. Functional annotation revealed alteration of the TGFb signaling [ACVR1C (×2.9), BMPER (×2.6) and DCN (×3.7)] and the leukocyte transendothelial migration [VAV3 (×2.5), MMP16 (×2.9), JAM1 (×2.3) and CLDN11 (x-8.3)], two major pathways that play a central role in the acquisition of the receptive endometrial phenotype, and therefore, in the implantation process.
Limitations, reason for caution: For each cell type, transcriptomes of 3 control shRNAs and 3 S100 shRNAs have been performed and must be repeated with more endometrial samples.
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. The authors of the study have no competing interests to report.
Trial registration number: Not applicable.

P-207 S100 protein family member involved in the acquisition of the receptive endometrial phenotype of fertile patients

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Study question: Using transcriptomic and proteomic technologies, we identified pertinent biomarkers of human endometrial receptivity. Functional analyses were performed in order to identify the role(s) of one of these biomarkers, belonging to the S100 family, in endometrial receptivity of fertile patients.
Summary answer: This candidate is crucial for the acquisition of the receptive endometrial phenotype because it plays a central role in both stromal and epithelial endometrial cells migration and decidualization.
What is known already: Numerous biomarkers of human endometrial receptivity have been reported by our group and others. However, few studies determined their role(s)/function(s) during the implantation window. We previously selected one candidate belonging to the S100 family and confirmed its overexpression during the implantation window in fertile patient's endometrial samples. This candidate was expressed in stromal and epithelial cells by western blot analyses of purified primary endometrial cells and immunofluorescence staining of sections from endometrium.
Study design, size, duration: Primary endometrial cell cultures of epithelial and stromal cells were performed. Then, we targeted the extinction of the candidate using shRNA technology (3 shRNAs, vector pLKO.1-puro-CMV-tGFP) in each endometrial cell type. The obtained phenotype was analyzed in regards of the morphology, proliferation, survival/death, adhesion, migration and decidualization.
Participants/materials, setting, methods: Stromal and epithelial endometrial cells were purified from biopsies performed during the implantation window of fertile patients. Following gene silencing in each cell type, migration assay by wound healing, adhesion assay using JAR trophoblastic spheroid cells and decidualization assay using 8-Br-cAMP treatment which mimics the progesterone signaling were performed.
Main results and the role of chance: Morphology was unchanged in infected cells. Mortality and proliferation rates over 24, 96 and 192 h were similar in candidate shRNA's and control. The adhesion rates of trophoblastic 3D spheroid to mono-layer of infected and non-infected endometrial cells were similar. Whereas, in both cellular fractions, candidate extinction significantly reduced cells migration over 24 h under in vitro conditions. Moreover, in infected cells, we observed (i) an attenuation of the cellular transformation as epithelial-like type associated with decidualization and (ii) a significant reduced expression of decidualization biomarkers, prolactin and connexin 43,

in stromal and epithelial endometrial cells. Therefore, by affecting migration and decidualization, two major biological pathways involved in the implantation process, we demonstrated that this candidate plays a key role during the implantation window.

Limitations, reason for caution: Validation of these results should be performed using purified primary endometrial cells obtained from a large cohort of fertile patients.

Wider implications of the findings: This study opens new perspectives in the understanding of the key role of the S100 protein in endometrial receptivity process of infertile patients.

Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by commercial/corporate company(ies), OVO Fertility Clinic, Ferring Pharmaceutical Company.

Trial registration number: N/A.

P-208 Dgcr8-dependent microRNAs are critical for uterine development and physiology in mice

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Study question: To examine whether deficiency of Dgcr8-dependent canonical microRNAs causes abnormalities in uterine development and physiology.

Summary answer: Characterization of reproductive phenotypes of PR-Cre driven uterine-specific Dgcr8 knock-out (uDgcr8 KO) mice demonstrates that uterine deficiency of Dgcr8-dependent microRNAs causes infertility due to multiple spectra of abnormalities in uterine development, reproductive cycle, and steroid hormone responsiveness in mice.

What is known already: MicroRNAs are critical for gene regulatory networks by directing the translational repression or degradation of complementary target mRNAs. Canonical microRNAs are produced from long primary transcripts by sequential complex events in which DROSHA-Dgcr8 complex and DICER are involved. While it is known that Dicer is critical for various reproductive events, it still remains unknown whether Dgcr8-dependent microRNAs are important for establishment of uterine environments for embryo implantation in mice.

Study design, size, duration: Dgcr8 conditional knock-out ($Dgcr8^{flox/flox}$) mice were crossed to PR-Cre mice to produce mice with specific deletion of Dgcr8 in female reproductive tracts (uDgcr8 KO). Reproductive phenotypes of uDgcr8 KO have been examined to understand function of microRNAs in uterine development and environments for embryo implantation in mice.

Participants/materials, setting, methods: RT-PCR, realtime RT-PCR, Western blotting and immunohistochemistry, BrdU assays, TUNEL staining were performed on uteri of wildtype and uDgcr8 KO mice. Gross morphology, histological analyses and total weight for uterus were examined. Mating and vaginal smear experiments were performed to examine fertility and estrous cycles of uDgcr8 KO mice.

Main results and the role of chance: Gross morphology, histology, and weight of uDgcr8 KO uteri, at 4-week-old stage when PR expression is significantly increased, started to show multiple uterine defects, and these deformities become severe onwards. The number of glands was reduced and thickness of myometrial layers is significantly lower in these mice. Administration of E_2 led to robust uterine epithelial proliferation in both ovariectomized control and uDgcr8 KO mice. However, stromal cells in uDgcr8 KO mice do not proliferate under the control of $E_2 + P_4$ treatment although they undergo normal proliferation in control mice treated with the same condition. Moreover, there are no antiproliferative effects of P_4 on E_2-induced epithelial proliferation in uDgcr8 KO mice. TUNEL staining demonstrated that apoptosis is significantly increased in stromal cells in Dgcr8 KO mice.

Limitations, reason for caution: Comparative analyses of reproductive phenotypes between uDicer KO and uDgcr8 KO mice are required to further refine major actions of microRNAs on uterine biology in detail.

Wider implications of the findings: The results will contribute to providing fundamental data to understand pathophysiology of gynecologic diseases such as endometrial cancers and infertility affected by E_2/P_4 possibly through regulation of microRNA expression in the endometrium.

Study funding/competing interest(s): Funding by national/international organization(s), National Research Foundation of Korea (NRF).

Trial registration number: N/A.

P-209 Macrophage migration inhibitory factor is required for ectopic endometrial tissue growth in vivo: a possible target for treatment of endometriosis

Abstract withdrawn by the author

P-210 The presence of endometrioma does not impair time lapse morphokinetic parameters and quality of embryos: a study on sibling oocytes

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Study question: Do the oocytes harvested from an ovary with an endometrioma give rise to embryos with aberrant timings of cleavage as assessed by time lapse monitorisation and poorer morphological quality compared to oocytes from the contralateral normal ovary in the same patient after ICSI?

Summary answer: Oocytes harvested from ovaries with an endometrioma <4 cm result in embryos with similar time lapse morphokinetic parameters and morphological quality compared to sibling oocytes from the contralateral normal ovary, after ICSI.

What is known already: Endometriomas need not necessarily be removed before IVF unless the size is above a certain threshold (i.e. >4 cm); since the overall chance of the cycle is not hampered by the endometrioma per se. It is still obscure whether the endometrioma might have an impact on the neighboring oocytes in the ovary that it resides, and if such an evidence can be documented clinically, a further argument can be put forward to remove them before IVF.

Study design, size, duration: Prospective observational study of 20 women (mean age: 32, range 23–41) undergoing ICSI treatment, who have an endometrioma <4 cm in one ovary while the contralateral ovary being normal in appearance, during the period of March 2013 to December 2013.

Participants/materials, setting, methods: Oocytes collected from both ovaries are inseminated by ICSI and placed in culture in Primo Vision time lapse embryo monitoring system utilizing 5% O_2, 5.7% CO_2, Life Global culture media and a trigas incubator as culture conditions. One or two embryos were transferred either on day 3 or day 5. Top quality embryos were defined as those with ≥7 even sized cells and ≤10% fragmentation on day 3 and with ≥3AA blastocyst morphology on day 5.

69 embryos from the endometrioma side and 59 embryos from the contralateral normal side were monitored.

Main results and the role of chance: Mean number of oocytes and MII oocytes collected were similar (5.3 vs. 4.3, *p*: 0.33 and 4.2 vs. 3.6, *p*: 0.49, endo + and – sides, respectively). Fertilization rates were also similar (*p*: 0.73). There were no differences, respectively between endo + and endo – ovaries in terms of the following time lapse morphokinetic parameters of embryos: (1) t5 (time of division to 5 cells after ICSI) (mean : 52.3 vs. 52.5 h, *p*: 0.95), (2) s2 (time between division to 3 cells and subsequent division to 4 cells) (mean: 3.9 vs. 2.6 h, *p*: 0.64), (3) cc2 (time between division to 2 cells and division to 3 cells) (mean: 8.9 vs. 9.9 h, *p*: 0.47), (4) t2 (division to 2 cells) (mean: 29.3 vs. 28.6, p: 0.19).

Furthermore the percentage of embryos that falls into the predefined optimal range for each morphokinetic parameter, from both groups, does not show any difference significantly. These percentages for t5 are 17.2% vs. 27.6%, *p*: 0.51; for s2, 13.3% vs. 13.3%, *p*: 0.81; for cc2, 12.2% vs. 20.0%, *p*: 0.26; and for t2, 16.7% vs. 24.4%, *p*: 0.28, for endometrioma +and – sides respectively.

Similarly the percentage of embryos with top morphological quality was similar in both groups (15% vs. 19%, *p*: 0.25, endo + and – sides respectively).

When embryos chosen for transfer were only from one side, 4 pregnancies were achieved in 7 transfers with endo – group and 4 pregnancies were achieved in 9 transfers with endo + group (*P*: 1.00).
Limitations, reason for caution: The findings of this study should be verified with larger patient groups and other morphokinetic parameters and assessments.
Wider implications of the findings: The findings of this study further strengthens the notion that removal of endometriomas up to a certain size before IVF is not a necessity in terms of the IVF outcome.
Study funding/competing interest(s): Funding by hospital/clinic(s), Atasehir Memorial Hospital, Istanbul.
Trial registration number: None.

P-211 Prospective randomized controlled study (Canadian task force classification) on hormonal or operative therapy of endometriosis – who wins the battle

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Study question: To evaluate three therapy strategies: hormone therapy, surgery, and combined treatment for genital endometriosis in a university based teaching hospital.
Summary answer: In the quest to find the most effective treatment of genital endometriosis, this clinical randomized study shows the lowest incidence of recurrence with combined surgical and medical treatment and improved pregnancy rate in any medically treated patients with or without surgery with the highest cure rate in the combined treatment group.
What is known already: Endometriosis is a systemic disease, which needs various treatments as up to date no single treatment is successful in every patient.
Study design, size, duration: A Prospective, randomized, controlled study, Canadian Task force classification I.

Four hundred fifty patients with genital endometriosis (2008–2011), stages I–III, aged 18 to 44 years, before first laparoscopy.
Participants/materials, setting, methods: Patients were randomly assigned to 1 of 3 treatment groups (University-based teaching hospital): hormone therapy, surgery, or combined treatment. Patients were reevaluated at second-look laparoscopy, at 2 to 2 months after 3-month hormone therapy in groups 1 and 3 and at 5 to 6 months in group 2 (surgical treatment alone). Outcome data were focused on the endometriosis stage, recurrence of symptoms, and pregnancy rate.
Main results and the role of chance: All treatment options, independent of the initial Endoscopic Endometriosis Classification stage, achieved an overall cure rate of 50%. A cure rate of 60% was achieved with the combined treatment, 55% with exclusively hormone therapy, and 50% with exclusively surgical treatment. Recurrence of symptoms was lowest in patients who received combined treatment. Significant benefit was achieved for dysmenorrhea and dyspareunia. An overall pregnancy rate of 55% to 65 % was achieved, with no significant difference between the therapeutic options.
Limitations, reason for caution: Although a good number of patients was included into this RCT no clear picture of an optimal therapy could be obtained. In this still complex and poorly understood disease therefore all know therapy concepts should be used for the treatment of patients.
Wider implications of the findings: After an initial diagnostic laparoscopy be careful with repetitive laparoscopic surgeries.
Study funding/competing interest(s): Funding by University(ies), The study was not funded.
Trial registration number: We do not have a registration number but, it was a clinical study with approval of our Ethic commission of the University Hospitals Schleswig-Holstein.

P-212 Alteration of the intrafollicular thiol-redox system in infertile women with endometriosis: relationship with oocyte and embryo quality

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Study question: Does alteration of intrafollicular biomarkers of the thiol-redox system in infertile patients with endometriosis affect the oocyte and embryo quality?
Summary answer: There may be an imbalance of the thiol-redox system and increased levels of inflammatory cytokines in the intrafollicular microenvironment of infertile patients with endometriosis, which may affect the qualities of the oocyte and embryo.
What is known already: Oxidative stress and chronic inflammation play important roles in the pathophysiology of endometriosis. The thiol-redox system participates in a variety of diseases related to oxidative stress.
Study design, size, duration: Sixty-five patients receiving IVF were included in this prospective observational study: 31 patients with endometriosis vs. 34 patients with unexplained infertility or infertility due to male or tubal factors (controls).
Participants/materials, setting, methods: Follicular fluid (FF) was obtained from a dominant follicle during oocyte retrieval and stored at –70°C. Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPX)-3, thioredoxin (TRX), TRX-binding protein (TBP)-2, and peroxiredoxin (PRX)-4 levels were measured with ELISAs to check oxidative stress. The inflammatory cytokines interleukin (IL)-1β, IL-6, IL-8, and tumor-necrosis factor (TNF)-α were also measured by ELISAs.
Main results and the role of chance: GSH levels were significantly lower and TBP-2 levels were significantly higher in the FF samples from the endometriosis group compared to the controls (12.73 ± 5.67 vs. 16.19 ± 6.94 μg/mL, $P = 0.033$; 219.97 ± 507.23 vs. 3.27 ± 6.14 ng/mL, $P = 0.042$). MDA, SOD, GPX-3, TRX, and PRX-4 levels were not significantly different between the two groups. IL-6, IL-8, and TNF-α levels were significantly higher in the endometriosis group compared to those in the control (16.97 ± 29.62 vs. 4.11 ± 2.89 pg/mL, $P = 0.022$; 216.26 ± 95.73 vs. 171.50 ± 72.06 pg/mL, $P = 0.037$; 0.93 ± 1.01 vs. 0.43 ± 0.33 pg/mL, $P = 0.036$, respectively). There were significant positive correlations among the four inflammatory cytokines. The levels of all of the inflammatory cytokines positively correlated with the levels of TRX in the FF samples. GSH levels were positively correlated with the number of high-quality embryos ($r = 0.299$, $P = 0.024$). GPX-3 and TRX levels were negatively correlated with the percentage of mature oocytes ($r = 0.275$, $P = 0.046$; $r = 0.398$, $P = 0.004$, respectively). TNF-α levels were negatively correlated with the cumulative embryo score per embryo ($r = 0.278$, $P = 0.025$). Logistic regression analysis revealed that the number of high-quality embryos was an independent factor predicting clinical pregnancy (OR 0.975, $P = 0.024$).
Limitations, reason for caution: The limitations of this study were that we cannot rule out the existence of minimal and mild endometriosis in the control group and results of FF samples from a single dominant follicle may not reflect the other follicles in the ovary.
Wider implications of the findings: Our findings will improve understanding of the intrafollicular microenvironment related to oxidative stress and chronic inflammation in infertile women with endometriosis
Study funding/competing interest(s): Funding by national/international organization(s), This work was supported by a grant from the Korea Healthcare Technology R&D Project, Ministry of Health and Welfare, Republic of Korea.
Trial registration number: NA.

P-213 Capn7 negatively regulates human endometrial stromal cell decidualization through binding to Foxo1

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Study question: What role does Capn7 exactly play in the regulation of human endometrial stromal cell (HESC) decidualization?
Summary answer: Capn7 negatively regulates HESC decidualization induced by 8-Br-cAMP and medroxy progesterone acetate (MPA) in vitro.
What is known already: Capn7 expression is much higher in endometria of women with endometriosis than in endometria of healthy women and an important feature of endometriosis is the defective endometrial decidualization, which indicates Capn7 probably participates in the regulation of human endometrial decidualization. Foxo1 is a key regulator of endometrial decidualization.

Study design, size, duration: Human endometrial stromal cells isolated from normal endometrial tissue of normal cycling women by endometrial biopsy were infected with Ad-CTL or Ad-Capn7 adenovirus at the indicated concentration, then stimulated by 1 μM MPA and 0.5 mM 8-Br-cAMP to induce decidualization. Experiments were conducted in HESCs repeatedly at least three times.
Participants/materials, setting, methods: Through experiments on adenovirus-mediated overexpression of Capn7 and siRNA-mediated knockdown of Capn7, with real-time PCR and ELISA, we studied the regulatory effect of Capn7 on HESC decidualization. Furthermore, immunoprecipitation and luciferase assay were conducted to examine interaction between Capn7 and Foxo1 and the impact of their interaction on HESC decidualizaton.
Main results and the role of chance: 8-Br-cAMP and MPA downregulated Capn7 expression in HESCs in a time-dependent manner ($P < 0.05$). Overexpression of Capn7 markedly attenuated PRL and IGFBP-1 mRNA expression and PRL protein secretion induced by 8-Br-cAMP plus MPA in HESCs ($P < 0.01$). Capn7 overexpression obviously inhibited the activity of PRL promoter (dPRL/310-Luc) and IGFBP-1 promoter (IGFBP-1-1021/232-Luc) induced by 8-Br-cAMP and MPA ($P < 0.01$). Meanwhile, Capn7 knockdown sharply enhanced PRL and IGFBP-1 mRNA expression ($P < 0.01$) and PRL secretion ($P < 0.05$) induced by 8-Br-cAMP plus MPA in a time-dependent manner. Moreover, Capn7 physically bound to Foxo1 in HESCs and Capn7 was able to suppress the transcriptional activation of PRL and IGFBP-1 induced by Foxo1 with the treatment of 8-Br-cAMP and MPA in a concentration-dependent manner ($P < 0.01$).
Limitations, reason for caution: All study above was only performed in human endometrial stromal cells isolated from normal women in mid-secretory phase by endometrial biopsy in vitro.
Wider implications of the findings: Our data firstly shows Capn7 as a novel negative regulator of HESC decidualization. To a step further, Capn7 might become a potential target for treatment of decidualization-associated diseases such as recurrent miscarriage, endometriosis.
Study funding/competing interest(s): Funding by national/international organization(s), this work was supported by The National Natural Science Foundation of China Grant 81171570.
Trial registration number: No.

P-214 Effect of pre-ovulatory progesterone elevation and duration of progesterone elevation on the pregnancy rate of frozen-thawed embryo transfer in natural cycles

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Study question: What is the incidence of progesterone elevation(PE) in natural cycle (NC)? Does PE impair the pregnancy rate of frozen-thawed embryo transfer – natural cycle (FET-NC), similar to that in stimulated in-vitro fertilization (IVF) cycles?
Summary answer: The incidence of PE in FET-NC was similar to that in stimulated cycles. PE for 2 days or more on or before the LH surge impaired the pregnancy rate of FET-NC while PE on the day of LH surge only did not have such an adverse effect.
What is known already: PE has an adverse effect on the pregnancy rate of stimulated IVF cycles, however, the incidence and its effects on the pregnancy rates in FET cycles have never been studied and remained uncertain.
Study design, size, duration: This is a retrospective analysis of all first FET-NC carried out between January 2006 and December 2011 and 610 cycles were included.
Participants/materials, setting, methods: Only the first FET-NC and women not pregnant in stimulated cycles were included. Achieved serum samples were assayed for progesterone concentrations from the day of LH surge up to 3 days before surge. The cut-off level of PE was defined as 5 nmol/L. Clinical and ongoing pregnancy rates were outcome measures.
Main results and the role of chance: The incidence of PE in FET-NC was 173/610 (28.4%). There were no significant differences in both the clinical and ongoing pregnancy rates (37.3% vs. 39.0%, $p = 0.702$; and 31.7% vs. 32.5%, $p = 0.845$, respectively) between those with or without PE on the day of LH surge. If PE lasted for 2 days or more, there was a significant reduction in the clinical pregnancy rate (39.4% vs. 20.7%, $p = 0.043$). Miscarriage rates were comparable between the two groups (13.3% vs. 15.4%, $p = 0.694$). Using multivariate logistic regression, women's age, progesterone rise for 2 days or more (OR(odd ratio) 0.333, 95% C.I. 0.117–0.949), and the number of top quality embryos were the significant factors for the clinical pregnancy rates in FET-NC cycles.
Limitations, reason for caution: Our study was a retrospective study and there may be some confounding factors that could not be totally controlled in our data analysis. Further prospective studies are needed to confirm our findings.
Wider implications of the findings: PE for 2 days or more impairs the pregnancy rates of FET-NC cycles. PE in NC may have an adverse effect on the endometrial receptivity. In stimulated cycles with PE, cycles can be cancelled and embryos can be replaced in subsequent FET cycles, but the strategy to deal with PE in FET-NC remains uncertain. The incidence of PE in repeated cycles and the strategy to deal with the PE in NC should be properly studied.
Study funding/competing interest(s): Funding by University(ies), The Department of Obstetrics and Gynaecology, the University of Hong Kong.
Trial registration number: This is a retrospective study, not a RCT, and so no trial number is available.

P-215 Prokineticin 1 leukemia inhibitory factor and Dickkopf 1 mRNA expression in the endometrium of women with unexplained infertility and idiopathic recurrent pregnancy loss

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Study question: How do the expression patterns of prokineticin 1 (PROK1), leukemia inhibitory factor (LIF) and Dickkopf 1 (DKK1) differ in peri-implantation endometrial tissue of unexplained infertility and idiopathic recurrent pregnancy loss (RPL) from normal fertile women.
Summary answer: The expression of PROK1 and LIF were statistically significantly increased in the endometrium of the women with idiopathic RPL than controls, whereas the expression of PROK1 was statistically significantly reduced in the endometrium of the women with unexplained infertility than control women.
What is known already: PROK 1, an angiogenic and permeability-enhancing factor, and LIF, pleiotropic cytokine of the interleukin-6 family, are expressed maximal in the mid-late secretory phase of menstrual cycle (window of implantation). Further, DKK1, antagonist in the canonical Wnt signaling pathway, which plays an important role in regulating proliferation and decidualization of the endometrium and in embryo-endometrial cross talk during implantation. We, therefore, suggest that dysregulation of PROK-1, LIF and DKK1 may be a contributing factor to infertility and/or RPL.
Study design, size, duration: Case-control study. The subjects consisted of 30 women with idiopathic RPL, 30 women with unexplained infertility and 30 healthy fertile controls.
Participants/materials, setting, methods: Endometrial pippelle biopsies were obtained from women with unexplained infertility ($n = 30$), idiopathic RPL ($n = 30$) and fertile women ($n = 30$) during mid-late secretory phase of menstrual cycle. The samples were analyzed by real-time polymerase chain reaction for expression of PROK1, LIF and DKK1 mRNA.
Main results and the role of chance: The expression of PROK1 was statistically significantly increased in the endometrium of the women with idiopathic RPL than controls. On the contrary, it was statistically significantly reduced in the endometrium of the women with unexplained infertility than control women. Increased LIF expression was observed in the endometrium of women with idiopathic RPL than controls, whereas there were no significant differences between women with unexplained infertility and controls. The expression of DKK1 did not alter among the groups.
Limitations, reason for caution: The study was a case-control and can only indicate relation but not a cause-effect association. Further studies are necessary to confirm our results.
Wider implications of the findings: Our results highlight that endometrial abnormalities are present in women with idiopathic RPL and unexplained

infertility. An increased expression of PROK1 and LIF could be one of the several abnormalities characterizing endometrium in women with RPL. Better understanding of the molecular mechanism underlying endometrial receptivity and implantation should guide clinicians through correct management and treatment of women with idiopathic RPL and unexplained infertility.
Study funding/competing interest(s): Funding by University(ies), This study was funded by the Scientific Research Projects Unit of Inonu University.
Trial registration number: None.

P-216 The effect of early timing of hCG triguring on pregnancy rate in patients stimulated with gonadotrophin releasing hormone (GnRH) antagonists for in vitro fertilisation (IVF)

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Study question: Does Earlier Administration Of Human Chorionic Gonadotropin (hCG) Improve Pregnancy In Cycles Stimulated With GnRH Antagonists?
Summary answer: Early HCG-triggering of final oocyte maturation is expected to result in lower progesterone levels on the day of hCG administration it might be assumed that such an intervention might result in an improved probability of pregnancy by leading to a less deranged and more receptive endometrium.
What is known already: HCG-Triggering of final oocyte maturation as soon as ≥3 follicles ≥17 mm are present on ultrasound has maximum probability of pregnancy in patients stimulated GnRH-antagonists for IVF.
Study design, size, duration: The purpose of this randomized controlled trial of 346 infertile patients between 2006 to 2013, below 42 years is to evaluate whether triggering of final oocyte maturation as soon as ≥3 follicles ≥16 mm are present on ultrasound or 1 day later affects the probability of pregnancy in patients stimulated GnRH- antagonists for IVF.

Primary Outcome Measures: Ongoing pregnancy rate.

Secondary Outcome Measures: number of MII oocytes, endometrial thickness, Progesterone levels and complications like OHSS.

Participants/materials, setting, methods: 346 infertile patients below 42 years included in this study to evaluate whether triggering of final oocyte maturation as soon as ≥3 follicles ≥16 mm are present on ultrasound or 1 day later affects the probability of pregnancy in patients on Antagonist IVF-ICSI were randomly divided into two groups of 173 patients each (group one-Early HCG and group two-Late HCG).
Main results and the role of chance: Significant differences were observed between the group One (early-hCG) and group Two (the late-hCG group) regarding – ongoing pregnancy rates (36.2% vs. 21.1%, respectively) and Non-Significant in E_2 (1560 ± 563 vs. 2.120 ± 931 pg/mL, respectively) and P (0.7 ± 0.5 vs. 1.2 ± 0.6 ng/mL, respectively) levels on the day of hCG administration and endometrial thickness (10.5 ± 2.6 mm vs. 7.6 ± 2.8 mm) on day of embryo transfer the number of metaphase II oocytes (6.4 ± 5.1 vs. 7.1 ± 5.8, respectively) and less complications like OHSS (3.2% vs. 4.7%).
Limitations, reason for caution: Till date limited studies.
Wider implications of the findings: Progesterone elevation has been associated with prolongation of the follicular phase in GnRH antagonists cycles by 2 days after the commonly used criterion of the presence of at least three follicles of > or =17 mm has been met. Such an intervention is associated with significantly lower ongoing pregnancy rates in GnRH antagonist cycles, without an apparent deterioration of embryo quality.

The adverse effect of P elevation on the day of hCG administration might be explained by the induction of differences at the histological level as well as at the gene expression level between endometrial samples exposed to varying concentrations of progesterone (P). Prolongation of follicular phase by delaying hCG administration for 2 days is associated with a higher incidence of endometrial advancement on the day of oocyte retrieval in GnRH antagonist cycles (Kolibianakis et al., 2005). Moreover, Vaerenbergh et al. (2011) demonstrated a distinct difference in endometrial gene expression profile between patients with progesterone serum concentration above and below the threshold of 1.5 ng/ml on the day of HCG administration.

Due to the fact that earlier triggering of final oocyte maturation is expected to result in lower progesterone levels on the day of hCG administration it might be assumed that such an intervention might result in an improved probability of pregnancy by leading to a less deranged and more receptive endometrium.

Study funding/competing interest(s): Funding by hospital/clinic(s), BTTB Centre.
Trial registration number: BTTB-2006/09.

P-217 Subserosal uterine endometriosis (SSUE) with deeply myometrial invasion: a variant of deeply infiltrating endometriosis (DIE) commonly mistaken as adenomyosis

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Study question: To provide further noninvasive ultrassonographic evidence to the evaluation of subserosal uterine endometriosis (SSUE) and adenomyosis. Furthermore, we desired to learn if there is a coincidental women's age incidence of these pathologies and identify if there is a common topographic location employing a large cohort of infertile women.
Summary answer: The opportunity to study a large group of infertile women suffering from DIE and adenomyosis diagnosed by a noninvasive technology enabled to properly treated women with SSUE; and, in addition to evaluate the small association between the two pathologies.
What is known already: TVSBP enables mapping of pelvic DIE and adenomyosis with accuracy. While clinical presentation is similar, endometriotic glands + stroma + fibrosis located outside the uterus characterizes DIE. Endometrial glands + stroma deep within the myometrium along with muscular hypertrophy + hyperplasia characterize adenomyosis.SSUE is a variant form of DIE, with an aggressive behavior, commonly mistaken as adenomyosis and it has not been recognized and treated. The association of both diseases has not been fully elucidated.
Study design, size, duration: Prospective observational study including 1585 consecutive infertile women evaluated for DIE via transvaginal ultrasound after bowel preparation (TVSBP). All patients underwent TVSBP by the same radiologist from 08/2010 to 12/2013. Two surgeons performed the videolaparoscopies (VLSC) at two University-affiliated hospitals; histopathology was performed by experienced pathologists. All women signed approved informed consents.
Participants/materials, setting, methods: Women aged 19–44 years old (yo) with infertility length varying 1–5 years underwent bowel preparation with low-residue diet, laxative and rectal enema. TVSBP was performed with Voluson E-8/5–9-MHz transducer. VLSC were carried out with and without robotic-assistance by two skilled surgeons. Statistics performed by *t*-test and chi-square; significance $p \leq 0.05$.
Main results and the role of chance: From 1585 women evaluated, 279 women were operated on and 99 women had pre-and 3 months post-op data available. From all patients' examined, 96 women (6.1%) aged 35.6 ± 4.8 years (average age ± SD) exhibited SSUE, whereas 255 women (16.1%) aged 38.7 ± 4.9 years ($p < 0.001$) were diagnosed having adenomyosis. For those suffering from SSUE, 66 women (68.8%) had posterior uterine wall disease (puw) and 30 (31.3%) had anterior uw. In the adenomyosis group, 111 (43.5%) women had adenomyosis in the aw and 118 (56.5%) in the pw ($p > 0.05$). Only 7 women showed concomitant diseases. The outcome of surgery for all women operated due to SSUE was excellent having only one patient had remained disease whereas half of the patients who had adenomyosis showed residual disease.
Limitations, reason for caution: TVSBP and extensive VLSC resections of DIE and adenomyosis were performed by trained specialists using first-rate technology therefore results could have been slanted. Limitations should be pointed to the late diagnosis, extensiveness of DIE and adenomyosis as well as that all women were infertile.
Wider implications of the findings: The main positive implication of this study relates to the excellent surgical outcome in women who underwent surgery for SSUE; whereas women with adenomyosis did not have the same chances to efficiently resect the disease process. Moreover, earlier the diagnosis is made for both diseases it will significantly impact on patients' fertility enhancement potential. Moreover, this information will contribute for a more comprehensive patient counseling and personalized treatment.

Study funding/competing interest(s): Funding by University(ies), Discipline of Gynecology, Medical School from the University of São Paulo, Brazil.
Trial registration number: None.

P-218 IVF outcome in patients with surgically treated deep infiltrating endometriosis

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Study question: To evaluate the ovarian response and IVF outcomes in women presenting with moderate-to-severe endometriosis.
Summary answer: Surgically treated deep endometriosis confers a higher risk of a poor controlled ovarian hyperstimulation response. The greatest predictor was previous ovarian cystectomy. Upon an ovarian stimulation response, pregnancy rates mirrored controls' rates.
What is known already: Several authors have evaluated IVF outcomes in women with endometriosis, but the results regarding the ovarian response and pregnancy rates are still controversial. This disagreement is mainly due to the heterogeneity of the studied populations, especially concerning the included patients' surgical history.
Study design, size, duration: In a four-year retrospective cohort study, data from 164 patients selected for IVF cycles were reviewed according to infertility aetiology.
Participants/materials, setting, methods: 82 patients with surgically treated deep endometriosis were matched to 82 patients with tubal infertility and no adnexal surgery. The two groups were matched by age, stimulation protocol and recruitment time.
Main results and the role of chance: Endometriosis patients had a higher risk of a poor response to IVF, with a significantly higher FSH dose ($P = 0.043$), a lower number of oocytes retrieved ($P = 0.011$) and more cycle cancellations ($P < 0.001$) than in the tubal control group. However, implantation (endometriosis vs. control group, 23.5 ± 3.4 vs. 22.8 ± 3.4; $P = 0.892$) and pregnancy rates were comparable between the two groups (clinical pregnancy rate: endometriosis vs. control group, 23.9% vs. 27.3%; $P = 0.507$; cumulative pregnancy rate: endometriosis vs. control group, 46.5% vs. 39.1%; $P = 0.203$). Prognostic factors evaluation showed that previous cystectomy (OR = 5.65, 95% CI: 1.44–22.12), basal AMH (OR = 0.62, 95% CI: 0.39–0.97) and patient age (OR = 1.24, 95% CI: 1.06–1.46) were the only independent predictors of a poor ovarian response to controlled ovarian hyperstimulation.
Limitations, reason for caution: This retrospective analysis might include some limitations in the selection of study groups, and in the definition of respective roles of endometriosis and surgery in the observed results.
Wider implications of the findings: The results of this study should encourage early, active ART care in surgically treated deep endometriosis. Good results in ART incite us to reassess IVF indications and triggering criteria for these patients showing a particularly inhibited ovarian response. It would also be interesting to assess the natural cycle in patients who are rejected from conventional stimulation.
Study funding/competing interest(s): Funding by hospital/clinic(s), Authors declare no competing interests.
Trial registration number: None.

P-219 A randomised control pilot study of the use of intrauterine human chorionic gonadotropin injection before embryo transfer in egg recipient cycles

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Study question: Does the administration of intrauterine human chorionic gonadotropin injection (hCG) before embryo transfer improves outcomes in IVF for women >40 receiving donor eggs?
Summary answer: This pilot study indicates a tendency for an increase in the probability of achieving pregnancy and an increase on implantation rates after administration of intrauterine hCG, for women >40 years old with multiple previous failed IVF cycles following IVF program with donor eggs.
What is known already: The important paracrine role of hCG in the preparation of implantation has been previously described. hCG has a direct effect on human endometrium, it increases the capacity of apical cell adhesion, it plays an important role in the proliferation of myometrial smooth muscle cells and reduces cell contractility, it increases progesterone receptors, it regulates the development of local immune tolerance and it is involved in the initiation of endometrial angiogenesis at the implantation site.
Study design, size, duration: A randomized control pilot study was initiated at a single IVF setting on July 2012–September 2013. Randomisation was performed in a 1:1 fashion to one of two groups: with or without hCG intrauterine injection. Adequate allocation concealment was assured from sequentially numbered, opaque, sealed envelopes prepared from a computer-generated list.
Participants/materials, setting, methods: A total of 194 IVF women all >40 years old receiving donor eggs were recruited. Patients were stratified for zero, 1–2 and >3 previous failed donor-cycles. hCG was administrated intrauterine before embryo transfer. Data analysis: Fischer's exact test (GraphPad Prism).
Main results and the role of chance: In all categories administration of hCG did not result in statistically significant pregnancy rates [0 previous failed D attempts CPR: 67% hCG group, CPR: 64% Control group ($P > 0.05$)/1–2 previous failed donor-cycles CPR: 73% hCG group, CRP: 58% Control group ($P > 0.05$)/>3 previous failed donor-cycles CPR:69.5% hCG group, CPR:47% Control group ($P > 0.05$)].
In hCG patients, IR was found statistically significant in patients with previous multiple failed donor attempts (IR: 44% $P = 0.023$ vs. Control group IR = 20%), while IR was not statistically significant for patient with 0 previous failed donor-cycles (IR:47% hCG group IR:42% Control group), 1–2 previous failed donor-cycles (IR:38% hCG group, IR:36%).
Limitations, reason for caution: Our pilot study involves a limited sample size in order to evaluate the preliminary data before conducting a larger trial. Selection bias was controlled by randomization. Blinding of patients was not possible in this study, but since the outcome (pregnancy) is robust, blinding would have been unlikely to affect the results.
Wider implications of the findings: A tendency of a positive effect on the pregnancy and implantation rates following the administration of intrauterine hCG injection before embryo transfer was indicated. A larger RCT will assess, with more confidence, the effect of hCG on the probability of pregnancy.
Study funding/competing interest(s): Funding by hospital/clinic(s), GENESIS ATHENS CLINIC.
Trial registration number: None.

P-220 Endometriosis health profile (EHP-30) scores and their association with surgical diagnosis in premenopausal women

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Study question: Is there any association between Endometriosis Health Profile (EHP) – 30 scores and severity of endometriosis as assessed by surgical diagnosis using the ENZIAN-score and the American Society of Reproductive Medicine's (ASRM) classification?
Summary answer: The EHP-30 is a user-friendly tool to evaluate quality of life and health status in women with endometriosis, but none of the five dimension scores of the EHP-30 core questionnaire correlated significantly with surgical diagnosis as assessed using the ASRM or ENZIAN score in our cohort.
What is known already: Endometriosis is known to affect different aspects of life in women of reproductive age. Besides physical disturbance and health status, quality of life is often impaired but hardly measurable. Therefore, the 30-item Endometriosis Health Profile (EHP-30) was introduced, addressing the impact of disease on the physical, psychological and social aspects of life.
Study design, size, duration: Premenopausal women with histologically confirmed endometriosis presenting at the University of Ulm between 2006 and 2012 were included and were asked to answer the EHP-30 core questionnaire. The questionnaires were filled out by the patients themselves during their hospital stay, returned via mail or completed via telephone interview.

Participants/materials, setting, methods: The EHP scores were analyzed to obtain the five dimension scores (pain, control and powerlessness, emotional well-being, social support, self-image). These results were correlated with surgical findings categorized using ENZIAN and ASRM scores. Statistical analysis was performed using the spearman rank correlation.
Main results and the role of chance: In total, 50 completed questionnaires were available for preliminary analyses. The mean age was 30.7 years (range 19–45). Medians and ranges obtained for the five dimension scores were 20.0 (0–77) for the pain score, 25.0 (0–96) for the control and powerlessness score, 33.0 (0–83) for the emotional well-being score, 22.0 (0–94) for the social support score, and 25.0 (0–92) for the self-image score. No significant correlations were found between the ASRM or the ENZIAN score and any of the five EHP-30 dimension scores (all $p > 0.9$, all $p > 0.7$, respectively).
Limitations, reason for caution: The number of included questionnaires was rather small. However, these were preliminary findings and we are planning to include at least another 50 women.
Wider implications of the findings: Although no correlation was found, we still believe that the EHP-30 is a useful tool to evaluate the quality of life in women with endometriosis. Our results confirm the clinicians' awareness that the degree of intraperitoneal lesion does not necessarily correspond to the dimension of subjective discomfort.
Study funding/competing interest(s): Funding by University(ies), none.
Trial registration number: Not applicable.

POSTER VIEWING

ETHICS AND LAW

P-221 Barriers to the evidence-based management of obese, infertile women

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Study question: What are the barriers to the evidence-based management of obese, infertile women?
Summary answer: There are major ethical concerns relating to the violation of women's autonomy; distributive justice; non-beneficence and maleficence. Epistemological and pedagogical concerns also emerged in that health-care professionals demonstrated poor knowledge, skills and attitude regarding the subject matter; and complained of time, organisational and logistic constraints; and lack of readily-available evidence.
What is known already: Health-Care Professionals (HCPs) have an ethical duty to identify the barriers to the evidence-based management of obese infertile women prior to implementing guidelines.

I posit that this ethical duty is engaged, given the world-wide concerns about the obesity pandemic, the costs to the exchequer and the reproductive and long-term general health consequences including cancer, diabetes, heart disease and increased mortality; which have triggered restrictive statutory guidelines and funding strategies with regards to women with BMI >30.
Study design, size, duration: Building on Kitson's implementation paradigm [((SI = *f*(E, C, F)); where SI = Successful Implementation; E = Evidence; C = Context; F = Facilitation; and *f* = Function]; I hypothesised that by studying the barriers to Evidence, Context and Facilitation respectively; it might be possible to improve Successful Implementation.
Participants/materials, setting, methods: Based on Kuhn's scientific empiricist paradigm, I focussed on health care professionals, their organisations and other stake-holders involved in managing these women.

I used a multi-modal and mixed methodology including: content analysis; contextual analysis; ethical analysis; coding; thematic analysis; questionnaires; semi-structured, qualitative interviews; and focus groups.
Main results and the role of chance: The major ethical barriers were concerns about violation of obese women's autonomy; distributive justice; non-beneficence and malificence. I also found major concerns about lack of robust evidence, particularly, readily-available synthesised evidence. This evidence gap possibly contributed to the intention-behaviour gap that existed. Epistemological and pedagogical barriers included poor knowledge, skills and attitude regarding the evidence-based management of obese infertile women. Health care professionals were worried about receiving complaints; cultural barriers; lack of time, funding, facilities and equipment; training; privacy (e.g. dedicated room and staff for weighing and calculating BMI); attrition; co-morbidity; organisational and logistic constraints; and appropriate lines of responsibility. These barriers led to detachment and a sense of "futility and avoidance."
Limitations, reason for caution: The sampling for the present study was purposive and uni-centred. As such, larger, possibly multi-centred studies are required to confirm these findings.
Wider implications of the findings: Robust ethical studies are needed to reassure health-care professionals about the ethical implications of the evidence-based management of obese infertile women. These studies should be underpinned by a programme of quantitative and qualitative evidence-synthesis. Accessibility to such reliable evidence; as well as training, time, funding, organisational and logistic support could improve the fidelity of health care professionals, organisations and stakeholders to evidence-based guidelines (such as NICE in the UK) on the management of these women.
Study funding/competing interest(s): Funding by University(ies), Kellogg College, University of Oxford.
Trial registration number: N/A.

P-222 On gametes and guidelines: a proceedings report of a symposium on the use of empirical bioethics to inform the regulation of reproductive technologies

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Study question: The aim of the symposium was (1) to explore congruence among experts about the admissibility of the use of empirical data for the development of guidelines; and (2) to work towards the development of an agenda for empirical research that can provide evidence to inform the regulation of reproductive technology.
Summary answer: To develop the agenda, we delineated points of agreement (consensus) and disagreement in the group of experts regarding the use of (different types of) empirical data for (different types of) guidelines.
What is known already: There is a growing body of empirical data on the experience of stakeholders in Assisted Reproductive Technologies (ART). Some studies tend to offer more than a description of attitudes and practices. They can influence ethical guidelines proposed by national and international professional organizations. For example, the UK Human Fertilisation and Embryology Authority took into account such evidence as public opinion when drawing up its recommendations on sex selection of embryos for social reasons.
Study design, size, duration: The symposium was held at the Brocher Foundation (Geneva, Switzerland) from 17–18 February 2014. It brought together (bio)ethicists, empirical scientists, medical professionals, and representatives of (international) medical societies and regulatory bodies (World Health Organization; European Society of Human Reproduction and Embryology; and the Human Fertilisation and Embryology Authority).
Participants/materials, setting, methods: Contributions of speakers who had experience with the use of empirical insights in the development of regulatory policy in the field of ART were combined with structured discussion sessions. The results of the symposium will be collated into a proceedings report to encourage the debate about guideline development in ART.
Main results and the role of chance: First, we extracted lessons to be learned from examples of policy making with the use of empirical research data, in both national and international contexts within different organizational settings. Second, methodological-theoretical questions regarding the use of empirical data for normative reflection in reproductive medicine were addressed. Third, we explored the role of empirical data in examples of policy making.

We developed (1) a research agenda to fill the knowledge gaps regarding families created by ARTs (particularly the children resulting from these technologies) and to consider the theoretical aspects of policy making and the relation between ethics, evidence and policy; and (2) an outline of main questions, and points of agreement (consensus) or disagreement regarding the use of empirical data to formulate guidelines.
Limitations, reason for caution: This presentation does not intend to offer a representative view of the field given the small group of experts participating. However, the small size of the meeting as well as the structured discussion sessions made it possible to provide ample opportunity for a thorough discussion.
Wider implications of the findings: This symposium brought together many of those professionals, working in different European countries with the aim of sharing insights and experience regarding policy development. The need for an inter-European forum is becoming increasingly evident given the rapid development of new technologies and the rising cross-border reproduction within Europe.
Study funding/competing interest(s): Funding by national/international organization(s), We would like to thank the Brocher Foundation for funding the symposium.
Trial registration number: None.

P-223 Cross-border reproductive care for law evasion: should local physicians be allowed to help infertility patients evade the law of their own country

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Study question: Should local physicians be allowed to help infertility patients evade the law of their own country?
Summary answer: Local physicians should be allowed to help their patients cross borders to obtain treatment forbidden at home. It is immoral to prohibit them to offer information, there is a general obligation to treat complications and physicians should even be allowed to do part of the treatment.
What is known already: A conservatively estimated 8000 law evading treatment cycles take place across Europe annually. The majority of cross-border infertility patients receive help from their own doctor, even when they are evading restrictive laws. Cross-border cooperation between physicians is one of the most important determinants for patient experience and it increases safety.
Study design, size, duration: Examples from France (warning that physicians risk 5 years imprisonment and a fine of 75.000 if they inform patients about the possibility of making use of egg donation abroad), Turkey (1–3 years imprisonment for help with gamete donation), Ireland and Switzerland (information about PGD).
Participants/materials, setting, methods: Based on respect for autonomy and the best interest of the patient, it is argued that physicians should help patients before treatment (informing) and after treatment (complications). Physicians should also be allowed to help during treatment (drug prescription, cycle monitoring, partial reimbursement, …), but they also have the right to refuse.
Main results and the role of chance: Helping patients in this manner has been described as acting against the spirit and essence of the law. By supporting CBRC for law evasion, physicians are essentially complicit in immoral behaviour. However, assisted reproduction is an area of contested morality. CBRC for law evasion can be a way to be tolerant to different attitudes towards these issues. If patients have the right to travel abroad for infertility treatment, they should also have the right to obtain information about that treatment from their physician. Some physicians help patients obtain partial reimbursement for their law evading treatment cycle. However, it is only justifiable for a physician to game the system for the benefit of the patient if this system is flawed.
Limitations, reason for caution: The ethical analysis is based on the adoption of certain moral principles. Although the principles accepted for our analysis are fundamental (patients' rights) there is still the possibility that others disagree.
Wider implications of the findings: Many policies prohibiting local physicians to help cross-border patients evade the law of their own country are not justified.
Study funding/competing interest(s): Funding by University(ies), Ghent University.
Trial registration number: None

POSTER VIEWING

FEMALE (IN)FERTILITY

P-224 New pre-mix protocol for AMH gen II assay: can this method be relied upon to determine clinically significant reference ranges for IVF patients

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Study question: Is it possible to establish new reliable reference ranges for Anti-Müllerian hormone (AMH) determinations with Beckman Coulter's AMH Gen II ELISA Assay's (Brea, California, US) new premix protocol in correlation with clinical data.
Summary answer: The bias evaluation of the previous protocol and the current pre-mix protocol for the AMH Gen II Assay yielded results with a coefficient of variation too great to successfully correlate with clinical data.
What is known already: Beckman Coulter identified that complement interference was responsible for falsely low AMH results with their AMH Gen II Assay. The revised protocol includes premix of all samples with assay buffer prior to adding sample to the microplate. They have been found to have a bias of up to 70% between both techniques. Bias evaluation is recommended for each laboratory. There is no international standard for AMH testing and no validated references ranges for use in fertility patients population. External quality control programs are only now emerging.
Study design, size, duration: 197 patient samples were tested using both protocols (sample pre-mix with assay buffer vs. non-premix) on the same day and within the same run after storage at −20°C between 1 to 37 days.
Participants/materials, setting, methods: Tested samples were from non-pregnant women referred to the clinic in Montréal for fertility issues. Blood samples were collected for AMH, follicle stimulating hormone and Estradiol. Patients underwent an antral follicle count ultrasound on the same day. Collection, handling and processing of AMH samples were conducted as per manufacturer's recommendations.
Main results and the role of chance: We found that the new premix protocol AMH results increased by an average of 85% over the previous protocol where complement interference is suspected. The mean of the coefficient of variation is 2.06 with a standard deviation of 1.6. Without an international calibrator, it is difficult to draw conclusion from the bias evaluation and impossible to determine new reference ranges for clinical practice. The results of concentration of AMH with the premix protocol give a mean of 2.41 ng/ml, sample range is 30 ng/ml and a standard deviation of 3.21 ng/ml. With the nonpremix protocol the mean is 3.98 ng/ml with a standard deviation of 4.14 ng/ml. The sample range is 23 ng/ml. The old nonpremix protocol has a better correlation with clinical marquers and clinical response to ovarian stimulation.
Limitations, reason for caution: Lot-to-lot variations in reagent kits must be considered. Although serum aliquots were stored at −20°C for less than 2 months before testing, we cannot exclude the possibility of complement degradation during sample storage.
Wider implications of the findings: Clinicians have been depending on AMH results as a reliable predictor of poor or excessive ovarian response to ovarian stimulation in IVF cycles. The new pre-mix protocol yields results which often do not correlate with other clinical findings. The non-premix method looks to correlate better with the clinical observation. It will be important to have international reference for AMH range. The technique need to be improved to correlate clinical data with the clinical data.
Study funding/competing interest(s): Funding by hospital/clinic(s), none.
Trial registration number: None.

P-225 The impact of obesity on the outcome of IVF – restrospective study at Caen's university hospital

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Study question: To examine the effects of overweight and obesity on the outcomes of *in vitro* fertilization (IVF).
Summary answer: Compared with normal weight women, obese and morbidly obese women had a significant lower live birth rate.
What is known already: The adverse effects of obesity on general health, perinatal morbidity and spontaneous fertility are now well known. However the impact of obesity on IVF results is still controverted.
Study design, size, duration: A retrospective cohort study was carried out on 1,666 women who underwent 3,277 IVF cycles between 2004 and 2010.
Participants/materials, setting, methods: Patients were classified in 4 groups: 1,178 had a normal weight (18 ≤ BMI < 25 kg/m^2), 327 had an overweight (25 ≤ BMI < 30 kg/m^2), 128 were obese (30 ≤ BMI < 35 kg/m^2), 33 were morbidly obese (BMI ≥ 35 kg/m^2).
Main results and the role of chance: The live birth rate was significantly lower in obese women with a BMI between 30 and 34.9 kg/m^2 (OR = 0.65, 95% CI = 0.46 to 0.90) and in morbidly obese women with a BMI ≥ 35 kg/m^2 (OR = 0.44, 95% CI = 0.21 to 0.89) compared to women with normal BMI between 18 and 24.9 kg/m^2 (OR = 1). There was no significant differences with overweight women BMI between 25 and 29.9 kg/m^2 (OR = 1.02, 95% CI = 0.83 to 1.25). The same results were observed for the clinical pregnancy rate, total number of oocytes retrieved, fertilization rate, total number of embryos obtained and the number of frozen embryos.
Limitations, reason for caution: No data concerning smoking and the BMI of the men were available while these are two well-known factors interfering with fertility.
Wider implications of the findings: Obesity is associated with unfavorable IVF outcome as evidenced by lower live birth rate, however these findings did not apply for overweight women. It is recommended that obese women undergo counselling in order to lose weight before the initiation of an IVF cycle.
Study funding/competing interest(s): Funding by University(ies), no funding.
Trial registration number: None.

P-226 Array comparative genomic hybridization improves outcomes in women of advanced maternal age compared to standard IVF/ICSI

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Study question: Does array comparative genomic hybridization (array CGH) analysis improve the outcomes in women of advanced maternal age?
Summary answer: Women of advanced reproductive age who seek IVF/ICSI treatment have overall poor outcome. In this study aCGH group performed better in terms of Clinical pregnancy rate Implantation and Live birth rate per embryo transferred. There was a slightly lower miscarriage rate and a high cancellation rate compared to the standard group.
What is known already: In human embryos chromosome aneuploidy, is a major cause of IVF failure and miscarriage and can result in affected live births. Array CGH aims to improve implantation and live birth rates, by identifying euploid embryos to transfer.
Study design, size, duration: A retrospective comparative study between the outcomes of women with age range 40–45 years, who had aCGH between 1st August 2010 and 1st August 2013, to the outcomes of women in the same age with good ovarian reserve who had standard IVF/ICSI. A total of 536 IVF/ICSI treatment cycles were analysed.
Participants/materials, setting, methods: The outcomes of women between 40-45 who had good ovarian reserve underwent treatment in Care fertility Nottingham. 0f 536 IVF/ICSI treatment cycles carried out, 96 cycles had aCGH planned with day three embryo biopsy and 440 underwent standard IVF/ICSI, with controlled ovarian hyperstimulation.
Main results and the role of chance: There was no difference in the average ages (41.8 ± 2.1 aCGH vs. 41.4 ± 2.09 non aCGH) and BMI's (25.4 ± 4.6 aCGH vs. 24.8 ± 4.7 non aCGH) in the two groups. In the standard group of 440 cycles started 430 underwent oocyte retrieval and 385 had embryo transfer. Among the 96 array CGH cycles started 93 had egg collection and 56 had any suitable embryos to transfer.

This showed high cancellation rate in aCGH compared to standard group 41.6% vs. 10.2% respectively, $P < 0.0001$). Clinical pregnancy rate per embryo transfer was 44.6% in Array CGH group but only 21.3% in standard IVF/ICSI, which was statistically significant ($p < 0.0001$). Implantation Rate in aCGH group and in standard group was 42.7% and 12.5% respectively ($p < 0.0002$). Live-birth rate per ET was 35.7% aCGH and 14.3% standard ($p < 0.0004$) which showed to be statistically significant.
Limitations, reason for caution: It is a retrospective analysis. Array CGH group showed high cancellation rate due to the absence of a suitable embryo to transfer While Array CGH group the embryos transferred were all euploid blastocyst and the standard group had either day three or a day five embryo transferred.
Wider implications of the findings: Among women of advanced reproductive age with good ovarian reserves it might be appropriate to offer Array CGH. However appropriate counseling is mandated due to high cycle cancellation rate.
Study funding/competing interest(s): Funding by hospital/clinic(s), Care Fertility.
Trial registration number: N/A.

P-227 A drop in serum estradiol after hCG administration in oocyte donors is predictive of lower oocyte yield but does not impact pregnancy rates

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Study question: Should ovum donors with a significant estradiol (E2) drop (>15%) following administration of hCG during an IVF cycle be canceled?
Summary answer: Oocyte retrievals need not be routinely canceled solely based on estradiol drop post-hCG administration. Clinicians should assess the total number of mature follicles, estradiol levels before and post- hCG and percentage of estradiol drop when deciding to proceed with the retrieval.
What is known already: Data on estradiol levels after hCG administration are limited in ovum donor cycles. A retrospective cohort study of 1712 IVF cycles, 122 cycles with >10% decrease in estradiol was associated with 40–50% reduction in clinical pregnancy and live birth rates. Two other retrospective studies (844 and 222 cycles) showed no difference in IVF success with declining estradiol levels.
Study design, size, duration: We conducted a retrospective cohort study. 587 anonymous donor oocyte cycles from January, 2005 through April, 2012 were reviewed.
Participants/materials, setting, methods: Shared ovum donor cycles in an academic medical center were divided into 3 groups: E2-rise, E2-plateau, E2-drop. An E2-rise was defined as a >15% increase in estradiol levels post-hCG, an E2-drop was >15% decline. ANOVA was used for statistical analysis. $P < 0.05$ was considered significant. Data reported as mean ± SEM.
Main results and the role of chance: 784 recipients intended to share a donor's oocytes with one other recipient. Cycles not resulting in a transfer in at least one of the recipients were excluded. Cancellation to single recipient after retrieval due to low oocyte yield were similar (5.6% in E2-rise, 4.6% in E2-plateau, 4.7% in E2-drop, $p > 0.05$). IVF outcomes of 720 recipients who shared oocytes from a donor cycle and underwent fresh embryo transfer were analyzed separately [Rise ($n = 538$), plateau ($n = 162$) and drop ($n = 20$)]. Within the donor and recipient groups baseline characteristics were similar. The mean number of oocytes per recipient in the E2-drop group was significantly lower than plateau and rise (8.95 ± 0.47, 10.84 ± 0.29 11.15 ± 0.18, $p < 0.001$, $p < 0.0001$ respectively). Fertilization rates, implantation rates, clinical pregnancy and live birth rates per transfer cycle were similar.
Limitations, reason for caution: Percent drop levels ranged between 15.1–47.3. Five (0.9%) donors were canceled due to estradiol drop post-hCG. Lowest estradiol decline resulting in pregnancy was 23.9% (both recipients had live births at term). Higher percent drop of estradiol likely signifies loss of quality and quantity of majority of oocytes and may warrant cancellation.
Wider implications of the findings: An estradiol decrease following hCG was correlated with lower egg yield but didn't alter donor/recipient pregnancy rates. Our findings would also extend to good responder IVF patients. Oocyte donors with a decline in estradiol following hCG administration can still undergo oocyte aspiration at a clinician's discretion. The effect of estradiol decline on endometrium remains to be elucidated.
Study funding/competing interest(s): Funding by University(ies), The Ronald O. Perelman Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York.
Trial registration number: none.

P-228 Lipid intravenous infusion for treatment of recurrent implantation failure or recurrent pregnancy loss in women with elevated levels of peripheral natural killer cells

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Study question: To evaluate the efficacy of intravenous lipid infusions (ILP) in achieving a pregnancy in women with previous recurrent pregnancy loss (RPL) or recurrent implantation failure (RIF) with elevated levels of peripheral Natural Killer cells (NKc) and a potential reduction of NKc activity shown in the peripheral blood NKc Inhibition panel.
Summary answer: In this selected group of patients with previous RIF and RPL, a 34.1% ongoing pregnancy rate was achieved with the use of ILP.
What is known already: Immunomodulatory treatment for RPL and RIF has been implemented for many years in the ART field in the form of steroid tablets and Intravenous Immunoglobulin Infusions (IVIg).

Very few studies have been published on the potential beneficial effect of ILP in the above groups of patients during fertility treatment.
Study design, size, duration: Retrospective, Case Controlled Study, with each patient acting as their own control 95 women with RPL/RIF screened for high levels of NKc with the use of NKc inhibition panel. 42 were found to have elevated NKc levels and ILP was administered prior to ET. Duration: May 2012–December 2013.
Participants/materials, setting, methods: The study included patients undergoing fresh IVF or Frozen embryo replacement cycles with own oocytes. Oocyte Recipient(OR) cycles were also included, provided the patients had had at least 1 previous failed attempt or 1st trimester pregnancy loss after a previous OR cycle. Study Setting: London Women's Clinic, London, UK.
Main results and the role of chance: Mean age for IVF/FER group was 39.22 years (32–44 years) and the patients averaged 2.58 failed previous attempts. Mean age for oocyte donors to the OR group was 27.4 years, and mean age of oocyte recipients was 42.6 years. Ongoing pregnancy rates (beyond 12 weeks gestation) and pregnancy loss rates were reported for 41 out of 42 patients given an ILP infusion. For 1 ILP patient the embryo failed to thaw. Total ongoing pregnancy rate for all groups was 34.1% (14/41). Total pregnancy loss rate was 24.1% (10/41). For the IVF/FER group we recorded an ongoing pregnancy rate of 32.1% (9/28) and a pregnancy loss rate of 25.0% (7/28). For the OR group an ongoing pregnancy rate of 38.4% (5/13) was achieved, with a pregnancy loss rate of 15.3% (2/13).
Limitations, reason for caution: During the study period, it has not been possible to accumulate an adequate number of women to act as control group, in order to validate our results. Embryos were not screened for Chromosomal anomalies (PGS). As a result, fraction of the pregnancy losses could not be avoided despite ILP therapy.
Wider implications of the findings: This study attempts to highlight the beneficial effect of immunological ILP treatment on this group of ART patients with RPL and RIF. ILP therapy could offer a safer and cheaper therapeutic option compared to IVIg infusions. Larger trials are needed to validate the results.
Study funding/competing interest(s): Funding by hospital/clinic(s), The London Women's Clinic, 113-115 Harley Street, London W1G 6AP, UK.
Trial registration number: Not applicable for this type of study.

P-229 Comparison of β-hCG serum levels in pregnancies achieved after fresh embryo transfer versus first or second choice thawed embryo transfer in egg-donation-IVF cycles

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Study question: To demonstrate the existence of significant differences in the determination of β-hCG in pregnancies obtained after fresh embryos vs. thawed embryo transfer when there is a singleton ongoing pregnancy.
Summary answer: No significant differences were found between the analytical determination of β-hCG in patients with ongoing single pregnancies after egg donation fresh IVF cycles and thawed embryo transfer. β-hCG levels were similar irrespective of age and BMI, although a negative relationships can be suspected.
What is known already: Vitrification is a safe technique in IFV laboratories. Together, fresh and thawed embryo-transfer, help to increase accumulated pregnancy rates in IFV-cycles. But there are several confounding factors which could modify β-hCG levels. Age and body mass index could modify them. We do not know about the effects of vitrifying embryos on β-hCG levels. Some groups suggest a more erratic rising of the hormone.
Study design, size, duration: Retrospective 3 years long cohort study, in egg donation cycles who achieved a single ongoing pregnancy after fresh compared vs. first or second choice thawed embryo transfer.
Participants/materials, setting, methods: Maximum number of embryo transferred was two. β-hCG was measured on 2 sharp weeks after estimated ovulation. Twins or triplets, non ongoing pregnancies were excluded. We included 58 pregnant women in the fresh group and 27 in the vitrified group. A t-test was obtained.
Main results and the role of chance: We found no differences in β-hCG levels between both groups ($p = 0.402$, IC 95% (–78.046–31.620). Age did no affect β-hCG levels ($p = 0.339$). There were no significant differences in β-hCG when comparing body mass index, but there was a trend to a negative relationship ($p = 0.066$).
Limitations, reason for caution: The main limitation of this study was the sample size, but only singleton ongoing pregnancy can be included to answer our question.
Wider implications of the findings: For specialists, it is important to know if there may be or not differences in β-hCG levels when we transfer fresh or thawed embryos, to counsel patients about the prognosis of that pregnancy. It is also important to know if age and body mass index may change the hormone level to avoid misinformation to patients.
Study funding/competing interest(s): Funding by hospital/clinic(s), IVI Zaragoza.
Trial registration number: This is not a RTC.

P-230 A new lower limit of single mid-luteal serum progesterone level as indicator for ovulation and pregnancy outcome

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Study question: a) To identify mean ovulatory mid-luteal serum progesterone level suggestive of probable ovulation. b) To investigate if mid-luteal serum progesterone is predictive of pregnancy outcomes.
Summary answer: This is the first study where timed mid-luteal serum progesterone levels in cycles with confirmed ovulation have been investigated. We suggest mid-luteal serum progesterone level of 20.53 nmol/l (2.5th centile cut-off) indicates probable ovulation. Also, higher mid-luteal serum progesterone of above 60 nmol/l does not appear to be associated with better outcomes in early pregnancy.
What is known already: To date many small studies have tried to ascertain a lower limit cut-off value for ovulatory serum progesterone in natural cycles. This has resulted in a wide variation in the levels used as the criterion for ovulation ranging from 3.2 to 38 nmol/l with 30 nmol/l being used as the cut-off widely. Early pregnancy serum progesterone has also been advocated as a tool in the diagnosis of early pregnancy failure with levels above 25 nmol/l 'likely to indicate' and above 60 nmol/l are 'strongly associated with' pregnancies subsequently shown to be normal. It has been suggested that the higher progesterone concentrations are seen in the luteal phase of conception cycles, these cycles were not followed through to early pregnancy.
Study design, size, duration: Retrospective observational cohort study of 405 cycles of ovulation induction followed by intrauterine insemination for unexplained sub-fertility or use of donor sperm undertaken in a tertiary level fertility centre.
Participants/materials, setting, methods: Study subjects were in the reproductive age group of 23–40 years, non-smokers with BMI of 18–30. All cycles were induced for ovulation with clomifene citrate and follicular growth was tracked using ultrasound. Ovulation test kits were used to confirm luteinizing hormone surge to time insemination. No human chorionic gonadotropin trigger was used in any of the cycles and luteal phase was not supported with progesterone supplements. Serum progesterone level was tested 7 days later. Ultrasound

scan at 6–7 weeks confirmed clinical pregnancies. There were 66 singleton conception and 337 non-conception cycles.

Main results and the role of chance: Mean age group of patients was 32.4 years (range: 21–40 years). All data were normally distributed and the mid luteal serum progesterone levels showed a range from 4.2–190 nmol/L with a mean of 57.5 nmol/L. Centile distribution of the mid-luteal serum progesterone levels showed that 2.5th centile (20.53 nmol/L, 95% CI = 14.55–23.25) detected 97.5% of ovulatory cycles. There were 97.1% of conception cycles and 97.9% of non-conception cycles above this cut-off. Furthermore, in the conception and non-conception cycles, the mean mid luteal serum progesterone level was 28.27 nmol/L and 76.84 nmol/L, respectively. This difference in mid luteal serum progesterone for the two groups was statistically significant ($p \leq 0.0001$). Subgroup analysis of the conception cycles showed mean mid luteal serum progesterone value 79.08 nmol/L and 79.53 nmol/L for live births and early pregnancy loss (includes biochemical miscarriages, no ectopics in this group) respectively ($p = 0.977$). There was 48% conception cycles and 60% non-conception cycles above the value of 60 nmol/L. Higher midluteal serum progesterone values does not appear to be associated with better pregnancy outcomes. This needs to be validated with higher numbers.

Limitations, reason for caution: There is an argument that luteal phase serum progesterone all cycles could be higher due to the use of clomifene possibly due to multifollicular ovulation; but in the analysed group there were singletons which excludes this possibility. The finding of early pregnancy and luteal phase progesterone needs validation with bigger numbers.

Wider implications of the findings: The findings from this study are adaptable to the current practice in the infertility settings. As this is the first study where cycles were tracked and tested for ovulation, the values derived from this study are more valid in deducing the lower limit for single measurement of mid-luteal serum progesterone to indicate probable ovulation. Although not statistically significant; early pregnancy loss appears to be associated with higher luteal serum progesterone as opposed to the idea of luteal phase defect in these patients. This interesting finding opens a new area for further research.

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

Trial registration number: Not applicable.

P-231 Clomiphene citrate and follicle stimulating hormone for controlled ovarian stimulation in intrauterine insemination (INeS trial)

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Study question: How does the use of follicle stimulating hormone (FSH) compare to clomiphene citrate (CC) when performing intra uterine insemination with controlled ovarian hyperstimulation (IUI-COH) in couples with unfavorable fertility prospects and unexplained or mild male subfertility in terms of chances of ongoing pregnancy?

Summary answer: Ongoing pregnancy rates were 40% after IUI-FSH and 35% after IUI-CC. Multiple pregnancy rates were 9% after stimulation with FSH and 0% after stimulation with CC.

What is known already: Results from a Cochrane systematic review and meta-analysis pooling evidence from seven studies including 556 patients with unexplained or male factor subfertility suggest that ovarian stimulation with FSH leads to higher pregnancy rates than stimulation with CC. Studies included in this analysis used various stimulation/cancellation protocols, and multiple pregnancy rates were high. Only one study aimed to achieve a maximum of two dominant follicles.

Study design, size, duration: We performed a secondary analysis of the INeS trial, comparing *in vitro* fertilization with a single embryo transfer or in a modified natural cycle to IUI-COH in couples with unfavorable fertility prospects and unexplained or mild male subfertility. For this analysis only couples who were allocated to IUI-COH were included.

Participants/materials, setting, methods: Couples received 100 mg CC or 75 IU FSH daily, according to local protocol. Final oocyte maturation was induced with HCG when one follicle of 17–18 mm was present. Cancellation criteria were more than three follicles of at least 16 mm, or more than five follicles of 12 mm.

Main results and the role of chance: 207 couples were allocated to IUI-COH and 194 couples started at least one cycle of IUI-COH. 809 cycles of IUI-COH were performed; 675 cycles with FSH and 134 cycles with CC.

Insemination was not performed in 5.2% of the cycles stimulated with CC and of 9.9% in the cycles stimulated with FSH. Cancellations due to multifollicular growth occurred in 47% of the cycles after FSH stimulation and 1.2% after CC stimulation. Ongoing pregnancy rates were 40% after FSH stimulation and 35% after stimulation with CC, with an odds ratio of 1.3 (95%CI: 0.68–2.4) adjusted for female age and diagnosis of male subfertility. There were 6 multiple pregnancies in the FSH group (9%) and none in the CC group (0%).

Limitations, reason for caution: Couples had not been randomized to stimulation with FSH or CC, but were treated according to local protocol of the participating centres (17 centres).

Wider implications of the findings: In the INeS trial, where a strict cancellation protocol was used in IUI-COH, we found comparable pregnancy rates after stimulation with FSH and CC. In the FSH group multiple pregnancy rates were higher. As this is not a randomized comparison these results need to be verified in a randomized controlled trial to investigate possible differences in (multiple) pregnancy rates between these stimulation methods.

Study funding/competing interest(s): Funding by national/international organization(s), The study was supported by a grant from ZonMW, the Netherlands Organization for Health Research and Development, and a grant from Zorgverzekeraars Nederland, the Netherlands association of health care insurers.

Trial registration number: The trial was registered at the Dutch trial registry (NTR 939).

P-232 Correlation between polymorphisms in genes associated with the estrogen synthesis and its concentrations in blood and follicular fluid

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Study question: Are the estradiol concentration correlated with polymorphisms in *CYP17A1, CYP19A1, CYP2C19, COMT, HSD17B1, ESR1 (PvuII* and *XbaI)* and *ESR2* (*AluI* and *RsaI*)? Are the genotypes correlated to human reproduction results?

Summary answer: There were two polymorphism that stood out in the statistical analyses, *CYP2C19* and *HSD17B1* polymorphisms, both are in the P-450 cytochrome and have important role in the estradiol synthesis. For both polymorphisms the mutated homozygous genotype prevailed in the group of idiopathic infertility when compared with the others groups.

What is known already: The composition of follicular fluid and routes of estrogen synthesis and metabolism are influenced by many genes and enzymes belonging to the main CYP and COMT family of enzymes. Moreover the action of the hormone on its target tissue is also regulated by binding sites, or the receptors present in each cell, represented in these study by *ESR1* and *ESR2*.

Study design, size, duration: Cohort study. A total of 375 women between cases and control were accompanied from the ovarian function procedure, when blood and follicular fluid were taken for DNA and hormone analysis to the pregnancy results. Samples have been collected since 2012 until now.

Participants/materials, setting, methods: Case group was composed by infertile women with diagnosis of: endometriosis, unexplained infertility and tube peritoneal factor. Control group was composed by healthy woman that

also undergoing to the high fertilization treatment due to partner infertility. For all patients we performed estradiol measurement on blood and follicular fluid and real time PCR for genotyping.
Main results and the role of chance: We observed a statistically significant difference between the unexplained infertility and Endometriosis groups compared to controls for the incidence of *CYP2C19* (p = 0,041) and *HSD17B1* (p = 0,036) polymorphisms. This analysis hypothesizes a possible correlation between the presence of genotype and the chance of unexplained infertility, increasing in 2× the risk. Besides, follicular estradiol levels showed to be higher in unexplained infertility than in samples from endometriosis and peritoneal tube factor groups (p = 0,040). Embryo parameters showed a statistical tendency to difference concerning the number of retrieved oocytes and embryos quality but we need to confirm the findings when completing sample analysis.
Limitations, reason for caution: The study is not concluded. 75% of the proposed sample was evaluated. Partial results were shown only as scientific report for the financial sponsor (FAPESP).
Wider implications of the findings: With the improvement of *in vitro* techniques of fertilization, biochemical markers to better predict the embryo quality have been investigated. However few studies show results associating genetic and metabolic profile observed in the follicular fluid. Our results corroborate the idea that metabolic and genetic profile can contribute significantly in fertility.
Study funding/competing interest(s): Funding by national/international organization(s), FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo).
Trial registration number: Not applied.

P-233 Perifollicular blood flow and its impact on follicular fluid cytokines in *in vitro* fertilisation cycles

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Study question: Is there a correlation between perifollicular blood flow (PBF), patient demographic, follicular fluid (FF) pro- and anti-angiogenic cytokines and oocyte developmental potential?
Summary answer: Maximal PBF measured at the time of ultrasound directed oocyte retrieval (UDOR) positively correlates with intrafollicular pro-angiogenic cytokine levels, oocyte maturity and developmental potential. Thus PBF, assessed at the time of oocyte retrieval may be incorporated into the decision making process when selecting an embryo for transfer.
What is known already: A heterogeneous cohort of follicles are recruited containing oocytes of variable maturity and fertilisation capabilities following controlled ovarian hyperstimulation (COH) in *in vitro* fertilisation (IVF) cycles. The oocyte and somatic cells of the ovarian follicle communicate via cytokines, many of which have pro- and anti-angiogenic properties. The stromal vascularity supports synthesis of oestrogen precursors by granulosa cells whilst the vascularity surrounding the follicle supplies the oocyte with oxygen, nutrients and hormones.
Study design, size, duration: 25 women undergoing consecutive COH IVF/IVF-ICSI cycles were recruited prospectively for power Doppler PBF grading (Chui classification) immediately prior to UDOR. All subfertility related aetiology were included in this group. A subset of 9 participants denoted 'healthy women' with no identifiable pathology requiring treatment for male factor subfertility also donated individual FF for analysis.
Participants/materials, setting, methods: Oocyte/embryo culture in single droplets enabled longitudinal tracking of each oocyte to its natural developmental fate. The FF and plasma cytokine levels (interleukin (IL)-6, tumour necrosis factor (TNF)-α and interferon (IF)-γ) were determined by multiplex immunoassay. Data were analysed using Chi- square test and mixed-model logistic regression analysis.
Main results and the role of chance: PBF from 302 follicles was assessed. High-grade PBF positively predicted normal fertilization and clinical pregnancy following embryo transfer. Women with endometriosis and PCOS were significantly more likely to have a low PBF grade for the majority of their follicles ($p < 0.001$). Women with a high BMI were also more likely to have a low PBF grade for the majority of their follicles whilst in the 'healthy women' subset (BMI range 20–30) a low-normal BMI positively correlated with a high PBF grade for the majority of their follicles ($p < 0.001$). 71 FF samples were analysed (cross section of PBF grades and follicle/oocyte fates). Pro-angiogenic FF IL-6 combined with high grade PBF was predictive of greater oocyte maturity whilst, conversely, anti-angiogenic interferon-γ together with low grade PBF was negatively associated with oocyte maturity ($P < 0.05$).
Limitations, reason for caution: This was a pilot study and it is possible that type 2 errors exist due to the small sample size. Whilst a single operator (ENB) performed all PBF measurements, classification remains subjective. The gold standard for cytokine analysis would be duplicate/triplicate to accommodate variability; in this study only one analysis was performed per sample.
Wider implications of the findings: We demonstrate that PBF can be assessed in the clinical setting using power Doppler ultrasound. Follicular vascularity relates to individual patient demographics, particularly BMI, and also to FF cytokine levels and these in turn, impact oocyte maturity and developmental potential. PCOS and endometriosis are associated with reduced oocyte viability; a compromised PBF may be the cause or effect and further studies are warranted. We propose that use of PBF may assist in selecting the embryo for transfer in conjunction with traditional morphological methods.
Study funding/competing interest(s): Funding by hospital/clinic(s), Leeds Teaching Hospitals NHS Trust.
Trial registration number: Leeds East Regional Ethics Committee (Ref: 07/H1306/151).

P-234 The natural conception rate in couples with unexplained or mild male subfertility scheduled for treatment with IVF-SET, IVF-MNC or IUI-COH (INeS trial)

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Study question: What is the natural conception rate in couples with unexplained subfertility scheduled for treatment with *in vitro* fertilization with a single embryo transfer (IVF-SET), IVF in a modified natural cycle (IVF-MNC) or intrauterine insemination with controlled ovarian hyperstimulation (IUI-COH)?
Summary answer: For natural cycles in between treatment the chance of natural conception varied between 3% to 4%, potentially leading to a cumulative percentage of 36% to 48% after 12 months.
What is known already: In couples with unexplained subfertility natural pregnancy chances are known to be substantial. It is however unclear whether natural conception chances can compete with actual treatment.
Study design, size, duration: The INeS study was a randomized controlled trial that included couples with unfavorable fertility prospects and unexplained or mild male subfertility who were allocated to IVF-SET, IVF-MNC or IUI-COH. The primary outcome was a healthy singleton, resulting from a pregnancy achieved within 12 months of follow up.
Participants/materials, setting, methods: Couples with unexplained or mild male subfertility were eligible, and allocated to 3 cycles of IVF-SET, 6 cycles of IVF-MNC or 6 cycles of IUI-COH until a live birth within a time horizon of 12 months.
Main results and the role of chance: We included 602 couples in the trial: 201 in the IVF-SET group, 194 in the IVF-MNC group and 207 in the IUI-COH group, and no large differences were found. In the three arms, there were 2096 untreated cycles that resulted in 74 pregnancies. Per cycle the chance of natural conception varied between 3% to 4%, potentially leading to a cumulative percentage of 36% to 48% after 12 months.
Limitations, reason for caution: The per cycle findings were extrapolated to estimates after 12 months. A more sophisticated modeling of these findings is presently being developed.
Wider implications of the findings: A large proportion of couples with unexplained subfertility would reach a successful pregnancy without fertility treatment. To fairly evaluate the cost-effectiveness of fertility treatment

in these couples we need to compare treatment with an expectant management policy.
Study funding/competing interest(s): Funding by national/international organization(s). The trial was supported by a grant from ZonMW, the Netherlands Organization for Health Research and Development, and a grant from Zorgverzekeraars Nederland, the Netherlands association of health care insurers.
Trial registration number: The INeS trial was registered at the Dutch trial registry (NTR 939).

P-235 Endometrial scratching in single implantation failure patients improves pregnancy rates in vitrified embryo transfer

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Study question: Does endometrial scratching in the previous cycle to the embrionary transfer yields an increase in pregnancy rates in patients with previous single implantation failure?
Summary answer: The pregnancy rates achieved in vitrified embryos transfers were higher in patients who underwent endometrial scratching, compared to control group, who did not receive any treatment.
What is known already: Achieving pregnancy in an assisted reproduction program is a multifactorial process where many factors as embryo quality, endometrial receptivity, practitioner experience, etc. can play a crucial role. The importance of embryo-endometrium dialogue, even if not fully understood, is widely accepted. The effect of endometrium scratching in this process is not established.
Study design, size, duration: Retrospective study including 119 patients who underwent a ICSI cycle and a subsequent frozen embryo transfer, between 2012 and 2013.
Participants/materials, setting, methods: A retrospective study was carried, including 119 patients with a single ICSI failure between 2012 and 2013 who had vitrified embryos for a second transfer. 24 patients were subjected to endometrial scratching (study group) and 95 patients (control) underwent transfer without scratching. Study was carried out in Ginemed Clínicas, Seville, Spain.
Main results and the role of chance: Pregnancy rates in scratching group achieved 58.3% (14/24) whereas in control group they remained at 46.3% (44/95).
Limitations, reason for caution: Sample size was unequal, so the results can only be interpreted as a trend towards improvement. The use of a placebo treatment in the control group should be considered for future studies.
Wider implications of the findings: Results obtained show a trend towards an improvement in the pregnancy rates achieved in frozen embryo transfers in patients undergoing endometrial scratching in the previous cycle to the embrionary transfer. For patients with unexplained implantation failure, endometrial scratching can be a offered as a strategy towards increasing pregnancy chances in a second embryo transfer.
Study funding/competing interest(s): Funding by hospital/clinic(s), Fundación Ginemed.
Trial registration number: None.

P-236 Effect of fibroids on fertility outcomes in donor oocyte recipients with normal intrauterine cavity: a cohort study

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Study question: In donor oocyte recipients with normal uterine cavity, does the presence of intramural and/or subserous fibroids affect the implantation, clinical pregnancy and live birth rates?
Summary answer: We found no evidence of a deleterious effect of intramural and/or subserous fibroids on the implantation, pregnancy and live birth rates of donor oocyte recipients with normal uterine cavity.
What is known already: Two recent meta-analyses showed that intramural and/or subserous fibroids could have a negative effect on pregnancy and live birth rates of women undergoing *in vitro* fertilisation (IVF). Nevertheless, the quality of the evidence is low, the number of patients included is limited, and results have not been analysed in the donor oocyte recipient subgroup. It is therefore important to contribute to the improvement of knowledge with specific research in this subgroup.
Study design, size, duration: All consecutive patients that received a donor oocyte in the period 2010–2013 were included in this retrospective cohort study. A total of 245 procedures in 140 women were analysed. Seventy four women had intramural and/or subserous fibroids and 66 had not. There were no losses to follow-up.
Participants/materials, setting, methods: The setting was a University Hospital in Buenos Aires, Argentina. All patients were studied with ultrasound and hysteroscopy. Intrauterine pathological findings were resolved previous to embryo transfer. All fertilisation procedures were done using introcytoplasmic sperm injection technique. We analysed data with a random effects logistic regression model accounting for correlation.
Main results and the role of chance: Overall, the mean age was 43.2 years (SD 4.42) with a range from 31 to 54 years. The crude implantation rate was 50.0% (95%CI 38.3%–61.7%) for women with fibroids and 57.4% (95%CI 44.8%–69.3%) for those without (*p*: 0.377). The crude pregnancy rate was 47.4% (95%CI 35.8%–59.2%) and 52.9% (95%CI 40.4%–65.2%), respectively (*p*: 0.504). The live birth/ongoing pregnancy rate was 30.3% (95%CI 20.2%–41.9%) and 38.2% (95%CI 26.7%–50.8%), respectively (*p*: 0.313). There were no differences in the odds of outcomes between groups in all the variables included in the random effects model, except for pregnancy rate in women with intramural and subserous fibroids (OR 0.294; 95%CI 0.102–0.850). Nevertheless, this association was lost when other variables were considered altogether, and in the regression model analysing live birth rates.
Limitations, reason for caution: The study is a retrospective cohort. Nevertheless, we included all consecutive patients and had no losses to follow-up. Although we considered most important confounders and correlation with a random effects model, potential confounders not yet known could still be present. The study could be underpowered for small associations.
Wider implications of the findings: These results are generalisable to women from the same subgroup: donor oocyte recipients with normal uterine cavity. We believe that other studies that did not systematically assess intrauterine cavity with the gold-standard procedure, hysteroscopy, could have observed confounded abnormal results in the fibroid subgroup due to undetected intrauterine pathology. In addition, our results are the first in a Latin-American population. They are consistent with studies performed in the same subgroup in other populations.
Study funding/competing interest(s): Funding by hospital/clinic(s), Hospital Italiano de Buenos Aires, Argentina.
Trial registration number: None.

P-237 Acetylcholinesterase in the ovary

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Study question: Acetylcholine (ACh) may be a signaling molecule in the ovarian follicle and corpus luteum but whether the ACh-degrading enzyme ACh-esterase (AChE) is present and could restrict actions of ACh or exert other functions in ovarian cells was unknown.
Summary answer: AChE is a granulosa cell (GC) derived enzyme which can counteract ACh-actions in the ovary, and one of the three AChE variants (R-AChE) also acts as a signaling molecule involved in cell death of human GCs.
What is known already: Proteomic studies revealed the ACh-degrading enzyme butyrylcholinesterase in human follicular fluid (FF). It may degrade ACh, which is formed by human GCs. Known ACh-actions include activation of muscarinic receptors of GCs, entailing increased calcium levels, activation

of the transcription factor egr-1, ion channel activation and regulation of gap junctional communication between GCs, as well as cell proliferation. Ovarian expression of AChE, which also degrades ACh and has additional functions, was however not known.

Study design, size, duration: FFs of 15 IVF-patients and cultured human GCs derived from IVF-patients were used for analysis of AChE activity, mRNA expression and protein analysis. To verify the observations, human, monkey and rat ovarian sections were studied by immunohistochemistry.

Participants/materials, setting, methods: Besides immunohistochemical analysis of rat, monkey and human ovarian sections, we tested human IVF-derived FFs for esterase activity (Ellman-assay). We used cultured human GCs for RT-PCR and Western blotting. The R-AChE specific peptide (ARP) was synthesized and tested for biological activity in human GC using video live cell imaging.

Main results and the role of chance: Immunohistochemistry revealed AChE in GCs of follicles and the corpus luteum. In human FFs, enzymatically active AChE and BChE were measured, with AChE activity accounting for about 50% of the overall activity. In freshly isolated human GCs and in cultured human GCs, AChE and BChE proteins and mRNAs were found. RT-PCR strategies followed by sequencing allowed us to identify three AChE splice-variants in human GCs: the read through (R), erythrocyte (E) and synaptic (S) AChE variant. In contrast to a control peptide, ARP increases cell death in cultured human GCs. Evidence for a special form of cell death, namely regulated necrosis (necroptosis) are mounting. In ongoing experiments we study whether necrostatin-1 may block the ARP-dependent increase of cell death in human GCs.

Limitations, reason for caution: Results were obtained in cultures of human luteinizing GCs, which may be a model for the ovulatory follicle and corpus luteum only.

Wider implications of the findings: The results of this study shed light on the unexplored ACh-system of the ovary. GCs are a source for ACh and for the ACh degrading enzyme AChE, which by its enzymatic activity may influence GC communication and proliferation. In addition, non-enzymatic actions of the soluble R-AChE variant suggest for the first time that it may serve as a signaling molecule involved in the regulation of cell death in the human follicle/corpus luteum.

Study funding/competing interest(s): Funding by national/international organization(s), DFG MA1080/19-1.

Trial registration number: Not needed.

P-238 The association of serum anti-Mullerian hormone (AMH) and 25-hydroxy vitamin D (25(OH)D) levels in women at young reproductive ages

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Study question: The active form of vitamin D upregulates AMH production in cultured prostate cell. Vitamin D may have an effect on AMH concentration in blood. The aim of our study was to determine whether 25(OH)D level correlates with AMH levels in women at young reproductive ages.

Summary answer: We did not find any association between 25(OH)D with AMH serum levels in young reproductive aged women.

What is known already: It was shown in cDNA microarray analysis of a prostate cancer cell line that AMH gene was regulated by vitamin D via functional vitamin D response elements that bind the vitamin D receptor. A novel relationship was reported between circulating 25OH-D and AMH in women aged over 40 years, suggesting that 25 OH-D deficiency might be associated with lower ovarian reserve in late-reproductive-aged women.

Study design, size, duration: This is a prospective study including 151 women which was carried out at Yeditepe University Hospital Obstetrics and Gynecology Department between May 2013 and September 2013

Participants/materials, setting, methods: 151 women with ages between 18 to 35 years were included in the study. Serum samples were obtained from the patients on the third day of their menstrual period. The 25(OH)D and AMH levels were measured. Linear regression analysis was performed.

Main results and the role of chance: There was a correlation between age and serum AMH levels. However we did not observe any association between age and 25(OH)D. We compared AMH levels with 25(OH)D. We performed linear regression analysis. We did not find any association between serum levels of 25(OH)D and AMH.

Limitations, reason for caution: The size of our study is small and the study. Also we investigated the correlation of vitamin D and AMH levels only in women at young reproductive ages.

Wider implications of the findings: Currently available data identify vitamin D as a key component in processes involved in reproductive success. Vitamin D was proposed as a positive regulator of AMH production in adults and a novel relationship was reported between circulating 25OH-D and AMH in women aged over 40 years. However our results suggest that vitamin D might be involved in reproductive success processes other than regulating AMH expression in young reproductive aged.

Study funding/competing interest(s): Funding by University(ies), Yeditepe University Hospital.

Trial registration number: This study is not an interventional study.

P-239 Analysis of ART procedures before (2007-2008) and after (2010-2011) the constitutional court change of Italian law on ART: records from the Italian assisted reproductive technologies register

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Study question: In 2009, Italian Constitutional Court outlawed some restrictions set out in the law (40/2004) regulating ART, leaving up to clinicians to choose in the interest of women. Our goal is to study differences in fertility treatments and outcomes performed before and after the change.

Summary answer: Our analysis on data collected from all ART Italian clinics showed that after the modification there was an increase in pregnancy rate per fresh cycles, higher number of frozen cycles and an significant growth in cumulative pregnancy rates (CPR). Moreover a positive trend towards a reduction of triplets was observed.

What is known already: Previous studies compared cycles before and after the change of law 40/2004. They showed an improvement of pregnancy rates in those cycles performed after the change. However, none of them examined the positive impact of frozen cycles on ART outcomes by CPR and only data from a single clinic were reported. Conversely, we present results from all ART clinics in Italy.

Study design, size, duration: We analyzed retrospectively 4 years of fertility treatment activity (excluding 2009, year of the law change), namely 218223 IVF cycles: 84091 fresh, 6278 frozen oocytes and 1217 frozen embryos cycles from 2007 to 2008 and 108747 fresh, 4948 frozen oocytes and 8942 frozen embryos from 2010 to 2011 after law modification.

Participants/materials, setting, methods: All ART clinics (203) were included which reported data at least once during the study period. Parameters regarding number of cycles, treatments indications, number of transferred embryos, pregnancies were analyzed. Data were statistically analyzed using IBM SPSS statistics version 21.

Main results and the role of chance: Small differences were observed in pregnancy rate per cycle, on patients with comparable infertility causes (19.9 vs. 20.2; $p = 0.086$) and comparable distribution of age (35.85 years vs. 36.41 years). Before modification of the law the number of frozen cycles was 8.2% (7495) of all ART cycles performed versus 11.3% (13890) afterwards. In particular the number of cycles with frozen embryos was very small (16.2% of all frozen cycles vs. 64.4% afterwards). As a consequence of the increase of cycle numbers using frozen oocytes/embryos, cumulative pregnancy rate increased from 21.0% to 22.2% ($p < 0.001$). Also, there was a downward trend in "triple or more" embryos transfer (42.3% vs. 40.8%; $P < 0.001$) thus the triplet pregnancies diminished from 3.5% of the total recorded pregnancies to 2.0% ($p < 0.001$).

Limitations, reason for caution: In compliance with Italian law data were collected in aggregate form only. This may have limited the variety and depth of our analysis.
Wider implications of the findings: Our analysis is a study that includes a very large number of IVF cycles and thus contributes to the discussion on the different policies induced by law 40/2004. It shows the negative impact of these restrictions on the success and the safety of fertility treatments (i.e. number related to pregnancy rate, cumulative pregnancy rate and triplets).
Study funding/competing interest(s): Funding by national/international organization(s), Italian ministry of health.
Trial registration number: None.

P-240 Effect of r-LH addition in previous unexpected poor ovarian response

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Study question: Poor ovarian response (POR) is most frequently linked to the condition known as diminished ovarian reserve, but it can occur in the absence of pathological ovarian reserve tests ('unexpected' POR). Possible explanations include theca cell deficiency. We evaluated the effect of recombinant luteinizing hormone (r-LH) administration in 'unexpected' poor responders.
Summary answer: Patients with non-pathological ovarian reserve tests and previous 'unexpected' POR seem to benefit from r-LH addition in subsequent cycles, without the need to increase the recombinant follicle stimulating hormone (r-FSH) starting dose.
What is known already: Oocytes collected remain the key marker of prognosis in women undergoing assisted reproduction technology (ART) treatments, because a poor ovarian response frequently determines a poor outcome regardless patient's age. The Bologna Criteria Consensus still mixes patients with different prognoses, indeed patients who display a low ovarian reserve as assessed by biochemical and ultrasound status have a much lower prognosis than those who experience an inadequate response to FSH without pathological markers of ovarian reserve.
Study design, size, duration: This retrospective, single-centre cohort study was conducted from January to December 2012 at the *In Vitro* Fertilization (IVF) unit of the San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milano. 65 patients were screened with basal hormonal assessment and ovarian reserve tests before the first cycle of stimulation.
Participants/materials, setting, methods: 65 patients with Anti-Mullerian Hormone (AMH) >0.5 ng/ml and/or Antral Follicular Count (AFC) >7 with POR in their first cycle were enrolled. Patients underwent a second IVF cycle with same protocol and starting dose of r-FSH used before and 150 IU of r-LH daily from day 1.
Main results and the role of chance: Compared to the first cycle, r-LH addition in the second cycle determined an increase in number of oocytes retrieved ($p < 0.001$), number of metaphase II oocytes ($p < 0.05$), estradiol (E_2) levels at human chorionic gonadotropin (hCG) triggering ($p < 0.001$) and number of embryos transferred ($p = 0.002$). A 15% clinical pregnancy was observed. Our results suggest that patients with non-pathological ovarian reserve tests and previous 'unexpected' POR seem to benefit from r-LH addition in subsequent cycles, without the need to increase the r-FSH starting dose.
Limitations, reason for caution: Our population was identified through a retrospective analysis based on an unexpected poor ovarian response and IVF failure in the first cycle with r-FSH-only ovarian stimulation. Moreover, we did not evaluate any biochemical and clinical markers supportive of r-LH addition as first line treatment in a specific sub-group of patients.
Wider implications of the findings: This is the first study that has identified a clinical category of patients, the unexpected inadequate responders to r-FSH with non-pathological ovarian reserve tests, that in a subsequent treatment cycle may benefit from a tailored strategy of ovarian stimulation based on addition of r-LH without the need to increase the r-FSH dose starting dose, resulting in an improvement of relevant parameters of a IVF cycle without detrimental effects.
Study funding/competing interest(s): Funding by hospital/clinic(s), San Raffaele Scientific Institute, Milan.
Trial registration number: Not required.

P-241 Freeze-all in poor prognosis patients: clinical outcomes of single vitrified euploid blastocyst transfers

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Study question: Is fresh transfer cancellation and single vitrified blastocyst transfer an effective procedure in poor prognosis patients, such as those approaching Preimplantation Genetic Screening (PGS)?
Summary answer: Freeze-all approach and cycle segmentation with single vitrified euploid blastocyst transfer guarantees successful and reproducible results in terms of implantation rate (>12 weeks of gestation) in poor prognosis patients population due to advanced maternal age (AMA).
What is known already: The theory of cycle segmentation entails cryopreserved single embryo transfer (ET) on a non stimulated receptive endometrium with no risk of hyperstimulation. The resulting evidence is that single blastocyst transfer guarantees the same cumulative live birth rate as double one already after the first cryopreserved ET together with a fall in twin pregnancy risk. This strategy has been proposed for good prognosis patients. No data is available on poor prognosis patients.
Study design, size, duration: Longitudinal-cohort-study. Freeze-all PGS cycles with elective vitrified blastocyst transfer between January 2012–September 2013 were considered. Patient indication for PGS was purely AMA (female age ≥37 years). The outcome measure was implantation rate (>12 weeks of gestation).
Participants/materials, setting, methods: Transvaginal oocyte retrieval was performed 36 h after hCG administration. Fertilization was achieved with intracytoplasmic sperm injection. Trophectoderm biopsy was performed at day 5/6 of embryo development, blastocysts were immediately vitrified, comprehensive chromosome analysis was conducted by a referral genetic centre. Only single vitrified euploid blastocyst transfers in natural cycles were performed.
Main results and the role of chance: In this study 174 patients (mean female age 40.6 years, range 37.0–45.7) undergoing 193 cycles were included; out of 1485 MII (mean number per patient 8.2, range 1–24) injected, 577 (38.8%; mean number per patient 3.0, range 1–13) reached the blastocyst stage and were biopsied. Among them 208 were euploid (36.0%; mean number per patient 1.1, range 0–5). At least one transferable blastocyst was available in 115 cases (59.5% and 66.1% per cycle and per patient, respectively). At present, 82 blastocysts were warmed, 81 survived (98.8%) and were transferred. The implantation rate was 50.6% (41/81)
Limitations, reason for caution: This is a proof of principle study aiming at verifying the feasibility of cycle segmentation in a poor prognosis patients population. Larger prospective studies with appropriate control groups are necessary to evaluate the possible advantages of this approach.
Wider implications of the findings: High blastocyst survival and implantation rate after vitrification can be obtained in AMA population, thus maintaining after cycle segmentation high chances of pregnancy even when a low number of embryos is available. This observation paves the way to extend this strategy also to poor prognosis patient population. Our findings are particularly important for the implementation of new technologies for embryo assessment that often require high turn-around time of analysis not compatible with fresh ET.
Study funding/competing interest(s): Funding by hospital/clinic(s), none.
Trial registration number: None.

P-242 How thick is enough - association of success rates and endometrial thickness in oocyte donation cycles - a retrospective study of 4,070 cases

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Study question: To know which is the optimal and the minimum endometrial thickness before starting with progesterone priming in cycles with hormonal replacement.

Summary answer: Success rates are similar if progesterone priming is started with an endometrial thickness between 5 and 12 mm.
What is known already: Hormone replacement therapies have helped us improve endometrial receptivity but some causes of unsuccessful treatments and the optimal way to prepare it are still unknown. There is still no agreement in the relationship between endometrial thickness and success rates.
Study design, size, duration: Retrospective study of 4,070 fresh cycles of IVF treatment with oocyte donation and hormone replacement therapy.
Participants/materials, setting, methods: Cycles were with endometrial stimulation and priming. Ultrasound control was between days 7 and 9. If the endometrium was trilaminar and between 5 and 12 mm. the donor's egg retrieval was programmed. Transfer was on day +3 between days 10 and 15 of the cycle.
Main results and the role of chance: The results were analysed in a continuous logistic regression model. Groups for each thickness were assigned with 8 groups between 5 and 12 mm. All groups were homogeneous for donors' and patients' age, years of infertility, and number of embryos transferred. The average thickness was 7.24 mm. No significant differences were found between endometrial thickness and pregnancy rates, success rates, number of gestational sacs and miscarriage rates.
Limitations, reason for caution: The main limitations of this study are due to its retrospective nature. These are reduced due to the size of our study population.
Wider implications of the findings: Resources are spent on repeat ultrasounds and strategies to increase endometrial thickness. This study suggests that a single ultrasound with an endometrial thickness of 5 mm. is sufficient to program egg retrieval. This can save time and resources to the health system.
Study funding/competing interest(s): Funding by hospital/clinic(s), Instituto Marques.
Trial registration number: N/A.

P-243 Hysterosalpingo-Foam Sonography (HyFoSy): a less painful procedure for tubal patency testing during the fertility work-up, compared to (serial) hysterosalpingography. A Randomized Clinical Trial

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Study question: Is a hysterosalpingo-foam sonography (HyFoSy) a less painful procedure as a first office tubal patency test in the fertility work-up, compared to a serial hysterosalpingography (HSG)?
Summary answer: This is the first prospective RCT evaluating pain scores between the recently introduced sonographic tubal patency test HyFoSy and HSG. Our trial showed that HyFoSy is more patient friendly compared to HSG, with significantly lower Visual Analogue Scale (VAS) pain scores in favour of HyFoSy (1.5 cm versus 4.3 cm $p < 0.01$).
What is known already: In our pilot study we compared the VAS pain scores experienced during HyFoSy and HSG in women with proximal occluded fallopian tubes after hysteroscopically inserted Essure® devices. This study, which included 23 women, showed a 75 % lower VAS pain score during HyFoSy compared to HSG. To our knowledge, this was the only study which compares pain scores between a HyFosy procedure and a HSG till now.
Study design, size, duration: A two-center, prospective open RCT between January and October 2013. In order to demonstrate a difference of 50% in VAS pain scores between a HyFoSy and a HSG, a total number of 40 patients were required. Eligible women were randomly allocated to undergo a HyFoSy procedure or a HSG.
Participants/materials, setting, methods: Women were eligible when aged between 18–41 years, had a low risk for tubal pathology and had an indication for tubal patency testing during their fertility work-up. Both examinations were equally performed in the VU University Medical Center and Spaarne Hospital. Visual Analogue Scale was used to assess pain scores.
Main results and the role of chance: The mean VAS score for pain perception during a HyFoSy procedure was 1.5 cm (standard deviation 1.1) compared to 4.3 cm (standard deviation 2.5) during HSG ($p < 0.01$). Mean difference 2.7 cm (95%CI 1.5–4.0). The HyFoSy procedure also showed a significantly shorter procedure time compared to a HSG; median 5.0 min (interquartile range 3.0) for HyFoSy versus 12.5 min (interquartile range 16.0) for HSG ($p < 0.01$).
Limitations, reason for caution: To internalize a HyFoSy procedure, you need to perform five HyFoSy's. In our study two gynecologist, already familiar with this technique, performed all HyFoSy's. Possibly a HyFoSy is less painful and less time consuming when performed by an experienced gynecologist compared to a resident. HSG were all performed by residents.
Wider implications of the findings: Since there is no need for radiography during a HyFoSy procedure this examination can be performed by a single operator in an outpatient clinic during regular office hours. During HyFoSy women will not be exposed to irradiation. Furthermore, there are no costs for the department of radiology, which makes HyFoSy a less expensive examination compared to HSG. Replacing HSG by HyFoSy as a first office tubal patency test could save millions for health care.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Grant support was received for the intervention from IQ Medical Ventures BV, Delft, The Netherlands.
Trial registration number: The Netherlands National Trial Register: NTR3457.

P-244 Intra-uterine insemination with donor semen in non-stimulated cycles: a large retrospective cohort study

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Study question: We performed a retrospective cohort analysis on the outcomes of 4415 intra-uterine inseminations with donor semen (D-IUI) in non-stimulated cycles performed over a 2-year period (January 2011–December 2012).
Summary answer: Retrospective analysis showed that D-IUI in non-stimulated cycles results in good OPRs (8.2%), high COPRs (40.2% after 6 cycles) and very low MPRs (<0.3%). Success of D-IUI is related to age of the patient. D-IUI in non-stimulated cycles is patient friendly (non-invasive and practically medication free) and involves low cost.
What is known already: D-IUI is a widely accepted and successful fertility treatment for patients suffering from severe male infertility or genetic problems. Single or lesbian women are also treated with D-IUI. The majority of D-IUI is performed in stimulated cycles. Data from the ESHRE consortium show a 13.4% delivery rate with 10.3% twins and 0.5% triplets (29 235 D-IUI cycles, 2009).

Few studies have focused on the success, safety and efficacy of D-IUI especially when performed in non-stimulated cycles.
Study design, size, duration: Primary outcome was ongoing pregnancy rate (OPR) defined as presence of gestational sac with fetal cardiac activity at 12 weeks post IUI. Secondary outcome was multiple pregnancy rate (MPR) and cumulative ongoing pregnancy rate (COPR) after 6 cycles of D-IUI. Influence of female age on D-IUI outcomes was also studied.
Participants/materials, setting, methods: D-IUI procedures were performed according to hospital specific protocols (private fertility center) using ultrasound and urine LH peak detection for ovulation timing. A 0.4 ml suspension of frozen/thawed prepared donor semen was inseminated 24–36 h post hCG injection or 24 h post LH peak. No luteal phase support was given.
Main results and the role of chance: A total of 4415 Donor-IUIs in non-stimulated natural cycles were performed during the study period. 364 healthy ongoing pregnancies were recorded, resulting in an OPR of 8.2%. Nine twin pregnancies were noted (MPR < 0.3%). COPR after 6 cycles was 40.2%. Mean patient's age was 35.9 years (range 21–45, 4 years; SD: 4.4 years; median: 36.3 years).

A 10% OPR and a COPR of 46.9% was noted for patients <25 years; 13.5% OPR and 58.1% COPR when between 25 and 30 years; 10% OPR and 46.9% COPR when between 30 and 35 years; 8.5% OPR and 41.3% COPR when between 35 and 40 years and 2.8% OPR and 15.7% when patients over 40 year.
Limitations, reason for caution: Present study is a retrospective cohort analysis on a large data set. There is a real need for prospective randomized trials comparing the efficacy of IUI with donor semen in both stimulated and non-stimulated cycles.

Wider implications of the findings: This retrospective large cohort study demonstrates that D-IUI in non-stimulated cycles results in good OPRs (8.2%) and high COPRs (40.2% after 6 cycles) with a very low risk for a multiple pregnancy (<0.3%). Use of D-IUI in non-stimulated cycles may require more cycles of treatment for obtaining a pregnancy especially with patient's age >40 years. Donor-IUI in non-stimulated cycles should be considered as a first line treatment before going to IVF with donor semen.
Study funding/competing interest(s): Funding by hospital/clinic(s), Stg Geertgen.
Trial registration number: N/A.

P-245 Dehydroepiandrosterone (DHEA) administration prior to IVF in poor responders. A prospective cohort study

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Study question: To evaluate whether administration of DHEA in women with history of poor ovarian response provides any additional benefit.
Summary answer: Supplementation with DHEA does not have any significant impact on women with poor prognosis undergoing controlled ovarian hyperstimulation (COH) for IVF.
What is known already: The use of DHEA improve ovarian stimulation outcomes in a woman of advanced reproductive age and could reduce embryo aneuploidy.
Study design, size, duration: Prospective cohort trial. In the period between June of 2008 and July of 2012, 48 patients diagnosed with poor ovarian response received supplementation with DHEA. Changes in baseline hormonal profile (FSH, E2 and AMH) before and after treatment, stimulation characteristics, stimulation and clinical outcome (clinical pregnancy and take home baby rates) were reported.
Participants/materials, setting, methods: Women diagnosed with poor ovarian response were offered supplementation with DHEA for at least 12 weeks. These women were compared to a group of poor responders (113) who did not receive any supplementation. During the study period, patients had measurements of day 2 FSH and estradiol on a monthly basis. Evaluation of AMH was performed before the initiation of treatment and just before the subsequent stimulation.
Main results and the role of chance: Supplementation with DHEA for at least 12 weeks resulted in a modest increase of AMH levels and a decrease in baseline FSH which were statistically significant. DHEA administration had no impact in any of stimulation parameters nor were there any differences in clinical pregnancy rates and live birth rates between the two groups.
Limitations, reason for caution: The study is not randomised and as such, it suffers with all inherent problems of studies of its kind and patients in our study had worse prognosis than the ones reported in previous studies.
Wider implications of the findings: There is no benefit in the administration of DHEA in women with a previous poor ovarian response to controlled ovarian hyperstimulation (COH). Patients should be counseled adequately regarding the uncertain effectiveness and potential side effects and cost of this treatment.
Study funding/competing interest(s): Funding by hospital/clinic(s), 2nd Department of Obstetrics and Gynecology, "Aretaieion" Hospital, University of Athens, Greece.
Trial registration number: None.

P-246 Impact of circulating levels of total and bioavailable serum vitamin D on pregnancy rate in an egg donation program

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Study question: Does lower bioavailable serum 25 OH vitamin D correlate to lower pregnancy rate in recipients of donated oocytes after ICSI and good quality embryo transfer?
Summary answer: Vitamin D status evaluated by serum levels of bioavailable 25-OH vitamin D, which is a better marker of the status than total 25-OH vitamin D, do not correlate with pregnancy rate in recipients of donated oocytes.
What is known already: Vitamin D deficiency has been linked to poorer implantation rate. A difference up to 41% in pregnancy rate has been described between vitamin D replete and non-replete oocyte recipients in ART cycles. Vitamin D status in oocyte donors does not seem to influence ART outcome, while in IVF cycles data are controversial. No previous data on bioavailable vitamin D exists in oocyte recipients that could help us to understand its impact on the endometrium.
Study design, size, duration: Retrospective, cohort study in 256 patients who were referred to our Clinic for oocyte donation from June to December 2013. Serum samples were obtained after 2 weeks under hormonal replacement therapy, and then kept frozen until analysis.
Participants/materials, setting, methods: Of all patients, 14% (n = 36) were vitamin D replete (vitamin D > 30 ng/ml), 48.5% (n = 124) had vitamin D deficiency (20–30 ng/ml) and 37.5% (n = 96) insufficiency (<20 ng/ml). Vitamin D binding protein was performed by a commercial ELISA kit (R&D system). Vitamin D was determined by chemiluminescence by Advia Centaur analyzer (Siemens). Albumin determination was realized by Cobas Mira Plus analyzer (Roche). Bioavailable vitamin D was calculated using Vermeulen validated formula. Student's t test and ROCC curves were used as appropriate.
Main results and the role of chance: Implantation rate and pregnancy rate were similar among patients with normal, insufficient or deficient total serum 25-OH vitamin D levels (26% and 69.4%, 41% and 69.4%, and 34% and 74%, respectively). No statistically significant differences were showed. Ongoing pregnancy rates were also comparable among the three groups. Predictive value of total vitamin D regarding pregnancy rate was analyzed by ROCC. Area under the curve (AUC) was 0.506, and bioavailable 25-OH vitamin D AUC was 0.467, showing that the analysis of either vitamin D or bioavailable vitamin D AUC was not informative.
Limitations, reason for caution: Being a retrospective design and the fact that only 36 patients (14.5%) had normal vitamin D levels may bias the results.
Wider implications of the findings: Vitamin D insufficiency and deficiency are a frequent condition in our infertile population. In contrast with previous studies, vitamin D non-replete patients do not have a lower chance of becoming pregnant with egg donation. We do not know if this highly prevalent vitamin D insufficiency may impair ovarian reserve and/or quality. At this stage, there is insufficient evidence to recommend vitamin D status screening in infertile patients.
Study funding/competing interest(s): Funding by hospital/clinic(s), IVI Madrid-Madrid-Spain.
Trial registration number: No.

P-247 Vaginal core body temperature assessment identifies pre-ovulatory body temperature rise and detects ovulation in advance of ultrasound folliculometry

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Study question: Is body temperature rise a post-ovulatory phenomenon and if not, could a novel vaginal temperature measurement based algorithm predict ovulation; what is the sensitivity (Se), Specificity (Sp) positive predictive value (PPV), negative predictive value (NPV) and accuracy of such an algorithm, with ultrasound folliculometry as the accepted gold standard?
Summary answer: Body temperature starts rising pre-ovulation, in a consistent predictable fashion. A novel algorithm, utilising multiple overnight core body (vaginal) temperature measurements, predicts ovulation one day

in advance, in 89% (Se) of cycles confirmed ovulatory by ultrasound; figures for Sp, PPV, NPV and accuracy, were 88%, 96%, 72% and 89%, respectively.
What is known already: The post-ovulation progesterone rise, results in a documented rise of body temperature, due to progesterone's thermogenic effect. Skin surface temperature measurements are prone to technical error and bias, and therefore cannot describe an accurate temperature rise, representative of ovulatory activity. Prior laboratory studies have shown that assessment of core body temperature however, which is not influenced by the same inconsistencies, can predict ovulation.
Study design, size, duration: Prospective observational study, approved by the Leeds Research Ethics Committee, U.K and by the Medicines and Healthcare products Regulatory Agency, U.K. 21 participants entered the study and contributed a total of 81 cycles over 1 year.
Participants/materials, setting, methods: Vaginal temperature was measured every 5 min overnight with a sensor employing a thermistor with a 0.003°C resolution. Ultrasound folliculometry scans were performed in a dedicated ultrasound clinic to establish the ovulation date. The nature of the temperature curves and the mean ovulation date over the database were assessed.
Main results and the role of chance: Although the average body temperature for each participant varied, the temperature curve form for all ovulatory cycles was extremely consistent in shape and slope, with a clear "onset of phase change" followed by a consistent rise over a number of days to a peak measurement. Furthermore, the date of ovulation as established by ultrasound folliculometry was found to fall on a mean of three days after the onset of phase change of the curve, and with a Gaussian distribution - thereby clearly identifying the mean as having validity and not being due to chance. The novel algorithm developed, correctly identified ovulation one day in advance in 9 out of 10 ovulatory cycles, with high specificity as well. The overall accuracy of the method reached 89%.
Limitations, reason for caution: The database used to develop the algorithm is relatively small, with 81 cycles with full comparative ultrasound folliculometry results. However, a further 17 cycles of data added since from other users of the study device; all conform to the conclusions reached by use of the novel algorithm.
Wider implications of the findings: Vaginal core body temperature assessment detects ovulation a day in advance through a user controlled device, with accuracy comparable to serial ultrasound, without the expense and inconvenience. It gives a much more reliable and detailed picture of peri-ovulatory temperature changes which are consistent in different subjects; temperature starts rising before ovulation. More research is needed to elucidate association of such variation to exact peri-ovulatory progesterone change, which has been associated with the possibility of fertilisation.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Fertility Focus Ltd.
Trial registration number: 11/YH/0038.

P-248 Fresh single blastocyst transfers result in higher levels of initial β-hCG compared to fresh single cleavage embryo transfers

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Study question: Our objective was to compare initial serum β-hCG levels in pregnancies resulting from a fresh single blastocyst versus a fresh single cleavage embryo transfer.
Summary answer: Initial serum β-hCG levels in pregnancies resulting from fresh single blastocyst transfers were significantly higher than pregnancies resulting from fresh single cleavage embryo transfers. This difference remained significant after adjustment for maternal age, treatment protocol and micromanipulation (ICSI/IVF).
What is known already: There are conflicting results in the literature on the levels of serum β-hCG in pregnancies after transfer of blastocyst compared to cleavage-embryos. However, there has been no data comparing β-hCG levels after single cleavage or blastocyst embryo transfers.
Study design, size, duration: We retrospectively analysed 3449 fresh non-donor single embryo transfers (SET) between August 2009 and December 2013. All embryos were cultured using the same culture medium. For standardization purposes, only β-hCG values from day 16 after oocyte collection were included.
Participants/materials, setting, methods: Positive β-hCG values were compared for blastocysts and cleavage stage embryo transfers. Regression analysis for confounding variables included maternal age, treatment protocol, micromanipulation (ICSI/IVF).
Main results and the role of chance: 1340 non-donor fresh SETs resulted in a positive serum β-hCG. After including only day 16 β-hCG results, 974 fresh SETs were analyzed, 755 were blastocysts and 219 were cleavage embryos. The mean β-hCG levels for the fresh blastocyst transfer were 291 ± 200 IU/L and for the fresh cleavage transfer were 235 ± 196 IU/L ($p = 0.0005$). The difference between β-hCG levels remains ($p = 0.0015$) after adjusting for confounding variables including maternal age, treatment protocol and micromanipulation (ICSI/IVF). β-hCG levels were positively correlated with a clinical intrauterine pregnancy ($p < 0.0001$).
Limitations, reason for caution: The limitation of the study is in the retrospective nature of the study. β-hCG values were recorded as part of routine prospective patient follow-up.
Wider implications of the findings: Initial β-hCG level measured after single embryo transfers accurately reflect the implantation process. Higher initial β-hCG levels resulting from single blastocyst transfers can be explained by the higher trophoblastic mass in the extended cultured embryo or by earlier secretion of β-hCG. Analysing factors that influence the β-hCG levels could contribute to a better understanding of the implantation process and may provide an appropriate tool for follow up.
Study funding/competing interest(s): Funding by hospital/clinic(s), Department of Obstetrics and Gynecology, McGill University, Montreal, QC, Canada.
Trial registration number: N/A.

P-249 Single frozen embryo transfers result in similar initial β-hCG levels as single fresh embryo transfers

Abstract withdrawn by the author

P-250 Maternal obesity effects on oocyte quality and compromises developmental ability

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Study question: Does maternal obesity affect to the function of organelles, the redox status of oocyte, and embryonic development?
Summary answer: Maternal obesity reduced mitochondrial membrane potential (MMP) and induced the abnormality of MII spindle morphology in ovulated oocytes. The redox state of oocytes derived from obese mice was more oxidised than that of normal oocytes. In addition, developmental ability of oocytes was compromised by maternal obesity.
What is known already: Overweight and obesity increase the risk of many health problems. In reproductive outcomes of obese women, rates of infertility and pregnancy loss are higher than normal women. The lipid accumulation in oocytes derived from obese mice and the defect of embryonic developmental of these were reported. However, the mechanisms of the defects on oocytes from obese female have not yet been cleared.
Study design, size, duration: Female C57BL/6J mice were fed either a control diet (control) or a hi-fat diet (obesity) for 8 weeks. MMP, the spindle morphology, the redox status, and developmental ability were compared between control oocytes and those from obese mice.
Participants/materials, setting, methods: Morphology of MII spindle was examined immunohistochemically, MMP was evaluated with JC-1 dye. Redox state was assessed by dihydroethidium (DHE) staining. For evaluation of the developmental ability, oocytes were fertilized by ICSI and cultured in KSOM medium.
Main results and the role of chance: After 8 weeks, mice fed high fat diet were significantly heavier than control (28.3 ± 2.8 g vs. 21.2 ± 1.2 g, $p < 0.01$). There was no significant difference on oocyte number between two groups. However in obese mice, degenerative oocytes were observed frequently. In obese mice oocytes, the length of MII spindle was remarkably shorter and

MMP was significantly lower than control. Further, high level of reactive oxygen species (ROS) in oocytes from obese mice was observed by DHE staining. After ICSI, the rate of development to blastocyst stage was significantly lower among oocytes from obese mice vs. control (42.2% vs. 63.3%, $p < 0.05$).
Limitations, reason for caution: This study was conducted using a mouse model with artificially induced obesity. This finding does not directly represent human infertility.
Wider implications of the findings: This study shows clearly that the maternal obesity affected to the function of organelle in oocytes and impaired their developmental competence. Also, our results suggest that some defects in oocytes from obese mice were caused by oxidative stress. Therefore, this study may provide novel insights for understanding obesity-related oocyte dysfunction and new approaches for improvement of the developmental ability of oocytes derived from obese female.
Study funding/competing interest(s): Funding by hospital/clinic(s), There are no conflicts of interest to declare.
Trial registration number: Not applicable.

P-251 Transvaginal ultrasound-guided hydrosalpinx aspiration and fibrin sealant injection in infertile women with hydrosalpinges undergoing IVF treatment

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Study question: The effectiveness of transvaginal ultrasound (TVUS)-guided hydrosalpinx aspiration and fibrin sealant injection (AFSI) in infertile women undergoing IVF, compared with salpingectomy and no treatment of hydrosalpinx.
Summary answer: TVUS-guided hydrosalpinx aspiration and fibrin sealant injection (AFSI) is an effective alternative therapy for hydrosalpinges in infertile women with hydrosalpinges undergoing IVF treatment.
What is known already: Laparoscopic salpingectomy prior to IVF is beneficial in infertile patients with large hydrosalpinges. However, there are limited data with inconsistent results on hydrosalpinx aspiration in infertile patients undergoing IVF and moreover, transvaginal ultrasound-guided hydrosalpinx AFSI in infertile women undergoing IVF has not been investigated yet.
Study design, size, duration: In this retrospective cohort study, a total of 104 consecutive IVF cycles between January 2009 and May 2013 were included in 104 infertile women with hydrosalpinges.
Participants/materials, setting, methods: This retrospective cohort study included 104 infertile women with hydrosalpinges who were treated with TVUS-guided AFSI at the time of oocyte retrieval (27 cycles) or salpingectomy (36 cycles) or never received any treatment for hydrosalpinges (41 cycles, control group). If patients underwent two or more cycles of IVF during the study period, charts corresponding to the 1st IVF cycle were reviewed and data of other IVF cycles except 1st cycle were excluded from this analysis.
Main results and the role of chance: There were no significant differences in patient's characteristics among the AFSI, salpingectomy and control groups. Total dose of recombinant human FSH (rhFSH) used for COS were significantly higher in the salpingectomy group of 2497.2 ± 631.1 IU compared with 2038.9 ± 726.1 IU in the AFSI or 2082.2 ± 789.7 IU in the control group ($P = 0.017$). The numbers of oocytes retrieved, mature oocytes, fertilized oocytes and grade I or II embryos were similar among the three groups. Clinical pregnancy rate was significantly lower in the control group than in the AFSI or salpingectomy group ($P = .011$, $P = .035$, respectively). Embryo implantation rate was also significantly lower in the control group than in the AFSI or salpingectomy group ($P = .001$, $P = .030$, respectively).
Limitations, reason for caution: Our study has a limitation to evaluate the effectiveness of TVUS-guided AFSI of hydrosalpinges at the time of oocyte retrieval due to a small number of sample available and the characteristics of objects were heterogeneous due to its retrospective nature.
Wider implications of the findings: This is the first study on TVUS-guided hydrosalpinx AFSI in infertile patients with hydrosalpinges undergoing IVF and demonstrated that TVUS-guided hydrosalpinx AFSI is at least as effective as salpingectomy and patient-friendly therapy. Therefore, TVUS-guided hydrosalpinx AFSI can be an effective alternative in infertile patients with hydrosalpinges undergoing IVF.
Study funding/competing interest(s): Funding by University(ies), College of Medicine, University of Ulsan, Asan Medical Center, Seoul, Korea.
Trial registration number: No.

P-252 Serological testing for celiac and autoimmune thyroid diseases in infertile women suffering from endometriosis, unexplained infertility and recurrent miscarriage

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Study question: Is there any relation between celiac disease (CD) and endometriosis, recurrent miscarriage and unexplained infertility? What is the prevalence of autoimmune thyroid disease (ATD) in an infertile population?
Summary answer: We observed the highest prevalence of CD in women suffering from RM. There was no difference between women suffering from endometriosis, UEI and RM for the incidence ATD.
What is known already: CD is a chronic autoimmune disorder. CD involves immunologically mediated intestinal damage with consequent micronutrient malabsorption and varied clinical manifestations, and there is a controversial association with infertility. A non-significantly increased risk of CD in women undergoing assisted reproductive technology (ART) was reported.
Study design, size, duration: This prospective cross-sectional study was conducted at Firat University Hospital IVF Unit between April 2012–April 2013 to estimate the risk of developing CD for women who had been diagnosed with endometriosis, RM and UEI. The study population was consisted of 126 infertile women (UEI, $n = 42$; endometriosis, $n = 42$; RM, $n = 42$). RM cases with uterine anomaly were excluded from study.
Participants/materials, setting, methods: The prevalence of gastrointestinal symptoms classified as Rome III criteria were determined with questionnaires. Tests for blood serum antibodies to tissue transglutaminase (tTG) and to gliadin (AG) of IgA and IgG classes, endomysial antibody (EMA), total IgA, anti-thyroid peroxidase (TPO) and anti-thyroglobulin (Tg) were made. Thyroid stimulating hormone and free thyroid hormones were analysed. CD was confirmed with endoscopic bowel biopsies in women with (+) autoantibodies. Statistical analysis were performed with SPSS 16.0 version. The differences for continues variables were analysed with Kruskal-Wallis variance analysis and Mann Whitney U test. The differences for dichotomous variables were tested with chi-square test or Fisher exact test where applicable.
Main results and the role of chance: The mean age and body mass index of study population were 31 ± 5 years and 25.5 ± 4.4 kg/m^2 respectively. The incidence of Rome III criteria symptoms were highest in endometriosis, median in RM and lowest in UEI. The comparison of celiac autoantibodies in participants revealed out significant difference between UEI and RM for parameter of IgG AG. For thyroid function tests and thyroid autoantibodies, significant difference was only observed for parameter of free T3 between UEI and RM. The prevalence of CD in our population was 2% with a rate of 4.7% in RM cases.
Limitations, reason for caution: The study population could be expanded with including control cases.
Wider implications of the findings: Investigation of CD in infertile couples, resistant to recurrent ARTs, could improve fertility outcome.
Study funding/competing interest(s): Funding by University(ies). This study was supported by Firat University Scientific Research Foundation.
Trial registration number: None.

P-253 The external validity of Live birth Predictive model in ART requires a specific assessment: a new proposal based on stagewise nested hypotheses

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Study question: Predictive Live birth models are increasingly used in ART centers to evaluate the opportunity of an IVF/ICSI cycle. However, the prediction accuracy remains poor, as demonstrated by almost all the external

validations. Does it mean that no model is usable so far, or that the validation technique was inappropriate?
Summary answer: A center-specific validation comparing the expected and observed prediction has always been used in historical validation, but may only conclude in poor accuracy, for the simple reason that center effect constitutes the essential variation source. Instead, a stagewise validation successively testing the equality, constant and patient-mix effects will provide an adequate approach.
What is known already: Templeton model (TM) has long been considered as the best predictive model, although recently considered as at least as good compared with Nelson models (Tevelde, 2013). TM was considered as the best model, mainly because only external validation studies were performed. However, all these validations systematically failed, showing a very poor discrimination (area under the ROC curve AuR <0.6) and unsatisfactory calibration, highlighting under- or – over-estimates of predictions for all the validated models.
Study design, size, duration: Retrospective observational data were constituted by 1082 cycles, selected as the last recently documented IVF/ICSI cycles for which all the variables needed to test TM were available. This sample size was decided to detect a clinically meaningful difference of 10% with a power of 85%, at 95% two-sided confidence level.
Participants/materials, setting, methods: Patients were characterized by a median age of 32 [IQ = 30,36], mean BMI of 23.3 ± 4.4 kg/m^2, first attempt for 47%, 62.8% ICSI, mean AMH of 4.3 ± 2.8µg/L. 179 (17%) led to live birth. Our validation method consists in a hierarchically nested model considering the expected prediction as a fixed covariate in a generalized mixed model, 3-stagewise testing equality, intercept, and patient-mix main effects.
Main results and the role of chance: Fitting TM equality on our data provided a very poor discrimination (AuR = 0.59, [0.52–0.69]) and strongly underestimated high birth rates. The second stage testing intercept model provided highly significant AIC reduction with intercept estimates of 1.29 ([1.07, 1.43], $p < 0.001$). The third stage failed to find a significant effect of patient mix coefficients. By fitting the corresponding model to our data, the discrimination significantly increased (AuR = 0.76, [0.71–0.80]), with an almost perfect calibration showing a maximum difference of 2.1% between the true and predicted values, fitted line coinciding with the diagonal (slope = 0.93, [0.85–1.47]). Finally, by entering 3 additional variables (FSH, BMI, smoking), FSH main effect was identified (OR = 1.75 [1.06, 2.87], $p < .001$)
Limitations, reason for caution: This validation is not generalizable to other centers, but this was not the objective. Ideally a multi-center study should confirm the invariance of patient mix coefficient through an adequate mixed model. TM might depend on specificities like cultural disparities and should be compared among countries. In spite of a dramatic increase of discrimination, a value of 0.76 remains relatively modest for regular use in practice.
Wider implications of the findings: In addition to demonstrate the necessity of a specific external validation of a predictive model, our proposal highlights the need to standardize the model in selecting a referent population (in particular maternal age of 30 years). It also suggests the important clinical meaning of the intercept effect interpreted as the center performance, thus providing a unique method of Quality control and bench mark comparison instead of a simple external validation.
Study funding/competing interest(s): Funding by hospital/clinic(s). Funding by commercial/corporate company(ies), University Hospital of Nantes (France), Merck-Serono.
Trial registration number: Does not apply.

P-254 The impact of HLA-G levels and endometrial NK cells in the uterine flushing from primary and secondary unexplained female infertility

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Study question: To determine the value of human leukocyte antigen (HLA)-G levels and endometrial Natural Killer (NK) cell percentages in uterine flushing from primary and secondary infertile women.
Summary answer: This study, for the first time in literature, indicates that lower soluble (s)HLA-G levels and the decrease in endometrial CD56+ KIR2DL4+ NK cells in the uterine flushing are associated with primary unexplained infertility.
What is known already: NK cells are the dominant type of maternal immune cells in the endometrium and they play a major role in determining pregnancy outcomes. HLA-G molecules are non classical HLA class I antigens characterized by a tolerogenic function during pregnancy. In particular, they are expressed by cythotrophoblast and they bind the KIR2DL4 receptor expressed by the uterine NK cells. This interaction controls the activation of the uterine NK cells and promotes the formation of the placenta.
Study design, size, duration: Twenty women affected by primary ($N = 14$) or secondary ($N = 6$) unexplained infertility were recruited. The mean duration of infertility was 2.8 ± 1.7 years and the mean age of the patients was 35.4 ± 3.6 years. Uterine flushing was obtained during Hysterosalpingo-contrast sonography 7–9 days after menstruation.
Participants/materials, setting, methods: sHLA-G levels were tested in uterine flushing samples using HLA-G specific enzyme immunosorbent assay with a specific antibody (MEM-G9). The cells obtained from the uterine flushing pelletization were analyzed by flow cytometry with CD3, CD56, CD158d monoclonal antibodies. The data were compared by Mann Whitney U test and logistic regression.
Main results and the role of chance: sHLA-G levels were undetectable in the uterine flushing samples of primary infertile women, as opposed to women affected by secondary infertility ($p < 0.001$). Furthermore, lower CD56+KIR2DL4+ NK cell percentages were detected in the uterine flushing samples of primary infertile women compared to secondary infertile women ($p = 0.0005$). Hormonal and demographic parameters (FSH, LH, E2, progesterone, prolactin, TSH and FT4 levels, smoke habits, age and weight) proved not to be related with primary and secondary infertility.
Limitations, reason for caution: This study is based on a limited sample size. These data should be confirmed in a larger cohort of subjects.
Wider implications of the findings: These observations are in agreement with the important role of HLA-G molecules and NK cells during pregnancy. This is the first study demonstrating that primary and secondary unexplained infertility cases are characterized by different basal sHLA-G levels and CD56+KIR2DL4+ NK cell percentages. Thus, these factors concur to the achievement of an adequate uterine micro-environment for embryo implantation.
Study funding/competing interest(s): Funding by national/international organization(s). This study was supported by "Alessandro Liberati Young Scientists Award 2013 - Regione Emilia Romagna" PRUA1GR-2013-00000084.
Trial registration number: None.

P-255 A prospective, randomized, double-blind, placebo-controlled Ph1 study to characterize the safety, tolerability, pharmacokinetic/pharmacodynamic profile of NT100, a novel rhG-CSF being developed for use during pregnancy

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Study question: The primary objective of this Ph1 study was to characterize the safety, tolerability, pharmacokinetic (PK), and pharmacodynamic (PD) profile of NT100, a novel rhG-CSF being developed specifically for use in repeat implantation failure and recurrent miscarriage.
Summary answer: NT100 was well-tolerated at all dose levels, exhibited dose-dependent pharmacokinetics, and demonstrated rapid, selective, and drug-dependent up-regulation of circulating Treg cells and down-regulation of circulating Th1 cells consistent with systemic changes associated with maternal-fetal immune tolerance. Importantly, NT100 was not associated with development of anti-drug antibodies.
What is known already: NT100 is a novel drug and this was a first-in-human study. Mechanistically, Treg cells have been implicated in promoting maternal-fetal tolerance, and excess Th1 pro-inflammatory cells have been linked to negative pregnancy outcomes. Additionally, while the class

of rhG-CSF drugs is labeled and approved for use in cancer patients, recent small, single center, randomized trials have suggested a potential benefit of using rhG-CSF in patients with either recurrent implantation failure or recurrent miscarriage.

Study design, size, duration: This was a prospective, randomized, double-blind, placebo-controlled study in 48 healthy women between the ages of 18 and 40. The study included both single- and multi-dose cohorts across three escalating dose levels. Within each of the 6 cohorts, subjects were randomized 3:1 to receive NT100 or placebo.

Participants/materials, setting, methods: NT100 or placebo was administered as a subcutaneous injection either as a single dose or once daily for 10 days. PK samples were analyzed by ELISA, and PD samples were analyzed by multi-color flow cytometry. NT100 was produced by a proprietary rhG-CSF cell bank through recombinant protein manufacturing methods.

Main results and the role of chance: Overall, NT100 was well-tolerated. There was no clinical difference in the rates or severity of adverse events between the placebo and NT100 treatment arms. All events were mild to moderate in severity with no serious adverse events or drug discontinuations. The half-life of NT100 averaged from 2.69–7.62 h across cohorts on Day 1 and 8.20–11.40 h on Day 10. CL/F increased between Day 1 and Day 10 approximately 2.1–3.1 fold and V/F increased by 2.4–8.5 fold. AUC_{0-24} was 31%–45% on Day 10 compared to Day 1. NT100 up-regulated circulating Treg cells and down-regulated circulating Th1 cells in a drug dependent manner. These effects were observed only in the multi-dose cohorts with maximal effects seen by day 3 of dosing.

Limitations, reason for caution: This was a Phase 1 study that investigated the safety, tolerability, and PK/PD profile of NT100. While the pharmacodynamics results are encouraging and suggest a role for NT100 in creating a toleragenic maternal-fetal environment, definitive randomized, double-blind, multi-center, placebo-controlled studies are needed. Phase 2 studies are currently ongoing.

Wider implications of the findings: ART has revolutionized treatment options for couples suffering from infertility, with a focus on therapies and procedures to maximize embryo quality and quantity. Recently, there has been increasing recognition that maternal-fetal tolerance plays a complementary and critical role in the initiation and maintenance of successful pregnancy. These early studies suggest that NT100 may support maternal-fetal tolerance and has potential in the future to complement existing therapies to maximize overall pregnancy and live birth rates.

Study funding/competing interest(s): Funding by commercial/corporate company(ies). This study was funded by Nora Therapeutics.

Trial registration number: Nora Therapeutics NT-02.

P-256 The likelihood of spontaneous conception following cessation of IVF & ICSI treatments

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Study question: What is the likelihood of conceiving spontaneously following cessation of IVF treatment regardless of the IVF outcome and how long does it take for conception to occur?

Summary answer: Our study revealed that overall the cumulative conception rates and live birth rates over a 6-year period were 29% and 24% respectively. 82% of those who conceived did so within 2 years after discontinuation of IVF/ICSI treatments.

What is known already: Only a few studies have reported spontaneous conception following cessation of IVF and ICSI treatments. However the rates of conception varied between these studies due to differences in patient cohort, duration of follow-up and the treatment received.

Study design, size, duration: An anonymous internet-based survey. All registered users of www.ivf-infertility.com received an electronic questionnaire and covering letter over a 6-week period ending in July 2013. The letter invited users to participate if they had received IVF or ICSI in the past; the covering letter explained the background of the study and acted as informal consent.

Participants/materials, setting, methods: The questionnaire addressed issues relating to information before, during and after IVF treatment. Questions prior to treatment included: female age, duration of infertility, parity, if the female had her fallopian tubes checked and the cause of infertility. Details relating to the treatment: number of IVF & ICSI cycles, whether the patient conceived by IVF and the outcome. Details following treatment: did the patient conceive spontaneously, the time taken to conceive, and the outcome of the pregnancy.

Main results and the role of chance: 484 patients responded. Of these, 11 responded that they did not try to get pregnant again as their family was complete or there had been changes in circumstances. A further 70 couples had received IVF using donated egg, donor sperm, donor embryos or had surgical sperm retrieval. The remainder 403 patients met the criteria for inclusion of the study. Overall, the cumulative live birth rates over a 6-year period following cessation of IVF & ICSI were 22% following successful IVF and 31% following unsuccessful IVF. 87% of those who conceived did so within 2 years, 12% within 3–4 years, 2% within 5–6 years and only 1% after 6 years.

Limitations, reason for caution: Our study is retrospective and relied on patient self-reporting with a potential bias, as pregnant women are more likely to participate than disappointed patients. Also, as an internet based survey it may not be a representative of all infertile couples seeking IVF & ICSI treatment.

Wider implications of the findings: Most infertile couples believe that IVF/ICSI is the only option available to them to conceive whereas our study shows otherwise. For most couples if spontaneous conception does happen after cessation of treatment they will be happy. However, some may resent having IVF treatment with the physical, financial and emotional burden they have endured and may suspect that IVF was unnecessary. Furthermore, some couples may not consider contraception and subsequently may face unexpected pregnancy, which may raise many issues.

Study funding/competing interest(s): Funding by hospital/clinic(s). No funding was obtained.

Trial registration number: Following enquiry with NHS UK Health Research Authority, no ethics committee approval was required.

P-257 Advantages of three-dimensional sonographic hysterosalpingosonography with gel foam in the assessment of tubal patency

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Study question: Is the evaluation of the tubal patency and the tubal course with a 3D TVS HyCoSy with gel foam easy feasible?

Summary answer: 3D volume acquisition during gel foam injection allows the visualization of the tubal patency and the tubal course in the space and it is accurate and easy to perform.

What is known already: The TVS HyCoSy with air and saline was a reproducible, simple and low economic cost technique for a real time diagnosis of tubal patency although it was less accurate in tubal occlusion and on tubal course and operator dependent with the need of high experience in sonography. The 3D TVS HyCoSy with ultrasound dedicated contrast media overcame this limits but it was off label for tubal patency and expensive.

Study design, size, duration: A prospective analysis of 132 infertile patients (for a total of 264 tubes) who underwent TVS HyCoSy with Gel Foam (ExEm® Foam GynecologIQ. Farco-Pharma GmbH) and 3D volume acquisition to assess the feasibility of this technique in the evaluation of tubal patency and tubal course.

Participants/materials, setting, methods: 132 women undergoing 3D TVS HyFoSy for visualization of tubal course with two consecutive volume acquisitions during gel foam injection. 2D TVS real time HyCoSy by the detection of saline and air bubbles moving through the tube and around the ovaries was then performed to confirm finally the tubal status.

Main results and the role of chance: After both procedures (3D and 2D evaluations), bilateral tubal patency was observed in 105 patients, bilateral tubal occlusion in 3 patients and unilateral tubal patency in 24 patients. Concordance rate for tubal status between first and second 3D volume acquisition and the final 2D real time evaluation was 84.8% and 97.7% respectively. After the two initial injections and the 3D volume acquisition only 4 patients required further injections and 2D real time to assess final tubal status.
Limitations, reason for caution: No unknown side effects or unexpected concerns on safety of ExEm® were observed in the literature. On the other hand more informations are needed on the effects of the components on embryos. So the safest strategy is to avoid conception in the cycle of tubal patency testing.
Wider implications of the findings: TVS HyFoSy with 3D volume reconstruction of the tubes retains the advantages of conventional 2D HyCoSy and, at the same time, overcomes the disadvantages. 3D volume acquisition during gel foam injection permits the visualization of the tubal course creating images of the tubes on the coronal view and, obtaining a volume, the tubal course in the space can be therefore evaluated.
Study funding/competing interest(s): Funding by University(ies), Department of Obstetrics and Gynecology, University of Rome Tor Vergata, Rome, Italy, Department of Obstetrics and Gynecology, University of Siena, Siena, Italy.
Trial registration number: None.

P-258 Ovarian reserve alterations in premenopausal women with chronic inflammatory rheumatic diseases – impact of rheumatoid arthritis, Behcet's disease and spondyloarthritis on anti-Mullerian hormone levels

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Study question: This study aimed to investigate the potential effect of Behcet's disease (BD), rheumatoid arthritis (RA) and spondyloarthritis (SpA) on ovarian reserve (OR), as reflected by serum anti-Mullerian hormone (AMH) levels.
Summary answer: This is the first study to show the reduced OR in patients with RA, SpA and BD.
What is known already: Recent publications showed a negative influence of systemic lupus erythematosus on female ovarian reserve (OR). Other authors did not find a significant impact of Crohn's disease or early rheumatoid arthritis (RA) on anti-Mullerian hormone (AMH) levels.
Study design, size, duration: Single center, cohort analysis.
Participants/materials, setting, methods: Serum samples of 33 RA, 32 SpA and 30 BD patients without previous cytotoxic – especially cyclophosphamide – treatment were analyzed and compared to age matched, healthy controls. AMH was quantified using a standard ELISA with standard value 1–8 µg/l; values <1 µg/l defined as reduced, <0.4 µg/l as severely reduced fertility. All patients gave written informed consent and filled out a questionnaire on menstrual irregularities, lifestyle, pregnancy outcomes and contraception. For statistical analysis SPSS 19.0 was used and $p < 0.05$ considered statistically significant.
Main results and the role of chance: The median age was 26, 28.5 and 33 years and the disease duration was 6.0, 5.9 and 7.0 years for RA, SpA and BD patients, respectively. Compared to healthy controls the patients had significant reduced AMH levels with a median value for RA of 1.83 (control: 2.44; $p = 0.009$), SpA 1.46 (control: 2.3; $p = 0.013$) and for BD of 1.08 (control: 1.93; $p = 0.007$). Several of the patients even had severely reduced AMH levels. The mean number of children was 0.4 for RA, 0.52 for SpA and 1.0 for BD patients.
Limitations, reason for caution: The number of children in patients with rheumatic diseases is not only limited by the reduced ovarian reserve but by various other influencing factors like the fear of patients or counseling physician.
Wider implications of the findings: This is the first study to show the reduced OR in patients with RA, SpA and BD. Together with the findings in SLE we conclude a negative influence of chronic rheumatic diseases on OR.
Study funding/competing interest(s): Funding by University(ies), University Hospital Tuebingen.
Trial registration number: N/A.

P-259 Relationship of human chorionic gonadotropin levels after oocyte triggering with oocyte maturation and IVF outcomes

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Study question: Can serum hCG levels predict the oocyte clinical performance after trigger injection?
Summary answer: Post triggering hCG levels ≥185 mIU/ml portended improved oocyte quality as reflected in a higher blastocyst formation rate ($p = 0.006$) and a trend toward a better clinical pregnancy rate ($p = 0.013$)
What is known already: The exact mechanism of hCG triggering in maturation and fertilization competence of oocytes and subsequently outcome has remained a debated issue. Prospective multicentre studies have indicated that increased live birth rates and increased number of top-quality embryos when hCG action affect and improve follicular maturation, oocyte quality and fertilization competence in IVF patients.
Study design, size, duration: A prospective, diagnostic test study was conducted between January 2013 and December 2013, 145 patients aged 22–38 undergone IVF cycles except for male infertility and parenteral aneuploidy were included. Patients who have E2 level ranging between 1000–3500 pg/ml were eligible for the study.
Participants/materials, setting, methods: Tertiary academic hospital reproductive unit; women's medical centre. Blood samples to analyze hCG levels were obtained at 12 h following subcutaneously injection of 500 µg r-hCG. We performed logistic regression analysis to predict a cut-point of hCG levels for mature oocyte, fertilization, blastula formation, implantation, clinical pregnancy and miscarriage rates.
Main results and the role of chance: Multivariate regression analysis showed that a hCG titer of 185 mIU/ml or higher at 12 h after trigger injection could predict oocyte clinical performance. Patients ($n = 88$) with serum hCG levels ≥185 mIU/ml had significantly higher mature oocyte (68.8% vs. 42.5%, OR = 1.64, 95% CI 1.47–1.85, $p = 0.027$), blastocyst formation (57.2% vs. 32.8%, OR = 1.77, 95% CI 1.64–1.93, $p = 0.006$) and clinical pregnancy (56.2% vs. 33.7%, OR = 1.71, 95% CI 1.58–1.88, $p = 0.013$) rates. Serum hCG levels were also greater in women who had a low miscarriage rate (4% vs. 12.4%, OR = 0.31, 95% CI 0.23–0.44, $p = 0.003$). No significant difference were found in fertilization ($p = 0.88$) and implantation rates ($p = 0.54$).
Limitations, reason for caution: Our study has a limited sample size. Measuring serum hCG levels after hCG administration for predicting oocyte quality and their development potential has to be adjusted for potential confounders before conducting a clinical trial.
Wider implications of the findings: Research investigating the components of oocyte quality in relation with hCG concentration will enable to test for oocyte developmental competence. If needed adding hCG supplementation after trigger injection may promote a better oocyte maturation and improve developmental competence in IVF cycles.
Study funding/competing interest(s): Funding by University(ies). None.
Trial registration number: None.

P-260 Impact of plasma and follicular fluid BMP15 levels on ICSI-ET cycle outcome

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Study question: Do plasma and follicular fluid (FF) bone morphogenetic protein 15 (BMP15) levels have influence on prediction of fertilization, implantation, clinical pregnancy and live birth rates? Is there any difference between gonadotropin releasing hormone (GnRH) agonist and antagonist down-regulation for the parameters of plasma and FF BMP15 levels?
Summary answer: OPU BMP15 levels showed negative correlation with FF BMP15 levels. Plasma BMP15 level on the day of oocyte pick-up (OPU) showed negative correlation with fertilization rate near significant. OPU BMP15 levels showed inverse relation with grade 1 embryo number.

What is known already: BMP15, a differentiation factor, is secreted from primordial follicles. BMP15 has been shown to inhibit the expression of follicle stimulating hormone (FSH) receptor (FSHR) in rat granulose cells in an FSH-independent manner. In women, there was a direct correlation between an increase in follicular fluid (FF) BMP15 concentration and high oocyte and embryonic quality.
Study design, size, duration: This study was conducted at Firat University Hospital IVF Unit between May 2012–May 2013. The study population was consisted of 82 infertile women undergoing intracytoplasmic sperm injection-embryo transfer (ICSI-ET) cycle that were down-regulated either GnRH agonist (n = 38) or antagonist (n = 44). We excluded the couples with complaint of azoospermia from the study.
Participants/materials, setting, methods: Plasma levels of BMP15 on cycle day 3 (D3) and OPU and FF BMP15 level were analysed with immunosorbent assay. Statistical analysis were performed with SPSS 16.0 version. The differences for continues variables were analysed with Mann Whitney U test. The differences for dichotomous variables were tested with chi-square test or Fisher exact test where applicable.
Main results and the role of chance: There was no relation between BMP15 levels and Antral follicle count and total retrieved oocyte number. OPU BMP15 levels showed negative correlation with FF BMP15 levels (R = 0.25, p = 0.01). OPU BMP15 levels showed inverse relation with fertilization rate near significant (R = 0.22, p = 0.053). There was no difference between agonist and antagonist groups for parameters of D3, OPU and FF BMP15 levels. There was no difference between women became pregnant or not for levels of BMP15. OPU BMP15 levels showed inverse relation with grade 1 embryo number (R = 0.25, p = 0.02). OPU BMP15 levels showed positive correlation with pregnancy outcome (R = 0.35, p = 0.04).
Limitations, reason for caution: The study population could be expanded with including women with decreased ovarian reserve. The possible interaction between BMP15 gene polymorphism and BMP15 levels could be investigated.
Wider implications of the findings: The relation between grade 1 embryo number and OPU BMP15 levels could be improved to select the top quality embryo for obligatory single transfer.
Study funding/competing interest(s): Funding by University(ies). This study was supported by Firat University Scientific Research Foundation.
Trial registration number: None.

P-261 A novel and precise technique to evaluate the gynecology system in a non invasive modality: virtual hysterosalpingography in 10000 cases

Abstract withdrawn by the author

P-262 MIF as a potential biomarker of endometriosis

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Study question: What is the correlation between *MIF*, *CD74*, *COX-2* and *VEGF* in normal endometrium during menstrual cycle and pathophysiology of endometriosis?
Summary answer: The expression of *MIF*, *CD74*, *COX-2* and *VEGF* genes in ectopic, eutopic and normal endometrium during menstrual cycle are Varied. Also women with endometriosis had higher circulating levels of MIF protein as compared to normal controls.
What is known already: Macrophage Migration Inhibitory Factor (MIF) via its receptor, CD74, initiates a signaling cascade that leads to proliferation and survival of cells. MIF binding to CD74 activates p38 signaling pathways that lead to positive effect on the expression of *COX-2*. MIF, via binding to CD74, activates MAPK cascade, hence, resulting in secretion of some inflammatory cytokines and over expression of *VEGF*. Also COX-2 plays an important role in *VEGF*-induced angiogenesis via JNK kinase activation pathways.
Study design, size, duration: All women taking part in this study were between 20–45 years old, had no endometrial hyperplasia or neoplasia. 20 ectopic and 20 eutopic endometriosis tissue and 12 normal endometrium during menstrual cycle as control group were tested in this study. Peripheral blood samples were likely obtained from each group.
Participants/materials, setting, methods: Total RNA was extracted using the TRI reagent and reverse transcribed in the presence of random hexamers. Quantitative real-time polymerase chain reaction (Q-PCR) was performed using cDNA and primers for *MIF*, *CD74*, *COX-2* and *VEGF*. Also, protein level of MIF in blood serum was measured by ELISA assay.
Main results and the role of chance: The mean relative expression of *MIF*, *CD74* and *COX-2* genes were significantly higher in ectopic endometrium in compare to eutopic and control endometrium ($p < 0.05$). In eutopic endometrium of patients affected endometriosis, expression of *CD74*, *COX-2* and *VEGF* genes were significantly higher in compare to control group ($p < 0.05$). However, there were significantly variations in mRNA expression of these genes in normal, ectopic and eutopic endometrium during menstrual cycle ($p < 0.05$). Also women with endometriosis had significantly higher circulating levels of MIF protein as compared to normal controls ($p < 0.05$).
Limitations, reason for caution: Limitations of this study were number of studied endometriosis patients and women with no sign of endometriosis as control.
Wider implications of the findings: Higher expression of *MIF*, *CD74* and *COX-2* genes in ectopic and *CD74*, *COX-2* and *VEGF* genes in eutopic endometrium can be considered as a molecular biomarker for endometriosis development and pathophysiology. Variation in the expression of these genes in normal endometrium during menstrual cycle could play an essential role in reproduction, inflammation and endometrium reconstruction. High level of MIF in blood serum in endometriosis could act as a biomarker in the diagnosis of endometriosis patients.
Study funding/competing interest(s): Funding by national/international organization(s), Royan Institute.
Trial registration number: N/A. This investigation is a case controlled study.

P-263 Endometrial thickness and pregnancy rates after IVF: a systematic review and meta-analysis

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Study question: *Article submitted for publication. What is the clinical significance of endometrial thickness for IVF outcome?
Summary answer: The frequently reported cut-off endometrial thickness (EMT) of 7 mm is related to a lower chance of pregnancy, but it cannot predict pregnancy. EMT therefore has a limited capacity to identify women who have a low chance of conceiving after IVF.
What is known already: Ongoing pregnancy rates per IVF cycle vary between 8.6 and 46.2%. Maternal age has been shown to be a negative predictor for successful IVF outcome, while good morphological embryo quality is positively associated with the chance to conceive after IVF. Furthermore, endometrial pattern, (sub)endometrial bloodflow and EMT have been described as prognostic factors. Their value, however, must be interpreted with caution, as some studies could not find a significant association between these factors and IVF outcome.
Study design, size, duration: A systematic review and meta-analysis was performed. Electronic databases were searched until October 2013. The search resulted in 890 hits. After scrutinizing the titles, abstracts and full-text articles, 22 studies were selected for final inclusion.
Participants/materials, setting, methods: Studies were included that investigated the association between EMT and IVF outcome. Summary Receiver Operating Characteristics (sROC) curves were estimated and ORs with 95% CI were calculated using a Mantel-Haenszel random effect model. Meta-regression was performed to determine if female age and number of oocytes interacted in the estimated effect.
Main results and the role of chance: The overall quality of the 22 included studies was moderate. The estimated sROC curve indicated a virtually absent

discriminatory capacity of EMT in the prediction of pregnancy. A thin endometrium (≤7 mm) was observed in only 2.4% of the reported cases (260/10724). In these cases a trend towards lower ongoing pregnancy and live birth rates for women with EMT ≤7 mm was observed (OR 0.38 (95% CI 0.09 to 1.5)). The probability of clinical pregnancy was significantly lower compared to cases with EMT >7 mm (23.3% versus 48.1%, OR 0.42 (95% CI 0.27 to 0.67)). The relationship between the number of oocytes and female age on the one hand and pregnancy on the other hand was very weak making correction for these variables unfeasible.

Limitations, reason for caution: Many retrospective studies were included. Significant differences disappeared if only prospective studies were included. Although study heterogeneity was low, differences in study quality and population were present. Seven studies could be included in the meta-regression. Finally, the confounding role for oocyte number and female age could not be ruled out.

Wider implications of the findings: Based on the findings of the current review it can be concluded that cancelling IVF treatment cycles seems not to be justified based on solely a thin EMT. However, the results must be interpreted with caution because of methodological weaknesses of the included studies. Further research is needed to investigate the real significance of EMT in IVF. Furthermore, a histology study might be of value to unravel the (patho)physiology at the endometrium level.

Study funding/competing interest(s): Funding by hospital/clinic(s). No funding.
Trial registration number: Not applicable.

P-264 Oxidative stress in the follicle– is it responsible for low response in IVF in young patients

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Study question: This study aimed to determine the levels of oxidative stress (OE) markers, antioxidant enzymes and cytokines in the follicular fluid (FF) of young women with low response in controlled ovarian stimulation cycles compared with fertile oocyte donors, to assess the oxidative stress impact on ovarian reserve.

Summary answer: Our results demonstrate that different levels of OE markers, oxidant enzymes and cytokines in low responder patients as compared to oocyte donors may have a negative impact on ovarian reserve.

These findings support the idea that increased level of OE markers in FF may play an important role in fertility.

What is known already: In the female reproductive system, OE might be related to conditions that limit the success of assisted reproductive techniques.

The oocyte environment in FF is rich in antioxidant enzymes that help to protect the oocyte from oxidative damage. However, studies analyzing the relationship between OE and reduced ovarian reserve in women undergoing gonadotropin stimulation are lacking. Thus far, little is known as to how alteration of the follicular environment leads to a reduced ovarian reserve.

Study design, size, duration: This prospective study compared the levels of antioxidant enzymes, oxidative stress markers and cytokines in FF of 20 patients <35 years, low responder (defined as having five o fewer oocytes retrieved) undergoing *in vitro* fertilization (IVF), and 30 healthy fertile donors during the period of June to November 2013.

Participants/materials, setting, methods: Young low responder patients undergoing IVF treatment at Clinica Tambre and fertile oocyte donors were recruited to participate in this study.

FF samples were obtained on the day of oocyte retrieval and assessed for antioxidant enzymes (GPX, GST), oxidative stress markers (MDA) and cytokines (IL-6, IL-8, TNF- alpha, VEGF)

Main results and the role of chance: Significant variations between the two groups in levels of enzymes directly involved in OS have been observed. In particular, FF antioxidant enzyme glutathione transferase (GST) concentration was significantly decreased in young women with reduced ovarian reserve compared with oocyte donors ($p < 0.001$). However, glutathione peroxidase activity (GPX) was no affected by ovarian reserve.

FF malondialdehyde (MDA) as oxidative stress marker concentration increased in young women with low response compared with oocyte donors ($p < 0.05$). Furthermore, follicular fluid interleukin (IL-6) concentration was significantly higher in young women with reduced ovarian reserve compared with oocyte donors ($p < 0.001$).

No significant differences were found in FF concentrations of interleukin (IL-8), tumoral necrosis factor alpha (TNF-alpha) and VEGF between groups.

Limitations, reason for caution: Low response in ovarian stimulation cycles of young women is associated with increased OS. However, the relations of some antioxidant enzymes like GST and cytokines (IL-8, TNF-alpha, VEGF) with OE and its implication in ovarian reserve needs to be addressed in further studies on a larger sample of women.

Wider implications of the findings: This is the first study analyzing the relationship between oxidative stress and low response in young patients undergoing controlled ovarian stimulation cycles compared with fertile oocyte donors. The results show that the most plausible explanation for low response in young women is oxidative stress.

Knowledge of these perturbations could lead to develop antioxidant therapies for these poor prognosis women undergoing IVF treatment. Antioxidant enzymes could be beneficial by antagonizing the harmful oxygen free radicals.

Study funding/competing interest(s): Funding by national/international organization(s). Financial support received from Fundacion Tambre, Madrid, Spain. The authors report no conflicts of interest.
Trial registration number: FT-112.

P-265 Human spermatozoa are killed specifically by *candida albicans* hyphae

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Study question: The commensal and opportunistic pathogenic yeast *Candida albicans* is known to impair sperm functions. By comparison of *C. albicans* with the closely related species *C. dubliniensis* in sperm co-incubation experiments we set out to unravel specific fungal traits which are important for sperm-*C. albicans* interaction.

Summary answer: Spermatozoal killing by *C. albicans* was strongly associated with the production of fungal hyphae, which were only poorly produced by *C. dubliniensis*. Moreover, evidence suggested that specific filament co-regulated factors of *C. albicans* are crucial for sperm interaction, rather than hyphae *per se*.

What is known already: Various microorganisms, including *C. albicans*, are known to display adverse effects on human spermatozoa. Yet, little is known of the molecular basis of sperm-microbe interaction. *C. albicans* and *C. dubliniensis* are closely related, dimorphic yeasts which are frequent commensals and pathogens of vaginal mucosae. *C. albicans* is more virulent than *C. dubliniensis*. Hence, the comparison of the two species allows the identification of putative pathomechanisms, thereby offering new insights in sperm microbe interaction.

Study design, size, duration: not applicable/ experimental *in vitro* analysis for basic research.

Participants/materials, setting, methods: Human semen samples were obtained by masturbation from normozoospermic donors, provided with informed consent and ethics committee approval. Co-incubation experiments were conducted *in vitro* using freshly prepared human spermatozoa, *C. albicans* and *C. dubliniensis* fungal cells. Live and dead spermatozoa were discriminated by staining and fluorescence microscopy.

Main results and the role of chance: Co-incubation experiments discovered that *C. albicans* binds and kills spermatozoa more effectively than *C. dubliniensis*. In accordance, a *C. albicans* knock-out mutant, which is defective in hyphae formation, also displayed decreased sperm binding and killing activity. Interestingly, a *C. albicans* mutant, which is unable to form hyphae but is known to still express major hyphae associated proteins, maintained the ability of sperm attachment and killing. These findings suggest that hyphae specific factors are essential for sperm interaction, rather than *C. albicans* hyphae *per se*.

Limitations, reason for caution: The present results are obtained by *in vitro* analysis of human spermatozoa with fungal model organisms. In general, such basic *in vitro* findings may not directly be correlated with the complex *in vivo* situation but require future studies.
Wider implications of the findings: The female genital tract is colonized by diverse microbial species and frequently infected by pathogens which may also be transmitted by male ejaculate. Little is known on potential direct effects of commensal or pathogenic microorganisms on human spermatozoa. In this context, *C. albicans* has poorly been investigated. Our results underline the importance of *Candida* dimorphism for host-pathogen interaction and shed new light in a potential link between microbes and host infertility.
Study funding/competing interest(s): Funding by University(ies), Department of Obstetrics and Gynecology, University of Wuerzburg, IZKF Würzburg; Hans Knoell Institute Jena; no competing interests.
Trial registration number: No number available.

P-266 Association between genotype for a leukemia inhibitory factor (LIF) gene polymorphism in women and pregnancy outcomes after ART

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Study question: Is there an association between genotype for the leukemia inhibitory factor (LIF) single nucleotide polymorphism (SNP) T/G (rs929271) polymorphism in women and pregnancy outcomes after IVF/ICSI?
Summary answer: The G/G LIF genotype in women was associated with increased implantation and ongoing pregnancy rates after IVF/ICSI.
What is known already: LIF is a multifunctional cytokine produced and secreted by the endometrial glands of the uterus. LIF plays a critical role in embryo development and blastocyst implantation. LIF expression increases during the onset of implantation, acting to prepare the uterus to be receptive to the blastocyst.

The literature provides evidence that certain gene polymorphisms are associated with implantation failure and pregnancy loss after IVF/ICSI techniques. However, studies of LIF polymorphisms are still scarce.
Study design, size, duration: A prospective cohort study was conducted from 03/2012 to 07/2013 in 353 infertile women subjected to IVF/ICSI protocols. The patients were genotyped for the LIF SNP T/T (n = 139), T/G (n = 181) and G/G (n = 33). All procedures were performed under the same clinical/laboratory conditions.
Participants/materials, setting, methods: DNA was extracted from peripheral blood samples taken from each participant. The LIF SNP T/G (rs929271) was genotyped using real-time PCR. Cumulative results (fresh and frozen cycles) were analyzed. Fisher's exact, ANOVA, Kruskal-Wallis and student's T tests were used.
Main results and the role of chance: Hardy-Weinberg genotype distributions of the entire sample indicated concordance between the observed and expected frequencies. The results showed that the G/G genotype in women was associated with higher ongoing pregnancy rates (Table 1).
Limitations, reason for caution: Additional validation of the analyzed SNP (increasing the number of cases) will be important to provide more information about the potential use of this polymorphism. Differences in the genetic backgrounds of various ethnic populations should also be considered.
Wider implications of the findings: This SNP (rs929271) could be used as a susceptibility marker capable of predicting implantation efficiency. The ability to predict ongoing pregnancy rates using genetic markers during IVF/ICSI treatment can encourage patients to undergo additional cycles of ART. In the group with the best prognosis (genotype G/G), the number of transferred embryos could be reduced to avoid complications associated with multiple pregnancies.
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 authorized by the local ethics committee.

Table 1: Results.

	Women's genotypes			
	T/T	T/G	G/G	*P*
n	139	181	33	
Age (years)	36.0 ± 4.2	35.3 ± 4.2	34.8 ± 4.5	0.19
Transfers (*n*):Total	1.4 ± 0.8	1.4 ± 0.8	1.2 ± 0.4	0.28
Transfers (*n*):Fresh/Frozen	1.2 ± 0.6/0.3 ± 0.6	1.2 ± 0.6/0.3 ± 0.6	1.1 ± 0.4/0.1 ± 0.3	0.66/0.43
Embryos transferred (*n*):Total	3.2 ± 2.0	2.9 ± 2.0	2.7 ± 1.4	0.11
Embryos transferred (*n*):Fresh/Frozen	2.7 ± 1.7/0.5 ± 1.1	2.4 ± 1.6/0.5 ± 1.2	2.3 ± 1.3/0.5 ± 0.7	0.12/0.88
Implantation rate	16.1%[a] (72/447)	18.9%[b] (100/530)	30.7%[a,b] (27/88)	0.002[a]/0.01[b]
Clinical pregnancy rate/transfer	29.2%[a] (59/202)	30.9%[b] (80/259)	50%[a,b] (20/40)	0.01[a]/0.02[b]
Ongoing pregnancy rate/transfer	18.8%[a] (38/202)	22.4%[b] (58/259)	42.5%[a,b] (17/40)	0.003[a] /0.01[b]
Ongoing pregnancy rate/patient	27.3%[a] (38/139)	32.0%[b] (58/181)	51.5%[a,b] (17/33)	0.01[a]/0.04[b]

Values within the same row with the same superscript letter were significantly different.

P-267 Ongoing pregnancy rates in intrauterine insemination are affected by late follicular phase progesterone levels

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Study question: Do progesterone levels on the day of hCG administration affect clinical outcomes in intrauterine insemination (IUI)?
Summary answer: High serum progesterone levels on the day of hCG administration in intrauterine insemination cycles significantly decrease ongoing pregnancy rates.
What is known already: Progesterone is absolutely essential in the establishment and maintenance of pregnancy. It has been demonstrated that secretion of large amounts of estradiol and progesterone could negatively affect ongoing pregnancy rate by decreasing endometrial receptivity in assisted reproduction techniques. The influence of premature increase progesterone levels in stimulated intrauterine insemination cycles has been less studied, but this information could be useful in order to match the time of insemination with the window of implantation.
Study design, size, duration: A retrospective study was performed in 11 private clinics belonging to IVI group. Patients undergoing IUI cycles with recombinant FSH from January 2012 to August 2013 were included in the study (n = 2839). Blood samples were obtained on the day of hCG administration for estradiol (E2) and progesterone (P4) analysis.
Participants/materials, setting, methods: Patients were started on recombinant FSH on day 3. Presenting at least 1 follicle ≥17 mm in the scan was the criteria for hCG administration. Patients were subdivided into six groups depending on progesterone concentration: p90. Clinical outcomes of each subgroup were analyzed.
Main results and the role of chance: Mean age was 35.2 years (95% CI 35.0–35.3), mean BMI was 22.1 kg/m² (95% CI 20.3–23.9), mean follicles ≥17 mm was 1.3 (95% CI 1.25–1.35), mean days of stimulation were 9.1 (95% CI 10.0–10.3); mean total FSH dose administered was 568 UI (95% CI 558–578), mean estradiol and progesterone levels on the day of hCG were 396 pg/ml (95% CI 385–406) and 0.5 ng/ml (95% CI 0.48–0.53) respectively.

Progesterone concentrations were significantly higher ($p < 0.001$) as long as estradiol concentration and follicles ≥17 mm increased.

We observed significant differences in ongoing pregnancy rate ($p = 0.003$) among the different progesterone subgroups: p90 (P4 ≥ 1.1 ng/ml), 6.3%.
Limitations, reason for caution: One of the major limitations of this study is that it was an observational design, although the large number of cycles and different settings make the implications interesting.
Wider implications of the findings: The presence of significant differences in ongoing pregnancy rate when progesterone concentration on the day of hCG administration was elevated, may help clinicians to counsel patients about reduced success rates in intrauterine insemination, but also managing the time of insemination to match it with the window of implantation.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), IVI Madrid.
Trial registration number: None.

P-268 Should intrauterine inseminations be performed under ultrasound guidance

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Study question: Does the use of ultrasound guidance facilitate cervical catheterization and increase pregnancy and live birth rates in intrauterine insemination (IUI) cycles?
Summary answer: Ultrasound guidance does neither facilitate cervical catheterization nor significantly increases pregnancy and live birth rates in IUI cycles.
What is known already: Many studies have shown the interest of ultrasound guidance for intrauterine embryo transfer in *in vitro* fertilization (IVF) cycles. The main argument put forward by authors is that ultrasound guidance makes cervical catheterization easier, leading to less traumatic embryo transfer, allowing a reduction in uterine contractions and a subsequent increased pregnancy rate. Concerning IUI cycles, only 3 studies have examined the interest of ultrasound guidance, with contradictory results.
Study design, size, duration: This prospective, monocentric, randomized study was conducted between February 2011 and November 2012 in a total of 190 cycles performed in 142 patients eligible for intrauterine inseminations cycles.
Participants/materials, setting, methods: Participants were recruited among patients undergoing IUI cycles in the ART Unit of the University Hospital of Nantes. Patients were randomized at the time of IUI within 2 groups, i.e. ultrasound guidance or not. Ease of cervical catheterization, pregnancy and live birth rates were then compared between these 2 groups.
Main results and the role of chance: 92 IUI were performed under ultrasound guidance, whereas 98 were performed without ultrasound guidance. The proportion of IUI cycles with easy catheterization was similar in both groups (79 and 78.5%). Similarly, the proportion of IUI cycles requesting a change of catheter was comparable in both groups (5.4 and 9.18% respectively). Biochemical pregnancy rate and live birth rate were 21.7% and 16.3% in the ultrasound group, and 14.2% and 11.2% in the group without ultrasound. These differences were not statistically significant.
Limitations, reason for caution: Despite the relatively large size of the population, the number of patients that was necessary in order to reach statistical significance was not calculated a priori. Despite the monocentric setting, several operators performed the inseminations. However, all the operators were trained and skilled for IUI, according to local guidelines.
Wider implications of the findings: These results do not lead us to recommend the use of ultrasound in IUI cycles.
Study funding/competing interest(s): Funding by hospital/clinic(s), Department of Reproductive Medicine, ART unit, University Hospital of Nantes, France, Assisted Reproductive Technology Unit, Hospital of Saint-Nazaire, Cité Sanitaire, France.
Trial registration number: local IRB approved study.

P-269 Detection of follicle stimulating hormone receptor splice variants in women undergoing *in vitro* fertilization

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Study question: Do FSH receptor splice variants affect ovarian stimulation parameters and IVF outcome?
Summary answer: The group of women expressing the FSHR isoforms tended to have a higher number of follicles and a higher number of oocytes, taking into consideration the small number of participants in this pilot study.
What is known already: Although inactivating FSHR mutations result in severe reproductive phenotype, it seems reasonable that subtle genetic variations of the receptor, such as polymorphisms and alternative splicing, may contribute to functional perturbations, subfertility or infertility. Recently, investigators identified four abnormal FSHR splicing products in the FSHR mRNA. All of them affected the extracellular ligand-binding portion of the receptor without causing a frameshift.
Study design, size, duration: Pilot study, including a total of 15 women undergoing IVF. The study was completed within a 6-month period.
Participants/materials, setting, methods: Controlled ovarian stimulation was conducted through a short GnRH- agonist or- antagonist protocol. Cumulus cells were collected on the day of oocyte retrieval, RNA was extracted, cDNA was synthesized and RT-PCR was applied using specific primers, spanning all exons of the FSHR gene. Results were visualized after agarose gel electrophoresis.
Main results and the role of chance: All women expressed the G6PDH gene, which was used as control gene. Women were assigned into 2 groups, according to their expression of FSHR isoforms. Seven out of 15 women (46.7%) expressed one or more FSHR isoforms, whereas 8 out of 15 (53.3%) did not express any isoform. In the group of women expressing FSHR isoforms, 1 out 7 presented three splice variants, 2 out 7 presented two splice variants, and 4 out of 7 presented a single splice variant. The group of women expressing any of FSHR isoforms tended to have a higher number of follicles (p value = 0.07) and a higher number of oocytes (p value = 0.06).
Limitations, reason for caution: The limited number of participants in the study, may not allow drawing definite conclusions.
Wider implications of the findings: A larger scale study may elucidate whether the detection of FSH receptor isoforms is a useful tool in the prognosis of women undergoing IVF and will also determine whether certain isoforms have a better prognosis compared to others. In this context the study of FSHR splicing variants may offer a new way to interpret the genetic profile of women undergoing IVF.
Study funding/competing interest(s): Funding by University(ies), School of Medicine University of Athens.
Trial registration number: Master entitled Reproductive Regenerative Medicine.

P-270 Low progesterone value on day of hCG yields increased implantation and live birth rate in high responders compared to poor responders in GnRH-antagonist IVF cycles

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Study question: To examine the association between progesterone level on the day of hCG administration in poor, good and high responders with implantation and live birth rate in women undergoing rFSH/GnRH antagonist IVF treatment protocol.
Summary answer: Low progesterone (≤1.5 ng/ml) level on day of hCG administration yields increased implantation and live birth rate with single blastocyst transfer in high responder (number of retrieved oocytes >15) compared to poor responder (number of retrieved oocytes <4) women undergoing treatment with rFSH and GnRH antagonist during controlled ovarian stimulation.
What is known already: Elevated preovulatory progesterone is negatively associated with lower ongoing pregnancy rates in women treated with long GnRH agonist protocol, but no significant difference in live birth rate was observed in GnRH antagonist cycles. Previous studies indicated that the threshold of serum progesterone increases with the ovarian response. Recently it has been demonstrated that the chance of ongoing pregnancy is not compromised in high responders wherein elevation in progesterone levels occurs more frequently.
Study design, size, duration: In this retrospective cohort study carried out during January 2011 and December 2012, a single blastocyst transfer was done in 386 women with mean age of 31.0 ± 4.0 years at our private IVF centre.
Participants/materials, setting, methods: Using standard controlled ovarian stimulation protocol with recombinant FSH and GnRH antagonist, injection hCG 5000 IU was administered when at-least two lead follicles measured ≥18 mm diameter. Serum estradiol, LH, progesterone levels were measured on the same day. A single blastocyst was selected for transfer following accepted gradation scoring method.
Main results and the role of chance: Each of the poor, good and high responder groups was divided into two sub-groups (≤1.5 ng/ml and >1.5 ng/ml) based on serum Progesterone on day of hCG. No significant difference was found in LBR in two sub-groups of poor responders (P: 0.2341) and good

responders (*P*: 0.1166). However, significantly higher LBR was observed in low progesterone subgroup as compared to elevated progesterone subgroup in high responders (*P*: 0.0348). Intercomparison of high and poor responder groups with progesterone values showed significantly higher LBR in high responders with low progesterone (*P*: 0.0239). No significant difference was found in elevated Progesterone subgroups between poor responders and good responders or high responders.
Limitations, reason for caution: Smaller sample size of this study is a major limitation. Selection of single blastocyst for transfer has to be meticulous in order to avoid bias of compromising on quality from a limited number available in poor responder group as against multiple good-quality blastocyst availability in good/high responder group.
Wider implications of the findings: This study challenges the most recent finding that elevated P levels in high responders do not compromise with pregnancy rate. This is also the first study involving intercomparison of progesterone levels in poor, good high responder women undergoing single blastocyst transfer.
Study funding/competing interest(s): Funding by hospital/clinic(s), Vaunsdhara Clinic and Assisted Conception Centre, Nagpur, India.
Trial registration number: Not applicable.

P-271 Evaluation of biological efficiency by live birth per oocyte ratio of over 2500 oocytes collected in mild stimulation IVF cycles

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Study question: To evaluate the biological efficiency of oocytes collected in mild stimulation IVF cycles. Live Birth per Oocyte ratio was used as an indicator for biological efficiency of oocytes in different age and ovarian reserve groups.
Summary answer: Lower stimulation protocols appear to be beneficial for the women over the age of the 35 years in terms of preserving the health of the oocytes, increasing the Live Birth to Oocyte ratio.
What is known already: In a recent large single centre study in women undergoing conventional IVF it was concluded that only 5% of oocytes were intrinsically capable of producing a baby in women <35 years and that this number falls with age to <1% in women >40 years. It is common practice to increase the dose of gonadotropins for stimulation as the patient's age increases to overcome lower ovarian responsiveness in Controlled Ovarian Hyperstimulation (COH) cycles.
Study design, size, duration: We carried out a retrospective analysis of Live Birth per Oocyte (LBO) ratio in a 3-year period 2010–2012 in consecutive patients undergoing mild stimulation IVF. All women had low gonadotropin stimulation in antagonist cycles. The total dose of gonadotropins, average number of oocytes collected, live birth rate per cycle and LBO ratios were calculated for 4 different age groups and 5 different antral follicle count (AFC) groups which were measured in pre-IVF scans.
Participants/materials, setting, methods: There were 2576 oocytes from 440 embryo transfer cycles. The mean age of the patients was 35.9 ± 4.2 years.
Main results and the role of chance: The mean total dose of gonadotropins per cycle was 1473 ± 584 IU/L and the Live Birth to Oocyte ratio (as a percentage) was 8.1, 5.0, 5.0, and 1.9 for age groups <35, 35–37, 38–39, 40–42 years respectively. When births from frozen embryo transfer cycles were included the LBO ratio was 8.6, 5.5, 5.6, 2.1, 3.7 respectively. When patients were pooled as per the AFC, LBO ratios were 7.2, 8.9, 5.0, 3.9, 1.9, for AFC's of 1–9, 10–14, 15–20, 21–26, >27 respectively. The Live Birth per Cycle rate was 42.9, 34.7, 35.0, 15.7 and 7.1 for age groups as above respectively.
Limitations, reason for caution: Retrospective data analysis.
Wider implications of the findings: Lower stimulation protocols appear to be beneficial for the women over the age of the 35 years in terms of preserving the health of the oocytes, increasing the Live Birth to Oocyte ratio. This may explain why the live birth rates per cycle were not negatively affected by mild stimulation regimes where fewer oocytes are retrieved. As the baseline AFC increases the LBO falls, for the same age group, which may be an indirect proof that high numbers of follicles growing in the same ovary negatively affects the quality of a fraction of the oocytes.
Study funding/competing interest(s): Funding by hospital/clinic(s), Create Health Clinic, London.
Trial registration number: N/A.

P-272 Outcomes of 11 years of assisted reproductive techniques in HIV infected women

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Study question: To assess the impact of HIV infection on the outcomes of ART.
Summary answer: Our data suggests that HIV-infected women, who have a low viral load and good immunity status, have reasonable chances of becoming pregnant with ART. However, their clinical pregnancy rates and delivery rates were lower than the rate observed in uninfected patients.
What is known already: The fertility potential of HIV positive women is still controversial: several studies have suggested that HIV infection and antiretroviral treatment could be associated with impaired ovarian reserve as well as an earlier onset of menopause, whilst others have shown responses to ovarian stimulation comparable to uninfected patients.
Study design, size, duration: Cohort study of 95 couples in which the woman was HIV positive, who had IVF/ICSI treatment in our fertility clinic between 2002 and 2013.
Participants/materials, setting, methods: 242 cycles of IVF/ICSI were performed. The outcomes were compared to those of 8002 cycles of IVF/ICSI performed in 3725 uninfected couples.
Main results and the role of chance: The mean patient age was 35, 7 ± 4.5 years. Ninety percent of the patients had antiretroviral treatment before starting IVF. The viral load was undetectable in 77% of the cycles and the mean CD4+ count was 598.2 ± 204 cells/mm^3. Two hundred twenty oocyte aspirations were performed and 1202 oocytes were retrieved leading to 584 embryos. The clinical pregnancy rate and delivery rate per embryo transfer were respectively of 23.4% and 16.4% compared to 33.2% and 25.2% in the control population (significantly higher than those observed in the HIV population, $P = 0.03$). No significant correlation was found between the number of fertilized oocyte, embryo quality, clinical pregnancy rate, delivery rate and parameters of the HIV infection such as the use of the antiretroviral treatment, the viral load and the CD4 count.
Limitations, reason for caution: The limit of our study is its retrospective design. Several confounding factors of infertility could therefore not be taken into account.
Wider implications of the findings: Further studies and specially basic medical research are needed to better understand the mechanisms by which HIV and antiretroviral treatments could affect the fertility of infected people.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Fertility clinic and laboratory for Research on Human Reproduction and IVF centre of the Université Libre de Bruxelles, and the Immunodeficiency Treatment Unit of the Erasmus University Hospital, Brussels, Belgium.
Trial registration number: It is a cohort study.

P-273 Cervical surgery and the fertility effect- cSAFE results

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Study question: Treatment of precancerous lesions of the cervix reduces the risk of cervical cancer but there is concern regarding the potential impact of excisional treatments on future fertility. This study examines whether excisional cervical surgery has an impact on subsequent fertility and whether the size of the specimen removed influences outcome.

Summary answer: Fertility was similar in women who had one LLETZ procedure compared to controls. There was no increased time to conception. The size of specimen removed did not affect outcome. Women who had a cone biopsy or more than one LLETZ had fewer pregnancies but no increase in time to conceive.
What is known already: The obstetric impact of LLETZ surgery has been a source of concern with particular reference to preterm birth, with numerous studies and meta-analyses performed. Despite concerns that LLETZ treatment can produce anatomical changes which could disrupt fertility, few studies have focused on subsequent fertility. Most of the studies to date were not case controlled and were limited by size and time to follow up.
Study design, size, duration: Retrospective cohort study with institutional ethical approval of women 24 to 40 years, who attended the colposcopy services in the National Maternity Hospital between 2001 and 2007. Cases included women, who had cervical surgery, (LLETZ or cone biopsy); controls were women who had attended colposcopy but did not have surgery.
Participants/materials, setting, methods: A postal questionnaire was sent to 3590 women of reproductive age; 1795 of whom had at least one excisional treatment (cases) and 1795 had no treatment (controls). The records were reviewed to confirm the clinical details and volume of tissue excised.
Main results and the role of chance: 1355 women replied. 759 had a history of a one previous LLETZ, 37 had a cone biopsy, 22 had more than one LLETZ and 537 women had no excisional treatment. There was no difference in the proportion of women who had been pregnant since their visit – 434/759 women in the surgery group and 296/534 in the control group ($p = 0.53$). The reported time taken to conceive (TTC) was not significantly longer in the LLETZ group – 7.76 months versus 8.43 for control group ($p = 0.37$) and there was no significant correlation found between TTC and volume ($p = 0.69$) or depth ($p = 0.52$) of cervix removed. No difference was noted between the groups with regards to those who could have conceived but did not – 57 in LLETZ group versus 34 in control group ($p = 0.56$). Those women ($N = 59$) who had either a cone biopsy or more than one excisional treatment had significantly fewer pregnancies than non-treatment controls – 24/59 versus 296/534 ($p = 0.03$) However, no difference was reported in their TTC ($p = 0.54$) or difficulties conceiving ($p = 0.07$).
Limitations, reason for caution: One LLETZ surgery did not appear to have an impact on a woman's ability to conceive in this study. However caution must be advised as the pre-treatment length of a woman's cervix can vary and larger LLETZ treatments may have a greater impact in select cases.
Wider implications of the findings: In this largest study to date, women, who had one LLETZ, did not take longer to conceive, had similar numbers of pregnancies and reported no increase in subfertility compared to controls. For the first time the depth and volume of tissue removed was studied and did not impact subsequent fertility. These results are reassuring for women having one LLETZ. More study is required in those having repeat treatment or cone biopsy and on pre-operative factors such as cervical depth and volume.
Study funding/competing interest(s): Funding by hospital/clinic(s), Merrion Fertility Clinic funded this study.
Trial registration number: NCT01944241.

P-274 Intrauterine administration of autologous mononuclear cells (AMC) before embryo transfer (ET) in blastocyst stage: Is it useful approach in cases with recurrent implantation failure (RIF)

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Study question: Intrauterine administration of hCG-primed autologous mononuclear cells or hCG before embryo transfers has recently been proposed as an alternative approach in order to improve pregnancy rates in fresh or frozen ET cycles. This study questions whether this approach can be comparable or superior over blastocyst stage embryo transfers.
Summary answer: Although positive results can be obtained in isolated cases, overall, intrauterine administration of AMC in blastocyst transfers do not bring any significant benefit in terms of clinical outcome in RIF cases over blastocyst stage ET.
What is known already: Blastocyst-stage ET and intrauterine administration of autologous mononuclear cells has recently been proposed as successful alternative treatment approaches in both fresh and frozen ET cycles for RIF patients. While blastocyst-stage ET aims to select the best embryo for implantation and synchronize the endometrium, the latter approach is developed in order to improve the endometrial receptivity.
Study design, size, duration: This retrospective comparative study has been performed in Bahceci Fulya and Umut Assisted Reproductive Technologies Centres between November 2012–December 2013. It includes 639 cycles, in which couples has at least 2 unsuccessful trials with good embryo qualities in their previous trials, with no known endometrial pathologies.
Participants/materials, setting, methods: During study period, the technique has been offered to candidate couples and in 185 cycles (88 fresh and 97 frozen) intrauterine administration of AMC performed in combination with their embryo transfers. Four hundred fifty four cycles (181 fresh and 273 frozen) with the same inclusion criteria but have not undergone the procedure were used as controls. The procedure involved the isolation and culture of autologous mononuclear cells 5 days prior to embryo transfer and intrauterine administration of these cultured cells 3 days before ET.
Main results and the role of chance: Female age, previous trials, ovarian stimulation characteristics, cycle cancellation rates as well as general laboratory parameters (mean oocytes collected, fertilized, cell number during early cleavage stages etc.) were similar in both groups. In both study and control groups as well as intragroup comparisons for fresh and frozen ETs, similar biochemical (BPR) and clinical pregnancy (CPR) as well as implantation rates (IR) were obtained.

	Control group		Study group	
	Fresh ET	Frozen ET	Fresh ET	Frozen ET
Cycles (*n*)	181	273	88	97
BPR	45.3%	52.7%	46.5%	51.5%
CPR	39.7%	43.9%	40.9%	44.3%
IR	27.8%	33.4%	25.0%	28.8%

Limitations, reason for caution: The number of cases in the study should be increased in order to draw a firm conclusion. Also, this approach should also be examined in cleavage stage embryo transfers or the cases with higher previous failed trials.
Wider implications of the findings: Our results show that intrauterine administration of AMC do not bring any additional benefit over blastocyst selection and transfer in cases with at least 2 previous failed trials.
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: None.

P-275 Early embryo development in terms of body composition by multifrequency bioelectrical impedance analysis

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Study question: Is there any difference of early embryo development after cIVF or ICSI in terms of the body composition including the body protein ratio (BPR), body fat ratio (BFR), body water ratio (BWR) and body mineral ratio (BMR)?
Summary answer: In BPR, BWR and BMR, the 2PN rate in the lower group was significantly lower than that in the higher group. In BFR, the 2PN rate in the higher group was significantly lower than that in the lower group.
What is known already: Getting nutrition is essential for surviving. Moreover, the right kind of food is important not only for body but also for good-quality oocytes. Excessive weight in women undergoing ART has been associated with lower pregnancy rate, lower live-birth rate and fewer normal fertilized eggs. The patient's dietary consumption of proteins and carbohydrate influences the outcome of IVF. However, the effect in terms of body composition at early embryo development stage is still unclear.
Study design, size, duration: This is a retrospective study of early embryo development in 3,716 oocytes after cIVF or ICSI in 279 cycles from December 2012 to January 2014. BPR, BFR, BWR and BMR were measured by multifrequency bioelectrical impedance analysis (In Body 720) at the day of the oocyte retrieval.
Participants/materials, setting, methods: The 2PN rate (number of 2PN oocytes/number of oocytes retrieved) and the good-quality embryo rate on

the day 3 (number of good-quality embryos on the day 3/number of oocytes retrieved) were analyzed in terms of BPR, BFR, BWR and BMR.

Main results and the role of chance: In BPR, BWR and BMR, the 2PN rates in the lower group were significantly lower than those in the higher group. In BFR, the 2PN rate in the higher group was significantly lower than that in the lower group. Secondly, the good quality embryo rate on the day 3 in the lower group was significantly lower than that in the higher group in BWR. The good quality embryo rate on the day 3 in the lower group was significantly lower than that in the intermediate group in BMR.

Table 1: 2PN rate in terms of body composition parameters.

	Lower group (A)	Intermediate group (B)	Higher group (C)	
BPR	56.7% (123/217)	64.8% (2241/3459)	85.0% (34/40)	A vs. B:$P < 0.05$, A vs. C:$P < 0.01$, B vs. C:$P < 0.01$
BFR	64.6% (464/718)	65.0% (1863/2866)	53.8% (71/132)	A vs. C:$P < 0.05$, B vs. C:$P < 0.01$
BWR	56.9% (128/225)	64.9% (2103/3241)	66.8% (167/250)	A vs. B:$P < 0.05$, A vs. C:$P < 0.05$
BMR	48.8% (41/84)	64.7% (2323/3592)	85.0% (34/40)	A vs. B:$P < 0.01$, A vs. C:$P < 0.01$, B vs. C:$P < 0.01$

Table 2: Good-quality embryo rate on the day 3 in trms of body composition parameters.

	Lower group (A)	Intermediate group (B)	Higher group (C)	
BPR	17.5% (38/217)	22.6% (783/3459)	17.5% (7/40)	N.S.
BFR	22.0% (158/718)	22.6% (648/2866)	16.7% (22/132)	N.S.
BWR	18.2% (41/225)	22.2% (719/3241)	27.2% (68/250)	A vs. C:$P < 0.05$
BMR	11.9% (10/84)	22.6% (811/3592)	17.5% (7/40)	A vs. B:$P < 0.05$

Limitations, reason for caution: After nutritional guidance, change of body composition and early embryo development didn't be carried out.

Wider implications of the findings: In terms of body composition, the 2PN rate of patients with low body protein ratio, low body water ratio or low body mineral ratio was significantly low. The 2PN rate of patients with high body fat ratio was significantly low.

Study funding/competing interest(s): Funding by hospital/clinic(s). There are no conflicts of interest in this study.

Trial registration number: N/A.

P-276 Effects of the antifreeze proteins on the vitrification of mouse oocytes: comparison of three different antifreeze proteins

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Study question: Can antifreeze proteins (AFPs) from three different sources improve efficacy of vitrification?

Summary answer: AFPs treatment during mouse oocyte vitrification showed significant improvement in oocyte development, meiotic spindle organization and intracellular ROS levels. Among the three APF-treated groups (FfIBP, pLeIBP and TYPE III AFP groups), FfIBP and LeIBP groups showed significantly higher normal meiotic spindle rates and mitochondrial activity than Type III AFP group.

What is known already: Rubinsky discovered that upon vitrification of immature oocytes and two-cell stage embryos of mice AFGPs at 40 mg/ml produced dramatic improvements in the morphological integrity of the samples and suggested that AFPs have the ability to inhibit ice formation and to stabilize the plasma membrane.

Study design, size, duration: The MII oocytes were obtained from 4-week-old BD-F1 mice. AFPs from bacteria (FfIBP), yeast (LeIBP) and fish (Type III AFP) added to vitrification and warming solutions individually. Meiotic spindle and DNA double strand breaks, intracellular ROS and mitochondrial activity were analyzed.

Participants/materials, setting, methods: Vitrification of oocyte was performed with the CryoTop (equilibration 1: 7.5% EG and 7.5% PROH for 5 min, equilibration 2: 15% EG, 15% PROH and 0.5 M sucrose for 1 min). Warming was performed in three steps with decreasing concentration of sucrose (1, 0.5 and 0.25 M sucrose).

Main results and the role of chance: AFPs treatment can improve oocyte quality and embryo development. Especially, FfIBP and LeIBP treatment may be effective in lowering DSBs and maintaining normal meiotic spindle and mitochondrial activity in vitrified-warmed murine oocytes.

The FfIBP and LeIBP groups showed significantly higher survival rates. The FfIBP group showed the highest cleavage rates. Blastocyst rates were significantly higher in FfIBP group. In blastocyst, apoptosis rates were significantly decreased in the AFPs groups. A significantly higher cell count in blastocyst was noticed in the AFPs groups. The rates of normal meiotic spindle organization and chromosome alignment were significantly higher in the FfIBP and LeIBP groups. Intracellular ROS levels significantly decreased in the AFP treated group. When compared the mitochondrial activity, the LeIBP group showed significantly higher activities. DNA double strand breaks rates were significantly lower in FfIBP and LeIBP groups than those in the control or Type III AFP group.

Limitations, reason for caution: The origins of FfIBP and LeIBP were from bacteria and yeast. Therefore, these AFPs to human oocyte and embryo should be tested before clinical applications.

Wider implications of the findings: AFP can apply to human oocyte and ovarian tissue cryopreservation to improve efficacy of vitrification.

Study funding/competing interest(s): Funding by national/international organization(s), This study was supported by a grant of the Korea Healthcare technology R&D Project, Ministry of Health & Welfare, Republic of Korea.

Trial registration number: 2.

P-277 The presence of FSH receptor polymorphism –29 G > A is associated with poor ovarian response in IVF/ICSI cycles

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Study question: The tailoring of reproductive treatments is crucial in promoting a high chance of success and a reduction in hypo- or hyper-response. Could the presence of polymorphic variants of the –29 G > A, Thr307Ala, Asn680Ser FSH receptor gene (FSHR) be used for an improved tailoring of stimulation protocols in ART?

Summary answer: The presence of GA or AA variants in –29 G > A is associated with a poor ovarian response and this knowledge could be used for an improved personalization of treatments.

What is known already: In addition to age, the most frequently used biomarkers for choosing the stimulation protocol are basal FSH, anti-Müllerian hormone (AMH) and antral follicle count (AFC). Nevertheless, these biomarkers do not permit an optimal personalization of therapies for each patient. The analysis of the polymorphisms relating to FSHR –29 G > A, Thr307Ala and Asn680Ser (the latter two in linkage disequilibrium) have been extensively studied as predictive factors for ovarian response but with contradictory results.

Study design, size, duration: An observational, prospective study, in which the analysis of FSHR polymorphisms was performed in 140 IVF/ICSI patients during 2013 for severe male or tubal factors at the ANDROS Day Surgery Clinic, Palermo, Italy. Patients with AMH >4 and <1 ng/ml, basal FSH ≥12 mUI/mL, female age ≥42 years were excluded from the study.

Participants/materials, setting, methods: 140 patients underwent a long-protocol and the primary outcome (the number of oocytes retrieved) was classified as a poor (<5 oocytes) or good response (≥5 oocytes).

The starting doses (low 75–150 IU, medium >150 <225 IU and high ≥225 IU) were determined by: age, AMH, basal FSH, AFC.

Main results and the role of chance: The frequencies were 0.21 Asn/Asn, 0.55 Ser/Asn, 0.24 Ser/Ser, for Asn680Ser; 0.04 AA, 0.34 GA, 0.62 GG, for –29 G > A.

Multivariate analysis revealed no differences on basal FSH, AFC, AMH, starting dose, total rFSH units, follicles ≥16 mm for Asn680Ser. However, the AA variant had a lower AFC than the wild type (7.6 ± 3.8 vs. 11.4 ± 6.8 respectively) for –29 G > A.

The majority of patients with a poor response (57%) presented GA or AA variants whereas most patients with a good response (67%) presented a wild type ($\chi^2 = 5.53$, df = 1, $p = 0.02$).

Considering only those who had started with low and medium doses, patients with a poor response presented GA or AA variants in a higher percentage than patients with a good response (65 vs. 35%, $\chi^2 = 3.89$, df = 1, $p < 0.05$).

Limitations, reason for caution: These results should be validated in further, larger studies.

Wider implications of the findings: The results of this study show that the presence of FSHR –29 G > A polymorphism is associated with a poor outcome during IVF/ICSI. This knowledge could be introduced into routine clinical practice for providing specific counselling, for choosing the best stimulation protocol and for optimizing the starting dose of gonadotropins.

Study funding/competing interest(s): Funding by hospital/clinic(s), ANDROS Day Surgery Clinic, Palermo, Italy.

Trial registration number: None.

P-278 Correlation between antral follicle counts and the number of oocytes retrieved

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Study question: How well do stimulation day 1 (congruent to spontaneous cycle day 2 or 3) antral follicle counts (AFC) predict the number of oocytes retrieved (NOR) following ovarian stimulation in a gonadotropin-releasing hormone (GnRH) antagonist protocol?

Summary answer: AFC was confirmed to be a statistically significant predictor of the number of oocytes retrieved during ovarian stimulation in a GnRH antagonist protocol, but on average explains only 20% of the variation in the number of oocytes.

What is known already: AFCs are commonly used to predict the ovarian response during *in vitro* fertilization (IVF) cycles. They are used to help determine patients at risk for ovarian hyperstimulation, as well as for prediction of those at risk of a poor ovarian response.

Study design, size, duration: This is a pooled analysis by study comparing AFC on stimulation day 1 vs. NOR for subjects of 3 randomized controlled trials: Pursue (N = 1388), Engage (N = 1476), and Ensure (N = 395). All women underwent controlled ovarian stimulation in a GnRH antagonist protocol followed by hCG trigger prior to IVF/intracytoplasmic sperm injection.

Participants/materials, setting, methods: Women in Pursue (35–42 years, ≥50 kg) received 150 µg corifollitropin alfa (CFA) or 300 IU daily recombinant FSH (rFSH), in Engage (18–36 years, >60 kg) received 150 µg CFA vs. 200 IU rFSH, and in Ensure (18–36 years, ≤60 kg) received 100 µg CFA or 150 IU rFSH.

Main results and the role of chance: Per study, Pearson correlation coefficients (ρ) were calculated and linear regression performed for NOR with covariates AFC, treatment, and their interaction. Mean AFC was 10.7, 12.4, and 11.2 in Pursue, Engage, and Ensure, respectively. Correlation between AFC and NOR was stronger in Pursue (ρ = 0.53; $P < 0.0001$) than in Engage and Ensure (ρ = 0.34 and ρ = 0.27, respectively; $P < 0.0001$). In Pursue and Ensure, correlation tended to be stronger in the CFA than the rFSH group (ρ = 0.57 vs. ρ = 0.48 and ρ = 0.33 vs. ρ = 0.17, respectively). In Engage, correlations were similar in the 2 groups (ρ = 0.34 and ρ = 0.36, respectively). Multiple correlation coefficients ranged from 11% in Ensure to 28% in Pursue. AFC explained approximately 20% of the variation in NOR. Individual prediction errors ranged between –4.3 and +3.2 oocytes (25th, 75th percentiles).

Limitations, reason for caution: This was a retrospective analysis. Equipment used to measure AFC was not standardized across centers.

Wider implications of the findings: Use of AFC to predict the number of oocytes retrieved for individual patients should be done with caution as prediction errors are typically between –4 and +3 oocytes.

Study funding/competing interest(s): Funding by commercial/corporate company(ies), Financial support for this study was provided by Merck & Co., Inc., Whitehouse Station, NJ, USA.

Trial registration number: NCT01144416, NCT00696800, NCT00702845.

P-279 Ovarian reserve and thyroid autoimmunity. A cross-sectional analysis using age-specific AMH levels

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Study question: Is there a significant association between low ovarian reserve and hypothyroidism and/or thyroid autoimmunity.

Summary answer: Low ovarian reserve is not related with hypothyroidism or thyroid autoimmunity.

What is known already: One retrospective study supported a potential association between diminished ovarian reserve (DOR) and increased TSH levels. Nonetheless, according to this study, DOR was defined as high basal FSH (14 UI/L), low antral follicular count (AFC <5) and prior poor ovarian response to stimulation, without taking into account one of the most accurate markers of ovarian reserve, anti-Mullerian hormone (AMH).

Study design, size, duration: A cross-sectional study including 4690 women between 2009 and 2012.

Participants/materials, setting, methods: Overall 4690 women were included. Patients were eligible for inclusion if they:

1. Had their AMH, FT4, TSH and TPO-Ab levels tested on the same day and
2. Did not have any risk factor that could potentially compromise their ovarian reserve (e.g., ovariectomy, ovarian surgery for endometriosis or gonadotoxic chemotherapy).

AMH levels were plotted in relation to age for the whole patients' cohort and age-specific AMH values (per year) were considered in order to categorize women according to the level of ovarian reserve.

In this regard, patients were categorized as women with: 1. Low ovarian reserve (women with age-specific AMH below the 10th percentile of the values), 2. Normal ovarian reserve (women with age-specific AMH between the 10th and 90th percentile of the values), and 3. High ovarian reserve (women with age-specific AMH above the 90th percentile of the values).

Kruskal-Wallis test was performed to compare TSH and FT4 between different ovarian reserve categories.

Chi-square test was performed in order to examine differences in the number of patients with positive TPO-Ab (>34 0kU/l) and the number of patients with hypothyroidism (clinical or subclinical).

All analyses were performed in SPSS 22 statistical software.

Main results and the role of chance: Overall, 462 patients were women with low ovarian reserve, 3756 demonstrated normal reserve and 472 demonstrated high reserve. Age and BMI did not significantly differ between patients' groups.

Mean (SD) serum FT4 levels were comparable between groups: low ovarian reserve, 11.98 (1.91), normal reserve 12.03 (1.93) and high reserve, 12.05 (1.62), $p = 0.524$, whereas, in accordance, TSH levels did not demonstrate any difference in women with low [median (IQR), 1.56 (1.14–2.18)], normal [1.54 (1.12–2.17)] and high ovarian reserve [1.51 (1.05–2.31)], $p = 0.841$.

Four hundred and eighty-three (483) patients (10.4%) had positive TPO-Ab. The percentage of patients with positive TPO-Ab did not significantly differ between low (11.7%), normal (10.2%) and high (10.0%) in ovarian reserve patients, $p = 0.594$). Finally, no differences were observed between groups in the incidence of clinical or subclinical hypothyroidism as defined by serum FT4 and TSH levels (3.2% in low, 3.6% in normal and 2.5% in high ovarian reserve patients $p = 0.447$).

Limitations, reason for caution: Due to the retrospective design of this study we cannot exclude the presence of biases related to retrospective data collection. Thus, results should be interpreted with caution. However, cross-sectional analysis is considered by most the optimal study design to assess prevalence of conditions (such as hypothyroidism).

Wider implications of the findings: This cross-sectional analysis failed to demonstrate an association between thyroid autoimmune disease and ovarian reserve. The major strength of this study is that it includes a very large group of patients in whom serum AMH, FT4, TSH and TPO-Ab were measured on the same day for the whole patients' cohort. In addition we clearly defined ovarian reserve based on age-specific AMH values from the same cohort of patients and not based on other variables (such as FSH levels alone, antral follicle count or response to previous treatment) by using specific threshold which may indeed vary between different age groups.

Although, thyroid autoimmunity does not appear to be related to low ovarian reserve, future cohorts may need to focus in women with specific infertility cause in order to find potential associations with thyroid function.

Study funding/competing interest(s): Funding by University(ies), UZ Brussel.

Trial registration number: N/A.

POSTER VIEWING

MALE AND FEMALE CONTRACEPTION

P-280 Two randomised clinical studies investigating the endocrine and clinical effects of normalising testosterone levels during oral contraception

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Study question: To investigate the endocrine and clinical effects when testosterone and other androgen concentrations are normalized during combined oral contraceptive (COC) use by co-administration of dehydroepiandrosterone.

Summary answer: COCs containing ethinylestradiol (EE)/drospirenone (DRSP) or EE/levonorgestrel (LNG) strongly suppress endogenous ovarian and adrenal androgen concentrations and interfere with sexual function. Co-administration of dehydroepiandrosterone (DHEA) is able to restore total and free testosterone (T). Favourable clinical effects are observed on several aspects of sexual function and mood, especially menstrual cycle related symptoms.

What is known already: COCs reduce androgen levels, especially free T, with up to 61% by inhibiting ovarian and adrenal androgen synthesis and by increasing levels of sex hormone-binding globulin (SHBG). COCs are also known to interfere with sexual function and cause mood disturbances. The question is whether the loss of androgens is related to these side effects of COCs.

Study design, size, duration: Two randomised, double-blind studies: 1. Study 1 (n = 100): 3 cycles COC (EE/DRSP) followed by 6 cycles COC combined with either 50 mg/day DHEA or placebo, 2. Study 2 (n = 84): 5 cycles COC (EE/LNG or EE/DRSP) and 50 mg/day DHEA followed by 5 cycles COC and placebo or the reverse.

Participants/materials, setting, methods: The single-centre studies (AMC, Amsterdam and CHR Citadelle, Liiège) were performed in healthy women, aged 18–35 years and BMI below 35 without sexual or mood complaints. Androgens, other endocrine parameters, sexual function, mood, quality of life, skin and safety were assessed without a COC and thereafter several times during treatment.

Main results and the role of chance: In total 99 respectively 81 women were randomised and treated. Nine women discontinued early (Study 1: two, Study 2: seven). Results show that:

(1) All COC users experience a loss of androgens, especially free T (−68 and −81% vs. baseline for EE/LNG and EE/DRSP respectively; $P < 0.0001$).
(2) COC use has unfavourable effects on sexual function (MFSQ Global score: −4.1 [$P < 0.002$]; decrease arousability/desire [$P < 0.05$]).
(3) By adding DHEA to an LNG/EE COC the loss of androgens, especially free T, can be restored ($P < 0.0001$) without inducing side effects.
(4) Favourable clinical effects were observed on several aspects of sexual function and menstrual cycle related symptoms. The sexual function diary showed a significant improvement of the responsiveness of women to sexual initiatives of the partner with DHEA ($P < 0.05$).

Limitations, reason for caution: The clinical results require confirmation of efficacy and safety in larger studies. Since DHEA negates the beneficial effect of COCs on androgenic skin symptoms, such women should not be treated with a DHEA containing pill.

Wider implications of the findings: Since all COC users lose T, DHEA treatment is safe and because it is unpredictable who will experience side effects due to the loss of androgens, addition of DHEA to COCs seems a reasonable alternative and an improvement of COCs. Side effects may be decreased and compliance may be improved.

Study funding/competing interest(s): Funding by commercial/corporate company(ies), Pantarheii Bioscience.

Trial registration number: ISRCTN06414473 (Study 1) and ISRCTN03247616 (Study 2).

POSTER VIEWING

MALE AND FEMALE FERTILITY PRESERVATION

P-281 Measuring effects of cancer therapy upon female fertility

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Study question: Is AMH a reliable marker of ovarian reserve in reproductive age women with cancer?

Summary answer: Cancer patient prior to chemotherapy had already lowered AMH as compared to healthy population. At 12-month follow-up, 80% of patients had undetectable AMH. Fertility preservation was offered to less than 10% of cancer patients.

What is known already: AMH is a promising marker of ovarian reserve in women with malignancy. Women who receive certain chemotherapy regimens suffer reduced ovarian reserve as a side effect. At present only limited data are available to guide clinicians about individuals' likelihood of chemotherapy-induced menopause and therefore who may benefit from fertility preservation.

Study design, size, duration: Prospective longitudinal multicentre study in academic hospital: 198 female participants, aged 18–43 years, recruited between May 2010 and May 2012 from 13 centres in UK. We present results of serum AMH at point 0, 6, 9 and 12-month follow-up, in light of women's self-reported reproductive history and lifestyle.

Participants/materials, setting, methods: Prospective longitudinal multicentre study in academic hospital: 198 female participants, aged 18–43 years, recruited between May 2010 and May 2012 from 13 centres in UK. We present results of serum AMH at point 0, 6, 9 and 12-month follow-up, in light of women's self-reported reproductive history and lifestyle.

Main results and the role of chance: Based on logistic regression model cancer patients prior chemotherapy had significantly lower serum AMH levels than volunteers ($p < 0.01$). In our prediction model, a cancer patient had serum AMH levels approximately of 6 year older volunteer. Serum AMH levels decrease with age and differ between breast, lymphoma and control group. AMH recovered to pre-chemotherapy levels in 55.5% of women with Hodgkin's lymphoma (mean age = 25.7). In breast cancer group (mean age 37.7) 13.2% patient had detectable AMH at 12-month follow-up. Over 35% of women newly diagnosed with cancer still expressed desire to have children. 44.4% in subgroup of women ≤39 years. Only 9.2% had consultation with fertility specialist and discussion about fertility preservation options.

Limitations, reason for caution: The group of Hodgkin's lymphoma and non-Hodgkin's lymphoma patients was small. Women on hormonal contraception were not excluded.

Wider implications of the findings: Reproductive age women with malignancy appear to have lower ovarian reserve than age-matched normal volunteers even before any gonadotoxic treatment is commenced. At 12 month follow-up majority of breast patients had undetectable AMH, while in younger group of patients with Hodgkin's lymphoma in 50% cases AMH recovered to pre-chemotherapy levels. In view of those findings, AMH measurement

would be recommended prior to fertility preservation. Post chemotherapy measurements of AMH at 12 month follow-up in breast cancer patients do not seems to have a clinical value.
Study funding/competing interest(s): Funding by University(ies), Funding by commercial/corporate company(ies), Midland Fertility Services, University of Warwick.
Trial registration number: International Standard Randomised Controlled Trial Number Register: ISRCTN28988709; http://www.controlled-trials.com/ISRCTN28988709; UK Cancer Research Network (UKCRN:8445).

P-282 Natural and assisted fertility outcome in male cancer patient seeking fertility preservation

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Study question: What is the fertility outcome in male cancer patients following gonad toxic treatment.
Summary answer: Pregnancy was achieved in 55% (73) of men who tried to conceive (133) following gonadotoxic treatment. Natural conception occurred in 47 cases (35%). 60 men attempted assisted conception with 26 achieving successful outcome.
What is known already: Very limited information is available on long term fertility outcome in men seeking fertility preservation prior to cancer treatment. Scanty reports suggest that the natural conception varies between 0.63 and 17% with very low uptake of cryopreserved sperm for subsequent assisted conception.
Study design, size, duration: This is a cohort study of 560 patients who undertook fertility preservation in a tertiary Assisted Conception Unit over the period of 25 years.
Participants/materials, setting, methods: The information on long term fertility outcome was collected by postal questionnaire and/or review of patients records (when assisted conception was undertaken). The intention to conceive or not was identified in 46% of study population.
Main results and the role of chance: The fertility preservation was provided to 560 men over period of 25 years. 256 patients (45%) in this cohort had testicular cancer. Lymphoma was the second most common cancer in this group (17%). The average age was 33.8 ± 9.9. The mortality rate is 9%. The overall fertility intention was known in 46% of the studied cohort. At least 48% of patients still did not try to conceive mostly due to personal reason. Among 133 patients who tried to conceive, 47 (35%) reported natural conception. 60 patients underwent fertility treatment resulting in a successful pregnancy in 43%. In this study we found a higher number of spontaneous pregnancies than previously reported in this group of men, particularly in testicular cancer.
Limitations, reason for caution: Even though this is one of the biggest studies it is retrospective with information obtained from the questionnaire.
Wider implications of the findings: The information obtained in this study may be useful in counselling men seeking fertility treatment. As currently there is very limited data on fertility outcome in this group of patients.
Study funding/competing interest(s): Funding by Hospital/Clinic(s), Guys and St Thomas Hospital.
Trial registration number: Not applicable.

P-283 Efficacy of random – start controlled ovarian stimulation for emergency fertility preservation in cancer patients

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Study question: What is the efficacy of the emergency approach of random-start controlled ovarian stimulation (COS) in the late follicular or luteal phase of the menstrual cycle in comparison to conventional early follicular start for patients with cancer undergoing fertility preservation treatment for oocyte or embryo cryopreservation?
Summary answer: The efficacy of random-start COS is comparable to conventional start COS in patients with cancer undergoing fertility preservation treatment. This urgent approach is a viable option for cancer patients when there is an urgent need to commence potentially gonadotoxic cancer treatment.
What is known already: Controlled ovarian stimulation for oocyte or embryo cryopreservation is the established method for fertility preservation in cancer patients. However, as there is often an urgent need to start chemotherapy or radiotherapy, the random start approach of commencing controlled ovarian stimulation in the follicular or luteal phase has been proposed to cryopreserve oocytes or embryos. However, there is still a paucity of data regarding the viability of this approach for fertility preservation.
Study design, size, duration: Retrospective cohort study of cancer patients undergoing fertility preservation in a single centre over a 10-year duration. Patients who underwent random-start COS (N = 14) were compared to patients who underwent conventional follicular start COS (N = 115).
Participants/materials, setting, methods: Women with cancer referred for fertility preservation. Post-chemotherapy or radiotherapy patients were excluded. All patients underwent COS with the GnRH antagonist protocol. Random start COS was started in either the follicular or luteal phase of the menstrual cycle. Setting: University tertiary centre.
Main results and the role of chance: There was no difference in the ages, BMI or antral follicle count in the two groups. In the random-start group most patients had breast (n = 8), haematological and gynaecological cancers while in the conventional-start group most patients had breast cancer (n = 61) followed by haematological, gastrointestinal and gynaecological cancers. There was no difference in the amount of gonadotrophins required (random-start: 2512 IU vs. conventional-start: 2118 IU) or the number of days of stimulation in the two groups. The number of oocytes retrieved in the random-start group was comparable to the conventional-start group (median: 8; range: 5–13 vs. 11; 5–16). The number of mature oocytes retrieved also did not differ between the two groups (random-start: 6, range: 2–12 vs. conventional-start: 8, range: 4–12).
Limitations, reason for caution: As none of the random-start COS patients underwent thawing and embryo transfer, it is not yet possible to evaluate implantation and clinical pregnancy rates in this group.
Wider implications of the findings: Random start COS is an important and viable option in cancer patients when urgent fertility preservation treatment is required as controlled ovarian stimulation can be started in the late follicular or luteal phase thereby minimizing delays in commencing cancer treatment. This will help in counselling patients seeking urgent fertility preservation.
Study funding/competing interest(s): Funding by University(ies), Hammersmith Hospital, Imperial College London, UK.
Trial registration number: Not applicable.

P-284 Age adjusted incidence of poor response in fertility preservation patients is significantly higher compared to the standard sub-fertile population

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Study question: Is there a higher incidence of poor responders in fertility preservation (for oncological indications) compared to the sub-fertile population?
Summary answer: The incidence of poor responders was significantly higher in the fertility preservation group.
What is known already: There is lack of published data relating to the likelihood of poor response in fertility preservation for oncological indications.
Study design, size, duration: A 2 year retrospective comparative analysis of the age matched incidence of poor responders between two groups of patients:

(a) fertility preservation for oncological indications
(b) subfertile population in the same hospital setting

Participants/materials, setting, methods: Calculation of incidence of poor response (5 or less oocytes per EC) in 27 patients who underwent fertility preservation between 2011–2013 in a University Hospital Assisted Conception Unit.
Main results and the role of chance: Incidence of poor responders in the fertility preservation group was 25.6% which was significantly higher compared with the sub-fertile group.

Limitations, reason for caution: Small retrospective study. Different stimulation protocols (random start for fertility preservation and use of letrozole).
Wider implications of the findings: Need for larger prospective studies to confirm our findings. The negative systemic impact of cancer on the reproductive system should not be overlooked during fertility preservation treatment. Caution may be needed when counselling such patients regarding the likely outcome of a typical super-ovulatory cycle using data extrapolated from non cancer populations.
Study funding/competing interest(s): Funding by Hospital/Clinic(s), Epsom & St Helier University Hospitals NHS.
Trial registration number: N/A.

P-285 Exogenous gonadotropin administration in cancer patients may improve meiotic competence of vitrified germinal vesicle stage oocytes but not survival rates when compared with unstimulated cycle

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Study question: The present investigation aimed at assessing: (i) whether vitrification of immature oocytes retrieved from unstimulated cycles or after controlled ovarian hyperstimulation (COH) performed in cancer patients seeking fertility preservation have comparable survival (SR) and meiotic resumption rates (MRR) after *in vitro* maturation (IVM) (ii) the SR of germinal vesicle stage (GV) oocytes in cancer patients in comparison to those observed with metaphase 2 oocytes vitrified in healthy controls.
Summary answer: SR of oocytes vitrified at GV following IVM or stimulated cycles are similar and comparable with those of metaphase 2 stage oocytes. However, MRR of GV oocytes from IVM is significantly decreased compared with that of GV oocytes from stimulated cycles.
What is known already: The potential of vitrified mature oocytes obtained after COH in infertile patients is well documented. However, there is a remarkable lack of data regarding the SR and MRR of oocytes recovered at GV during unstimulated or stimulated cycles and vitrified after IVM in women suffering from cancer seeking fertility preservation. The recent emergence of IVM in the strategy of fertility preservation may offer an opportunity to address this issue.

The recent emergence of IVM in the strategy of fertility preservation may offer an opportunity to address this issue.
Study design, size, duration: From November 2013 to January 2014, 22 patients suffering from cancer, candidates to oocyte vitrification in unstimulated (IVM group, $n = 10$) or after COH (COH group, $n = 12$) were prospectively studied. In addition, 7 egg donors having vitrified metaphase 2 oocytes were used as a controls (control group).
Participants/materials, setting, methods: Thirty-two GV oocytes at day 1 recovered from IVM cycles and 34 GV oocytes at day 0 obtained after COH were vitrified using the cryotop device. Warming was immediately performed after freezing and oocytes having survived were cultured for 24 h into appropriate media. GV oocytes having reached metaphase 1 or 2 stages were considered as competent for meiosis. In the same period, 36 metaphase 2 oocytes vitrified/warmed from healthy controls allowing the calculation of a SR.
Main results and the role of chance: SR of vitrified oocytes after warming were of 88.0, 86.3 and 88.1% in the IVM, COH and control groups, respectively. Although SR were similar in all groups (overall p value: $p = 0.88$), the MRR of GV oocytes in IVM group was significantly lower when compared with COH groups (16.5 vs. 56.8%; $p = 0.018$).
Limitations, reason for caution: Results presented in this study should be confirmed on a larger series of oocytes in all groups of patients.
Wider implications of the findings: Our findings surprisingly indicate that GV vitrified oocytes recovered from unstimulated or after COH in cancer patients display similar SR as metaphase 2 oocytes of healthy women. Furthermore, in women suffering from cancer, the meiotic competence of vitrified GV oocytes is dramatically reduced when oocytes are recovered during an unstimulated cycle. Further investigation is needed to distinguish possible direct deleterious effects of vitrification procedures or an overall decreased intrinsic meiotic competence of oocytes retrieved from small antral follicles.
Study funding/competing interest(s): Funding by University(ies), Centre Hospitalier Universitaire Jean Verdier. Université Paris 13.
Trial registration number: Not needed.

P-286 Influence of the culture system on the establishment of the estrogenic or progestagenic phenotype of human luteinizing granulosa cells

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Study question: We aim to standardize a chemically defined culture system, in order to reverse the luteinization process of GCs obtained from IVF cycles, pre-luteinized by hCG, for use in experimental *in vitro* maturation of preantral ovarian follicles and as a model for studies in the human GC differentiation.
Summary answer: Changes in culture system can induce different phenotypes on human GCs. Thus, TCM-cells changed their phenotype to fibroblast-like cells during plate culture and also acquired the functional characteristics of terminally differentiated cells, committed with luteinization process. On the other hand, in chemically defined medium, GCs do not complete *in vitro* the process of luteinization triggered by hCG, and tend to undifferentiation through the luteinizing process, assuming an estrogenic GC phenotype.
What is known already: Human granulose cells culture is already well documented, but granulose-luteinized cells obtained from IVF cycles tend to differentiate in luteinized cells.
Study design, size, duration: An experimental study was conducted with human granulose cells (CG), obtained from 10 women undergoing IVF, from July 2012 to June 2013.
Participants/materials, setting, methods: The GCs were cultured during 144 h in α-MEM containing IGF-I, ITS, FSH androstenedione and PVP-40 (chemically defined medium) or TCM containing FSH and serum. After 48, 96 and 144 h of culture the time course of GCs morphology, and secretion of E2, P4 and relaxin was analyzed, as well as FSH/LH receptors ratio. Other functional markers of luteinization as the expression of steroidogenic enzymes were investigated by RT-PCR at the end of culture. The relationship between cell shape, cell-plating density, and GC steroidogenesis *in vitro* was further explored.
Main results and the role of chance: The morphology of α-MEM cells rather than TCM-cells resembled closely that seen *in vivo* and they secreted 8 to 16-fold more estradiol and less progesterone (6 to 14-fold) than TCM-cells. Additionally, relaxin secretion was significantly reduced in α-MEM cultures. The P4/E2 receptors ratio was strongly affected by cell-plating density in both cultures and TCM-cells manifested a more luteinized state at higher cell densities. Expression of *LHCGR*, *FSHR*, *HSD3*, *CYP17* and *CYP19* genes was detected in both TCM and α-MEM-cells at 144 h of culture. GCs from αMEM are estrogenic and expressed the *CYP19* gene demonstrating a closely association between enzyme expression, aromatase activity and estrogenic capacity. In αMEM, the total amount of *CYP17* expression increased about 8-fold over the levels found in TCM-cells ($p < 0.01$).
Limitations, reason for caution: Cells were obtained during oocyte retrieval in IVF cycles, though the cultures were not obtained at the same moment.

Also, many cultures (30) were performed in order to obtain 10 samples for inclusion, because the concentration of granulose cells recovered after culture for PCR analysis was not always enough.
Wider implications of the findings: Intending to use granulose cells culture as a support for co-culture in pre antral follicles culture in order to mimic *in vivo* environment, the luteinized cells aspirated during oocyte retrieval is not a good option, as they behavior as luteal phase cells. This new protocol that permits undifferentiation to earlier stages of follicular development (follicular phase phenotype) may allow the use of this cells for this IVM purpose, mainly in cases of fertility preservation, when primordial follicles isolated from cryopreserved ovarian tissue may be cultured.
Study funding/competing interest(s): Funding by University(ies), Funding by National/International Organization(s), FAPESP and FAEPA/HC-FMRP/USP, Brazil.
Trial registration number: Not applicable.

P-287 Oocyte cryopreservation after ovarian stimulation with gonadotropin and letrozole to save fertility in breast cancer

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Study question: Is mature oocyte cryopreservation efficient and safe to save fertility in patients with estrogen receptor-positive breast cancer?
Summary answer: Oocyte cryopreservation is efficient and safe to save fertility in breast cancer patients as a mean number of 5 mature oocyte could be cryopreserved after an ovarian stimulation with no supraphysiological increase of estradiol level and 1 birth of an healthy male was achieved in 1 thawing.
What is known already: The mature oocyte cryopreservation in breast estrogen receptor-positive cancer patients is debated because of the risk related to the often supraphysiological estradiol levels achieved in ovarian stimulation. To avoid excessive estrogen increase, the association of aromatase inhibitors and gonadotropins have been proposed in the past few years to perform embryo cryopreservation as a fertility preservation strategy in breast cancer patients. Trials with oocyte cryopreservation recently started.
Study design, size, duration: This study aims to evaluate the feasibility of oocyte cryopreservation in breast cancer patients undergoing gonadotoxic treatments, in terms of estradiol levels reached during stimulation, number of oocytes retrieved and cryopreserved, pregnancies achieved. 44 patients were examined between 2008 and 2013.
Participants/materials, setting, methods: Out of 44 breast cancer patients, 19 women with estrogen receptor-positive cancer underwent ovarian stimulation with gonadotropins and letrozole for oocyte cryopreservation. The oocytes retrieved were cryopreserved. To date one patient asked for oocyte thawing cycle and achieved pregnancy**.** AMH, FSH, AFC mean basal values were evaluated.
Main results and the role of chance: The mean age of the patients in the study group was 36.2 ± 5.3. The mean FSH and AMH basal levels resulted, respectively, 10.6 ± 6.7 UI/dL and 2.1 ± 1.1 ng/mL. AFC (antral follicle count) was 6.7 ± 2.5. The mean total dose of gonadotropins used for patient was 2.484 ± 1370 IU, whereas the mean peak of estradiol reached during the stimulation was 334 ± 319 pg/mL. The mean number of oocytes retrieved for patient was 8.4 ± 5.0 and the mean number of oocytes cryopreserved was 6.3 ± 4.5. One patient thawed the oocytes after 3 years from cryopreservation and achieved a pregnancy with the birth of a healthy male.
Limitations, reason for caution: Data are still very limited and a larger population of breast cancer patients are needed to confirm the efficiency and safety of oocyte cryopreservation.
Wider implications of the findings: The new therapies of malignancies led to an increase in cancer survival rates. Unfortunately gonadotoxic therapies can induce premature ovarian failure. The possible options to preserve fertility were embryo cryopreservation and ovarian tissue cryopreservation so far. In comparison to those option, oocyte cryopreservation is minimally invasive, efficient, safe and ethically acceptable and can be considered as a first choice strategy to save fertility in estrogen receptor-positive breast cancer patients.
Study funding/competing interest(s): Funding by University(ies), Funding by Hospital/Clinic(s), IVF Center University Medical Center Sant'Orsola-Malpighi Bologna, Italy.
Trial registration number: No registration number.

P-288 Vitrification does not increase the apoptosis incidence and caspase 3/7 activity in human ovarian tissue

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Study question: Does vitrification increase the apoptosis incidence and caspase 3/7 activity in human ovarian tissue?
Summary answer: Vitrification does not increase the apoptosis incidence and caspase 3/7 activity in human ovarian tissue.
What is known already: Slow freezing of human ovarian tissue is method of choice for clinical usage but recently vitrification method received more attention because of it's of simplicity and affordability.
Study design, size, duration: Human ovarian tissue biopsies were obtained from 30 women undergoing elective caesarean sections and transported to the laboratory with pre-warmed and equilibrated Leibovitz L-15 medium within 2 h. Then they were cut into small pieces and divided into 2 (vitrified and non-vitrified control) groups.
Participants/materials, setting, methods: Apoptosis incidence was assessed by light microscope and by other assessments such as TUNEL assay, DNA Laddering, and evaluation of the level of caspase 3/7 protein by luminescent assay in non-vitrified and vitrified human ovarian tissue.
Main results and the role of chance: No sign of apoptosis was seen morphologically in vitrified and non-vitrified samples. No apoptosis signal by TUNEL staining and also DNA Laddering pattern were seen in vitrified group. The caspase 3/7 activity was 2294 ± 169.19, 2231 ± 89.271 RLU/µg protein in non-vitrified and vitrified groups respectively and there were no significant difference between these groups.
Limitations, reason for caution: There is some limitation about obtaining human ovarian tissue biopsies.
Wider implications of the findings: The vitrification technique could become an alternative for ovarian tissue preservation.
Study funding/competing interest(s): Funding by National/International Organization(s), Tarbiat Modares University and Iran National Science Foundation.
Trial registration number: 5290881.

P-289 Optimizing ovarian tissue quality prior to cryopreservation for fertility preservation – effects of mechanical separation on cell damage

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Study question: Does the procedure of separation of medulla from the cortex affect tissue viability in ovarian cortex from patients undergoing cryopreservation for fertility preservation? If yes, what is the underlying mechanism? What is the best method to separate the medulla from ovarian cortex in terms of tissue viability?
Summary answer: Mechanical separation of ovarian medulla from the cortex induces cell apoptosis in the cortical stroma but not in follicles. When comparing three different methods to remove medulla from cortex: microsurgical scissors, scalpel blade or slicer, the two former induce a significantly higher degree of apoptosis than the latter.
What is known already: The thickness of the ovarian cortical strip is a critical factor influencing cryoprotectant penetration and cooling rate when cryopreserving ovarian tissue: the thicker, the less viability of the tissue. Different methods have been published to separate the ovarian medulla and cortex (decortication), but to our knowledge there is not study describing the effect of de-cortication on cortical viability and there is no comparative study aiming to detect which method is better, if any.
Study design, size, duration: *In vitro* study. Forty ovarian tissue pieces from patients undergoing fertility preservation where randomly allocated to the following de-cortication groups: scratching with a scalpel blade (B), cutting with microsurgical scissors (M), separation using a slicer(S) or no separation (control). Stromal and follicular viability were evaluated after 4 h of culture.
Participants/materials, setting, methods: Participants: patients undergoing ovarian cortex cryopreservation for fertility preservation.
Setting: University Hospital. Animal facilities.
Methods: Ovarian tissue was allocated to the above-mentioned experimental groups. After de-cortication and incubation at 37°C in M199 medium, follicles underwent morphological classification and apoptosis was evaluated in stromal tissue and follicles with the TUNEL assay.
Main results and the role of chance: Ten oncologic patients (Breast cancer, $n = 8$; Hodgkin lymphoma, $n = 1$ and Ewing sarcoma, $n = 1$) aged 28.6 (range 15–34) were enrolled in this study. Four ovarian strips from each patient

were allocated to the experimental groups. Follicular densities didn't differ between any patients neither between experimental groups. The proportion of good quality follicles (type I and II according to Martinez-Madrid et al., 2004 classification: Morphologically normal, TUNEL negative oocyte and less than 10% apoptotic Granulosa cells) didn't differ between groups (C: 100 ± 0.0%; B: 98.6 ± 6.9%; M: 100 ± 0.0%; S: 100 ± 0.0%; n.s.). The proportion of stromal TUNEL+ cells/tissue area was higher after de-cortication with the scalpel and scissors when compared to the control group (C: 0% [0–15.85%]-reference; B: 0% [0–19.28%]-$p = 0.011$; M: 0% [0–10.33%]-n.s; S: 4.51% [0–36.08%]-$p < 0.001$).

Limitations, reason for caution: This is an *in vitro* study and our findings must be confirmed after long-term evaluation, preferably after xenotransplantation in an *in vivo* model. We are currently performing such experiment.

Wider implications of the findings: Ovarian de-cortication is a necessary step prior to cryopreservation. Increased stromal damage has been proven to compromise tissue viability therefore endocrine activity and pregnancy rate after ovarian cortex retransplantation. In our study we have shown that de-cortication with a scalpel or microsurgical scissors are more deleterious for the stroma than the slicer. If such findings are translated to the clinical practice, results of ovarian cortex cryopreservation and transplantation may improve.

Study funding/competing interest(s): Funding by University(ies), Funding by National/International Organization(s), This study was partially founded by Fundación Dexeus Salud de la Mujer (2013 Round).

Trial registration number: IIS La Fe Identifier: 2013/0377.

P-290 *In vivo* optical imaging of immature mouse testicular tissue engineering by three-dimensional scaffolds for pre-pubertal male fertility preservation

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Study question: Pre-pubertal cancer boys for fertility preservation remains controversial due to unable sperm production for cryobanking. Dose the biocompatibility and biodegradability of three-dimensional scaffolds applied for immature testicular tissue engineering impact on the efficiency and efficacy of the transplantated immature murine testicular tissue *in vivo* for maintaining pre-pubertal male fertility preservation?

Summary answer: Bioresorbable polyesters, Poly-L-lactic acid (PLLA), designed to nano-fibrous membrane by electrospinning, was served as fine and coarse scaffolds. The regeneration of immature murine testicular grafts in fine scaffold was much better than the coarse one and fresh graft control without scaffolds which were tracked by *in vivo* bioluminescence imaging (BLI).

What is known already: As a pilot study, we demonstrated the significant better outcome of scaffold loaded immature murine testicular tissue than fresh grafts without scaffold *in vivo* at 2013 ASRM Annual Meeting. In our previous study (the first place prize poster at ASRM), we have proved the way that the scaffold applied in immature murine testis was feasible and effective by *in vivo* optical imaging. The novelty is promising for interdisciplinary strategy fertility preservation of pre-pubertal cancer boys.

Study design, size, duration: 4-week-old wild-type recipients were transplanted age-matched donor (FVB/N-Tg (*Pol*II-luc) Ltc transgenic mice) testicular tissue to the castrated scrotum with fine, coarse scaffold (PLLA) ($n = 5$) loaded and control without scaffold, followed by tracking by BLI in a longitudinal model to monitor transplanted grafts *in vivo* as long as 56 days.

Participants/materials, setting, methods: FVB/N-Tg (*Pol*II-luc) Ltc transgenic male mice were used as donors. Sexually immature inbred FVB/NJNarl wild-type mice were used as recipients. Before the experiment, the age-matched recipient mice underwent bilateral orchiectomy to leave scrotum as the grafted site. Bioluminescence imaging (BLI) was utilized to measure the testicular tissue development after transplantation.

Main results and the role of chance: Based on the quantity of BLI analysis, the scaffold loaded immature testicular tissue demonstrated better survival than tissue on day 7 after transplantation. The fine scaffold used in testis tissue transplantation increased cell regeneration rendered the best outcome compared with the coarse scaffold and tissue alone especially on the 35 days after transplantation afterward. Therefore, polymer scaffolds are often modified with bioactive molecules or treated with extracellular matrix (ECM) proteins to improve cell attachment and nutrition extension to promote cell adhesion and improve cytocompatibility. PLLA provides better surfaces for promoting immature testicular engineering.

Limitations, reason for caution: This study pilots the way to the interdisciplinary strategy by the utility of scaffolds and *in vivo* optical imaging by BLI as a tool to track the development of testicular grafts. The mechanism of the scaffold structure on testicular tissue regeneration needs to be clarified by future study.

Wider implications of the findings: Understanding the diversity of scaffold that mimics tissue subjects may support the testicular transplantation for pre-pubertal male fertility preservation. Due to their excellent shaping and molding properties, research interest in developing polyester nano-fibrous based scaffolds may serve a new trend to study immature testicular tissue engineering for pre-pubertal fertility preservation. BLI also makes it possible to track the relative amounts and locations of tissue over time and adheres to the "3 R's" in animal research.

Study funding/competing interest(s): Funding by National/International Organization(s) (National Science Council) NSC 102-2314-B-038-045, Taiwan, ROC.

Trial registration number: LAC-101-0090.

P-291 The effects of angiopoietin-2 on the transplanted mouse ovarian tissue

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Study question: Does Angiopoietin-2 (ANG-2) have positive effects on follicle integrity and revascularization of the transplanted mouse ovarian tissue (OT)?

Summary answer: ANG-2 administration significantly increases the intact follicle ratios and blood vessel numbers of the OT grafts. However there was no remarkable improvement in the mean follicle numbers, apoptotic follicle ratio and serum FSH levels.

What is known already: The follicle loss of transplanted OT is caused by ischemia and slow revascularization. To shorten the ischemic period and promote angiogenesis, some angiogenic factors have been treated for transplanted tissues. ANG-2 is one of the major angiogenic factors and has been reported to promote blood vessels and increase vascular permeability in the ischemic and/or hypoxic environment. However, the exact role and effect of ANG-2 in OT grafts are not well known yet.

Study design, size, duration: The 5-week-old B6D2F1 female mice were divided into 3 groups (a control and two ANG-2 groups) followed by ovary collection and vitrification. After warming, the ovaries were autotransplanted into kidney capsules with/without ANG-2 injection (50 or 500 ng/kg), and then killed at day (D) 2, 7, 21 and 42 after transplantation.

Participants/materials, setting, methods: Total 2437 follicles in OT grafts were assessed for the follicular density, integrity and classification by hematoxylin and eosin staining. Apoptosis and revascularization were evaluated by Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate Nick End Labeling assay and CD31 immunohistochemistry respectively. Serum FSH levels were measured by Enzyme-linked immunosorbent assay.

Main results and the role of chance: All the ANG-2 groups (50 and 500 ng) showed remarkable increase of morphologically intact follicle ratio across all the grafting duration except D21 (no statistical difference). The numbers of CD31 positive vessels (the sum of 3 fields at ×400 magnification) were significantly increased in both ANG-2 groups compared with the control group at all the grafting duration. Especially at D42, the 500 ng ANG-2 group showed significantly more vessels than the 50 ng ANG-2 group as well as the control group. However the mean follicle numbers of grafts, apoptosis ratio and serum FSH levels showed no significant difference among the groups.

Limitations, reason for caution: The limitation of this study was the absence of *in vitro* fertilization using oocytes obtained from ANG-2 treated OT grafts, which should be performed to confirm whether the ANG-2 treatment improve the ischemic damage and promote revascularization. And the ANG-2 pathway in OT grafts should be identified for further studies.
Wider implications of the findings: OT transplantation can be used for fertility preservation of cancer survivors as well as animal experiments to assess malignant recurrence after OT retransplantation and follicular developmental potential. It is necessary to promote angiogenesis for the success of OT transplantation. In this study, remarkably well preserved follicles and larger amount of vessels were appeared in ANG-2 treated groups. So we thought that ANG-2 treatment is effective for OT transplantation and improve transplantation outcomes.
Study funding/competing interest(s): Funding by National/International Organization(s), This study was supported by a grant of the Korea Healthcare Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI12C0055).
Trial registration number: basic science (not clinical trials), total mouse ovarian follicles: 2437.

P-292 Physiological initiation of early follicular growth in fresh and frozen/thawed prepubertal murine ovarian tissue after dynamic *in vitro* culture

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Study question: Can dynamic *in vitro* culture support physiological initiation of follicular growth in frozen/thawed prepubertal murine ovaries?
Summary answer: The follicular growth pattern after dynamic *in vitro* culture of fresh as well as frozen/thawed prepubertal murine ovaries is equivalent to the physiologic growth observed *in vivo*.
What is known already: Although ovarian tissue cryopreservation followed by autotransplantation is a very promising strategy for fertility preservation, in certain malignancies, autotransplantation still bears the risk of reseeding malignant cells. *In vitro* culture techniques are objectives of many ongoing research projects as they might be the only save option for these patients to use their stored ovarian tissue. However, predominantly conventional static culture techniques are applied with exchange of culture medium once every 24 to 48 h.
Study design, size, duration: Ovaries from fresh (n = 30) and frozen/thawed (n = 20) 8-day-old BALB/c mice were cultured in a dynamic system for 4 days. After culture, fresh (n = 11) and frozen/thawed (n = 10) ovaries were isografted into adult female BALB/c mice. Ovaries from 8-day-old (n = 17) and 12-day-old (n = 13) animals served as baseline and in-vivo control respectively.
Participants/materials, setting, methods: To generate dynamic culture conditions a peristaltic pump continuously flushed culture-chambers with medium leading to a constant medium-exchange within 24 h. Follicular growth was evaluated by histological follicle classification and counting. After 30 days of isografting, expression of proliferating cell nuclear antigen (PCNA) was evaluated by immunohistochemistry.
Main results and the role of chance: After dynamic culture the percentage of secondary follicles was 10.63 ± 4.89% in fresh and 11.09 ± 7.75% in frozen/thawed ovaries. These results were significantly higher than the percentages in fresh (5.02 ± 2.90%, $p < 0.01$) and frozen/thawed 8-day-old baseline controls (5.2 ± 5.55%, $p < 0.01$). Compared to 12-day-old in-vivo controls (9.6 ± 6.0%) the percentage of secondary follicles in fresh and frozen/thawed ovaries were equivalent (p = n.s.). After isografting 10 of 11 fresh grafts and 7 of 10 frozen/thawed grafts could be recovered. Preliminary analyses of the grafts showed antral follicle development and PCNA expression.
Limitations, reason for caution: This study was designed to compare dynamic culture to in-vivo conditions. Therefore this technique remains to be compared to conventional static culture systems. Further, the impact of dynamic culture on larger follicular growth still has to be investigated *in vitro*, although it has been shown by isografting.
Wider implications of the findings: The described dynamic culture system supports the initiation of *in vivo*-like follicular growth in fresh and frozen/thawed murine ovarian tissue and might be useful for human ovarian tissue culture in the context of fertility preservation. Secondly, dynamic culture provides the possibility to test pulsatile administration of culture medium supplements as well as fractionated sampling of conditioned medium and can therefore be a valuable tool for basic research.
Study funding/competing interest(s): Funding by National/International Organization(s), the authors have no conflicts of interest. This study was funded by the Tyrolean Research Foundation. K. W. was recipient of a DOC-fForte Fellowship of the Austrian Academy of Sciences.
Trial registration number: Not applicable.

P-293 A history of abvd (adriamycin/bleomycin/vinblastine/dacarbazine) therapy for hodgkin lymphoma alters the number of oocyte cryopreserved after *in vitro* maturation in candidates for urgent fertility preservation

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Study question: Whether a medical history of Hodgkin lymphoma (HL) treated with ABVD is associated with poor results of *in vitro* maturation of oocytes (IVM) in women seeking urgent fertility preservation (FP) after relapse of the hematologic disease or second malignancy?
Summary answer: Despite similar markers of the ovarian reserve, women having received ABVD for HL and candidates for FP using IVM after relapse of the disease or breast cancer, have reduced number of immature oocytes recovered and total number of mature eggs cryopreserved when compared with cancer patients without history of chemotherapy.
What is known already: Chemotherapy can result in ovarian failure and premature menopause. Despite a paucity of data, patients facing infertility after cancer treatments may be exposed to poor outcome of assisted reproductive technologies. ABVD chemotherapy administered for HL before the age of 30 years is usually associated with low rates of premature ovarian insufficiency. However, some women may face a relapse of the hematologic disease or another type of cancer. The question of FP should therefore be considered.
Study design, size, duration: Forty-four women, aged 22–39 years, suffering from HL or breast cancer were enrolled in this prospective, observational study conducted between 2009 and 2013. After measurement of serum anti-Müllerian hormone (AMH) and antral follicle count (AFC), all women were candidates for urgent FP using oocytes cryopreservation after IVM.
Participants/materials, setting, methods: Twenty-two patients diagnosed, at least 2 years after ABVD, with relapse of HL or breast cancer underwent IVM (ABVD group). Results were compared with 22 cancer patients without history of chemotherapy, matched for age, BMI, AMH, AFC and the phase of the cycle at which oocytes were retrieved (Control group).
Main results and the role of chance: By design, in ABVD and control groups, women were comparable in terms of age (28.5 ± 1.31 vs. 28.68 ± 1.22 years, respectively), BMI (22.7 ± 0.8 vs. 22.3 ± 0.7 Kg/m^2) and markers of the follicular ovarian status (serum AMH levels (2.95 ± 0.3 vs. 3.07 ± 0.4 ng/mL; and AFC (16.9 ± 1.3 vs. 17.3 ± 1.3 follicles, respectively). Despite similar AMH and AFC, the number of immature oocytes recovered was significantly lower in the ABVD group when compared to controls (6.45 ± 1.0 vs. 8.95 ± 0.9 oocytes, $P < 0.05$, respectively). Although the overall maturation rate was similar in both groups (65.2 ± 3.5 vs. 61.0 ± 7.3%, NS, respectively), the total number of *in vitro* matured oocytes cryopreserved was significantly decreased in women having received chemotherapy (4.1 ± 0.8 vs. 5.7 ± 0.7, $P < 0.05$, respectively).
Limitations, reason for caution: The cohort of patients treated for HL is relatively small. In addition, we only provide information on the overall number of oocyte cryopreserved after IVM. Therefore, the potential of these frozen oocytes is still unknown.
Wider implications of the findings: The present investigation suggests that oocyte cryopreservation after IVM may be altered by previous ABVD therapy, even though markers of the follicular ovarian status remain in the normal ranges. These findings should be taken into account in the strategy of FP of

women with relapse of HL or second malignancy. Therefore, ovarian tissue cryopreservation should be considered systematically in combination with IVM in women having received ABVD therapy.
Study funding/competing interest(s): Funding by University(ies), Funding by Hospital/Clinic(s), University Paris XII, Bobigny, France, Hôpital Jean Verdier, Bondy, France.
Trial registration number: Not applicable.

P-294 Xenotransplantation of ovarian tissue into SCID-mice: does stimulation with human gonadotropins and GnRH agonist enhance and accelerate the developmental capacity of oocytes?

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Study question: Does stimulation with human gonadotropins and GnRH agonist enhance and accelerate the developmental capacity of oocytes in human ovarian tissue xenografted into SCID mice.
Summary answer: After xenotransplantation, human primordial follicles can be matured to metaphase II oocytes even without stimulation. Administering human gonadotropin and GnRHa does not enhance the developmental capacity of oocytes in xenografts. The optimal stimulation schedule for grafted tissue remains unknown.
What is known already: Ovarian tissue xenografting has proved to be a useful model to examine ovarian function and follicle development. To improve the developmental competence of oocytes within xenografts, stimulation with exogenous hormones has been used. Gonadotropins can upregulate angiogenic growth factors in the ovary and thus play a role in ischemia-reperfusion injury. The most effective gonadotropin preparation for stimulating follicles in ovarian tissue grafts, as well as the optimal dosage and duration of gonadotropin stimulation, still remains unknown.
Study design, size, duration: The aim of the study was to enhance the developmental ability of oocytes in human ovarian tissue xenografted to SCID mice, by using gonadotropins. Xenograft survival, follicular atresia due to apoptosis, follicular proliferation, and cancer cell transmission were examined in cryopreserved ovarian tissue from 17 patients with various malignancies after xenografting the tissue into 88 female immunodeficient mice.
Participants/materials, setting, methods: Cryopreserved human ovarian tissues were grafted into i.m. pockets of the neck muscle of bilaterally oophorectomized SCID mice. The mice were divided into three groups. Group A were treated with HMG alone (receiving HMG every 2 days, starting from the day of transplantation for a maximum of 24 weeks). Group B received in addition to HMG a GnRH-agonist depot every 4 weeks and group C were not treated and served as a control group.
Main results and the role of chance: Similar degrees of follicle survival and development were noted in group 1 compared to group 2 ($p > 0.5$). Comparing the unstimulated group vs. group 1 and 2, a similar rate of follicular survival and development was noted. Regarding an interaction between stimulation protocols and grafting duration, no statistic significant results were observed ($p > 0.5$). Metaphase II oocytes ($n = 3$) were observed in follicles in grafts from three patients. Two MII oocytes were harvested without stimulation, one even without HCG administration. In addition, corpora lutea was present in 4 grafts. None of the mice presented symptoms of reintroduced malignancy nor did microscopic evaluation of the grafts raise any suspicion of residual malignant disease.
Limitations, reason for caution: A major difficulty in the transplantation studies was that the total numbers of follicles in the patients' grafts were low. The low number of follicles in human ovarian tissue grafts can be explained by the age-related decline of follicles, interpatient variation, and in particular the use of small grafts.
Wider implications of the findings: A better understanding of the intraovarian factors that are involved in the regulation of the resting follicle pool is needed in order to positively influence clinical outcomes. Human gonadotropins and GnRH-agonists were not found to enhance the developmental capacity of oocytes in xenografts. Further research is needed on the influence of gonadotropin stimulation on follicles in ovarian grafts, and there is continuing debate regarding the value of stimulation for achieving pregnancy in patients with autologous transplantation.
Study funding/competing interest(s): Funding by National/International Organization(s), Wilhelm Sander Foundation (reference no. 2008.086.1 and 2012.127.1), Munich, Germany, and the Deutsche Forschungsgemeinschaft (DI 1525/4-1), Bonn, Germany.
Trial registration number: None.

P-295 39 transplantations of cryopreserved ovarian tissue – experience of the network FertiPROTEKT

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Study question: What is the success rate in terms of endocrine activity, pregnancy rate and delivery rate following transplantation of ovarian tissue in a network of many different centers?
Summary answer: Autotransplantation of frozen-thawed ovarian tissue restored ovarian function in 36 cases, pregnancies were achieved in seven cases and deliveries in 4 (by end of 2013). With a pregnancy rate of 18% these results confirm those of other centers showing that cryopreservation and retransplantation is a good method to restore fertility.
What is known already: Cryopreservation of ovarian tissue with subsequent retransplantation following a period of recurrence-free survival is a promising technique for fertility preservation in patients facing gonadotoxic treatment. Around thirty pregnancies have been reported worldwide from this procedure and it can be expected that more cancer patients who have been cured of their disease are likely to request transplantation of frozen/thawed ovarian tissue. However, there are still many unanswered questions regarding this subject.
Study design, size, duration: Retrospective clinical case series of 39 cancer patients (mean age 34.9) transplanted with their own frozen/thawed ovarian tissue (between 2007 and 2013), after completed cancer treatment in a network of 100 centers involved in fertility preservation in Germany, Switzerland and Austria (FertiPROTEKT, www.fertiprotekt.eu).
Participants/materials, setting, methods: Ovarian tissue was cryopreserved with slow freezing protocols. The cryopreserved ovarian tissues were retransplantated orthotopically in the vicinity of the ovary (either in the pelvic wall ($n = 29$), the ovary ($n = 5$), or both sites ($n = 5$)). Endocrine function was assessed by monthly blood tests (FSH, LH, E2, progesterone) and ultrasound after transplantation.
Main results and the role of chance: 36 patients regained ovarian function between 6 and 24 weeks (mean 16 weeks) after transplantation, as shown by follicle development and estrogen production. Only in 3 patients no activity of the transplant could be seen 6 month after transplantation. In seven patients with planned IVF, oocytes from the transplanted ovarian tissue could be retrieved and fertilized without ovarian stimulation. Of all 39 patients, seven got pregnant; six conceived spontaneously, one after IVF and the transfer of the embryo. So far four healthy live births have been reported.
Limitations, reason for caution: Although cryopreservation enables patients to conceive when they have overcome their primary disease, the number of transplantations is still too low to give general recommendations regarding the decision which centers provides the highest success rates, the best site for retransplantation and the longevity of the tissue.
Wider implications of the findings: Transplantation of ovarian tissue is an encouraging method of re-establishing menstrual cycles and achieving pregnancy in women with iatrogenic POI. Fertility preservation should only be performed within well organized and professional networks and in highly specialized centers.
Study funding/competing interest(s): Funding by University(ies), Funding by National/International Organization(s), University Hospiatl Erlangen, Erlangen, Germany, FertiPROTECT, www.fertiprotekt.eu, The authors declare that they have no competing interests.
Trial registration number: None.

P-296 Follicle development after xenotransplantation of cryopreserved/thawed human ovarian tissue in SCID mice within 4 different observation periods

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Study question: How long should the xenotransplantation of cryopreserved/thawed human ovarian tissue be conducted to evaluate the dynamics of follicular growth?
Summary answer: After 4 weeks of grafting, secondary follicles can already be observed; antrum formation is present within 12 to 16 weeks of xenotransplantation.
What is known already: Ovarian tissue cryopreservation and transplantation are promising options for fertility preservation in young female cancer patients. Many studies have been performed to evaluate the follicular growth in cryopreserved/thawed human ovarian tissue. Xenotransplantation into SCID (severe combined immunodeficient) mice is a good tool to assess follicular growth and morphology prior to autotransplantation. However, the optimal observation period of follicular growth after xenotransplantation of cryopreserved/thawed human ovarian tissue into SCID mice is still unclear.
Study design, size, duration: Cryopreserved/thawed human ovarian tissue obtained from cancer patients ($n = 8$) was xenotransplanted into 6-week-old SCID mice ($n = 39$) and was randomized to 4 groups of observation periods: 4, 8, 12 and 16 weeks.
Participants/materials, setting, methods: Two 3-mm-pieces of cryopreserved/thawed ovarian tissue donated by 8 cancer patients were transplanted into a subcutaneous neck-pouch of 6-week-old ovarectomized SCID mice for 4, 8, 12 and 16 weeks. By the end of observation periods, grafts were recovered and processed for histomorphological analysis. Tissue directly after thawing served as pregraft-control.
Main results and the role of chance: 38 out of 39 mice (97.4%) survived the operation. So far all grafts have been recovered and were macroscopically comparable to pregraft-controls with visible vascularization. Seven mice are still under observation. After 4 weeks of xenografting, the percentage of primordial follicles was reduced to 41.7% ($n = 11$) compared to pregraft-controls (88.2%, $n = 9$). A reduced percentage of primordial follicles was also observed after 8, 12, and 16 weeks of grafting (27.0, 14.0, 10% respectively, $n = 4$ per group). Whereas the percentage of growing follicles increased after 4, 8, 12 and 16 weeks of grafting (30.9, 43.2, 64.2, and 50%) compared to only 1% in pregraft-controls. Antrum formation was already observed within 12 and 16 weeks.
Limitations, reason for caution: The inhomogeneous follicle distribution may make difficulties in comparing the follicle numbers between patients. It is also still difficult to extrapolate the results from xenografting experiment to patient's conditions, however it still is the closest experimental approach.
Wider implications of the findings: The results from this study can be implemented to select the optimal observation period in cryopreserved/thawed human ovarian tissue xenotransplantation. So that future xenotransplantation experiments might be performed more efficiently. The information from this study might also be used for future application, for example *in vivo* oocyte maturation in conditions where cryopreserved/thawed human ovarian tissue retransplantation cannot be carried out.
Study funding/competing interest(s): Funding by National/International Organization(s), This study is funded by the Tyrolean Research Foundation. S. Ayuandari receives scholarship from Indonesian Directorate General of Higher Education (DIKTI).
Trial registration number: Not applicable.

P-297 Ovarian response among adolescent and young reproductive-aged women undergoing fertility preservation compared with healthy oocyte donors

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Study question: How does ovarian response to stimulation differ among women less than age 25 undergoing oocyte cryopreservation (mOC) for medically-indicated fertility preservation (FP) compared with healthy oocyte donors (OD)?
Summary answer: Controlled ovarian hyperstimulation (COH) was effective for all patients undergoing mOC. The youngest patient in this group was 12 years of age and menstruating, confirming that OC can represent a viable FP option, even for very young pubertal girls.
What is known already: Chemotherapy and/or radiation treatment frequently render young reproductive-aged women infertile with greatly diminished or absent ovarian function. The advent of OC techniques has provided one option among several for preserving a young woman's fertility, although few data exist regarding FP outcomes in early pubescent and adolescent girls.
Study design, size, duration: Chemotherapy and/or radiation treatment frequently render young reproductive-aged women infertile with greatly diminished or absent ovarian function. The advent of OC techniques has provided one option among several for preserving a young woman's fertility, although few data exist regarding FP outcomes in early pubescent and adolescent girls.
Participants/materials, setting, methods: Patients < age 25 years who underwent mOC at our center were compared with healthy OD in the same age range. Data were analyzed using student's *T*-test and are presented as mean ± standard deviation. Significance threshold was $p < 0.05$.
Main results and the role of chance: The starting dose of gonadotropin (mOC: 340 ± 90 vs. OD: 240 ± 52 IU/day) and total gonadotropins administered (mOC: 3000 ± 1500 vs. OD: 2400 ± 840 IU) were greater for the mOC than the OD patients ($p < 0.05$). The total number of oocytes retrieved (mOC: 22 ± 13 vs. OD: 24 ± 11) and days of cycle monitoring (mOC: 7 ± 3 vs. OD: 7 ± 1 days) were not significantly different between the two groups. The number of mature (MII) oocytes (mOC: 15 ± 10 vs. OD: 20 ± 9) and the percentage of retrieved MII oocytes (mOC: 72% vs. OD: 83%) were significantly lower in the mOC vs. the OD patients ($p < 0.05$). Notably, MII oocytes for cryopreservation were successfully retrieved in all mOC cycles.
Limitations, reason for caution: This study was limited by its retrospective design. The average age of the mOC group (21.4 ± 3.1 years; age range: 12–25) was younger than that of the OD (23.3 ± 1.3 years; age range: 21–25; $p < 0.05$) as FP is offered to girls ≥12 years and donors must be at least 21 years of age.
Wider implications of the findings: These findings suggest that young pubertal and adolescent patients undergoing FP for medical indications can safely achieve adequate, albeit perhaps slightly diminished responses to gonadotropin COH as compared to healthy young ODs. Importantly, all young patients had mature oocytes cryopreserved, affording them an opportunity for future attempts at fertility when desired. Successful FP in our youngest patient (12 years) indicates that OC can represent a promising and potential option, even for newly pubertal girls.
Study funding/competing interest(s): Funding by University(ies), New York University Langone Medical Center.
Trial registration number: Not applicable.

P-298 The effect of FSH and activin A activation on Akt and pAkt protein levels of bovine ovarian follicles cultured *in vitro*

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Study question: This study was designed to test whether activation of bovine ovarian follicles by recombinant human follicle stimulating hormone (rhFSH) or recombinant human Activin A (rhActA) during *in vitro* follicle culture affects Akt or pAkt protein levels of the ovarian follicles.
Summary answer: The results of the study suggest that, the use of rhActA as activator of bovine ovarian follicular development *in vitro*, results in higher pAkt protein level of the ovarian follicles with respect to rhFSH or combined treatment of rhFSH and rhActA.
What is known already: rhFSH and rhActA have been used in mammalian ovarian follicle culture systems for activation of follicular growth *in vitro*. Treatment of the follicles by these activators in combined form resulted in bigger follicle sizes but unhealthy oocyte development. It is also suggested that these activators might be responsible for primordial follicle survival through Akt pathway. The effects of these hormones to follicles were generally evaluated by only morphological criteria after *in vitro* culture.

Study design, size, duration: In this study, five experimental groups existed: Cortical strips obtained from fresh bovine ovaries: (1) or cortical strips cultured *in vitro* without any activation (2) or with activation of rhFSH (3), rhActA (4) or combination of these activators (5). Each group contained the pool of follicles from five different ovaries.
Participants/materials, setting, methods: Ovarian cortical strips isolated from heifers were cultured *in vitro* for 6 days with addition of rhFSH (50 ng/mL), rhActA (100 ng/mL) or combination of these hormones. After culture period, strips including a number of secondary follicles were lysed for western blotting. Akt and pAkt protein levels were determined for each group.
Main results and the role of chance: 20% of control group of cortical strips cultured included secondary follicles. There were 23 and 26% of secondary follicle containing strips in rhFSH and rhActA treated groups respectively, whereas secondary follicle containing cortical strip ratio was 18% in combination group of rhFSH and rhActA.

Although, total Akt levels of different groups were found to be similar, pAkt level of the cortical strips exposed to a combination of rhFSH and rhActA was significantly ($P < 0.05$) lower than other groups. The control group of fresh cortical strips displayed significantly the highest pAkt level. rhActA group cortical strips had also a significantly higher level of pAkt with respect to other culture groups rhFSH group had also a higher level of pAkt with respect to control group of culture.
Limitations, reason for caution: These results only display the effect of follicle activating hormones to one step of possible regulators of follicular survival that includes active form of Akt protein. However, there are many other molecules to be evaluated after *in vitro* follicle culture procedures for establishment of optimal culture systems.
Wider implications of the findings: Akt is participated in anti-apoptotic mechanisms that prevent follicular atresia. Hence, reduced levels of pAkt in rhFSH and rhActA combination group might be the reason of lower numbers of follicles reaching secondary stage. In previous studies rhActA was shown to have role in early stage follicle survival and this approach might explain higher levels of pAkt in rhActA group. The results obtained from this study may suggest an evaluation criteria for *in vitro* cultured follicles.
Study funding/competing interest(s): Funding by National/International Organization(s), The Scientific and Technological Research Council of Turkey (TUBITAK).
Trial registration number: 112S390.

P-299 Natural ovarian stimulation (NATOS): an innovative estradiol-sparing, multiple follicle protocol suitable for fertility preservation in women with breast cancer

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Study question: It is suitable, for fertility preservation candidates with breast cancer, to recovering multiple mature eggs while avoiding hyper-estrogenism, an inherent by-product of controlled ovarian hyperstimulation (COH). Therefore, we aimed at testing an innovative COH protocol that dissociated E2 production from multiple follicle development using strong and sustained GnRH antagonist doses.
Summary answer: This pilot study indicates that NATOS is effective because it ensures serum E2 levels within the physiological range, strong follicle production and viable eggs, as reflected by high pregnancy rates. Therefore it constitutes a therapeutic option for women with breast cancer.
What is known already: Supraphysiologic E2 levels are unwelcome in fertility preservation candidates with breast cancer. Aromatase inhibitors have been used during COH to avoid hyper-estrogenism but serum oestrogens levels can exceed 600 pg/mL when numerous oocytes are recovered. Therefore, we tested an innovative COH protocol: we virtually curtailed endogenous LH levels by using strong and sustained GnRH antagonist doses while maintaining standard exogenous FSH-only administration.
Study design, size, duration: Prospective pilot study. We studied 5 IVF-ET candidates aged 28–35 years who volunteered to undergo NATOS. No exogenous E2 supplementation was performed during COH.
Participants/materials, setting, methods: On day 2 of their menstrual cycle, patients started daily recombinant FSH (doses fitting antral follicle counts, SC) paralleled with daily GnRH antagonist (3 mg/day, SC) administration. Ovulation triggering with hCG was performed when routine criteria of follicle maturation were met and IVF-ET was done according to our routine procedures.
Main results and the role of chance: On dhCG, serum E2 and LH levels were 343.8 ± 170.8 pg/ml and <0.1 mIU/ml, respectively. We obtained 16.2 ± 7.3 mature ovocytes and 12.2 ± 6.8 embryos. Endometrium thickness was preserved (9.2 ± 1.5 mm at day hCG) as well as fertilisation rates (73.0 ± 13.2%). Serum estradiol levels at day hCG per ovocyte retrieved was 21.5 ± 7.6 pg/mL. We can note the remarkable discrepancy between serum E2 levels on the day of hCG administration and oocyte and embryo availability. All 5 NATOS patients underwent ET and 4 of them achieved an ongoing pregnancy. Pregnancies evolved uneventfully for the time being 2 patients delivered healthy babies.
Limitations, reason for caution: These results need to be confirmed in prospective randomized comparative trials including a large number of patients. Dose-finding studies are needed to establish the effectiveness of lower GnRH antagonist doses.
Wider implications of the findings: These pilot results indicate that: 1. Profound LH suppression by strong and sustained GnRH antagonist doses in the presence of multiple growing follicles maintains E2 levels serum around the physiological range; 2. NATOS challenges the hypothesis that sizeable LH amounts are required for follicle and oocyte competence; 3. NATOS offers a new therapeutic option for stimulating women undergoing IVF-ET, in particular, those with breast cancer seeking fertility preservation or those suffering from repeated failures.
Study funding/competing interest(s): Funding by University(ies), None.
Trial registration number: None.

P-300 Transplantation-induced follicle activation as a cross-species phenomenon

Abstract withdrawn by the author

P-301 Use of combined oral contraceptive (COC) pill to schedule ovarian stimulation with letrozole for fertility preservation in breast cancer patients

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Study question: Time between surgery and the initiation of adjuvant chemotherapy is critical in patients with early stage breast cancer. We evaluated the use of COC pill to synchronise ovarian stimulation with letrozole to avoid delay in initiation of chemotherapy.
Summary answer: In patients with invasive breast cancer undergoing fertility preservation, use of COC pill to synchronise ovarian stimulation with letrozole is a potential option to manage timing between surgery and chemotherapy.
What is known already: Ovarian stimulation for oocyte and/or embryo cryopreservation is an established option for fertility preservation. Classically, antagonist cycles commence with menstruation. Oktay et al. (2008) reported time between surgery and chemotherapy of 45 days with letrozole cycles starting with menstruation. Waiting for menses may result in unacceptable delay between surgery and chemotherapy. Proposed strategies to avoid delay include random start, GnRH antagonists in luteal phase and ovulation triggering followed by GnRH antagonists in late follicular phase.
Study design, size, duration: We performed a retrospective analysis, between June 2007 and September 2013, of 35 patients with invasive breast cancer. They were referred to the Swiss French-Speaking Fertility Preservation Network for consideration of cryopreservation of oocytes and/or zygotes between surgery and adjuvant chemotherapy.
Participants/materials, setting, methods: Women of reproductive age were reviewed by a fertility specialist. COC (0.03 mg ethinylestradiol/0.15 mg

levonorgestrel) was started between day 2–5 of the cycle. Once ovarian stimulation was scheduled, COC was stopped. Two days later letrozole (5 mg/day) was introduced. A further 2 days later ovarian stimulation was started using an antagonist protocol.
Main results and the role of chance: Median age at stimulation was 31 years (25–39) and 82.9% were nulliparous. Cancer characteristics: stage (IA: 40%; IIA: 34.3%; IIB: 11.4%; IIIA: 11.4%; IIIC: 2.9%), grade (G1: 5.9%; G2: 26.5%; G3: 67.6%), receptors expression (oestrogen: 62.9%; progesterone: 57.1%; HER2 3+: 27.3%). Median anti-Müllerian hormone level: 17.3 pmol/l (1.0–78.2). Median duration of COC (7 days, 2–73), stimulation (10 days, 6–15), dose of stimulation (2025 IU, 862.5–3900), oestrogen level at trigger (1.6 nmol/l, 0.34–5.9), number of oocytes collected (11, 2–77) and mature (7, 1–52). 40% of patients choose to cryopreserve (median number) oocytes (7.5, 1–52), 45.7% zygotes (4.5, 1–12) and 14.3% both (6 (5–11) oocytes and 7 (3–10) zygotes). The median fertilisation rate was 83.3% (14.3–100). The median time from surgery to chemotherapy was 35 days (18–63).
Limitations, reason for caution: This is a retrospective analysis with a small sample size. Further evaluation of the potential role and safety of COC to synchronise ovarian stimulation with letrozole requires a prospective study with adequate length of follow up and outcomes such as use of preserved oocytes/zygotes, pregnancy rate, and cancer recurrence.
Wider implications of the findings: It is crucial not to delay chemotherapy when deciding to perform fertility preservation in patients with invasive breast cancer. COC pill allows flexibility of ovarian stimulation scheduling during the planning of fertility preservation. Since timing is important, it is also essential to refer patients to discuss fertility preservation as early as possible, preferably before surgery.
Study funding/competing interest(s): Funding by Hospital/Clinic(s), Lausanne University Hospital.
Trial registration number: None.

P-302 Conventional versus slush nitrogen vitrification of human ovarian tissue

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Study question: Does slush nitrogen vitrification improve the morphofunctional preservation of ovarian slices compared with conventional vitrification?
Summary answer: Results demonstrate that compared to conventional vitrification, slush nitrogen improves the preservation of follicles and stromal cells and decreases ultrastructural damage of oocyte mitochondria.
What is known already: Controversial data in literature about the efficiency of vitrification of human ovarian tissue can be due to limitative factors as the speed of freezing, the choice of supports and the use of high concentrations of cryoprotectants. The use of slush nitrogen that avoids the Leidenfrost effect increasing the cooling rate to 135,000°C/min, has been shown to improve the clinical efficacy of human oocyte vitrification.
Study design, size, duration: We performed a control vs. treatment study on human ovarian biopsies collected during laparoscopy from seven consenting patients. Cortical strips (2 mm × 4 mm × 1 mm) of each patient were processed for histology, confocal microscopy and ultrastructure either as fresh controls (F) or after vitrification in liquid (LN2) or slush nitrogen (SN2).
Participants/materials, setting, methods: Ovarian strips were treated at RT 5 min in 25%, 5 min in 50%, and 1 min in 100% vitrification solution (VS: MEM, HSA 20 mg/ml, DMSO 10%, EG 26%, PVP 2.5%, sucrose 1 M), plunged in LN2 or SN2 and thawed at 37°C in culture medium with 1 M (15 s), 0.5 and 0.25 M sucrose (5 min).
Main results and the role of chance: A total of 978 follicles (F, $n = 206$; LN2, $n = 468$; SN2, $n = 304$) were studied. The percentages of grade 1–3 follicles in F were not affected by SN2 vitrification (50.3 vs. 50.1, 18.9 vs. 21.1, 30.8 vs. 28.8, NS) whereas a highly significant decrease of grade-1 follicles (28.1%) and increase of grade-2 follicles (37%) ($P < 0.01$) occurred after vitrification in LN2. Preliminary ultrastructural data showed that LN2 follicles ($n = 6$) had increased signs of cryoinjuries, i.e., a disorganized layer of granulosa cells (GC), oocyte with extracted cytoplasm and damaged mitochondria (F, 32.4; SN2, 38.6; LN2, 65%; $P < 0.01$) compared to both fresh ($n = 5$) and SN2 follicles ($n = 5$). Stromal cells appeared heterogeneous in all samples.
Limitations, reason for caution: The ultrastructural data are preliminary and the study is *in vitro* evaluation that should be warranted by post thawing *in vitro* culture and xenotransplantation.
Wider implications of the findings: SN2 vitrification, allowing a better preservation of grade 1 follicles, 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 S.*p*.A. Rome.
Trial registration number: None.

P-303 The research on the xenotransplantation of human frozen-thawed ovarian tissues in vitro culture in the presence of gdnf

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Study question: Whether could human ovarian follicles better grow and develop when the human frozen-thawed ovarian tissues were xenotransplanted to nude mices after *in vitro* culture in the presence of GDNF.
Summary answer: The follicles of 100 ng/ml GDNF grafted group have the better growth and development potential than that of the grafted group.
What is known already: Recently, glial cell line-derived neurotrophic factor (GDNF) was found playing an important role in male and female reproductive system. It has been confirmed to promote follicular development in mice, porch and sheep. But the effects of GDNF on human follicular development have not yet be demonstrated by related reports.
Study design, size, duration: We collected 30 normal ovarian cortex tissues. These ovarian tissues were cryopreservated by the direct cover vitrification. The frozen-thawed human ovarian tissues were cultured *in vitro* for 6 days, by using the basic culture medium (the grafted group) and the 100 ng/ml GDNF culture medium (100 ng/ml GDNF grafted group). In the two groups, the human ovarian tissues were xenotransplanted under kidney capsule of nude mice. We had finished this experiment for more than 2 years.
Participants/materials, setting, methods: In the above two groups, the human ovarian tissues were xenotransplanted under the left and right kidney capsule of nude mice. After the xenotransplantion for 1 week, we tested the histology and the PCNA and CD34 immunohistochemical staining. And we also tested the histology and the PCNA and CD34 immunohistochemical staining in the frozen-thawed human ungrafted ovarian tissues (ungrafted group).
Main results and the role of chance: We used 15 nude mice in this experiment. 13 nude mice survived (survival rate 86.67%). We recovered 25 grafts (recover rate 96.15%). In grafted group and 100 ng/ml GDNF grafted group, the percentage of primordial follicles was significantly lower and the percentage of primary follicles was significantly higher than that in ungrafted group. Among the three groups, there was statistical significancy between each two groups ($P < 0.05$). The percentage of secondary follicles present in 100 ng/ml GDNF grafted group was significantly higher than that in control ungrafted group ($P < 0.05$). The PCNA positive rate in 100 ng/ml GDNF grafted group was highest among the three groups ($P < 0.05$). The microvascular density in 100 ng/ml GDNF grafted group was higher than that in the grafted group ($P > 0.05$).
Limitations, reason for caution: Although we demonstrated that the follicles of 100 ng/ml GDNF grafted group have the better growth and development potential than that of the grafted group, we could not make preantral follicles *in vitro* maturation.
Wider implications of the findings: We demonstrated that GDNF could promote the frozen-thawed human preantral follicular development.
Study funding/competing interest(s): Funding by University(ies), Funding by Hospital/Clinic(s), the First Affiliated Hospital of Zhengzhou University and Health Department of Henan Province.
Trial registration number: No.

P-304 Knowledge, attitudes and awareness regarding fertility preservation among cancer patients, oncologists and clinical practitioners in Lebanon

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Study question: What are the attitudes, knowledge and awareness of fertility preservation and its options among patients', oncologists' and clinical practitioners' (CPs) in Lebanon?
Summary answer: The attitudes and knowledge of FP among health professionals and cancer patients in Lebanon is poor, awareness therefore should be promoted. Many barriers to FP knowledge among patients and physicians were identified. Moreover difficulties were identified among health practitioners towards the discussion of FP options with patients.
What is known already: Fertility Preservation (FP) is a rapidly developing medical field aiming to help women, men and children overcome the problems of infertility associated with cancer treatments such as radiation and chemotherapy. Increasing survival rates of cancer patients emphasizes the significance of quality of life concerns that include preservation of fertility. In Lebanon, little is known about FP practices directed towards cancer patients, and even less about the attitudes, knowledge and awareness of FP among health professionals.
Study design, size, duration: This was a cross sectional study involving surveys administered to clinical practitioner's (CPs, $n = 88$), and patients ($n = 141$) at the American University of Beirut Medical Center (AUBMC), Lebanon and oncologists ($n = 53$) all over Lebanon between March 2012 and February 2013. There were no interventions in this study.
Participants/materials, setting, methods: Three different surveys were administered to CPs, patients' and oncologists, with the main outcome measures being the attitudes, awareness and knowledge score towards fertility preservation.
Main results and the role of chance: *Awareness of FP*: Approximately 90% of CPs and 94% of oncologists agree that FP should be discussed with the patient before their cancer treatment. Patients were found to be undecided towards the acceptance of FP religiously (32.6%), its cost (34.8%) and success rate (37.6%). *Attitudes of FP*: there is evidence towards the existence of gender stigmatization in relation to informing patients of their FP options. *Knowledge of FP*: there was conflicting knowledge of FP options available in Lebanon among oncologists. CPs were more likely to have accurate knowledge of FP options and treatment. The mean FP knowledge score among patients was 7.57 ± 3.20, with a maximum score of 18 possible.
Limitations, reason for caution: The main limitation of this study was the structure of the questionnaire. This limited the potential for an in depth analysis of the practice behavior of oncologists and CPs; patient characteristics as barriers towards FP and system barriers to FP.
Wider implications of the findings: AUBMC captures 15.3% of the cancer patients in Lebanon; currently the fertility rate in Lebanon is 1.76-below the replacement rate. More is needed to be done to ensure that oncologists and CPs are communicating and discussing FP with their patients and among themselves. A proactive approach needed to educate, train, and increase the awareness of current FP options in relation to cancer treatment in order to empower patients to seek fertility preservation.
Study funding/competing interest(s): Funding by Hospital/Clinic(s), American University of Beirut Medical Center (AUBMC). However this study received no funding, and there are no competing interests to declare.
Trial registration number: Not applicable.

P-305 Acute requests for cryopreservation of oocytes: a challenge for IVF-clinics

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Study question: How can IVF-clinics identify organizational obstacles inherent to acute cryopreservation and how can IVF-clinics develop a new clinical pathway to overcome these obstacles?
Summary answer: 'Strengths, Weaknesses, Opportunities and Threats' (SWOT)-analyses proved useful both to explore organizational obstacles and to evaluate a clinical pathway for acute requests for cryopreservation of oocytes.
What is known already: Obstacles limiting the access to fertility preservation (FP) have been described from the perspective of the patient and the health care professional. No studies have described obstacles of organizing FP from the perspective of the IVF-clinic. Cryopreservation of oocytes is the most common form of FP and requires interdisciplinary collaboration, adequate information provision and early referral. Because IVF-clinics usually organize elective care, setting up a new clinical pathway for acute cryopreservation of oocytes is a challenge.
Study design, size, duration: Quality management project, executed from May 2011 until July 2013.
Participants/materials, setting, methods: For this project, which included one IVF-clinic and two oncological sites, a four-step strategy was used: (1) monitoring baseline referral process of women with an acute request for cryopreservation of oocytes, (2) a baseline SWOT analysis, (3) the set-up of an acute clinical pathway for cryopreservation of oocytes and (4) a final SWOT analysis.
Main results and the role of chance: Acute cryopreservation of oocytes was requested for a total of 28 women. Most requests ($n = 16$; 70%) concerned women with breast cancer. The mean age of women with an acute request was 32.0 years (range 23–41 years). Five requests concerned FP for women younger than 18 years (all women with mosaic Turner syndrome). The implementation of information leaflets and pre-consultation questionnaires for women and referring professionals improved the quality of first FP consultation as evaluated by final SWOT-analysis. Collaboration with oncological centres and information about FP for professionals improved the referral process.
Limitations, reason for caution: This quality management project did not allow for benchmarking with other IVF-clinics. Examining the effectiveness of the clinical pathway in offering other FP treatments to all women with an acute indication was not possible. This project did not assess the quality dimension of patients.
Wider implications of the findings: The systematic approach of this quality management project led to the set-up of a separate clinical pathway for acute cryopreservation of oocytes and is advised to other IVF-clinics.
Study funding/competing interest(s): Funding by National/International Organization(s), Foundation NutsOhra (project number CE201003) and Virtutis Opus.
Trial registration number: Not applicable.

P-306 No effect of VEGF and bpV(HOpic), a potent inhibitor of PTEN, on primordial follicle growth activation in a human ovarian tissue xenotransplantation model

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Study question: Is it possible to optimize a SCID-mouse xenotransplantation-model by the use of Vascular Endothelial Growth Factor (VEGF) and bpV(HOpic) to the extent of allowing complete human folliculogenesis with a sufficiently large, growing follicular cohort to assess intra-ovarian mechanisms during early growth and differentiation?
Summary answer: The xenotransplantation model yielded mature and apparently healthy oocytes. An enhancing effect of bpV(HOpic) on the activation rate of human primordial follicles after xenotransplantation could not be detected. Likewise, no effects of VEGF treatment on neovascularisation were found.
What is known already: Human ovarian tissue transplanted into immunodeficient mice is likely to engraft, however, necrosis may occur due to insufficient neovascularisation. VEGF plays an angiogenenic, but also nonangiogenic role, by stimulating the production of growth factors and mobilization of progenitor cells.

Deletion of genes in the PTEN-PI3K-Akt-Foxo3 pathway exhibited premature activation of dormant follicles and incubation of mouse ovaries *in vitro* with a PTEN inhibitor (bpV(HOpic)) and a PI3K activating peptide may activate dormant follicles.
Study design, size, duration: Prospective, controlled study on administration of VEGF to mice and treatment of human ovarian tissue with bpV(HOpic) before xeno-transplantation. Ovarian tissue was donated by two patients (30 and 26 years), cut, randomly allocated to 5 experimental groups and transplanted to 20 female SCID mice (two pieces per animal) for 16 weeks.
Participants/materials, setting, methods: 40 cortex fragments were randomly allocated to five experimental groups. Group 1: tissue was cultured before transplantation for 24 h in 100 mM bpV(HOpic). Group 2: tissue cultured without bpV(HOpic). Group 3: 500 ng VEGF were administered to the mice. Group 4: administration of VEGF solvent. Group 5: combination of group 1 and group 3. After 16 weeks of transplantation the ovarian tissue was removed and visible follicles were counted and opened to extricate

oocytes. The tissue and oocytes were fixed for histological evaluation and immunohistochemistry.

Main results and the role of chance: After the transplantation period 12 oocytes (group 1: 3 germinal vesicle (GV); group 2: 1 metaphase I (MI), 1 GV; group 3: 1 GV; group 4: 1 metaphase II, 2 MI, 3 GV; group 5: no oocytes) from the xenografted tissue could be extracted. The immunohistochemical analysis of the oocytes showed normal and aberrant oocytes. The oocyte quality was independent from the experimental group and the developmental stage of the oocyte.

In all experimental groups the number of antral follicles increased while the number of primordial follicles decreased. The proportion of follicles with high morphological quality was not statistically significant between the experimental groups ($p = 0.891$). The proportion of proliferating ($p = 0.759$) and apoptotic ($p = 0.08$) follicles did not differ between the experimental groups. The immunohistochemically assessment of the number of newly formed blood vessels is still in progress.

Limitations, reason for caution: Differences between groups in various outcomes may not have reached statistical significance due to sample size constraints. Human ovarian tissue with high primordial cell count is difficult to obtain. The xeno-transplantation model appears still to be limited by significant necrosis despite fresh transfer of cortex after retrieval.

Wider implications of the findings: The previously described activation of dormant follicles by a PTEN inhibitor could not be replicated in this human-mouse model system. An optimized SCID mouse human follicular growth model would allow the study of early events in recruitment and folliculogenesis, as well as clinical applications in the context of quality control and method optimization of fertility preserving techniques.

Study funding/competing interest(s): Funding by National/International Organization(s), Funding by Commercial/Corporate Company(ies), unrestricted educational grant by Ferring GmbH, Deutsche Forschungsgemeinschaft (DFG), FOR 1041, Germ Cell Potential.

Trial registration number: For basic science a trial registration number is not required.

P-307 Efficacy of random-start controlled ovarian stimulation in cancer patients

Abstract withdrawn by the author

P-308 Morphological and apoptotic evaluation of vitrified human ovarian tissue

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Study question: The aim of this study was to develop a vitrification procedure for human ovarian tissue cryopreservation in order to optimally preserve the morphological characteristics of ovarian tissue. To evaluate the efficiency of this method Light Microscopy (LM), Transmission Electron Microscopy (TEM) and TUNEL assay were used.

Summary answer: After vitrification stromal compartment maintained morphological and ultrastructural features similar to fresh tissue, and any apoptotic changes were observed. Regarding follicles, the vitrification did not induce nuclear fragmentation in the granulosa cells as well as in the oocyte; however the oocyte cytoplasm appeared slightly negatively affected by vitrification.

What is known already: Vitrification might be an emerging alternative procedure for the cryopreservation of ovarian tissue. Many studies support vitrification as the method of choice for ovarian tissue cryopreservation, providing similar results to conventional freezing, with the additional advantage of preserving the ultrastructure of stromal tissue, that is usually affected by freezing. Although these results are encouraging, some authors report that vitrification caused extensive morphological and functional damages to primordial follicle in human ovarian tissue.

Study design, size, duration: The preservation of ovarian tissue was evaluated by LM, TEM and TUNEL assay on fresh and vitrified/warmed tissue of 5 female to-male transgender subjects suffering from Gender Identity Disorder undergoing sex reassignment surgery by hysterectomy-ovarectomy and who have donated their ovarian tissue for research.

Participants/materials, setting, methods: Ovarian biopsy was cut into large size sample and cryopreserved using the vitrification/warming protocol, based on two incubation solutions at different cryoprotectant concentrations and on the use of an open system. Both fresh and vitrified/warmed tissues were processed for LM, TEM and TUNEL assay.

Main results and the role of chance: By LM, the vitrified samples showed oocyte nucleus with slightly thickened chromatin and irregular shape, while granulosa cells were well preserved. The ovarian stromal maintained the features of fresh tissue. By TEM, the vitrified oocytes showed nucleus with slightly irregular shape and finely dispersed chromatin. Clear vacuoles were seen in the oocyte cytoplasm and a slight clarification of the cytoplasmic matrix as well. Cytoplasmic organelles were altered in shape and distribution. Irregularly shaped mitochondria were found scattered in the cytoplasm or around large vacuoles. Granulosa cells were adherent to oocyte. Stromal cells showed dispersed chromatin and homogeneous cytoplasm with slight vacuolization. TUNEL assay demonstrated the lack of apoptosis induction by vitrification in ovarian tissue.

Limitations, reason for caution: A limitation of the present study was the lack of functional tests that allowed to assess if the damage to oocytes was reversible and transitory or not. Further investigation such as tissue culture and/or xeno-transplantation might give information about viability of the follicle and the stroma after vitrification.

Wider implications of the findings: As cryopreserved ovarian tissue can be transplanted orthotopically or heterotopically to restore both steroidogenic and gametogenic functions, the use of a well-preserved ovarian tissue is a fundamental prerequisite to obtain great results in clinical application. Therefore, the optimization of the vitrification protocol could allow to switch from conventional freezing to vitrification for human ovarian tissue cryopreservation.

Study funding/competing interest(s): Funding by University(ies), University of Bologna.

Trial registration number: Clinical trial no. 61/2007/O/Tess.

P-309 How do female patients experience fertility preservation counselling and decision-making

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Study question: How do female patients experience fertility preservation (FP) consultation (FPC) with a specialist in reproductive medicine and do patient experiences relate to decisional conflict and regret with regard to the FP decision?

Summary answer: Patients indicated room for improvement with respect to their experiences with various aspects of FPC. Negative experiences were associated with decisional conflict, whereas decisional conflict related to decision regret.

What is known already: When confronted with a need for gonadotoxic treatment for either cancer or a benign disease, girls and young women will have to make an irreversible choice with regard to FP within a short period of time. Patients may experience difficulties in decision-making (decisional conflict) with respect to this decision and develop regret during follow-up. Patients' knowledge about FP and their opportunities to ask questions during FPC have previously been inversely related to decisional conflict.

Study design, size, duration: A questionnaire on experiences with FPC – designed after qualitative research – was retrospectively distributed to a cohort of 146 FPC patients counselled between July 2008 and July 2013. Aiming to minimise recall bias, we defined a subgroup of patients counselled since 2011 and who did not try to conceive.

Participants/materials, setting, methods: Patients were aged ≥16 years when we distributed the questionnaire and had received FPC for either cancer or a benign disease in a single university hospital in the Netherlands. Apart from patient experiences, patient characteristics, follow-up, decisional conflict, and decision regret were assessed in the questionnaire.

Main results and the role of chance: A total of 79 patients (54%) responded. Patients indicated room for improvement with respect to their FPC experiences. Out of 64 patients to whom FP was offered, 60 evaluated their decisional conflict. Negative experiences were associated with decisional conflict regarding the FP decision (not enough time for counselling: $p < 0.0001$; not having the opportunity to ask all questions: $p < 0.0001$; not feeling supported by the counsellor: $p = 0.0003$; not all applicable FP options were discussed: $p = 0.0001$; benefits and disadvantages of FP options were not clearly explained: $p = 0.0005$). Decisional conflict was related to decision regret ($p < 0.0001$). In the subgroup of patients counselled after 2011 who did not try to conceive ($n = 33$), patient experiences remained negatively associated with decisional conflict, whereas decisional conflict was related to regret ($p < 0.0001$).

Limitations, reason for caution: Given a retrospective design, we are not informed about the causality of the associations observed. We studied Dutch patients counselled in a single centre who were at least 16 years old when filling in the questionnaire. This may limit the generalisability of our data to other settings and populations.

Wider implications of the findings: More attention should be paid to the importance of patients' experience with FPC and the association between patients' FPC experiences and decisional conflict. Future research should focus at the causality of the associations between patient experiences, decisional conflict and decision regret. Interventions aiming at improving patients' comprehension of the topic of FP and their feelings of being supported, such as written information material, a decision aid, or additional contact with the FP counsellor, are advisable.

Study funding/competing interest(s): Funding by University(ies), Nijmegen Centre of Evidence Based Practice.

Trial registration number: Not applicable.

P-310 Referral of female cancer patients for fertility preservation

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Study question: What are the current referral rates for newly diagnosed female cancer patients, aged 0–39 years, to a reproductive medicine specialist for fertility preservation (FP) counselling (FPC) and what changes can be detected in the numbers and characteristics of patients receiving FPC during time?

Summary answer: Although the absolute number of patients receiving FPC increased during time, only 7.1% of all patients (age 0–39) were referred for FPC in our region (2011). Referral disparities were found with respect to age and cancer diagnosis. Changes were seen in patients' age, relationship status and FP choices during time.

What is known already: With FP prior to the start of gonadotoxic cancer treatment, an attempt is made to safeguard patients' future reproductive function. Despite patients' preferences, guideline recommendations, and rapid evolvement of FP techniques, referral rates for FPC are low. Barriers for referral and referral disparities have been identified in previous studies. However, these were conducted either in a non-European setting or without providing information about patients who did *not* receive FPC.

Study design, size, duration: Data on all female cancer patients receiving FPC at the Rumc – a Dutch university hospital – were retrospectively collected ($n = 233$; 2001–2013). Referral rates were calculated for 2009–2011, based on hospital files and data from the Dutch Cancer Registry ($n = 1487$).

Participants/materials, setting, methods: The characteristics of patients receiving FPC ($n = 233$) were described in the light of the introduction of new FP techniques, i.e., ovarian tissue cryopreservation and the vitrification of oocytes. Referral rates were calculated for patients with various diagnoses and ages.

Main results and the role of chance: A total of 1487 female cancer patients (age 0–39) were diagnosed with invasive cancer in the Rumc's region (2009–2011) of whom 91 (6.1%) received FPC at the Rumc ($n = 82$) or at an affiliating hospital ($n = 9$). In 2011, the percentage of patients referred for FPC was 7.1%. Referral disparities were found with respect to age and diagnosis, since patients aged 20–29 years or diagnosed with breast cancer, lymphoma, and leukaemia were referred more frequently compared to patients under the age of 20 years or diagnosed with neurological cancer, cancer of the head, neck, lung, skin, urinary tract, thyroid or adrenal gland, or eye. In the last years, patients tended to be younger, more frequently lacked a (stable) relationship and more frequently chose to perform FP.

Limitations, reason for caution: Referral rates might be underestimated as patients may not have an interest in FP, receive FPC elsewhere, or receive ovarian transposition without adjuvant FPC.

Moreover, we were uninformed about other characteristics than diagnosis and age of patients who did not receive FPC and could not investigate all possible referral disparities.

Wider implications of the findings: The low referral rates and referral disparities reported in the current study indicate that there are opportunities to improve referral practices. Future research should focus at (the implementation and evaluation of) interventions to improve referral practices, such as information materials for patients at oncology departments, discussion prompts, or methods to increase the awareness of physicians and patients of FP techniques and guidelines.

Study funding/competing interest(s): Funding by Hospital/Clinic(s), Funding by National/International Organization(s), Radboud University Medical Center, Nijmegen, The Netherlands, Nijmegen Centre for Evidence Based Practice.

Trial registration number: Not applicable.

P-311 Desire for children and self-reported mental health in 484 female and male survivors of cancer in reproductive age

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Study question: Is the desire for children among cancer survivors consistent with the preferences they had when cancer treatment and potential fertility preservation was planned?

Summary answer: Among survivors without a desire for children at time of cancer treatment, one out of six changed their mind and wished to have children 3–7 years later.

What is known already: Cancer patients with no desire for children at diagnosis are less likely to receive information about the impact of their planned treatments on future fertility and about fertility preservation. Cancer survivors that become involuntarily childless report more psychological distress and worse mental health compared to survivors that are able to conceive following treatment.

Study design, size, duration: Cross-sectional survey of survivors identified in national population-based cancer registries. Inclusion criteria: diagnosed 2003–2007 with a malignancy requiring a treatment recognized as gonadotoxic and/or treatment directed at the reproductive organs and being of reproductive age (18–45 years) at diagnosis. Eligible survivors ($N = 810$) were approached by a postal questionnaire during 2010.

Participants/materials, setting, methods: A total of 484 survivors, 156 men and 328 women (60% response rate) completed the postal survey, which included study-specific items on reproductive variables and the validated Swedish version of the Short Form 36 Health Survey (SF-36). Face validity and feasibility of study-specific items were confirmed in a pilot study.

Main results and the role of chance: Most survivors who had a desire for children at time of cancer treatment still wanted children 3–7 years later. In addition, a substantial group ($n = 55$, 17%) with no initial desire for children had changed their mind about wanting children after treatment. This group was characterized by young age ($p < 0.001$) and being childless at cancer diagnosis ($p < 0.001$); while most of the men in this group had banked sperm, none of the women had used fertility preservation. About a third of the survivors with a desire to have children had experienced difficulties achieving a pregnancy after the cancer treatment. An unfulfilled desire to have children was associated with worse mental health in the survivors.

Limitations, reason for caution: While no response bias was detected regarding age at diagnosis or time since diagnosis, the relatively low response rate may be a threat to the external validity. In addition, some caution is advised when drawing conclusions from retrospective data.

Wider implications of the findings: Health professionals in cancer care need to be aware that patients' plans for future children may change over time, particularly if they are young and childless at diagnosis. All patients of reproductive age should be provided with adequate information about the impact of cancer treatment on future fertility and fertility preservation.

Study funding/competing interest(s): Funding by National/International Organization(s), The Swedish Cancer Society.

Trial registration number: N/A.

P-312 Human ovarian tissue viability before and after cryopreservation in a major European centre

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Study question: What is the effect of cryopreservation and thawing of ovarian tissue following the protocols of a major European centre on the viability of ovarian tissue from oncological patients opting for fertility preservation?
Summary answer: Ovarian tissue cryopreservation and thawing significantly impairs the viability of stromal cells as well as follicles in the ovarian tissue of oncological patients opting for fertility preservation.
What is known already: Worldwide, cryopreserved ovarian tissue from at least 2500 patients is stored at a limited number of highly experienced centres. Pregnancies following ovarian tissue thawing and autotransplantation, have demonstrated that at least part of the ovarian tissue survives the cryopreservation and thawing procedure. It remains unknown, however, whether there is any room for improvement of frequently used protocols for cryopreservation and thawing in order to achieve higher pregnancy rates.
Study design, size, duration: This prospective cohort study was conducted from January through November 2012 and included 25 cancer patients opting for ovarian tissue cryopreservation at a Cryobank from a university hospital. For each patient, ovarian tissue viability was assessed before and after cryopreservation and thawing according to the Cryobank's protocols.
Participants/materials, setting, methods: 3-mm biopsies were taken from the ovarian tissue before and after cryopreservation/thawing, and subjected to various follicle viability assays (histology; Calcein viability assay, follicle steroid hormone production *in vitro*). In addition, glucose uptake *in vitro* was used to determine overall tissue viability.
Main results and the role of chance: Cryopreserved/thawed ovarian tissue showed a significant decrease in glucose uptake when compared to fresh (control) tissue (p = 0.004 during day 0–4 of the *in vitro* culture; p = 0.002 during day 4–7), indicating a negative influence of cryopreservation/thawing on the tissue's overall viability. With respect to follicle viability, statistically significantly lower E2 and progesterone production (day 4–7; p = 0.004 after correction for biopsy weight; p = 0.025 after correction for follicle viability count) were found for cryopreserved/thawed tissue. A lower percentage of the follicles was intact (histology) after cryopreservation/thawing (73 vs. 96% in fresh tissue). The numbers of viable follicles as determined by the Calcein viability assay were similar for fresh and cryopreserved/thawed tissue.
Limitations, reason for caution: Since only small biopsies were available, the number of follicles that could be evaluated was limited, which may have led to sample bias. Therefore, the exact consequences of our study for pregnancy rates after autotransplantation of ovarian tissue are not certain.
Wider implications of the findings: This study emphasizes the importance of comparing and optimizing protocols that are clinically used for cryopreservation and thawing of ovarian tissue. By optimising protocols, the viability of ovarian tissue after cryopreservation and thawing – and conceivably the clinical outcome after ovarian tissue autotransplantation – may be improved in the near future. Efforts to improve protocols should not only assess the viability of the follicles, but also the viability of the stromal cell compartment.
Study funding/competing interest(s): Funding by Hospital/Clinic(s), Funding by National/International Organization(s), Funding by commercial/corporate company(ies), 3.
Trial registration number: Not applicable.

P-313 Breakthrough in vitrification methods – a highly secure, safe and efficient aseptic method of vitrifying and storing oocytes for oocyte banking and all IVF indications

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Study question: Do we have to be afraid of aseptic vitrification protocols for oocyte cryopreservation and does this fear justify the wide spread use of open devices disregarding the guidelines of the EU-directives?
Summary answer: We developed a method for aseptic vitrification of oocytes which is, compared to 'open' non-aseptic devices a highly secure and shows efficient results. Our protocol fulfills all requirements to comply with the strict regulations in the EU and is applicable for egg donation and for other IVF indications.
What is known already: Despite the fact that non-aseptic vitrification implies risk of contamination with pathogens and – even more critical – direct contact with reactive chemical compounds present in liquid nitrogen during the whole storage period, this technique is applied in many centers worldwide. The aim of this study was to show that aseptic vitrification is not only working for zygotes and blastocysts but also for metaphase-II oocytes and it should be seen as state-of-the-art in the IVF-Lab.
Study design, size, duration: The data were collected in course of a multicenter study in three European IVF-centers. During 3 years 1.174 oocytes from 83 egg donation cycles and 64 IVF cycles were aseptically vitrified and warmed with our protocol. First endpoint was embryo development, second endpoint implantation and birth outcome.
Participants/materials, setting, methods: MII-oocytes derived from egg donation as well as from IVF-patients were aseptically vitrified and warmed using VitriSafe devices and protocol optimized for the closed device. After fertilization by ICSI or IMSI embryo development was monitored. Transfer took place either on day 3 or day 5. Pregnancy outcome was evaluated.
Main results and the role of chance: After vitrification/warming a survival rate of 85.6% was observed as evaluated by vital and morphologically normal oocytes 2 h after warming. Thereby, no statistically significant difference between donor oocytes and oocytes derived from the heterogeneous pool of IVF-patients was found. After ICSI or IMSI a fertilization rate of 77.6% was obtained, which was comparable to fresh cycles in the same time period. Further, a cleavage rate on day 3 of 95.9% was reported. After transfer of either cleavage stage embryos or blastocysts, the implantation rate ranged between 13.8% with day 3 embryo transfer and 24.1% after blastocyst transfer. Further a birth rate of 35.1% was documented. No minor or major birth defects were reported.
Limitations, reason for caution: The results demonstrate that our aseptic vitrification protocol is highly secure and efficient, not only for donor oocytes, which have the best chances to survive the stressful cryopreservation procedure, but also for oocytes from IVF-patients with impaired oocytes quality.
Wider implications of the findings: Some earlier investigations applying closed devices for vitrification reported reduced survival rates and poor embryo development; however these studies used different vitrification protocols. When using aseptic straws protecting gametes from direct contract with liquid nitrogen, it has to be taking into account that the cooling rates (but not the warming rates!!) are reduced which has to be overcome by an adapted vitrification protocol in term of media and timing of exposure to CP solutions.
Study funding/competing interest(s): Funding by Hospital/Clinic(s), None.
Trial registration number: None.

P-314 GnRH agonist Leuprolide acetate does not protect human ovary and granulosa cells from chemotherapy induced damage

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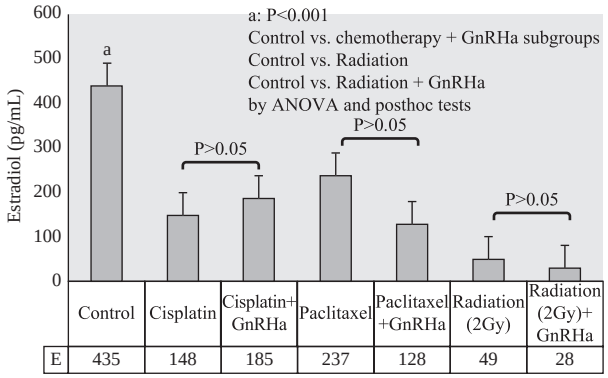
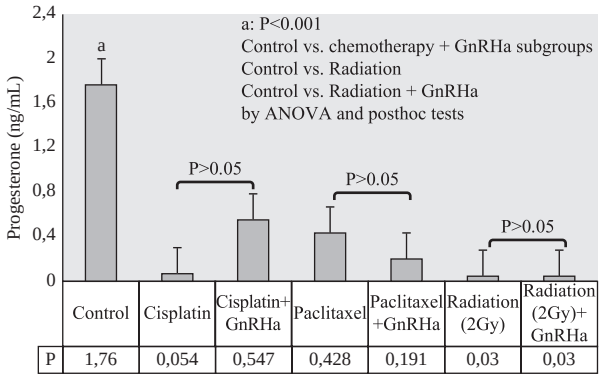
Study question: Do GnRH agonists really protect human ovary from chemotherapy induced damage?

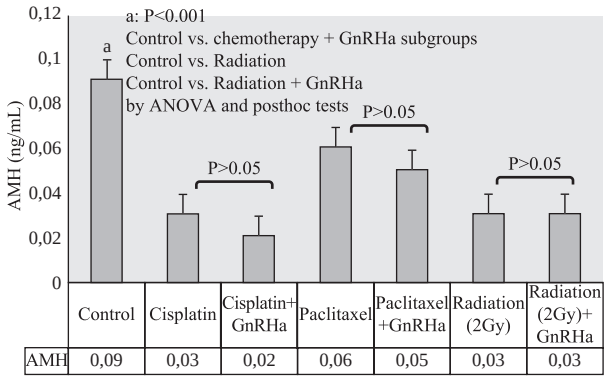
P-314 – Figure 1A



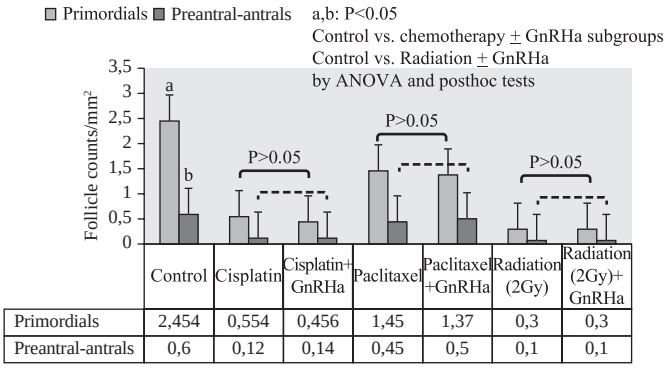
	Control	Cisplatin	Cisplatin+ GnRHa	Paclitaxel	Paclitaxel +GnRHa	Radiation (2Gy)	Radiation (2Gy)+ GnRHa
E	435	148	185	237	128	49	28



	Control	Cisplatin	Cisplatin+ GnRHa	Paclitaxel	Paclitaxel +GnRHa	Radiation (2Gy)	Radiation (2Gy)+ GnRHa
P	1,76	0,054	0,547	0,428	0,191	0,03	0,03

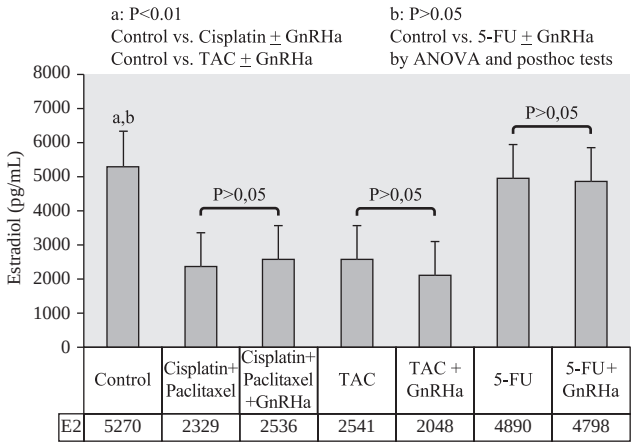


	Control	Cisplatin	Cisplatin+ GnRHa	Paclitaxel	Paclitaxel +GnRHa	Radiation (2Gy)	Radiation (2Gy)+ GnRHa
AMH	0,09	0,03	0,02	0,06	0,05	0,03	0,03

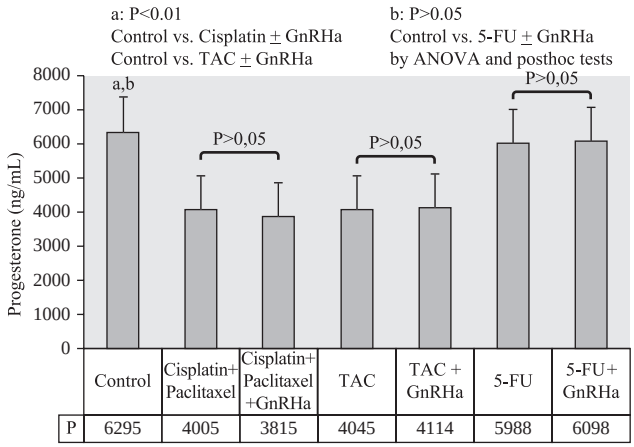


	Control	Cisplatin	Cisplatin+ GnRHa	Paclitaxel	Paclitaxel +GnRHa	Radiation (2Gy)	Radiation (2Gy)+ GnRHa
Primordials	2,454	0,554	0,456	1,45	1,37	0,3	0,3
Preantral-antrals	0,6	0,12	0,14	0,45	0,5	0,1	0,1

Figure 1B



	Control	Cisplatin+ Paclitaxel	Cisplatin+ Paclitaxel +GnRHa	TAC	TAC + GnRHa	5-FU	5-FU+ GnRHa
E2	5270	2329	2536	2541	2048	4890	4798



	Control	Cisplatin+ Paclitaxel	Cisplatin+ Paclitaxel +GnRHa	TAC	TAC + GnRHa	5-FU	5-FU+ GnRHa
P	6295	4005	3815	4045	4114	5988	6098

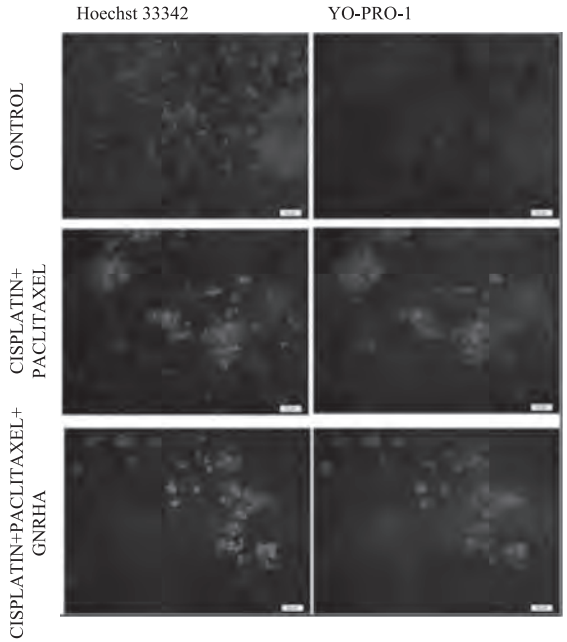
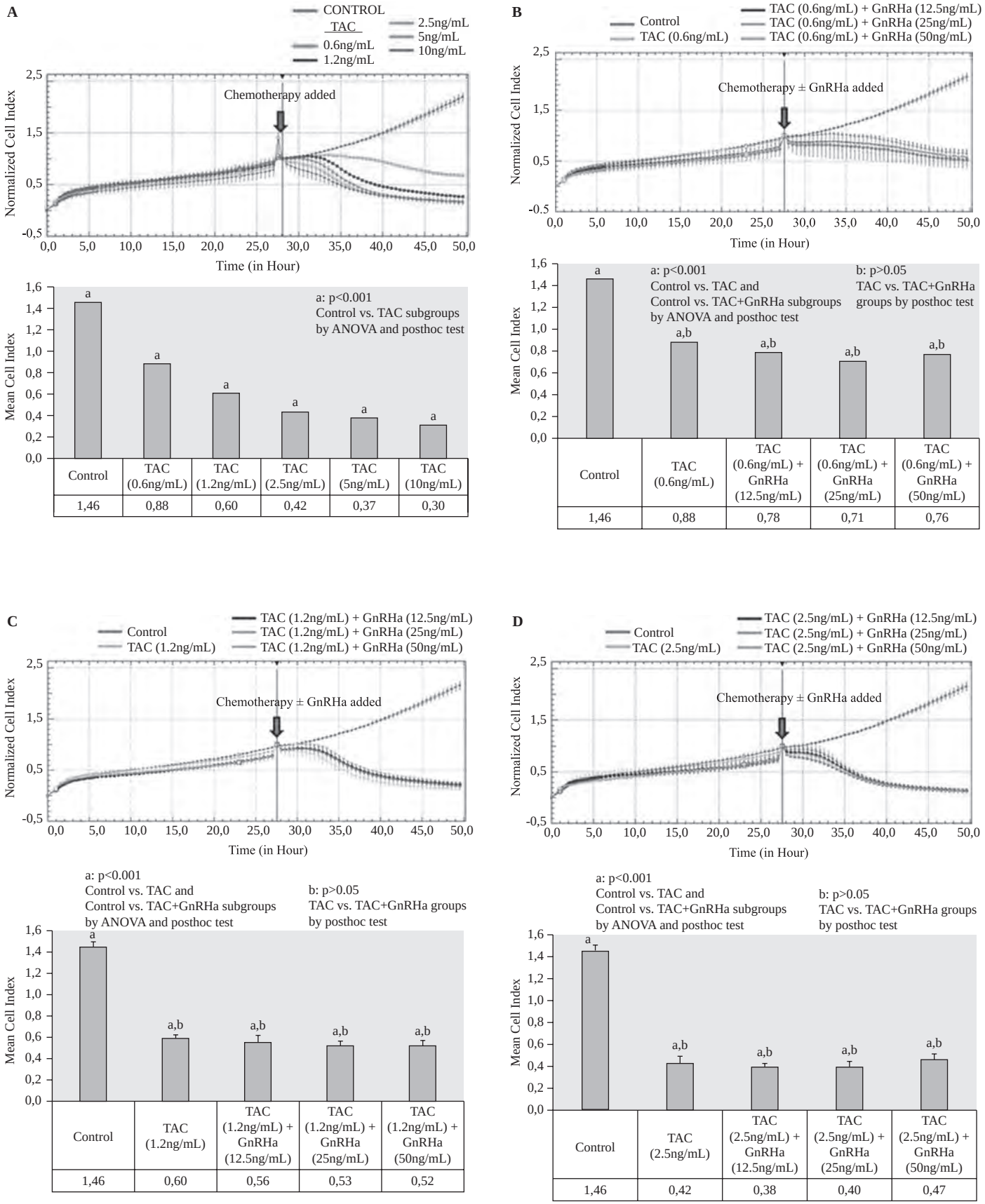


Figure 1C


A
CONTROL
TAC
0.6ng/mL
1.2ng/mL
2.5ng/mL
5ng/mL
10ng/mL
Chemotherapy added
Normalized Cell Index
Time (in Hour)
Mean Cell Index
a: p<0.001
Control vs. TAC subgroups
by ANOVA and posthoc test
Control | TAC (0.6ng/mL) | TAC (1.2ng/mL) | TAC (2.5ng/mL) | TAC (5ng/mL) | TAC (10ng/mL)
1,46 | 0,88 | 0,60 | 0,42 | 0,37 | 0,30
B
Control
TAC (0.6ng/mL)
TAC (0.6ng/mL) + GnRHa (12.5ng/mL)
TAC (0.6ng/mL) + GnRHa (25ng/mL)
TAC (0.6ng/mL) + GnRHa (50ng/mL)
Chemotherapy ± GnRHa added
a: p<0.001
Control vs. TAC and
Control vs. TAC+GnRHa subgroups
by ANOVA and posthoc test
b: p>0.05
TAC vs. TAC+GnRHa
groups by posthoc test
Control | TAC (0.6ng/mL) | TAC (0.6ng/mL) + GnRHa (12.5ng/mL) | TAC (0.6ng/mL) + GnRHa (25ng/mL) | TAC (0.6ng/mL) + GnRHa (50ng/mL)
1,46 | 0,88 | 0,78 | 0,71 | 0,76
C
Control
TAC (1.2ng/mL)
TAC (1.2ng/mL) + GnRHa (12.5ng/mL)
TAC (1.2ng/mL) + GnRHa (25ng/mL)
TAC (1.2ng/mL) + GnRHa (50ng/mL)
Chemotherapy ± GnRHa added
a: p<0.001
Control vs. TAC and
Control vs. TAC+GnRHa subgroups
by ANOVA and posthoc test
b: p>0.05
TAC vs. TAC+GnRHa groups
by posthoc test
Control | TAC (1.2ng/mL) | TAC (1.2ng/mL) + GnRHa (12.5ng/mL) | TAC (1.2ng/mL) + GnRHa (25ng/mL) | TAC (1.2ng/mL) + GnRHa (50ng/mL)
1,46 | 0,60 | 0,56 | 0,53 | 0,52
D
Control
TAC (2.5ng/mL)
TAC (2.5ng/mL) + GnRHa (12.5ng/mL)
TAC (2.5ng/mL) + GnRHa (25ng/mL)
TAC (2.5ng/mL) + GnRHa (50ng/mL)
Chemotherapy ± GnRHa added
a: p<0.001
Control vs. TAC and
Control vs. TAC+GnRHa subgroups
by ANOVA and posthoc test
b: p>0.05
TAC vs. TAC+GnRHa groups
by posthoc test
Control | TAC (2.5ng/mL) | TAC (2.5ng/mL) + GnRHa (12.5ng/mL) | TAC (2.5ng/mL) + GnRHa (25ng/mL) | TAC (2.5ng/mL) + GnRHa (50ng/mL)
1,46 | 0,42 | 0,38 | 0,40 | 0,47

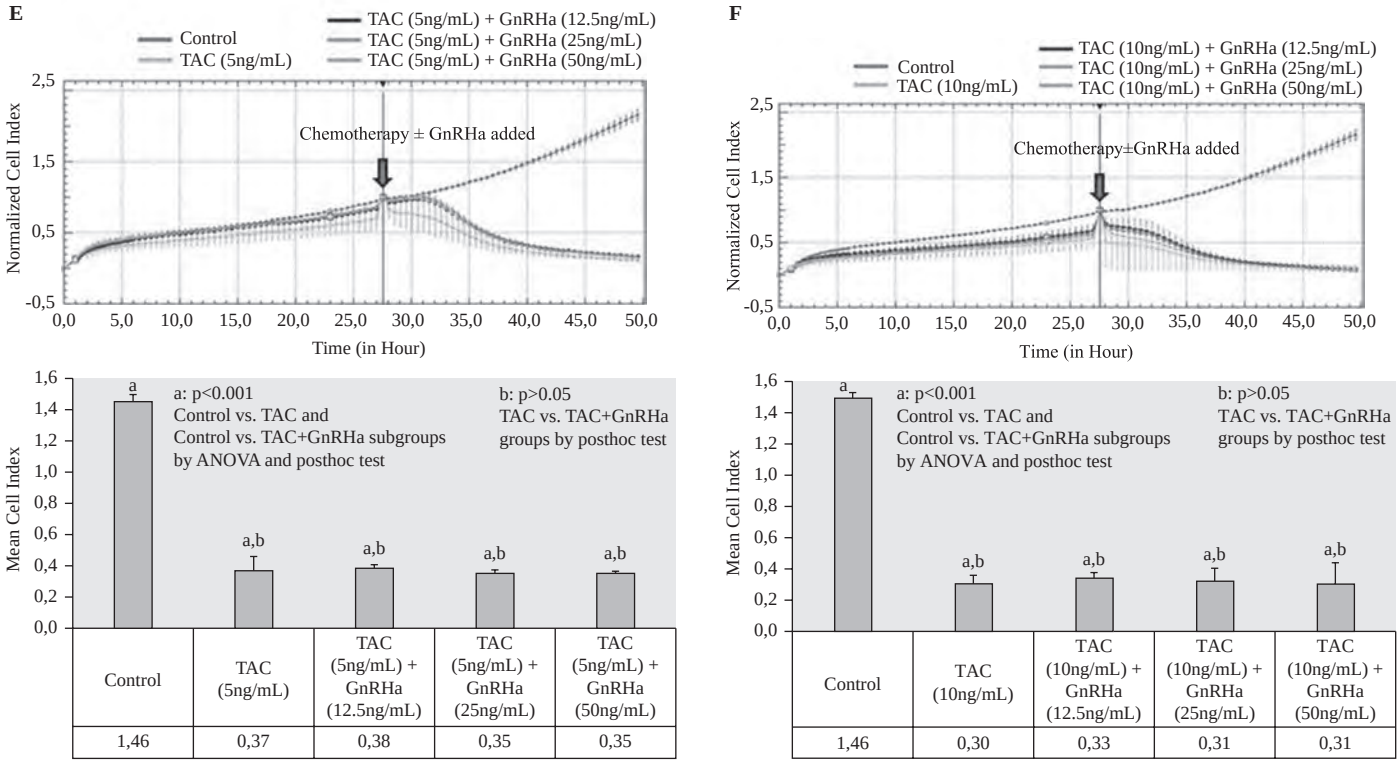
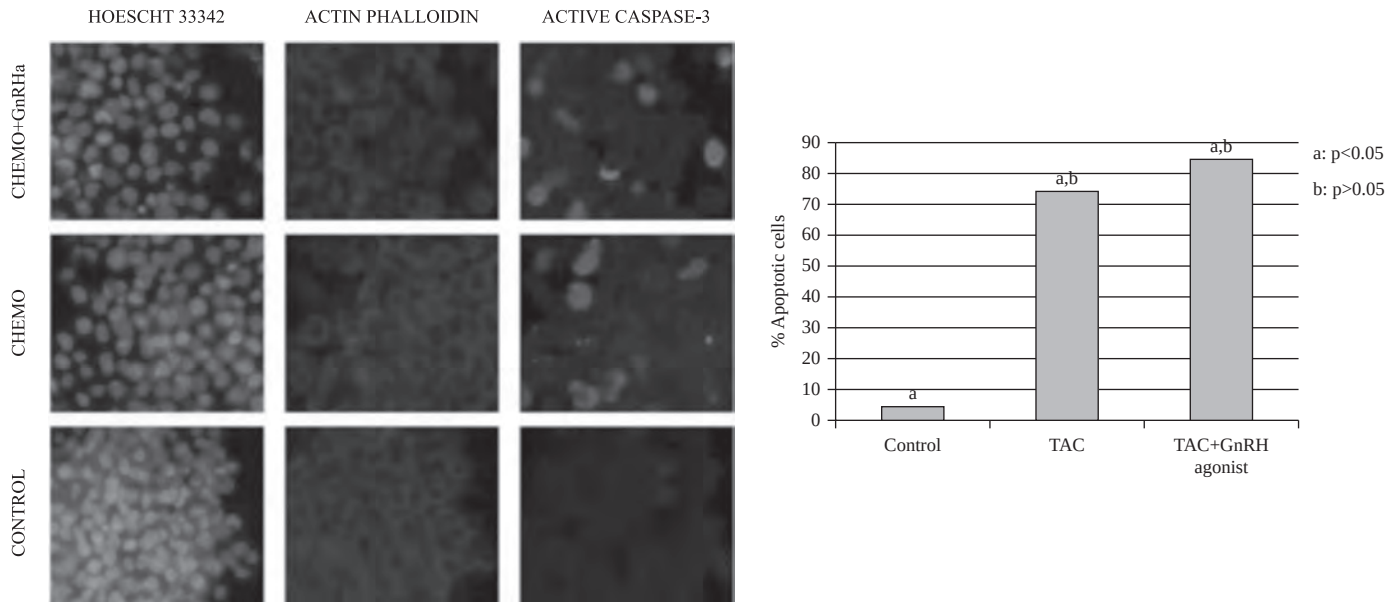


Figure 1D



Summary answer: No, GnRH agonist (GnRHa) leuprolide acetate does not offer any protection against chemotherapy induced damage in human ovary.

What is known already: Randomized controlled trials assessing whether or not the administration of GnRH agonists protect the ovaries of female cancer patients exposed to gonadotoxic chemotherapy regimens yielded conflicting results. We therefore hypothesized that if GnRH agonists really protect ovarian follicles from cytotoxic chemotherapy regimens via their cognate receptors in the ovary they should offer at least some degree of protection when used *in vitro*.

Study design, size, duration: An *in vitro* histomorphometric human study with quantitative hormonal, cell proliferation and apoptosis markers

Participants/materials, setting, methods: Ovarian cortical samples obtained from young patients (mean age 32 ± 2.4) undergoing laparoscopic excision of benign ovarian cysts were dissected equally into 1 cm × 0.5 cm pieces and cultured in 24 well format culture plate for 24 h. Luteal granulosa cells recovered from follicular fluid during oocyte retrieval procedure were plated (50,000 cells/well) in 24 well format culture plate for 24 h. Culture medium consisted of DMEM-F12 culture medium supplemented with 10% FBS. Chemotherapy agents with different gonadal toxicity were choses. Cisplatin (40 µg/mL), paclitaxel (2000 ng/mL), 5-FU (100 ng/mL) TAC combination (docetaxel, adriamycin, cyclophosphamide, 0.6 thru 10 ng/mL) were used at doses corresponding to their therapeutic blood levels. Since LD_{50} for human oocytes is 2 Gy this dose was chosen for radiation. Immortalized proliferating granulosa cells (COV434) were plated in E96 well plate (50,000 cells/well). When the cells reached the log phase, they were treated TAC chemotherapy regimen at five different concentrations (0.6–1.2–2.5–5–10 ng/mL) corresponding to the lowest and peak blood levels of the drugs. For each concentration of the combination, GnRH agonist

Leuprolide acetate was given at 3 different concentrations (12.5–25 and 50 ng/mL), which reflect the minimal blood and the highest intrafollicular concentrations of the drug. The effect of TAC chemotherapy regimen ± GnRHa on granulosa cells were monitored in a real-time quantitative manner using × Celligence system up to 50 h. The presence of GnRH receptor in granulosa cells were validated using RT-PCR method. The culture fluids of ovarian cortical samples and granulosa cells were assayed for estradiol, progesterone and AMH production. Granulosa cells incubated with chemotherapy agents ± GnRHa were processed for apoptosis marker cleaved caspase-3 expression and YO-PRO-1 staining under immunofluorescence microscope (Olympus IX71).

Main results and the role of chance

Ovarian cortical samples: *In vitro* E_2, P and AMH production from cortical samples incubated with cisplatin, paclitaxel and radiation were not different from their counterparts treated with GnRHa (Figure 1A). But compared to control their hormone production were significantly lower regardless of the addition of GnRHa. Similarly, neither the number of dormant primordials nor the growing follicle fractions (primary, preantral and antral) differed between chemotherapy and chemotherapy ± GnRHa groups. Control samples contained significantly higher number of follicles compared to chemotherapy/radiation ± GnRHa subgroups.

Luteal granulosa cells: E_2 and P productions of luteal granulosa cells exposed to antimetabolite chemotherapy drug 5-FU ± GnRHa were comparable to control cells whereas those cells treated with cisplatin, taxol, and TAC with and without GnRHa produced significantly less amount of E_2 and P than control cells. In accordance, the proportion of dead cells as evidenced by YO-PRO-1 staining were significantly higher in the cells exposed to toxic cisplatin and TAC chemo regimens. Addition of GnRHa did not decrease the number of dead cells (Figure 1B).

Immortalized granulosa cells: The cells exposed to TAC regimen at five different concentrations exhibited a dose dependent growth arrest and apoptosis. Concurrent GnRHa administration did not rescue the cells from apoptosis as shown by their downward growth curves and quantitative analysis of the cell proliferation indices (Figures 1C,D).

Consistent findings observed in ovarian samples and two different types of granulosa cells practically rule out the possibility that these results were obtained by chance.

Limitations, reason for caution: None.

Wider implications of the findings: These results strongly suggest that GnRH agonists does not protect human ovary and granulosa cells from chemotherapy and radiation induced damage *in vitro*, providing first time a molecular evidence against its protective effects on the ovary.

Study funding/competing interest(s): Funding by University(ies), Koc University School of Medicine, Istanbul Turkey.

Trial registration number: None.

P-315 Will women use their cryopreserved oocytes – a follow up study on reproductive choices and outcomes after freezing oocytes for medical reasons

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Study question: What are the reproductive choices and outcomes in women who cryopreserved their oocytes for medical reasons?

Summary answer: Women who cryopreserved oocytes for medical reasons and tried to become pregnant, first attempted natural conception and next resorted to assisted reproduction with fresh oocytes. So far, none of the cryopreserved oocytes were used. Half of the women attempting to conceive, became pregnant; most of them by natural conception.

What is known already: Women confronted with a risk of premature ovarian insuffiency (POI) due to gonadotoxic therapy, ovarian surgery or genetic predisposition, have an indication to cryopreserve oocytes. Many of these women will retain ovarian function, thus will have a chance of natural conception. The added value of cryopreserved oocytes to reproductive outcomes is unknown as there is a lack of follow-up of women who cryopreserved oocytes for medical reasons.

Study design, size, duration: A follow-up study of a cohort of 85 women who cryopreserved their oocytes for medical reasons between 2009 and 2012.

Participants/materials, setting, methods: Medical data from women who cryopreserved their oocytes at a university clinic in the Netherlands were extracted and self-report paper-pencil questionnaires were disseminated. The collected data considered: demographics, outcomes of ovarian stimulation, undergone fertility-threatening treatment, menstrual cycle changes, pregnancy attempts and outcomes and intended plan with cryopreserved oocytes.

Main results and the role of chance: Sixty-eight women, followed-up for an average 25.3 months, returned the questionnaire (response rate: 77%). None of the women had used her cryopreserved oocytes although 16 women tried to conceive. Eight of them were trying to conceive naturally, five conceived naturally within 2 months and three women conceived with assisted reproduction not requiring cryopreserved oocytes (two women with conventional IVF because of tubal pathology and endometriosis, one woman with IUI because of polycystic ovary syndrome). Three out of a total of eight pregnancies resulted in live birth, two in miscarriages and three were ongoing. Most women (71%) intended to conceive with their cryopreserved oocytes as last resource option.

Limitations, reason for caution: Transferability of our findings is challenged by the small sample but positively affected by our high response rate. As the time span between cryopreservation of oocytes and follow-up was short, follow-up of the cohort should be repeated in 2 years.

Wider implications of the findings: It is unclear whether starting assisted reproduction while having cryopreserved oocytes is the most appropriate clinical decision. Our findings emphasize the relevance of taking chances of natural conception into account in counselling women on live birth rates after cryopreserving oocytes.

Study funding/competing interest(s): Funding by Hospital/Clinic(s), Academic Medical Centre.

Trial registration number: Not applicable.

P-316 Altering life goals: a qualitative longitudinal study exploring experiences of fertility and parenthood after cancer

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Study question: How do men and women experience life with regard to fertility and parenthood in connection with a cancer diagnosis and 2 years later?

Summary answer: Among patients with an initial desire for children, the experience of cancer diagnosis and treatment triggers worries and may lead to a devaluation of the desire to have children or a struggle to obtain a central life goal.

What is known already: Infertility is a well-known side effect of cancer treatment and retrospective studies indicate that cancer survivors regret not having had discussions about infertility risks or access to fertility preservation at time of diagnosis. Concerns regarding fertility after cancer may change from being of no importance to becoming a problem when returning to everyday life. Few studies have investigated newly diagnosed cancer patients' experience of fertility and parenthood and how their perspective develops over time.

Study design, size, duration: Using a longitudinal qualitative design, seven men and nine women were interviewed at two time-points; during initial cancer treatment and 2 years later. The interviews focused thoughts and feelings about fertility and parenthood following cancer. Data were analyzed using qualitative content analysis.

Participants/materials, setting, methods: Informants were recruited between 2009 and 2011 at three different wards specialized in hematology and oncology in Sweden. Inclusion criteria: newly diagnosed with cancer, receiving curative treatment with a potential negative impact on fertility, aged 20–45 and being able to communicate in Swedish.

Main results and the role of chance: Our results indicate three preliminary main themes. In the theme 'Continue on chosen path' thoughts and feelings about fertility and parenthood remained unchanged and mostly concerned an unaltered decision about not having children or a persisting desire to have children sometimes in the future. The theme 'Devalued desire for children' describes how experiences of worries regarding fertility and parenthood essentially had reduced the desire to have children 2 years later. In the theme 'Struggle towards life goal' wanting children was described as an increasingly important part of life, and planning and struggling to have children had become a major project 2 years after diagnosis. Those who were unable to have children described great efforts to come to terms with infertility and to find possible solutions.

Limitations, reason for caution: As common in qualitative research, the results cannot readily be generalized to larger populations, but are judged to be applicable to women and men with cancer in the same context.

Wider implications of the findings: Our preliminary results suggest that the experience of cancer in reproductive age may lead to an alteration of life goals, including a devaluation of future children or an intensified desire and struggle to become a parent. It is important to provide young adult cancer patients with valid information and appropriate health care and support, including referral to specialist care if necessary.
Study funding/competing interest(s): Funding by University(ies), Funding by National/International Organization(s), The Swedish Cancer Society, Karolinska Institutet Faculty Funds.
Trial registration number: Not applicable.

POSTER VIEWING

PARAMEDICAL - LABORATORY

P-317 Comparison of data following *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) from split cycles in cases of no moderate or mild male factors

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Study question: To examine which is better – IVF or ICSI – in cases of no moderate or mild male factors, especially in new patients.
Summary answer: Split cycles is the better method at the first attempts in patients of no moderate or mild male factors.
What is known already: It is said that embryos derived from IVF have better clinical outcomes than embryos derived from ICSI.
Study design, size, duration: Subjects were 373 couples (382 cycles) with no moderate or mild male factors at Kyono ART clinic from January 2012 to February 2013. These were divided into two groups: IVF in 1536 oocytes and ICSI in 1435 oocytes.
Participants/materials, setting, methods: We assessed the fertilization, D3 good quality embryo, blastulation, pregnancy, and miscarriage rates in IVF and ICSI in the same patients and the same cycles. A single embryo transfer was performed in all cycles.
Main results and the role of chance: Fertilization rates were 64.3% (987/1536) vs. 76.7% (1101/1435) (p = 0.01); D3 good quality embryo rates were 45.3% (398/879) vs. 47.7% (467/979) (p = 0.30); and blastulation rates were 48.0% (383/798) vs. 39.8% (357/896) (p = 0.01) in IVF vs. ICSI, respectively. Clinical pregnancy rates and miscarriage rates were 34.7% (26/75) vs. 24.5% (23/94) (p = 0.15) and 19.2% (5/26) vs. 21.7% (5/23) (p = 0.89) in IVF vs. ICSI of fresh cycles and 52.8% (76/144) vs. 43.2% (73/169) (p = 0.09) and 15.8% (12/76) vs. 21.9% (16/73) (p = 0.34) in IVF vs. ICSI of vitrified-warmed cycles. In cases of mild male factor, fertilization rates were 63.5% (113/178) vs. 88.2% (149/169) (p = 0.01), and blastulation rates were 50.6% (41/81) vs. 38.3% (41/107) (p = 0.09) in IVF vs. ICSI, respectively.
Limitations, reason for caution: None.
Wider implications of the findings: 'Split' is the better method at the first attempts in patients of no moderate or mild male factors. However, IVF is stressless for embryos and gave better results. Consequently, IVF should be performed in more oocytes of split cycles. Further studies are needed.
Study funding/competing interest(s): Funding by Hospital/Clinic(s), Kyono ART Clinic.
Trial registration number: This study is not RCT.

P-318 Delivery of healthy babies derived from metaphase ii oocytes with smooth endoplasmic reticulum clusters

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Study question: Do smooth endoplasmic reticulum clusters (SERCs) in human metaphase II (MII) oocytes affect embryonic development and clinical pregnancy rates?
Summary answer: Embryos derived from SERC oocytes were not prone to malformation.
What is known already: Reportedly, SERCs may be associated with an increased risk of abnormal outcomes. However, other findings indicate that embryos derived from MII oocytes with visible smooth endoplasmic reticulum can develop normally and give rise to babies with no major malformations.
Study design, size, duration: Consecutive intracytoplasmic sperm injection (ICSI) cycles with SERC(+) oocytes or SERC(−) oocytes were retrospectively analyzed with regard to embryological and pregnancy outcomes; outcomes from SERC(+) oocytes were compared with those from SERC(−) oocytes. The procedure and protocol were approved by an Institutional Review Board (IRB).
Participants/materials, setting, methods: We analyzed 252 SERC(+) cycles and 3326 SERC(−) cycles, and we divided SERC(+) cycles into two subgroups: those with SERC(+) oocytes vs. those with SERC(−) oocytes. Student's t and chi square tests were used for the statistical analysis. P values of <0.05 were considered statistically significant.
Main results and the role of chance: Clinical outcome data for SERC(+) cycles did not differ significantly from that for SERC(−) cycles. However, fertilization and implantation rates were significantly lower in the SERC(+) MII oocytes sub-group than in the SERC(−) MII oocytes sub-group.

Using fresh embryos originally derived from SERC(+) MII oocytes, three patients became pregnant (one patient had a miscarriage), and each of the 14 other patients successfully conceived with transfer of a single vitrified-warmed blastocyst derived from an SERC(+) MII oocyte; however, two of these patients had miscarriages. Ultimately, 14 of the 17 patients with SERC(+) MII oocytes gave birth to 14 healthy babies without any congenital abnormalities.
Limitations, reason for caution: None.
Wider implications of the findings: The SERC(+) MII oocytes were significantly lower in fertilization rates and implantation rates than that of SERC(−) MII oocytes. Nevertheless, SERC(+) oocytes can produce healthy babies by both fresh and vitrified-warmed embryo transfer.
Study funding/competing interest(s): Funding by Hospital/Clinic(s), Kyono ART Clinic.
Trial registration number: This study was not a randomized controlled trial (RCT).

P-319 Clinical efficacy of multinucleated blastomeres (mnb)

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Study question: The nuclear status assessed after the first embryo segmentations according to the presence and the proportion of visible nuclei in blastomeres has been considered an important factor in implantation potential, although its true influence on the probability of birth is unknown.
Summary answer: MNB tend to have a lower development rate and lower implantation rate in comparison to non-MNB.
What is known already: One study has shown the negative impact of multinucleation on implantation and birth. It has been reported that MNB has negative impact compared with non-MNB in many clinics or facilities.
Study design, size, duration: We selected two pronuclei derived from ICSI (3456 embryos) and IVF (1263 embryos) in 2012 at our clinic, and these were divided into four groups: (1) non-MNB (ICSI/IVF), 2) MNB derived from ICSI with fresh sperm, (3) MNB derived from ICSI with frozen sperm, (4) MNB derived from IVF.
Participants/materials, setting, methods: We compared multinuclear incidence rate, good quality blastomere rate at day 3, good quality blastocysts at days 5–6 and implantation rate. We adopted Veek and Gardner's morphological rating system, and implantation rate was determined by the number of gestational sacs per embryo transferred.
Main results and the role of chance: Compared with group 2 + 3, the multinuclear incidence rate of group 4 was significantly lower: 4) 1.36% (17/1246), 2 + 3) 2.40% (81/3375); $P < 0.05$. Furthermore, the multinuclear incidence rate of group 3 was significantly higher compared with group 2: 3) 2.2% (12/273), 4) 1.36% (17/1246); $P < 0.05$.

Group 2 was lower compared with non-MNB in good quality blastomere rate at day 3, and other groups had no significant differences at days 3 and 5–6. There was no significant difference between any of the groups in implantation rate. Although two cases of transferred MNB led to deliveries with no abnormalities.

Limitations, reason for caution: We excluded data of IVM, oocyte activation and TESE
Wider implications of the findings: MNB in good quality blastomere rate at day 3 was lower in group 2 only. But, all groups showed similar results in terms of clinical efficacy. While no transfers of fresh MNB led resulted in delivery, transfers of vitrified MNB resulted in live birth. However, the numbers of cases are still few and we need to consider further investigation.
Study funding/competing interest(s): Funding by Hospital/Clinic(s), Kyono ART Clinic.
Trial registration number: None.

P-320 Assisted hatching on rewarmed blastocysts significantly increases their ability to hatch

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Study question: Does assisted hatching (AHA), performed on rewarmed vitrified blastocysts, affect their ability to hatch?
Summary answer: Assisted hatching significantly increased the hatching rate of rewarmed blastocysts.
What is known already: Assisted hatching (AHA) has been used in by numerous investigators to try to increase pregnancy rates. The effects of AHA has mainly been studied in women receiving fresh embryos and the results have shown only minor effect of the treatment in selected groups. However, little is known on the effects of AHA on rewarmed blastocysts that have been vitrified using artificial collapse prior to vitrification (creating a hole in the zona pellucida (ZP)).
Study design, size, duration: The study was performed during the autumn of 2013. This was a pre-clinical prospective controlled study where embryos were allocated into control and treatment (AHA) groups. The data was analyzed using Cox regression for clustered data for time to hatching AHA vs. control group adjusted for known confounders.
Participants/materials, setting, methods: The treatment consisted of laser assisted opening of the ZP (approximately 20% of the ZP total circumference). The hatching process was observed using time-lapse using 'time to hatching' as the primary end-point.
Main results and the role of chance: Eighty-nine (89) blastocysts, 46 randomized to AHA and 43 to control group, from 45 women were included. Women were 34.0 years (25–40) and 34.0 (26–38) respectively. Already after 24 h, 35 (76%) blastocysts were hatched in AHA compared to 5 (12%) in control group, $p < 0.0001$. At the end of follow-up, 40 (87%) were hatched in AHA group vs. 12 (28%) in control group. Median follow-up time was 11.4 and 48 h for AHA and control group respectively, and HR (95% CI) was 6.61 (3.35–13.03), $p = <0.0001$. Results remained similar after additional adjustment for day of freezing, expansion grade, ICM and TC.
Limitations, reason for caution: This study is a preclinical trial and thus any possible effects of using AHA clinically is subjected to speculation.
Wider implications of the findings: As AHA in rewarmed blastocysts increases hatching effectiveness pre-clinically, we speculate that this may help at least a proportion of embryos that may be hindered by their ZP *in vivo*. AHA has not been seen to generally improve implantation but may be particularly important in blastocysts that have previously been artificially collapsed using either laser or pipette creating a very small opening through which the blastocyst attempts to hatch (and getting stuck).
Study funding/competing interest(s): Funding by Hospital/Clinic(s), Fertilitetscentrum, Göteborg.
Trial registration number: N/A.

P-321 Current use and quality assessment of IVF culture media – questionnaire study addressed to embryologists and culture media companies

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Study question: What are the opinions of embryologists and culture media companies regarding the current use and quality assessment of *in vitro* fertilization (IVF) culture media and is there sufficient exchange of information between them?
Summary answer: There is cross-talking between the culture media companies and the embryologists but there are areas that could be improved such as disclosure of full composition and follow up of outcomes with structured reporting systems. Also, both the companies and the embryologists feel that the quality assessment processes are not optimal.
What is known already: Nowadays there are numerous commercially available culture media whose exact composition is not revealed due to patent reasons. There is some evidence suggesting that IVF media can have an effect on the pre and post-implantation development and that suboptimal culture conditions could induce epigenetic changes affecting the future health of the offspring. Assisted reproductive technology (ART) media are now classified as class III medical devices in the European Union (EU) and should be CE marked.
Study design, size, duration: Separate online surveys were addressed to the embryology team of 826 IVF units in EU and Switzerland and to eight culture media companies. Both questionnaires were designed around four thematic axons to facilitate comparisons: current use of culture media, composition, quality assessment and follow up of outcomes.
Participants/materials, setting, methods: Fertility centres from 29 countries were invited to participate. In order to create a complete mailing list of IVF units, the ESHRE members who participated in the data collection for ART in Europe (2009) in the relevant countries were contacted and a thorough computerized search was performed for every country.
Main results and the role of chance: Seven out of eight culture media companies responded, all at high standards. 138 embryologists completed the survey. 14 questionnaires completed at low standards were excluded. 5/7 companies stated that they disclose full composition but only 29.5% of embryologists agree that this is always the case. The vast majority of the embryologists believe that IVF culture media can have an effect on the future child but few believe that the quality assessment processes are optimal; the companies agree that they are neither optimal nor standardized. 79% of embryologists answered that companies do not request any follow up information. However, the companies commented that IVF units may be reluctant to share their results and only 3 feel that their media are used as instructed by the embryologists.
Limitations, reason for caution: The response rate for IVF units was relatively low (16.1%) and some countries are over-represented. For instance, 24/124 embryologists' responses that were included in the analysis came from the United Kingdom. Also, the responses given from company representatives do not necessarily represent the official policy of the culture media company.
Wider implications of the findings: This survey highlighted that the embryologists feel that culture media can have an effect on the IVF offspring. However, there is lack of transparency regarding composition. Also, the postmarket surveillance and traceability, basic pillars of CE marking, are possibly neglected in real life. Therefore, a constant dialogue should be established between the IVF units and the culture media companies and emphasis should be given to full disclosure of composition and to monitoring of outcomes.
Study funding/competing interest(s): Funding by University(ies), No external funding was either sought or obtained for this work.
Trial registration number: Not applicable.

P-322 A study on the reliability of sperm counts measured in capillary-loaded and in coverslip counting chambers

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Study question: Does the Segre-Silberberg effect render sperm counts in capillary-loaded counting chambers less representative, as opposed to counting chambers with a coverslip?
Summary answer: There is no significant difference in the outcome and reproducibility of sperm counts in 20 µm capillary-loaded counting chambers and 20 µm coverslip counting chambers.
What is known already: In computer aided semen analyses counting chambers with a fixed depth, either loaded capillary or by covering a drop with a coverslip, are being used. Because of the viscosity of semen, the spermatozoa would be unevenly distributed in a capillary-loaded chamber, causing a misrepresentation of the sperm count, the so-called Segre-Silberberg effect. Sperm counts in chambers

in which a coverslip is placed on a drop might be more representative of the sample.

Study design, size, duration: 5 µl samples of 21 ejaculates and of 21 suspensions of motile spermatozoa were placed in both a 20 µm capillary-loaded blue chamber (Leja, the Netherlands) and a 20 µm coverslip red chamber (2X-CEL, HamiltonThorne, U.S.A.). Per chamber 20 microscopic fields were counted.

Participants/materials, setting, methods: Semen samples of low viscosity were used randomly from patients visiting our fertility clinic in the 2nd half of 2013. Statistics included correlations between the chambers, the paired-samples *T*-test, range of counts within the chambers, sample size analysis (Statistical Solutions, LCC, U.S.A.).

Main results and the role of chance: Correlations between the capillary-loaded and coverslip chamber are highly significant. For semen, the sperm count in the red chamber = 0.945 × the blue chamber (r^2 = 0.942), for suspensions red = 0.937 × blue (r^2 = 0.961). For semen nor suspension the difference in sperm count between the chambers is significant (*T*-test). For semen and suspensions the range of counts within the chamber is the same in both types of chambers. Our results showed no differences between spermatozoa in semen samples of normal viscosities and suspensions of spermatozoa in a watery liquid.

Limitations, reason for caution: That the differences between the chambers were not significant might be attributed to the number of samples being too small. However, a significant difference appears improbable, because sample size calculations, based on the current means and standard deviation, predict 281 samples be needed, which is about ten times as much as we measured. At the same time, it cannot yet be excluded that coverslip counting chambers also filled unevenly.

Wider implications of the findings: The results of this study makes a large Segre-Silberberg effect unlikely: we found no significant differences between the capillary-loaded and the coverslip chambers; nor between semen and suspension. Also the range of counts within a chamber was the same for both types of chambers and for both tested materials. Therefore, it would seem that for good laboratory practice capillary-loaded chambers and coverslip chambers are equally suitable.

Study funding/competing interest(s): Funding by Hospital/Clinic(s), University Medical Centre, Groningen.

Trial registration number: NA.

P-323 Genotype for a herpes virus-associated ubiquitin-specific protease (HAUSP) gene polymorphism in women and pregnancy outcomes after ART

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Study question: Can genotype of the herpes virus-associated ubiquitin-specific protease (HAUSP) G/A polymorphism (rs1529916) in women be used to predict pregnancy outcomes in ART?

Summary answer: There is no association between genotype for the HAUSP G/A polymorphism in women and implantation or pregnancy rates after IVF/ICSI. The genotype for the HAUSP G/A (rs1529916) polymorphism appears to not affect the chances of achieving pregnancy.

What is known already: HAUSP stabilizes and cleaves ubiquitin from its substrates. HAUSP is a direct antagonist of MDM2, the E3 ubiquitin ligase for the tumor suppressor protein TP53. The literature is not clear regarding whether the HAUSP G/A (rs1529916) polymorphism is associated with female fertility. However, studies on the HAUSP gene are rare.

Study design, size, duration: A prospective cohort study was conducted from 03/2012 to 07/2013 in 354 infertile women who were subjected to IVF/ICSI protocols. Patients were genotyped for the HAUSP polymorphism A/A (*n* = 36), G/A (*n* = 141) and G/G (*n* = 177). All procedures were performed under the same clinical/laboratory conditions.

Participants/materials, setting, methods: DNA was extracted from peripheral blood samples taken from each participant. The HAUSP G/A (rs1529916) SNP was genotyped using real-time PCR with Taqman Universal PCR Master Mix and a Taqman SNP genotyping assay.

Cumulative results (fresh and frozen cycles) were analyzed. Fisher's exact, ANOVA, Kruskal-Wallis and student's *T* tests were used.

Main results and the role of chance: Hardy-Weinberg genotype distributions of the entire sample indicated concordance between the observed and expected frequencies. No correlation was observed between genotype for the HAUSP G/A (rs1529916) polymorphism in women and clinical outcomes after IVF/ICSI (Table 1).

Table 1: Results.

	Women's genotypes			
	G/G	**G/A**	**A/A**	***P***
N	177	141	36	
Age (years)	35.7 ± 4.5	35.1 ± 4.0	36.2 ± 3.6	0.28
Transfers (*n*): total	1.4 ± 0.8	1.4 ± 0.7	1.4 ± 0.7	0.86
Transfers (*n*): fresh/frozen	1.2 ± 0.6/0.2 ± 0.5	1.1 ± 0.5/0.3 ± 0.6	1.1 ± 0.5/0.2 ± 0.6	0.35/0.15
Embryos transferred (*n*): total	3.1 ± 2.2	2.8 ± 1.7	3.1 ± 1.7	0.38
Embryos transferred (*n*): fresh/frozen	2.7 ± 1.8/0.4 ± 1.0	2.3 ± 1.4/0.6 ± 1.2	2.7 ± 1.5/0.4 ± 1.2	0.10/0.23
Implantation rate	17.9% (99/552)	20.1% (81/403)	15.9% (18/113)	0.54
Clinical pregnancy rate/patient	44.1% (78/177)	45.4% (64/141)	44.4% (16/36)	0.97
Clinical pregnancy rate/transfer	30.8% (78/253)	32.0% (64/200)	32.6% (16/49)	0.94
Ongoing pregnancy rate/patient	31.1% (55/177)	34.0% (48/141)	25.0% (9/36)	0.58
Ongoing pregnancy rate/transfer	21.7% (55/253)	24.0% (48/200)	18.4% (9/49)	0.67

Limitations, reason for caution: Additional validation of the analyzed SNP (increasing the number of cases) will be important to provide more information about the potential clinical use. Despite recruiting all eligible participants during the study period, the sample size was limited. Differences in the genetic backgrounds of various ethnic populations should also be considered.

Wider implications of the findings: Although an association between the HAUSP G/A (rs1529916) polymorphism and human fertility has previously been reported, there appears to be no relation between genotype and implantation/pregnancy rates after IVF/ICSI. The genotype of this polymorphism in prospective mothers will likely not be useful as a susceptibility factor for predicting the chances of achieving pregnancy. To obtain a consistent pregnancy predictive value, additional genotyping should be combined with analyses of other factors that may influence pregnancy outcomes.

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. An ethics committee authorized this study, and written informed consent was obtained from all participants.

P-324 Embryonic genotype for a herpes virus-associated ubiquitin-specific protease (HAUSP) gene polymorphism and pregnancy outcomes after ART

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Study question: Is there an association between embryonic genotype for the HAUSP G/A (rs1529916) polymorphism and pregnancy outcomes after IVF/ICSI?

Summary answer: There is no association between an embryo's HAUSP rs1529916 genotype and implantation or pregnancy rates after IVF/ICSI.

What is known already: Implantation depends on functionally related genes with coordinated expression between maternal and fetal tissues. The literature

is not clear regarding whether the HAUSP gene G/A (rs1529916) polymorphism is associated with female fertility, although the G allele has been correlated with the best fertility prognosis. Research is still needed to clarify this issue as well as determine the relevance of paternal and embryonic genotypes.
Study design, size, duration: A prospective cohort study was performed on 354 couples submitted to IVF/ICSI and recruited from 03/2012 to 07/2013. The couples were divided into two groups according to their HAUSP genotype combinations: GGxGG (because the G allele is correlated with the best fertility prognosis) and all of the other genotype combinations.
Participants/materials, setting, methods: DNA was extracted from peripheral blood samples taken from each participant. The HAUSP G/A single nucleotide polymorphism (SNP) (rs1529916) was genotyped by real-time PCR. Cumulative results (including fresh and frozen cycles) were analyzed. Hardy-Weinberg genotype distributions of the entire sample indicated concordance between the observed and expected frequencies.
Main results and the role of chance: Characteristics such as age, infertility etiology, number of transfers and number of transferred embryos were not significantly different ($P > 0.05$) between groups. There was no association between couples with embryos carrying only the HAUSP G/G genotype and implantation rate, pregnancy rate or miscarriage rate (Table 1).

Table 1: Results.

General features and clinical outcomes	Couples' genotype combinations		
	G/GxG/G	A/AxA/A, G/AxA/A, G/G x A/A, G/AxG/A, G/GxG/A	*P*
n	96	258	
Embryo genotype	Only G/G	G/G, G/A, A/A	
Implantation rate	18.6% (52/279)	18.5% (146/789)	1.0
Clinical pregnancy rate/patient	39.6% (38/96)	46.5% (120/258)	0.27
Clinical pregnancy rate/transfer	29.4% (38/129)	32.2% (120/373)	0.58
Miscarriage rate	28.9% (11/38)	29.2% (35/120)	1.0
Ongoing pregnancy rate/patient	28.1% (27/96)	32.9% (85/258)	0.44
Ongoing pregnancy rate/transfer	20.9% (27/129)	22.8% (85/373)	0.71

Limitations, reason for caution: Additional validation of the analyzed SNP (increasing the number of cases) will be important to provide more information about the potential use of this polymorphism. Differences in the genetic backgrounds of various ethnic populations should also be considered.
Wider implications of the findings: Although an association between the HAUSP G/A polymorphism and human fertility has previously been reported, there appears to be no relation between embryonic genotype for this polymorphism and implantation or pregnancy rates after IVF/ICSI. The HAUSP polymorphism genotypes of potential parents seem not to be a susceptibility factor for predicting the chances of achieving pregnancy.
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 authorized by the local ethics committee.

POSTER VIEWING

PARAMEDICAL - NURSING

P-325 An unknown world: midwives and nurses' perspectives of their role in infertility care

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Study question: The main objective of this study was to investigate midwives and nurses' perspective about their education/training in infertility care and their role in Infertility centers.
Summary answer: There is a need for further investigation in this area; the professionals involved in the study seemed to play marginal roles in patients' care and let emerge a dearth in their training as concerns Infertility care.
What is known already: Differently from other European countries, in Italy there are no specific trainings, in Nursing and Midwifery educational programs, regarding Assisted Reproductive Technology. Consequently, it is hard to find specialized staff of nurses and midwives, whose role can be crucial in women and families' care, in Infertility centers in Italy.
Study design, size, duration: An exploratory survey was conducted during in two consecutive days in November 2013 distributing semi-structured questionnaires to midwives and nurses working in Italian infertility centers. The main items of the questionnaires were focused on the educational level, professional behaviors, and clinical activities of the participants.
Participants/materials, setting, methods: Questionnaires were distributed to a purposive sample of Italian nurses and midwives, in the context of a national congress about Infertility. Only midwives and nurses were eligible and only the professional who gave consent in the study were recruited.
Main results and the role of chance: Questionnaires were distributed to 120 professionals. The response rate was 62.5%. The 28% of the participants were registered nurses, whilst 72% midwives. 54/75 (72%) of respondents never attended specific courses in Assisted Reproductive Technology during their graduate/post graduate training. 43 out 75 (57%) of respondents work in infertility center: 58% worked in public hospital centers whilst 42% in a private center.

Nurses and midwives were involved in families' care mainly by assisting doctors in medical procedures (e.g., oocyte retrieval) and diagnostic tests (e.g., sonohysterosalpingography). Few of them (16%) had an active role in cycle-planning and counselling. In six cases, the respondents also had organizational or administrative roles.
Limitations, reason for caution: This was an exploratory study with a limited and purposive sample size; further research is necessary in order to have more insights about the phenomenon. However, this study offers a contribution in the current dearth of information regarding the role of nurses and midwives in Infertility centers in Italy.
Wider implications of the findings: The findings of this study have the potential to inform future research, Midwifery and Nursing education and practice, in the specific context of Infertility care.

Midwives are increasingly expanding their horizons and practicing in the plethora of areas of their professional profile. A reconsideration of their role in Infertility centers looks therefore necessary considering the actual situation and the pressure on the Italian Health service to conform more to European standards.
Study funding/competing interest(s): Funding by Hospital/Clinic(s), Centro Procreazione Assistita Demetra Firenze.
Trial registration number: N/A.

P-326 The impact of a group preparation seminar for Japanese couples considering the use of donor insemination (DI)

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Study question: Does a group preparation seminar for couples considering DI have positive impact on participants' confidence about their use of DI and intention to share information about DI with offspring?
Summary answer: Findings suggested that a preparation seminar providing comprehensive information about DI have positive impact on participants' attitude toward sharing information about their genetic origin with offspring. Also findings showed significant differences between pre and post-test result of female participants' self-esteem score, state anxiety score, and loneliness score.
What is known already: Several studies (Daniels & Thorn, 2007; Thorn & Daniels, 2003) have suggested that a group preparation program on parental confidence and attitude about sharing the information with offspring conceived as a result of DI.
Study design, size, duration: One group pre-post design was performed. Forty Japanese heterosexual couples considering DI participated in a preparation seminar provided 2010–2011.
Participants/materials, setting, methods: Couples considering DI participated and provided comprehensive information about DI. Using questionnaires administered pre and post seminar, we examined participants' self-esteem (Rosenberg Self-Esteem Scale), anxiety (STAI: State-Trait Anxiety Inventory), feelings of isolation (revised UCLA Loneliness Scale), and attitudes toward sharing information about DI with offspring.

Main results and the role of chance: Forty-seven (26 females and 21 males) completed the questionnaire (response rate 81.0%). The average age was 34.1 years for males and 32.9 years for females.

The findings shows that the male attitudes measuring by 4-point Likert scale regarding sharing information about DI with offspring shows significant differences between pre and post-test from 2.7 to 3.2 ($p = 0.007$), while the female attitudes from 2.9 to 3.4 ($p = 0.000$). The STAI state score of females shows an increase from 46.0 to 41.4 and significant difference ($p = 0.003$). The UCLA loneliness score shows no significant difference. The self-esteem score of females increased by 1.0 points, while that of the females increased by 1.1 points, however, not significant.

Limitations, reason for caution: This study was performed in a limited number of participants. Further studies are needed.

Wider implications of the findings: Japanese couples considering DI have a need to obtain sufficient information about DI and disclosure of DI with offspring to increase parental confidence, as well as previous studies. The role of professionals involving DI is to encourage them to make decision with no force.

Study funding/competing interest(s): Funding by national/international organization(s), Support for the study provided by Japanese Society of Fertility Nursing.

Trial registration number: Not applicable.

P-327 Successful pregnancies following assisted reproductive technology (art) for cancer survivors

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Study question: Can patients who have had cancer treatments such as radiotherapy and chemotherapy maintain their fertility?

Summary answer: Patients who have had cancer treatments are capable of being pregnant and delivering a healthy baby.

We need to create a situation which enables cancer patients to consult medical professionals about fertility preservation in relation to their treatment for malignant diseases.

What is known already: Although cancer treatments can affect women's fertility, in recent years, they have improved and resulted in maintaining a high quality of life (QOL) after recovery. It is important to maintain fertility for a woman's QOL.

Study design, size, duration: 69 patients who had experienced cancer treatments visited our clinic from August 2005 to September 2013. The treatments have been approved by the patients and the ethical committee. 68.1% (47/69) of patients received assisted reproductive technology (ART) treatment before or after the cancer treatments.

Participants/materials, setting, methods: The subjects had breast cancer (22), uterine cancer (6), leukemia (5), aplastic anemia (2), bone sarcoma (2), ovarian cancer (2), one for gastric cancer, tongue cancer, colon cancer, lung cancer, brain tumor, hypophyseal tumor, Hodgkin's disease, and myelodysplastic syndrome. Pregnancy and miscarriage rates and children's health at birth were examined.

Main results and the role of chance: Oocyte retrieval was performed for 47 patients (110 cycles). Fresh embryo transfer was performed in 46 cycles, and all oocytes in 9 cycles and embryos/blastocysts in 26 cycles were vitrified. Fertilization rate was 70.3% (244/347). Pregnancy rate per embryo transfer was 21.9% (21/96), and this resulted in 16 healthy births, 1 stillbirth, 1 postnatal death at day 59 (hydramnios and HELLP syndrome), 1 artificial fetal death (13-trisomy syndrome), 1 miscarriage, and 2 ongoing pregnancies. Average weight and height of babies at birth was 2933.3 ± 477.3 g and 49.6 ± 2.0 cm.

Intrauterine insemination (IUI) was performed for 6 women (10 cycles), and 3 patients became pregnant. One healthy baby was delivered, and 2 other outcomes were unknown.

Limitations, reason for caution: Since limited cases were used for this study, results may be slightly different when larger numbers are used for this study.

Wider implications of the findings: This study indicates that the survivors of cancer should not be discouraged from having children and can expect a good outcome of pregnancy. It is crucial to cooperate and communicate with oncologists, nurses, counselors and reproductive doctors about a patient's age, ovarian reserve, cause of infertility (male factor: yes or no), influence of chemotherapy and irradiation, pregnancy rate and miscarriage rate.

Study funding/competing interest(s): Funding by hospital/clinic(s), Kyono ART Clinic.

Trial registration number: Not applicable.

P-328 Effects of an educational program to foster fertility awareness in teenage girls: a pilot-study

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Study question: The objective of this study is to conduct an educational program on infertility for healthy, teenage, female university students, in order to provide them with knowledge regarding infertility and one's own life plan concerning pregnancy and childbirth, and to shed light on how such knowledge affects their behavior.

Summary answer: After the program, half of the participants recognized the necessity to move up their life plan and 20% became aware of the possibility that they might the infertile.

What is known already: The number of infertile patients in Japan is on the rise. Since infertility is related to a woman's age it is advisable to provide knowledge about infertility to women who desire to have a child in order to urge them to attempt to get pregnant at a suitable age. However, the focus of conventional sex education is on preventing pregnancy and preventing sexually transmitted diseases leaving little health education that aims to prevent infertility.

Study design, size, duration: One-group pretest-posttest design, in December 2013, 36 healthy female university students were subjected to a 60 min educational program on infertility after which the effects of the program were measured by having participants fill in a questionnaire.

Participants/materials, setting, methods: The participants were 36 healthy, single, female university freshmen. The program consisted of a lecture entitled 'Basic Information about Infertility and Treatment' and a discussion entitled, 'Your Life Plan Concerning Pregnancy and Childbirth'. The questionnaire asked questions related to knowledge and awareness about infertility. A statistical analysis was conducted.

Main results and the role of chance: All participants gave responses. The mean age was 18.6 ± 0.5. In response to questions about one's own fertility, the number of participants that answered, 'I might be infertile' rose from five before intervention to 12 participants after intervention thereby lacking a statistically significant difference ($p = 0.07$).

35 participants (97.2%) replied that they wish to get married and have a child.

Comparing responses from before and after the intervention, 28 participants (77.8%) experienced a change in their awareness in regards to infertility and treatment, and 17 participants (47.2%) made corrections to their life plan. The reason that many of the participants gave for making corrections was, 'I learned that it's better to get pregnant and have children at an earlier age'.

Limitations, reason for caution: The small sample size and lack of a control. Also, in order to measure the long-term outcome of intervention it is necessary to confirm the degree of knowledge several months and even several years later, as well as the rate of achievement of the participants' life plans.

Wider implications of the findings: If the implementation of health education about infertility can make women aware of their own fertility and promote health management that takes pregnancy into consideration, it may be possible to prevent infertility in the future. It is therefore useful to incorporate information on infertility and infertility treatment, as well as detailed proposals for life plans, into health education given to teenage girls.

Study funding/competing interest(s): Funding by University(ies), Ibaraki Prefectural University of Health Sciences.

Trial registration number: N/A.

P-329 Improving patients performance on self-medication in a Brazilian IVF centre: nurses on action

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Study question: What role nurses can meet in IVF centers? In Brazil, as demands grow, potential spaces develop. A systematized nursing consultation was designed to assist infertile patients with their daily treatment injections. The aim was to reduce patient anxiety/stress and to increase security avoiding wrong procedures or inappropriate handling of medications.
Summary answer: Fertility patients usually feel insecure handling/performing self-medication. Nursing consultation plays an important hole helping patients, reducing anxiety on patients concerning self-medication administration. Overall there has been 63% of adherence in nurse consultation showing that there is still place for patient's consciousness concerning the importance of the nurse assistance.
What is known already: Controlled ovarian stimulation significantly increased pregnancy rates and refinement resulted in multiple protocols that can be used alone or in different combinations. Patients are increasingly anxious about the amount of information received regarding the drugs and their profile: pre-loaded pens with medications; manually loaded injections with syringes needing different reconstituting; pre filled syringes with diluents plus lyophilized powder; pre filled syringe without need of reconstitution or a mixed of all of these possibilities.
Study design, size, duration: We set up a prospective longitudinal study to enroll the nurses in assisting patients with their daily fertility treatment injections. A systematized consultation was introduced in August, 2012 in the clinical routine for patients who would start ICSI cycles. Consultations were held from August 2012 to August 2013.
Participants/materials, setting, methods: Nursing consultation was offered to patients who would start ICSI cycles consisting of explanation the steps in ovulation induction and presentation of the medication using a special suitcase created by the nurses to enable patients to handle and simulate self-administration. A plastic tummy and an intra-muscular/subcutaneous pad were used.
Main results and the role of chance: Among 241 patients undergoing ICSI cycles, 147 nursing consultations were held representing 63% of adherence. In the first 3 months 12 consultations were performed (4/month). At first patients did not find important "to mark a schedule with the nurse" and some tried to minimize this query saying that "they had no time available". In the following 9 months, 135 nursing consultation were performed (15/month) showing a significant improvement. From November 2012 on a questionnaire was introduced to obtain feedback from nursing consultation. Patients were instructed to return the questionnaire on the day of oocyte aspiration. All questionnaires returned (62/136) pointed that the nurse consultation was clear and important and 36/62 yielded an extra comment marking it was outstanding for their treatment.
Limitations, reason for caution: All IVF patients were eligible to have a nurse consultation. Adherence was high (63%). However, 37% still resisted either because they didn't find it important or considered themselves would solve it well. The nurse's image as part of the team was not well situated for these patients, burdening the clinicians.
Wider implications of the findings: From the model of a bureaucratic leader away from contact with the patients, we are aimed at a different perspective to improve nurse's activities on the ART program assisting patients with their daily fertility treatment injections. As the role of nurses in IVF clinics in Brazil is not well established our study contributes suggesting that nurses can effectively act beside the clinician concerning daily fertility self-medication.
Study funding/competing interest(s): Funding by hospital/clinic(s), Fertipraxis Centro de Reprodução Humana.
Trial registration number: Not applicable.

P-330 Factors influencing pain sensation during oocyte aspiration with conscious sedation

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Study question: What are the factors that influence the pain experience during an oocyte retrieval for IVF or ICSI.
Summary answer: A younger age, a higher number of oocytes and the doctor performing the procedure were the only factors influencing the degree of pain.
What is known already: Most studies considering pain sensation during oocyte retrieval compare different regimens for pain relief or anesthesia.
Study design, size, duration: In 2012 and 2013 a prospective cohort study was performed, measuring the pain sensation of 644 women undergoing an IVF or ICSI procedure. Women who had general anesthesia (n = 5) or a paracervical block (n = 7) were excluded. All relevant data concerning the treatment were recorded in an electronic patient record.
Participants/materials, setting, methods: The primary outcome was the numeric rating scale (NRS), ranging from 0 to 10. Immediately after the procedure the patient was asked to assess the most severe pain during the procedure. One hour later and at the moment of discharge from the outpatient clinic the NRS was measured again. The standard premedication was 1000 mg of paracetamol and 20 mg of oxazepam given 2 h before the oocyte aspiration and 50 µg Fentanyl iv immediately before the procedure. If necessary, an additional dose of 25 µg was given. From July 2013 onwards one rectal suppository of 100 mg diclofenac was added to the premedication. A 16 gauge needle was used.
Main results and the role of chance: The average NRS during the procedure was 5.6 ± 2.0. One hour after the procedure the NRS was 2.8 ± 3.7 and at the time the patient went home 1.5 ± 1.2. There was no difference in pain sensation between IVF and ICSI. Neither was the diagnosis a significant factor, although there was a tendency for endometriosis and male factor the be most painful. The correlation between the female age and the NRS was −0.12 (P = 0.003) with women below 30 having het highest pain score. The correlation between the NRS and the number of follicles was 0.12 (p = 0.02), with women having less than 5 follicles having the lowest pain score. The puncture order nor the occurrence of pregnancy was of any importance. There was, however, an influence of the doctor who did the procedure. The average NRS between doctor could vary as much as one point.
Limitations, reason for caution: The average NRS was 5.6. This might look high, but should be seen in relation to the low use of general anesthesia for IVF and ICSI in the Netherlands. The pain diminished quickly after the procedure.
Wider implications of the findings: These findings might help the physician to select to proper patient for general anesthesia. Attention should be focused on proper instructions for doctors who perform the procedure.
Study funding/competing interest(s): Funding by hospital/clinic(s), Department of Obstetrics and Gynaecology, Jeroen Bosch Hospital's-Hertogenbosch, the Netherlands.
Trial registration number: N.A.

P-331 The knowledge of the increased risk of complications in multiple pregnancy does not affect the desire to transfer more than one embryo in IVF treatment

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Study question: How many embryos would the subjects participating in an online survey want to transfer in their IVF cycle?
Summary answer: Even being previously informed that the transfer of more than one embryo may result in multiple pregnancy and consequently risk to the mother and babies, the majority of subjects participating in this survey preferred to have 2 embryos transferred, followed by 3 embryos and 1 embryo, respectively.
What is known already: One of the main complications in IVF is multiple pregnancies. Increasing awareness of maternal and fetal complications has led to a reduction in the number of embryos transferred in IVF clinics, but the frequency of twin pregnancies is still high. A reduction in the number of embryos transferred might, however, contradict the patient's desire to achieve a successful outcome of the IVF treatment and thereby increase the necessity for further IVF attempts.
Study design, size, duration: This study was conducted in a Brazilian private assisted fertilization center. Individuals that accessed the center's website (www.fertility.com.br), from September 2013 to December 2013, have been asked to participate in the survey. The survey was based on important information concerning multiple gestations, followed by a single multiple choice question.

Participants/materials, setting, methods: Knowing that the transfer of one embryo reduces the chance of pregnancy; and the transfer of >1 embryo could result in multiple pregnancy, which brings risks to the mother and babies, answer: How many embryos would you transfer in your IVF cycle? Available answers were 1, 2, or 3 embryos.
Main results and the role of chance: A total of 365 subjects participated in the survey, 35 males (9.6%) and 330 females (90.4%). Mean age was 34.5 ± 6.3 years. Mean female age was 34.3 ± 5.9 years (range: 17–51) and mean male age 36.3 ± 9.0 years (range: 16–66). Regarding professions a total of 217 (59.4%) participants were related to human sciences, 59 (16.2%) to biological sciences, 50 (13.7%) to exact sciences and 39 (10.7%) were unemployed or students. The majority of the participants answered that they would like to have 2 embryos transferred (n = 188; female = 163, male = 25; 51.5%); followed by three embryos (n = 145; female = 142, male = 3; 39.7%), and one embryo (n = 32, female = 25, male = 7; 8.8%).
Limitations, reason for caution: A survey of this kind is subject to certain methodological limitations. The results may have been influenced by patient self-selection because (a) not everyone is connected; (b) the majority of participants are female; and (c) the fertility potential of the participants is unknown.
Wider implications of the findings: Men and women are not well aware or tend to underestimate the risks of complications associated with multiple embryo transfers and multiple gestations. The physician has to give necessary and qualified information to the couple to help them with the decision-making process regarding the number of embryos to be transferred. Nonetheless, it is the physician's responsibility to consider single embryo transfer as the method of choice and perform double/triple embryo transfers only in special circumstances.
Study funding/competing interest(s): Funding by hospital/clinic(s), Fertility – Centro de Fertilização Assistida.
Trial registration number: Not applicable, due to the design of the study.

P-332 Surrogacy in India: who are the surrogates?

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Study question: We investigated the sociodemographic characteristics and motivations of surrogates, who are part of the sample of the few studies on surrogacy in India (one of the top current international destinations for surrogacy), including our study in Mumbai.
Summary answer: Contrary to "predictions", the surrogates investigated are not among the poorest of the country/state/city, nor the least literate. Their motivations are nevertheless financial but as in other studies, there are other reasons for engaging in the surrogacy process.
What is known already: Surrogacy has become a burning issue, especially in southern countries such as India, where some experts denounce a higher risk of exploitation due to differences in socio-economic background between surrogates and intended parents. Surrogacy is nevertheless poorly investigated and documented with empirical data. Although India has become of special interest in local and international debates, only a few studies, mainly anthropological, have been carried out on surrogacy in the country.
Study design, size, duration: The presentation is based on 6 field studies on surrogacy conducted between 2006–2013 in different Indian states (Bangalore, Gujarat, Delhi, Punjab, Maharashtra) and cities (Ahmedabad, Anand, Jamnagar, Mumbai, Surat). Each study observed and interviewed the main protagonists, including surrogates (12 to 100 surrogates depending on the study sample).
Participants/materials, setting, methods: These 6 qualitative studies have collected interesting data and findings on the surrogates. Their sociodemographic characteristics (family situation, educational level, occupations, religion) will be gathered and compared with data available at local and national level and with studies in other countries (Canada, United Kingdom).
Main results and the role of chance: These observations and comparisons highlight three main issues: the common sociodemographic characteristics and motivations of surrogates in India, their possible specificity compared with the majority of women in India and their possible differences with surrogates investigated in more developed and Western countries. As there are no mandatory national or local records on surrogacy in India, as the practice in the country is still taboo and very controversial, it is very difficult to collect evidence on this issue. These 6 main studies provide relevant key elements for collection and comparison in order to better understand the practice in India.
Limitations, reason for caution: The analysis is not representative of the entire complex surrogacy practice in India (only a few clinics, cities/states and surrogates compared with the recent huge increase of agreements). Biases have to be considered: the clinics and surrogates investigated are only those who agreed to participate and may differ from non-participants.
Wider implications of the findings: This analysis provides preliminary evidence on surrogacy in India and allows us to understand the specificity (or otherwise) of the commitment of some women in the surrogacy process. It thus contributes to a better understanding of surrogacy practice and challenges in India. It may also warn against (or invalidate) the risk of possible exploitation that is always denounced in southern countries such as India.
Study funding/competing interest(s): Funding by national/international organization(s), for the study in Mumbai, funding was received from the European Commission (Marie Curie programme).
Trial registration number: Not applicable.

P-333 The role of boundary ambiguity: a new understanding on perinatal grief and psychological distress among Chinese women who experience miscarriage after IVF

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Study question: The current study attempts to examine the role of boundary ambiguity in perinatal grief, psychological distress and coping, as well as to explore the phenomenological experience of boundary ambiguity and family stress among Chinese women who experience miscarriage after IVF.
Summary answer: It was found that boundary ambiguity experienced by the miscarrying women after successful pregnancy through IVF, a perceptual state in which an individual is uncertain about who is in or out of the family system, could be able to predict perinatal grief and depression, difficulty in coping, as well as psychological despair.
What is known already: Research focusing on boundary ambiguity has been applied to a number of different populations and can be varied among different belief systems and family structures. Little is known among the role of boundary ambiguity in case of miscarriage, which is presumably associated with the psychological wish for a baby to be present when he/she is physically absent, causing maternal stress and impair individual functioning subsequently.
Study design, size, duration: A mixed method including a cross-sectional quantitative study and in-depth interviews was conducted. Structured questionnaires included Boundary Ambiguity Scale, Perinatal Grief Scale, and Hospital Anxiety and Depression Scale. Individual interviews were conducted in order to generate their subjective experience of boundary ambiguity and its association with psychological distress.
Participants/materials, setting, methods: 41 women (response rate = 46%, mean age = 30, SD = 1.3) experiencing first-trimester miscarriage after successful IVF were recruited at a university-affiliated hospital in Hong Kong. Informed consent was sought during hospital-stay after miscarriage treatment. Twelve agreed to participate a follow-up interview 2 weeks after discharge from hospital.
Main results and the role of chance: Quantitative analysis has shown that boundary ambiguity could significantly predict perinatal grief [$F(1,39) = 63.87$, $p < 0.001$] and depression [$F(1,37) = 4.416$, $p < 0.05$] in miscarrying women. This was consistent with the previous research on boundary ambiguity that the higher the subjective experience of boundary ambiguity, the higher the psychological distress and daily dysfunction among individuals. From the qualitative interviews, four meta-themes emerged as most representative of the phenomenological experiences of miscarriage after successful IVF cycle, namely as (i) difficulty in making sense of the sudden loss; (ii) confusion about the presence of physical body sensation and the absence of the baby; (iii) perceptual difference towards the experience among family member resulting higher ambiguity in family structure; and (iv) and the nature of disenfranchised grief in perinatal loss.
Limitations, reason for caution: The lack of available information about the premorbid status of the person before their loss, which allows the researcher to infer the person's level of functioning prior to pregnancy loss. Self-selection bias was also inevitable in questionnaire survey, and the cross-sectional nature of the study did not permit causal inferences.
Wider implications of the findings: The current study adds to the understanding of boundary ambiguity of miscarriage women and their acute perinatal grief in facing pregnancy loss. The nature of ambiguity in case of miscarriage

can shed light on the new perspective of counselling by acknowledging the perceptual discrepancy between the psychological presence and physical absence of the baby. The acute experience of perinatal grief and depression highlights the need of psychological support at this critical point of healthcare provision.
Study funding/competing interest(s): Funding by University(ies), The University of Hong Kong.
Trial registration number: Nil.

POSTER VIEWING

PSYCHOLOGY AND COUNSELLING

P-334 Alexithymia, coping and fertility related stress

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Study question: The aims of this study were to examine: (i) the prevalence of alexithymia in a sample of infertile women, and (ii) the association between alexithymia, coping strategies and fertility related stress, applying multivariate statistical techniques to control for the effects of demographic variables.
Summary answer: The prevalence of alexithymia in a sample of infertile women was 19.2%. Alexithymia was positively associated with avoidance coping and negatively associated with problem-management and problem-appraisal coping. Alexithymia and coping strategies were significantly related to fertility related stress after controlling for demographic variables.
What is known already: The investigation of the relationship between alexithymia and fertility related distress is a relatively neglected area of research. Only few studies have explored the role of alexithymia in infertility. Studies by Conrad et al., (2001) and by Lamas et al., (2006) have concluded that alexithymia rates are significantly higher in infertile group than in control group. These authors suppose raised the question of secondary alexithymia as a coping strategy in infertile patients.
Study design, size, duration: This was a cross-sectional study including 160 infertile women. This sample size was expected to produce at least the 119 participants needed to test the overall fit of a regression model with a medium effect size with up to 10 independent variables. The recruitment period lasted 8 months.
Participants/materials, setting, methods: The sample consisted of infertile women undergoing fertility treatment with in vitro fertilization in one of the largest public fertility clinics in Athens. Self-report instruments were used to measure alexithymia (TAS-20), coping (COPE) and fertility-related stress (FPI). Bivariate (Pearson's correlation, ANOVA) and multivariate statistical analyses (multiple linear regression) were used.
Main results and the role of chance: The response rate was 92%. 19.2 % of the sample scored in the high alexithymia range. High alexithymia score was positively associated with women's age ($p < 0.001$) and infertility duration ($p < 0.005$) and with low educational ($p = 0.008$) and low economical level ($p = 0.044$). Alexithymia was not significantly associated with marital status, aetiology of infertility and number of previous IVF trials. Alexithymia was positively associated with avoidance coping ($p < 0.001$) and negatively associated with problem-management ($p < 0.001$) and problem-appraisal coping ($p < 0.001$). Multivariate analysis showed that when controlling for demographic factors, high avoidance coping, low problem-appraisal coping and high alexithymia were positively associated with fertility related stress ($\beta = 0.309$, $p = 0.000$, $\beta = -0.203$, $p = 0.006$, $\beta = 0.151$, $p = 0.050$, respectively). The total proportion of variance in fertility related stress explained by all the independent variables was 33.9%.
Limitations, reason for caution: This study had some limitations. First, the study design was cross-sectional, which precludes dawning conclusions regarding the direction of relationships. Additionally, the present study did not control for other potentially important psychosocial factors (e.g. personality traits) and thus, the results obtained may be influenced by uncontrolled confounding.
Wider implications of the findings: Results of this study indicated that alexithymia prevalence in infertile sample was higher than Greek general population. In addition, the association between alexithymia and duration of infertility may be interpreted in the sense that secondary alexithymia acts as a coping strategy in infertile women. These findings are consistent with the findings of previous studies.
Study funding/competing interest(s): Funding by national/international organization(s), This study was partly funded by the Institute for Mental Health and Research and Treatment of Personality Disorders.
Trial registration number: This study was not a trial.

P-335 Sexual orientation and marital status of intended parents as seen by intended surrogates

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Study question: It's a preliminary report to clarify if the sexual orientation and marital status of intended parents are relevant for intended surrogates and might affect or influence their decision as for bearing a child.
Summary answer: Absolute majority of intended surrogates – 64.5% – consider that every intended parent regardless of sexual orientation or marital status has the right to parent a child and are willing to help.
What is known already: The general belief was that intended surrogates support most of all "traditional" families and oppose so called "new" families.
Study design, size, duration: The study has been conducted for 18 months from August 2012 to January 2014. Only gestational surrogacy was studied as traditional surrogacy, when own oocytes of a surrogate are used, is out of law in Russia since Jan. 01, 2012.
Participants/materials, setting, methods: 150 e-mail questionnaires were sent out to women who applied to European Surrogacy Center in Moscow to become a gestational surrogate in 2012. 124 participants completed the 30 question form by e-mail or in person.
Main results and the role of chance: The mean age of the entire group was 28.5 years. 51% were married, 16% divorced, 10% had a partner. All participants had children. 91% declared to be Orthodox Christians, 5.5% were Muslims, 2% did not indicate their religion, 1.5% were atheists.

64.5% declared that sexual orientation or marital status of intended parents was of no relevance for them. 11% wished to bear a child for a married heterosexual couple only. 2% could bear a child for any intended parents, though as they put it was desirable for them to deal with a married heterosexual couple. 2% could bear a child for a lesbian woman or for a lesbian couple, but not for a gay man or a gay men couple. 2% have nothing against single gay men but wouldn't bear a child for a gay couple. 18.5% would not consider gay men and gay couples as intended parents, though cohabiting heterosexual couples and single men and women being nevertheless eligible.
Limitations, reason for caution: A thorough psychological study of intended surrogates should be conducted before accepting them for a program for so called "new" families. Motives of non-acceptance of "new" families by intended surrogates and its implications should be thoroughly studied. Assignment of a "wrong" surrogate for a "wrong" program could lead to conflicts. Further studies of surrogates, their motivations and psychology should be conducted.
Wider implications of the findings: The main motive for the surrogates' refusal to accept singe men or gays as intended parents was not hatred towards gays, but a concern about the future of the child to be born. A psychologist consultation should be recommended for any surrogate starting the program, especially with gay parents. To avoid any conflicts during the implementation of the program or after the birth only surrogates who share the belief that everyone has the right to parent a child can be assigned for "non-traditional" families.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Reproductive Law and Ethics Research Center. Moscow, Russia.
Trial registration number: 082/12.

P-336 Quality of life assessment in German female infertile patients: a prospective cohort study conducted at a university-affiliated infertility care center

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Study question: This study aimed at assessing quality of life (QoL) by means of an objective measurement tool (FertiQoL) in German infertile patients

before a first IVF/ICSI cycle with ancillary assessment of changes in FertiQoL scores after a failed first cycle and the predictive capacity of FertiQoL scores of treatment discontinuation.
Summary answer: The mean FertiQoL score before first IVF/ICSI treatment was 73. Mean FertiQoL scores did not change after failure to achieve pregnancy in a first IVF/ICSI cycle. FertiQoL score did not significantly differ between patients who discontinued treatment after a first cycle vs. patients continuing treatment.
What is known already: FertiQoL is an internationally validated instrument to measure quality of life in individuals experiencing fertility problems. Subscales and total scales show high reliability and sensitivity of FertiQoL to well-established moderators of QoL. A validation in German patients has as yet not been performed. The potential uses of the FertiQol tool in a clinical context have not yet been fully explored.
Study design, size, duration: Prospective cohort study (duration: 12/2011-12/2013) on 118 female patients; fertility quality of life (FertiQoL) tool together with a questionnaire on sociodemographic variables was filled out at the time of first IVF/ICSI treatment initiation; follow-up assessment was offered at initiation of a second cycle in case of failure.
Participants/materials, setting, methods: Female patients (mean age 34.1 years, mean duration of infertility 4.2 years) were offered to fill out the core FertiQoL (24 items in emotional, mind-body, relational and social subscales) in addition to a sociodemographic questionnaire also including self perception of fertility status. Furthermore, all IVF/ICSI treatment outcomes were assessed.
Main results and the role of chance: Before a first IVF/ICSI cycle, the mean (SD) scores for subscales emotional, mind-body, relational and social items were 62 (18), 75 (17), 81 (13) and 78 (14), respectively and the total FertiQoL score was 73 (12). Twenty-one patients who did not achieve pregnancy and continued treatment showed a mean total score of 75 (9) at initiation of a second cycle versus 72 (12) at initiation of the first cycle. Patients who discontinued treatment after a failed first cycle had a mean total score at initiation of the first cycle of 74 (14) vs. 73 (11) in patients who continued treatment and 77 (12) in patients achieving live birth. All differences were not statistically significant.
Limitations, reason for caution: Patients who had decided for IVF/ICSI treatment might represent a positively selected cohort as compared to patients affected by infertility in general; hence the high mean scores of FertiQoL in this study. All comparisons between groups of patients are limited by small sample sizes.
Wider implications of the findings: The present study has established a reference level of QoL in the clinical setting of patients starting to attempt IVF or ICSI and may serve as basis for future studies. FertiQoL scores are unlikely to identify patients at high risk of discontinuation, and – contrary to intuition – FertiQoL scores did not change with the burden of a failed attempt, but larger data sets are necessary to corroborate this finding.
Study funding/competing interest(s): Funding by University(ies), University of Lübeck.
Trial registration number: N.A.

P-337 Is fertility counselling offered and accepted by patients undergoing assisted reproduction? Insight from a British cohort of mixed ethnicity

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Study question: Service evaluation of uptake and perception about counselling amongst patients undergoing Assisted Reproduction Techniques (ART) in a tertiary level fertility unit with dedicated NHS funded counselling services.
Summary answer: Counselling uptake is variable depending upon the family support, social and cultural values. Depending upon how and when the information is provided, it can help couples through their most stressful journey. Majority of the couples tend to seek support after an unsuccessful attempt.
What is known already: Most couples find assisted conception treatment stressful and HFEA (Human Fertilisation and Embryology Authority)-licensed clinics in the UK have to offer patients an opportunity to talk to a counsellor about the implications of the suggested treatment. There is wide variation in counselling services provided and patient uptake across different units. Therefore, we undertook this survey to determine the uptake and perception about counselling in our local population.
Study design, size, duration: A prospective anonymous 'questionnaire based survey' was implemented for 4 weeks (October 2013-November 2013) to all patients attending the clinic for ART. Those willing to participate filled the requested information. One hundred patients, both women and men, completed the questionnaire during the time period.
Participants/materials, setting, methods: The questionnaire was adapted from literature and included demographic details (gender, age, ethnicity, previous treatment); counselling uptake (awareness, information on accessing it, accepted or declined); perception (reasons for not accessing, infertility affecting self-esteem, self-confidence and relationship). Questionnaires were administered to all patients attending the unit, whilst waiting for their appointments.
Main results and the role of chance: Of the 100 patients, 70 were women and 30 men. 27 couples (54/100) were administered questionnaires to assess difference in gender uptake and perception. Mean age was 34.4 years, 53% were Caucasian and 47% ethnic minorities (35% Asians). 95% of the patients were aware about counselling but 20% of these did not know how to access. Counselling was offered to 86%, however, only 30% accepted. Interestingly, 23.5% reported that undergoing ART reduced their self-esteem (8 on a scale of 0–10) and 15% reported reduced self-confidence (7 on a scale of 0–10).

Qualitative themes included 'If I fail to conceive, I will take up counselling'; "not sure if counsellor would understand my family pressure"; "I came along only because my partner wanted to and it was useful."
Limitations, reason for caution: This study was done to evaluate our local service, therefore performed over a short period of time. It would be interesting to further investigate perceptions and uptake of counselling for a larger study sample with semi-formal interviews and focus-groups.
Wider implications of the findings: Counselling, if appropriately offered, is accepted by couples undergoing ART. It must be tailored according to the local population taking into consideration their social, cultural beliefs and family pressures. Men often decline counselling, however, they attend with partners and find it useful. The importance of counselling services for an assisted reproductive technology unit is valuable and should not be under-estimated.
Study funding/competing interest(s): Funding by University(ies).
Trial registration number: N/A.

P-338 Myths about fertility and art success rates – results of the survey

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Study question: Which factors affect the deferral of pregnancy? What significant myths regarding fertility can be observed?
Summary answer: The data show lack of awareness and education in the general population with regards to fertility issues, supported by false beliefs in the possibilities of reproductive medicine, technological progress and healthy lifestyle.
What is known already: Demographic developments over the last 15 years show a significant increase in the age of spontaneous first pregnancies and a shift to over 40 years of age for IVF seeking females.
Study design, size, duration: Survey of 1223 respondents from June to December 2013 gathered their estimated statistical chances of spontaneous pregnancy for age categories 35–60 and awareness of age related decline in IVF success rates. Furthermore the study collected knowledge of factors crucial for women in late 40s to 60s to become pregnant.
Participants/materials, setting, methods: Questionnaire based internet survey was addressed to the general public, non-anonymous. Respondents have been monitored for age, gender, social background, education and nationality. Obtained results were compared to literature sources.
Main results and the role of chance: The results show a number of misconceptions and myths about female fertility as well as complete lack of awareness and knowledge about age related fertility decline in the general population, both females and males, irrelevant to age group or educational background. This fact is also supported by the false beliefs in the possibilities of reproductive medicine and technological progress in our high-tech era.
Limitations, reason for caution: Conclusions from an internet based survey might be limited by voluntarily participation, unknown proportion of subjects

refusing participation, misunderstanding of the questionnaire or lack of contact with researcher. Extrapolations with the general population should be done with caution.
Wider implications of the findings: The data from the survey and experiences through psychological counselling correspond with an increasing trend in the delay of motherhood and family planning. An important factor is the misconception about fertility and IVF options. Media coverage of late pregnancies and media attention celebrating older mothers facilitate false hopes for unlimited genetic motherhood. Fertility awareness should be considered as part of biology education.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), No competing interests of authors.
Trial registration number: N.A.

P-339 Importance of psychological assessment in the screening of the sperm donors

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Study question: Through a 2 year review (2012–2013), we aim to determine the frequency of candidates excluded as a result of screening based in the psychological assessments of the sperm donors.
Summary answer: The clinics have to ensure the quality and safety of the process of gamete donation. The process must involve a careful and rigorous selection of donors. We must identify whether the donation could constitute a possible situation of risk to the health of third parties or risk to themselves.
What is known already: The sperm donor should be medically and psychologically healthy and be aware of the laws concerning reproduction. The donation has several implications and consequently, knowing about the sperm donor's personality and character are of importance at the time of recruitment as one means of predicting and evaluating the donor's decision and motives, as well as for assessing if his personality is stable. Anyway, relatively little attention has been paid to these implications.
Study design, size, duration: 206 sperm donors, between January 2012 and December 2013, after a first visit, in which a preliminary medical history was taken, and without significant medical problems were invited for an assessment with the psychologist.
Participants/materials, setting, methods: The donors undergo screening and examination in accordance with American Society of Reproductive Medicine (ASRM) guidelines, including physical and psychological examinations. Psychological screening involves the administration of a semi-structured interview and, if need more information or have suspicions about the existence of possible psychopathology a personality (NEO-FII) test is administered.
Main results and the role of chance: From 2012 to 2013, 206 candidates underwent on-site evaluation, of which 31.5% (65/206) were rejected in the psychological evaluation. Inside this group: symptomatology associated with clinical disorders Axis I (including substance abuse disorders) (29.23%); psychosocial and environmental problems (27, 69%); behaviors associated personality disorders and/ or mental retardation (18, 46) and family history disorders (16, 92%).
Limitations, reason for caution: It must be noted that information provided by donors was self-reported and thus must be interpreted with caution, as self-report may be likely to accentuate the level of exaggeration and self-marketing undertaken by donors.
Wider implications of the findings: Donations are made for reproductive purposes, are voluntary, altruistic, anonymous and not can never injure constituent rights neither donors nor users. It would not be then neither desirable nor permissible to include candidates with psychological or cognitive disorders, or a vital risk profile, which could be aggravated by the very act of donation, or by the interpretation that they themselves may make its role as a donor as well as the implications that may have this type of donation (ethical, social, emotional and cognitive).
Study funding/competing interest(s): Funding by hospital/clinic(s), Financial support received from Fundacion Tambre, Madrid, Spain. The authors report no conflicts of interest.
Trial registration number: N/A.

P-340 Psychological maternal-fetal attachment among pregnancies achieved with assisted reproduction treatments

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Study question: Analysis of maternal-fetal attachment during third trimester among pregnant women after Assisted Reproduction Techniques (ART) compared to spontaneously achieved pregnancies.
Summary answer: No differences were observed in maternal-fetal attachment between women pregnant after ART or spontaneously achieved pregnancies.
What is known already: The study of prenatal attachment -one of the main predictors of mother-child attachment developed after birth enables to detect possible difficulties for the mother when establishing an affective relationship with the fetus. This may affect the fetus development, delivery and future mother-child relationship. However, the studies that have assessed this construct among pregnant women through ART are few and have many methodological limitations.
Study design, size, duration: A total of 365 pregnant women, 47 of them after ART and 318 spontaneously pregnant, were evaluated at an University (University of Deusto, Spain) associated Assisted Reproduction Center (Quirón Bilbao, Spain) during a 6 month-period of time (between June and December, 2013). A cross-sectional study was performed.
Participants/materials, setting, methods: The Prenatal Bond Assessment Scale (PBAS) and an ad hoc developed questionnaire or reproductive background were used as assessment tools. The PBAS is a Likert scale composed of 24 items, with five options for each answer, divided in six subscales. The psychometric properties of the instrument were considered adequate.
Main results and the role of chance: The pregnant women attending Maternal Education classes of 33 groups of 18 public and private institutions of Bizkaia were evaluated. Participation rate was high: only two women refused to participate in the study. Maternal-fetal attachment through the third trimester of pregnancy was strong: the women fantasized about the fetus, they altruistically protected the fetus and interacted with him or her. Also, pregnant women sought information about the fetus, showing anxiety secondary to a real or imaginary loss. Nevertheless, the identification of the fetus as a different individual to pregnant women was moderate. No differences were observed in maternal-fetal attachment between those pregnant women through ART and those pregnant spontaneously. Maternal age, gestational age and previous spontaneous abortions were not related to prenatal attachment.
Limitations, reason for caution: The number of participants in this study was small. It is necessary to keep on assessing the prenatal attachment and the link between this affective relationship and other variables by analysing them over the three trimesters of pregnancy and after birth.
Wider implications of the findings: The results of this study are consistent with the findings of other authors who have investigated this issue. The use of an instrument with adequate psychometric properties to assess prenatal attachment provides robustness to the results. To our knowledge, this is the first approach to the assessment of this early affective relationship among Spanish population.
Study funding/competing interest(s): Funding by University(ies), University of Deusto.
Trial registration number: N/A.

P-341 Anxiety, depression and sexual dysfunction in women undergoing infertility treatment

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Study question: The aim of this preliminary investigation was to evaluate anxiety, depression and sexual dysfunction in the infertile women and to investigate the risk factors related to psychological difficulties.
Summary answer: Anxiety and depression were more prevalent in women seeking treatment for infertility. Infertile women might experience less orgasm compared to general fertile women. Psychological difficulties in the infertile women were not correlated with age, duration of marriage, duration of infertility, or presence of delivery history.

What is known already: Women undergoing infertility treatment is believed to suffer various physical, emotional and psychological difficulties at a higher frequency than the comparable general population. It is influenced personality factors, strength of family and support system in social cultural context. There is a lack of consensus regarding the risk factors of psychological difficulties, especially in northeastern Asia.
Study design, size, duration: Forty women who visited infertility clinic to receive infertility treatment between November and October 2013 were enrolled. Forty Healthy women seeking general health screening served as the control.
Participants/materials, setting, methods: Infertile women and control were asked to complete standardized validated questionnaires assessing anxiety, depression and sexual function. We administrated the Hospital Anxiety and Depression Scale (HADS) test, Depression Anxiety and Stress Scale (DASS), and Female Sexual Function Index (FSFI).
Main results and the role of chance: Mean HADS score was 6.35 for anxiety and 8.32 for depression. However, women with anxiety who scored more than 11 were 12.9%, and women with depression were 32.3%, which were significantly higher than control. Using DASS score, anxiety (25.8%), depression (29.0%) and stress (19.4%) were also more prevalent in the infertile women than control. Total FSFI score of infertile women was 22.33 similar with the score of control. The mean scores of desire (3.09), arousal (3.48), lubrication (4.39), satisfaction (3.94), and pain (3.96) were not statistically different. However, mean score of orgasm in infertile women was 3.16, which was significantly lower than that of control. Age, duration of marriage, duration of infertility or presence of delivery history did not influence HADS, DASS, or FSFI score.
Limitations, reason for caution: Because of the limited number of this study, further longitudinal studies with larger sample sizes are required to clarify the prevalence and risk factors of anxiety, depression, and sexual dysfunction in women undergoing infertility treatment.
Wider implications of the findings: This preliminary study revealed the more prevalent of anxiety and depression in the infertile women. Routine screening using standardized questionnaires to identify the vulnerable women is needed to provide proper counseling service before infertility treatments. Infertility and its treatment did not evoke the sexual dysfunction entirely. Infertile women may experience less orgasm. This study adds to the growing body of literature to study the relationship between psychological difficulties and infertility distress.
Study funding/competing interest(s): Funding by hospital/clinic(s).
Trial registration number: NO.

P-342 Patients' attitudes towards embryo donation: a web-based survey

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Study question: What are the attitudes of couples with expired remaining embryos towards embryo donation to other patients?
Summary answer: Couples intending to donate considered embryo donation more as a sort of gamete donation than giving up for adoption; patients indicated that the centre should consider criteria to allow people to receive donated embryos.
What is known already: Literature reports a considerable proportion of couples in favour of different forms of embryo donation, especially if restrictions can be set on recipient characteristics [Wånggren 2013]; however, most studies explored attitudes of patients whose intentions to donate were only hypothetical, since they did not have embryos in storage yet [Laruelle and Englert, 1995], had not necessarily completed IVF treatment [Lyerly et al., 2006] or were not allowed to donate by law [Mohler-Kuo 2009; Wånggren 2013].
Study design, size, duration: A one-time web-based survey addressed to 505 couples from August 2013 to December 2013.
Participants/materials, setting, methods: 505 couples with expired remaining embryos (after the maximum embryo storage period of 5 years by contract), were contacted to participate. The response rate was 46.34 %. Respondents were categorized according to their decision to either (1) destroy their embryos, (2) donate to research or (3) donate to infertile patients.
Main results and the role of chance: No significant difference was identified regarding the reason for not using the remaining embryos among the three groups. Religion and educational level significantly influence preferences, with higher educated people more likely to donate for research ($P = 0.003$) and the not religious more likely to donate to infertile patients ($P < 0.001$). Couples intending to donate were significantly more likely to experience embryo donation as a sort of combined oocyte and sperm donation, while couples preferring destruction related it more to giving up for adoption ($P < 0.001$). However, no difference was seen among the three groups regarding the views of patients towards the value of their embryos. The majority of the candidate embryo donors indicated that the centre should exclude embryo recipients with a history of drug and alcohol abuse.
Limitations, reason for caution: Further follow-up needs to evaluate the attitudes and motivation of patients that are willing to undergo further screening needed prior to the actual embryo donation.
Wider implications of the findings: As the survey was addressed to couples who had previously signed a contract for the disposition of their expired remaining embryos, the nature of the questions was not hypothetical. Results may therefore give indications about the real profile of embryo donors and provide centres interested in starting an embryo donation program, or countries intending to legalize it, with useful information.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Centre for Reproductive Medicine at UZ Brussel.
Trial registration number: Not applicable.

P-343 A preliminary study on the fertility quality of life questionnaire relational scale: an analysis regarding its relationship with marital adjustment measures in Italian infertile couples

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Study question: Is the *Fertility Quality of Life Questionnaire* (FertiQoL) relational scale linked to other measures of marital adjustment in a sample of infertile couples undergoing Assisted Reproductive Technique (ART), as a valid tool for measuring and improving couples' adjustment during infertility treatment?
Summary answer: Patients showing high relational quality of life (QoL) report high levels of couple commitment, dyadic adjustment, and marital satisfaction, including low levels of sexual concerns and need of parenthood. The FertiQoL relational scale could be useful for identifying infertile couples at risk of marital distress due to their infertile condition.
What is known already: Infertility has a significant impact on a person's QoL and his/her psychological distress. The FertiQoL has been validated in different countries and there are many studies regarding the relationship between FertiQoL and anxiety, depression and infertility distress. No previous studies have examined the validity of the FertiQoL relational scale and specifically its correlations with well-validated measures of a couple's marital adjustment, satisfaction and commitment.
Study design, size, duration: A cross-sectional study was conducted with 247 infertile couples undergoing infertility treatment from February 2013 to January 2014.
Participants/materials, setting, methods: 154 couples (response rate = 62.3%) undergoing ART treatment at the ANDROS Day Surgery Clinic in Palermo (Italy) completed the following questionnaires prior to the beginning of the cycle: the Dyadic Adjustment Scale (DAS), the Commitment Inventory, the FertiQoL, the ENRICH Marital Inventory and the Fertility Problem Inventory (FPI).
Main results and the role of chance: All the FertiQoL scales had acceptable internal consistency (Cronbach's alpha ranging from 0.70 to 0.84), with a mean inter-item correlation ranging from 0.26 to 0.47. Negative associations were found between the FertiQoL relational scale and marital commitment ($r = –0.39$, $r = –0.33$, $p < 0.01$ for Relationship Agenda and Couple Identity respectively), FPI ($r = –0.18$, $r = –0.14$, $p < 0.01$, for Need for parenthood and Sexual Concerns respectively). Moreover, the FertiQoL relational scale was positively associated with the DAS ($r = 0.29$, $r = 0.26$, $p < 0.01$ for Consensus and Cohesion respectively) and the ENRICH ($r = 0.25$, $p < 0.01$) scores. No differences in FertiQoL relational scale were found between women and men ($t = –1.38$, $p = 0.17$).
Limitations, reason for caution: The study participants were recruited only from an Italian private clinic and were all heterosexual; a broader multi-center study, including different groups, could verify the applicability of results.

A considerable number of couples did not complete the questionnaires, therefore a sample bias cannot be excluded.
Wider implications of the findings: The FertiQol relational scale seems to be an adequate and valid scale for measuring the couple relationship domain of the infertility-related quality of life for women and men. The FertiQoL relational scale can be used to identify women and men at risk of impaired marital adjustment and specific areas (commitment, communication, sexuality) where psychosocial support might be most beneficial.
Study funding/competing interest(s): Funding by hospital/clinic(s), ANDROS Day Surgery Clinic, Palermo, Italy.
Trial registration number: Unnecessary.

P-344 Women's attachment anxiety mediates the negative effect of the need for parenthood on psychological wellbeing: a cross-partner analysis

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Study question: Is a person's need for parenthood associated with theirs and their partner's worse psychological wellbeing? Is this association indirectly affected by attachment anxiety regarding the partner?
Summary answer: Need for parenthood was associated with higher attachment anxiety, which was associated with poor wellbeing for both the women and their partner. Men's need for parenthood was not associated with theirs or their partner's wellbeing (either directly or indirectly).
What is known already: Infertility is a stressful event that may threaten couples' wellbeing. Due to interpersonal nature of infertility, an individual's inability to conceive is likely to activate attachment anxiety (i.e. feelings of insecurity about their partner's availability), especially when individuals have a high need for parenthood. Higher attachment anxiety is known to be associated with worse psychological wellbeing. However, it is not known if the need from parenthood of one partner may affect the other partner's wellbeing.
Study design, size, duration: In this cross-sectional study, a sample of 45 Portuguese couples undergoing assisted reproduction treatment in a Portuguese public hospital were recruited from 2007 to 2008.
Participants/materials, setting, methods: Fifty-five couples (90 participants) were invited. Validated self report questionnaires assessed the need for parenthood (Fertility Problem Inventory), attachment anxiety (Adult Attachment Scale) and psychological wellbeing (WHO Quality of Life Questionnaire). Path analysis examined direct and indirect effects of need for parenthood on wellbeing for each and across both couple members.
Main results and the role of chance: Response rate was 82%. Couples were trying to get pregnant for about 4 years (M = 4.4, SD = 2.4) and had previously undergone one IVF treatment (M = 0.96, SD = 1.3). The analysis model showed a good fit to the data (X^2 = 0.94, df = 4; CFI = 1.000, RMSEA = 0.00). There were no significant direct effects of the women's and their partner's need for parenthood on her own or her partners' psychological well being. However, significant indirect effects were found. Women's need for parenthood was negatively associated with her own and her partner's wellbeing, via higher attachment anxiety (90% CI = –0.923 to –0.131 and CI = –0.875 to –0.053, respectively).
Limitations, reason for caution: Sample size was relatively small. Power analysis indicated enough power (0.80) to detect medium to large effects but not small effects. Due to the cross-sectional design causality cannot be established. However, response rate was high, the study investigated inter-couple effects and validated questionnaires were used to assess sound theoretical constructs.
Wider implications of the findings: In couples where women have a high need for parenthood both members of the couple are negatively affected by the experience of infertility. One identified mechanism is related with the insecurity women experience about the availability of her partner. These results support the view that both members of the couples should be involved in the treatment process and that care should be directed for promoting individual but also relational wellbeing.
Study funding/competing interest(s): Funding by national/international organization(s), MMR received a PhD fellowship (SFRH/BD/23152/2005) from the Portuguese Foundation for Science and Technology. There are no conflicts of interest to declare.
Trial registration number: The study is not a trial.

P-345 Couple relationship quality (CRQ) influences women's and men's quality of life during the course of infertility treatment

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Study question: Do women's and men's perceptions of Couple Relationship Quality influence their Quality of Life (QoL) and its potential variation during the course of infertility treatment (ART)?
Summary answer: Findings from two path models highlighted that both partners' levels of QoL increased from treatment intake to the day of insemination or embryo-transfer, and that only the women's QoL variation was influenced by their own and partner's CRQ perceptions.
What is known already: Previous studies have shown that infertility has distressing effects on QoL in infertile couples and that poor QoL is associated with poor psycho-social adjustment. Moreover, women undergoing an ART cycle have lower Qol levels than their partners. There is evidence that a woman's emotional response to IVF differs throughout treatment, but only a few studies have assessed both partners' QoL during the course of one treatment cycle and its relationship with patient's CRQ.
Study design, size, duration: This longitudinal study comprised two repeated measurements: before treatment (T1) and on the day of insemination or embryo-transfer (T2). The hypothesized models tested the effect of partners' perceptions of CRQ over change in QoL. 247 couples were consecutively referred for ART treatment from February 2013 to January 2014.
Participants/materials, setting, methods: QoL (FertiQoL) and the CRQ (ENRICH Marital Inventory, Commitment Inventory, Dyadic Adjustment Scale) of 98 couples (response rate = 39.7%), recruited at the ANDROS Day Surgery Clinic (Palermo, Italy), were evaluated. Change in QoL (RQoL) was calculated by regressing QoL-T1 over QoL-T2. Structural Equation Modelling was used to test the models.
Main results and the role of chance: Both men's and women's QoL significantly increased from T1 to T2 (t = 6.39, and t = 3.76, $p < 0.01$ respectively). The hypothesized models provided an adequate fit (χ^2 = 71.75; df = 27; χ^2/df = 2.66, CFI = 0.94, RMSEA = 0.08, for women, χ^2 = 60.57; df = 36; χ^2/df = 1.68, CFI = 0.96, RMSEA = 0.06, for men), showing that women's RQoL at T2 was negatively affected by their CRQ perception (β = –0.38, $p < 0.05$). Women with a lower CRQ, therefore, displayed a higher increase in QoL during treatment than women with higher CRQ, who tended to report a lower increase in QoL. In addition, women's RQoL was positively influenced by their partner's CRQ perception (β = 0.33, $p < 0.05$). Finally, men's RQoL at T2 was not affected either by their own perception of CRQ (β = 0.11, p = n.s.) or by their partner's CRQ perception (β = 0.14, p = n.s.).
Limitations, reason for caution: There are some limitations in this study: firstly, data were obtained only from one clinical site; and secondly, variables other than CRQ may have affected the couples' QoL.
Wider implications of the findings: Our results show that marital relationship has a different impact on couples' QoL during ART. We could argue that women who perceive a lower couple relationship quality tend to report a greater increase in QoL, possibly due to the positive effect of their involvement in treatment. Clinicians should focus psychological counseling on helping men to express their couple satisfaction to their partners early in the treatment process, given its influence on their partner's QoL.
Study funding/competing interest(s): Funding by hospital/clinic(s), ANDROS Day Surgery Clinic, Palermo, Italy.
Trial registration number: Unnecessary.

P-346 A randomised controlled trial assessing the impact of listening to music on women undergoing fertility treatment

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Study question: Whether listening to music at the time of embryo transfer improves anxiety levels in women undergoing assisted conception treatment.
Summary answer: Anxiety is significantly lower post-embryo transfer regardless of whether or not music is listened to. Listening to music during embryo transfer did not overall affect anxiety levels or clinical pregnancy rates. However in a subset of the most anxious patients, music therapy may help reduce anxiety levels.
What is known already: Fertility treatment may have a negative emotional impact on women. Lower levels of anxiety have been associated with improved treatment success but there is no standardised method for addressing these needs.

Music is a safe and beneficial non-pharmacological intervention in a number of medical fields. It may alter subjective and objective psychological anxiety as well as physiological functioning. However, little data exists surrounding the therapeutic use of music in fertility treatment.
Study design, size, duration: An assessor-blinded randomised control trial of 42 women recruited February-December 2013. Powered for a medium-effect size, two groups and one repeated measure. Randomisation was performed immediately prior to treatment was restricted using balanced-permuted-blocks of ten and four. Follow-up was conducted via participants' notes until pregnancy outcome was known.
Participants/materials, setting, methods: Women undergoing IVF/ICSI were recruited from a fertility clinic and randomised into a 'music' (listened to self-selected music during embryo transfer) or 'control' (no music) group.

Participants completed the Spielberger State Trait Anxiety Inventory Form prior to, and immediately following a post-treatment observation period. Total scores were compared.
Main results and the role of chance: 32 (76.2%) women were less anxious following treatment than before treatment (mean change in anxiety score 6.91 95% CI 4.19–9.63, $p < 0.01$) with no difference between controls (6.71 95% CI 2.28–11.14) and cases (7.10 95% CI 3.53–10.66) ($p = 0.46$). Adjusting for pre-treatment anxiety level and embryo quality did not alter results. Amongst controls, anxiety was proportional to pre-treatment anxiety levels; women with higher anxiety levels experiencing greater decreases in anxiety post-treatment ($r = 0.35$, $p < 0.01$). This was not the case within the music group. Clinical pregnancy rates (55.0%) did not differ between music and control groups ($p = 0.95$).
Limitations, reason for caution: The assessment of anxiety was validated and reliable method but is subject to the bias of any written, self-completed questionnaire. Double-blinding was not possible for this study, increasing the likelihood of experimenter effects. Music therapy was used only within the specific context of our study design.
Wider implications of the findings: Women report anticipatory anxiety before embryo transfer but this is resolved soon after. This study has not shown self-selected music to be an adequate anxiety reducing measure when used within the context of our study but a greater length of exposure to, or different types of music may have effects. Our results indicate that music may potentiate relief in vulnerable subpopulations and this could be explored in future research.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), The University of Southampton and Complete Fertility, Southampton. The authors have no conflicts of interest to declare.
Trial registration number: Regional Ethical Committee 12/SC/0689.

P-347 974,071 unique visitors to Mensfe.com, a self-help website for sub-fertile men, analysis of the 5 year period, 2009 and 2013

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Study question: What was the frequency of visits to the 10 most popular discussion groups on the Forum in relation to activity levels of the threads and posts over 5 years (2009–2013)?
Summary answer: The top 10 threads apparently posted by men revealed 5 themes: (1) Supporting their female partner (2) High stress levels (3) Good to share male perspective story (4) Seeking specific information (5) Concerns about sperm quality.
What is known already: To our knowledge, this is the first time an attempt has been undertaken to ascertain topics of interest, experiences and declared needs of the visitors on this self-help website for sub-fertile men.
Study design, size, duration: Study design: Observational, retrospective, Size: 974,041 apparently unique visitors to Mensfe.com; 254,257 page impressions to Forum, Duration: 1 January 2009–31 December 2013 (5 years).
Participants/materials, setting, methods: 974,041 apparently unique visitors to Mensfe.com occurred during a complete 5 year period. 254,257 page impressions to the Forum were recorded during this time to the 10 Discussion groups. The content of these 10 Discussion groups was analysed with respect to threads, and then ranked in relation to level of activity.
Main results and the role of chance: 254.257 (26%) Forum page views resulted from the 974,401 visits. 357 visitors registered as members of Mensfe.com to enable access to participate in the Discussion groups on the Forum. 812 threads were observed in the 10 Discussion groups, the top ten threads being visited on 110,265 occasions (43% of total Forum visits), adding 393 replies. The top 10 threads apparently posted by men revealed 5 themes: (1) Supporting their female partner (2) High stress levels (3) Good to share male perspective story (4) Seeking specific information (5) Concerns about sperm quality. Ratio of men to women members from the statistical data by the website provider is 9-1.
Limitations, reason for caution: The weaknesses of our study include lack of verification of unique visitor status thus failing to exclude the possibility of double counting or the important variable of gender.
Wider implications of the findings: The very high rates of apparently unique visitors to Mensfe.com reported here imply that online self-help websites are relevant to sub-fertile men. Furthermore, analysis of Forum activity may be relevant to fertility experts of all disciplines providing care for sub-fertile men. Nevertheless, the extremely high levels of activity seen on this website imply that there is a continuing need for resources in this aspect of infertility. We are planning to refine our data capture capacity, hopefully without compromising the interest of sub-fertile men and to a lesser degree their partners to consider Mensfe.com and similar websites as valuable in their quest to start a family.
Study funding/competing interest(s): Funding by national/international organization(s), Mensfe.com, which is a not for profit organisation.
Trial registration number: Not applicable.

P-348 Psychosocial factors associated with long-term emotional adjustment in couples who have ended fertility treatment

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Study question: Which psychosocial factors are associated with elevated anxiety and depressive symptoms in couples who have ended their fertility treatment 1 to 2 years ago?
Summary answer: In couples who have ended fertility treatment, elevated anxiety and depressive symptoms are associated with marital and sexual dissatisfaction, and the use of dysfunctional cognitive coping strategies.
What is known already: Little is known about the psychosocial needs of couples who have ended fertility treatment. Previous studies suggest that couples may experience symptoms of anxiety and depression, especially couples who remain childless. There is some evidence that marital satisfaction and cognitive coping strategies play an important role in the long-term emotional adjustment of these people.
Study design, size, duration: In April 2013, 680 former patients of the outpatient clinic of the Erasmus MC, Rotterdam, were invited to participate in this cross-sectional study. One-hundred thirty people agreed (response rate 19%).
Participants/materials, setting, methods: We invited all patients and their partners who had discontinued fertility treatment for various reasons at Erasmus MC, in 2011. Participants completed the following self-report questionnaires at home: Hospital Anxiety and Depression Scale, Maudsley Marital Questionnaire, Cognitive Emotional Regulation Questionnaire, and Illness Cognition Questionnaire. Hierarchical multiple regression analyses were performed.
Main results and the role of chance: Preliminary analyses showed that seven percent of the participants scored above the cut-off for possible depressive disorder, whereas fourteen percent scored above the cut-off for possible anxiety disorder. Depressive symptoms were positively associated with sexual dissatisfaction and catastrophizing about fertility problems, and negatively associated

with positive refocusing. Anxiety was positively associated with marital dissatisfaction and helplessness related to fertility problems, and negatively associated with acceptance of infertility. No associations were found between anxiety and depressive symptoms on the one hand, and having a biological child, rumination about fertility problems, and positive reappraisal on the other hand.
Limitations, reason for caution: The response rate of this study was low and few men participated, which may limit the generalizability of the results. Due to the cross-sectional design of this study, we cannot draw any causal inferences.
Wider implications of the findings: This study confirms findings from previous studies and its results suggests that psychosocial interventions targeted at people who experience anxiety and depressive symptoms after ending fertility treatment should focus on dysfunctional cognitive coping strategies, and sexual and relationship problems.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), No funding has been received for this study. J.S.E.L. has received fees and grant support from the following companies (in alphabetical order): Ferring, Genovum, Merck Serono, Merck Sharp and Dome, Organon, Serono, Shering Plough and Shering.
Trial registration number: N/A.

P-349 The impact of stress and anxiety during embryo transfer on pregnancy rates: a prospective trial

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Study question: To evaluate if stress and/or anxiety during embryo transfer could negatively affect pregnancy rate in IVF cycles.
Summary answer: There are no significant correlation between stress and anxiety during embryo transfer between pregnancy and no pregnancy in women undergoing IVF treatments.
What is known already: There is no confirmation regarding the impact of stress and anxiety on IVF outcomes, although some available previous studies have shown that acute stress during embryo transfer may impair the results of IVF cycles.
Study design, size, duration: Prospective cohort study at a private IVF Center. 79 infertile women, up to 35 years-old, with good prognosis for conception in IVF/ICSI cycles (5 to 20 oocytes collected, masturbation sperm recovery, and at least one good embryo transferred) were enrolled from January 2013 to January 2014.
Participants/materials, setting, methods: On the day of ovarian stimulation (at baseline) and 5 min before embryo transfer participants were requested to fill out a questionnaire (IDATE Test) to measure anxiety and arterial blood pressure, as well as heart rate between two periods: at baseline (at the beginning of ovarian stimulation) and 5 min before embryo transfer.
Main results and the role of chance: There was no correlation between anxiety (IDATE scale test) during embryo transfer and pregnancy ($p = 0.464$). Neither there were statistical differences in the variations of mean arterial blood pressure (systolic: 0.02, 95% CI: –5.2 to 5.1 and diastolic: –1.8, 95% CI: –7.1 to 3.5) and in the variation of mean heart rate (–0.4, 95% CI: –6.15 to 5.33) between the two periods. Furthermore, there were no differences in the mean maximum heart rate between pregnant (107.6 bpm) and non pregnant (108.6 bpm) women ($p = 0.771$).
Limitations, reason for caution: Small sample size could be the reason for not detecting possible negative impacts of anxiety and stress in pregnancy rates on IVF cycles.
Wider implications of the findings: The results of the present study are in contrast to those of smaller studies that used logistic regression modeling to assess the impact of anxiety and stress on pregnancy outcome. This study shows no differences in anxiety or stress between women who conceived and did not after IVF treatments.
Study funding/competing interest(s): Funding by hospital/clinic(s), Projeto Alfa-Aliança de Laboratórios de Fertilização Assistida.
Trial registration number: Not applicable.

P-350 The lived experience of oocyte donors in Iran: a phenomenological study

Abstract withdrawn by the author

P-351 Dropout rates in IVF with single embryo transfer, IVF in a modified natural cycle and IUI with ovarian hyperstimulation (INeS trial)

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Study question: How do dropout rates compare between in vitro fertilization with single embryo transfer (IVF-SET), in vitro fertilization in a modified natural cycle (IVF-MNC) and intra uterine insemination with ovarian hyperstimulation (IUI-COH)?
Summary answer: Dropout rates were significantly higher in the IVF-MNC group compared to IUI-COH. IVF-SET showed similar dropout rates compared to IUI-COH. Major reason for discontinuing treatment was the negative attitude of couples towards the allocated treatment.
What is known already: Dropout rates of fertility treatments are often considered as an adverse treatment outcome. We previously demonstrated that in couples with unexplained or mild male subfertility IVF-SET and IVF-MNC are non-inferior to intrauterine insemination with controlled ovarian hyperstimulation (IUI-COH) in terms of the birth of a healthy singleton. So far differences in dropout rates between IVF-SET, IVF-MNC and IUI-COH have not been investigated.
Study design, size, duration: The INeS study was an open-label, randomized controlled multicenter non-inferiority trial performed between January 2009 and February 2012. The primary outcome was a healthy singleton, resulting from a pregnancy achieved within 12 months of follow up.
Participants/materials, setting, methods: Couples with unexplained or mild male subfertility were allocated to 3 cycles of IVF-SET, 6 cycles of IVF-MNC or 6 cycles of IUI-COH. Reasons for dropping out were recorded in case record forms and classified as “actively censored” drop-outs (medical advice) and “passively censored” drop-outs (personal reasons, attitude towards treatment).
Main results and the role of chance: In total 602 couples were included in the analysis: 201 in the IVF-SET group, 194 in the IVF-MNC group and 207 in the IUI-COH group. The dropout rates were 15% in the IVF-SET group, 23% in the IVF-MNC group and 13% in the IUI-COH group, with a relative risk of 1.2 (95% CI: 0.71–2.0) for IVF-SET compared to IUI-COH and 1.8 (95% CI: 1.2–3.0) for IVF-MNC compared to IUI-COH. The majority of the couples was passively censored. The main reason for passive censoring was unfavorable attitude to treatment (e.g. lack of confidence in treatment success).
Limitations, reason for caution: Underlying factors may have influenced the passive and active censoring, for instance preference of the physician; these factors could not be extracted from these data.
Wider implications of the findings: As treatment success rates are negatively affected by discontinuation of treatment, dropout rates should be taken into account when comparing pregnancy rates. Reducing dropout rate is crucial to further improve the efficacy and cost-effectiveness of treatments. Our results

show similar dropout rates when comparing IVF-SET to IUI-COH, yet there were higher dropout rates for IVF-MNC compared to IUI-COH. This suggests that IVF-MNC, although often considered as IVF-light, presents a considerable burden to the patients.
Study funding/competing interest(s): Funding by national/international organization(s), the trial was supported by a grant from ZonMW, the Netherlands Organization for Health Research and Development, and a grant from Zorgverzekeraars Nederland, the Netherlands association of health care insurers.
Trial registration number: The INeS trial was registered at the Dutch trial registry (NTR 939).

P-352 Infertility self-efficacy: the mediator effect between shame and infertility-related stress and depressive symptoms

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Study question: Does the perception of self-efficacy to deal with infertility mediate the effect of shame on depression and infertility-related stress in infertile women?
Summary answer: The current study shows that infertility self-efficacy plays a mediator role on the association between internal and external shame and depression and infertility-related stress.
What is known already: The emotional impact of infertility and the relationship between depression, stress and the outcome of fertility treatment have been studied. Furthermore the importance of emotional processes such as internal and external shame to the understanding of psychopathological symptomatology associated with infertility has also been established. In addition, the perception of infertility self-efficacy involves several self-regulation processes (cognitive, affective and motivational) and determines the appropriate skills in order to deal with various situations.
Study design, size, duration: Cross sectional study. Data were collect in 4 public infertility centers and 3 private clinics between July 2008 and July 2011.
Participants/materials, setting, methods: One hundred and sixty-two women with a primary infertility diagnosis that have looked for treatment in Portuguese infertility public and private clinics. Participants completed the following set of standardized self-report measures: Others as Shamer, Experience of Shame Scale, Beck Depression Inventory, Fertility Problem Inventory, and Infertility Self-efficacy Scale.
Main results and the role of chance: Correlation analyses reveal that external and internal shame are significantly associated with infertility self-efficacy, depression and infertility-related stress. In turn infertility self-efficacy is strongly correlated with both depression and infertility-related stress. Mediator analyses results show that infertility self-efficacy fully mediates the impact of internal shame on infertility-related stress and depression and partially mediates the impact of external shame on these dependent variables. Furthermore, external shame still presents a direct effect on infertility-related stress and depression. Thus the perception of self-efficacy to deal with infertility and the demands of medical treatment seems to be a relevant target, as well as shame feelings, for psychological intervention in infertile women struggling with depression and stress.
Limitations, reason for caution: Our findings must be interpreted cautiously due to cross-sectional design and self-report data. This design limits robust causal conclusions to be drawn and points to the need of future replication studies with a longitudinal design, using other none self-report instruments such as semi-structured interviews.
Wider implications of the findings: Results suggest that it may be useful to address not only external and internal shame in psychological interventions tailored for infertile women but also to target self-efficacy perception to deal with infertility. In line with these results interventions such as the Mindfulness Based Program for Infertility, Acceptance and Commitment Therapy and Compassion Focus Therapy, which are specially designed to target such variables may improve the effectiveness of psychotherapeutic interventions.
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: N/A.

P-353 How much does infertility really affect couples' sexuality – 460 patients' appraisal of sexual difficulties relative to other effects of infertility

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Study question: How distressing is the presumed detriment in sexuality among infertile couples relative to other personal difficulties provoked by infertility?
Summary answer: Within a list of 9 emotionally adverse effects of infertility, patients ranked sexual difficulties as the least distressing. This result was maintained independently of gender, etiology of infertility, duration of infertility, and complexity of the reproductive treatment.
What is known already: Clinicians believe that infertility substantially deteriorates couples' sexual functioning. Available research shows mixed evidence. A higher prevalence of sexual dysfunctions compared to fertile population norms has been reported in some studies, as well as differences in some subscales of sexual function in group-comparison studies. However, the heterogeneity of study designs and the lack of control of variables prevent general formulations.
Study design, size, duration: This was a cross-sectional study conducted at the Instituto de Investigación Materno-Infantil, Hospital Clínico San Borja-Arriarán, Universidad de Chile, between January 2012 and May 2013. This center provides low and high-complexity (ART) treatments of infertility. Inclusion criteria for the 460 participants were primary infertility of at least 3 years duration.
Participants/materials, setting, methods: 276 women and 184 men answered a questionnaire where they ranked using a numerical scale (0–7) their perceived level of difficulty on 9 effects of infertility. ART patients were assessed upon enrollment. Qualitative interviews were conducted on 54 randomly selected couples by the principal investigator, a certified psychologist.
Main results and the role of chance: Mean scores of difficulty were, in decreasing order: anxiety (5.24), sadness (5.13), injustice (4.55), anger (4.48), social pressure (3.90), self-esteem (3.30), guilt (3.14), marital conflicts (1.94) and sexual difficulties (1.56). There were no differences between male and female scores. The score on 'sexual difficulties' did not vary as a function of etiology of infertility, duration of infertility, or complexity of reproductive treatment. Women with endometriosis did not report higher scores of sexual difficulty tan other women, nor did men with male-factor infertility compared to other men. In the psychological interviews, while many patients described having suffered occasional and temporary sexual problems at some point of their infertility trajectory, they declared having overcome these dysfunctions with the understanding and support of their spouse.
Limitations, reason for caution: Although the interviews were essential for understanding why patients assigned the lowest score to their sexual difficulties, one limitation of the study is that a moderate number were conducted ($n = 54$). Another limitation is that this study's findings may be culture-bound and not applicable to infertile populations in other countries.
Wider implications of the findings: The findings of this study contradict those of other publications and highlight the importance of the methodology employed. It is not the same to evaluate sexual disorders in isolation than within a global context where other undesirable effects of infertility can be appraised conjointly. Patients´ introspections shed useful insight on why they considered their sexual difficulties as their least concern. Future research might benefit from including qualitative inquiry as a complement to quantitative assessments.
Study funding/competing interest(s): Funding by hospital/clinic(s), Unidad de Medicina Reproductiva, Instituto de Investigación Materno-Infantil.
Trial registration number: N/A.

P-354 The value of children (VoC) to homosexual and heterosexual individuals

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Study question: What motivates people to become a parent? The aim is to find and describe underlying motifs and perceived costs and benefits associated with

having children and to see whether these motifs and perceptions differ between homosexual and heterosexual individuals.
Summary answer: Whereas the perceived benefits of children are similar between men and women as well as between homosexuals and heterosexuals, the perceived costs show some differences in the pattern of welfare dimensions as measured by the VoC-scales.
What is known already: Since the decision to have a child is often the result of a longer process of deliberation, perceived costs and benefits would have to be considered in trying to understand individuals' motivation towards parenthood. Even without concrete plans for parenthood, individuals have certain attitudes towards children which have to be negotiated in an attempt of realization. Those attitudes can influence the choice itself, timing, and methods used to achieve parenthood.
Study design, size, duration: Cross sectional analyses with focus on the Value of Children (VoC) scales, as they were incorporated into the pairfam panel.
Participants/materials, setting, methods: Data base for the homosexual population is an original dataset with 1,679 respondents from Germany. This data was collected in winter 2009/2010 via CATI and online questionnaire. Data for the heterosexual population stems from the second wave of the German pairfam-panel (N = 9,069). Methods include factor analyses and descriptive results.
Main results and the role of chance: First results show that perceived benefits of children seem to be similar for both homosexual and heterosexual individuals, but also when comparing men with women independently of their sexual orientation. Perceived costs of children, however, show differences in the pattern of welfare dimensions, as measured by the VoC-scales. Some of the items associated with comfort, stimulation, affect or esteem are placed into different dimensions after factor analysis of the scale in the comparison of homosexual vs. heterosexual individuals. Furthermore differences in the factor loadings hint towards differences in the weight of those dimensions for both homo- and heterosexual men and women.
Limitations, reason for caution: The comparison of perceived costs and benefits between homo- and heterosexual respondents is only possible with a refined scale with fewer items. The results for homosexuals, however, can be further analyzed by use of an earlier VoC-scale with more items, which should allow to draw more reliable conclusions.
Wider implications of the findings: Results may help to better understand the motifs of parents to be, especially considering the variety of pathways into parenthood, in order to assist those individuals with the realization of their wish to parent. The comparison between homo- and heterosexuals may also provide information about a more general idea of parenthood as a basic concept, regardless of an individual's sexual orientation.
Study funding/competing interest(s): Funding by University(ies), State Institute for Family Research at the University of Bamberg (ifb).
Trial registration number: None.

P-355 Impact of environment and tolerability of treatment on life satisfaction in infertile women

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Study question: What are the direct and indirect influences of environment and tolerability of treatment on life satisfaction?

How might environment and tolerability of treatment influence life satisfaction through anxiety?
Summary answer: There was a strong positive association between the environment of treatment and life satisfaction; however the correlation between tolerability of treatment and life satisfaction was marginally significant.
What is known already: A growing body of literature has explained patients' quality of life (FertiQoL) and has shown its significant relationship with gender, patient-centered care, negative psychological consequences, sexual and marital satisfaction and self-esteem. Although extensive research has been carried out on these relationships, no study has been found which simultaneously covers the association between treatment FertiQol, life satisfaction (SWLS) and Anxiety [Hospital Anxiety and Depression Scale (HADS)].
Study design, size, duration: This cross sectional study was conducted in Royan Institute, Tehran, Iran during November and December 2013. A total of 125 infertile women undergoing fertility treatment participated in the study.
Participants/materials, setting, methods: Women recruited for this study completed the questionnaire at the time of embryo transfer to be at the same point in the process of treatment. The FertiQol, HADS and SWLS were administered to the participants. Model testing was conducted using partial least square (PLS) analysis with the SmartPLS 2.0.
Main results and the role of chance: The PLS structural equation model revealed a good fit with the data. The most striking result to emerge from the data was that environment of treatment, as the most influential variable in the model, had a direct positive influence on SWLS (direct effect = 0.430; $p < 0.05$). Interestingly, environment of treatment had also a strong effect on tolerability of treatment (direct effect = 0.405; $p < 0.05$); although the indirect impact of tolerability of treatment on SWLS (through anxiety) was not significant (indirect effect = 0.031; $p > 0.05$), there was a borderline direct impact of tolerability on SWLS (direct effect = 0.159; $0.05 < P < 0.1$).
Limitations, reason for caution: With limiting the sample to Royan women, caution must be applied; as the findings might not be transferable to men or other Iranian fertility centers.
Wider implications of the findings: Environment and human body are more than just physical place and biomedical machine. Environment is a complicated scene where people perform social roles as women or men, black or white, mother or father. In medical centers the patient's dominate role, tied to other roles in everyday life, is one who has problem and needs help. The more environment and ways of treatment match patients' other roles without magnifying infertility, the more satisfaction comes by.
Study funding/competing interest(s): Funding by hospital/clinic(s), Reproductive Biomedicine Research Center of Royan Institute, Iran.
Trial registration number: The study was not a trial.

P-356 Embryologists' health: results from a nation wide on line questionnaire in Spain

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Study question: Is the embryologists' health status, including burnout syndrome, similar than that of a reference population?
Summary answer: The present study shows that physical embryologists' health status is better than that of a reference population. Embryologists, especially women, present poor levels of mental health related to burnout syndrome.
What is known already: Numerous studies have been made of the mental and physical health of health care personnel in general, and the prevalence of burnout syndrome has been reported to range from 4–40%. However, very few studies have been made in this respect of clinical laboratory staff and, to the best of our knowledge, no study has analysed these aspects among clinical embryologists.
Study design, size, duration: A cross-sectional design to conduct an online self-assessment survey. In May and June 2013, two e-mails were sent to all members of the Spanish Association of Clinical Embryologists, ASEBIR, (787, but only 731 active members) explaining the aims of the research.
Participants/materials, setting, methods: Embryologists who are members of ASEBIR. The questionnaire contained sociodemographic and occupational questions and two standard instruments: 'Short Form-12 Health Survey (SF-12)' as a measure of physical (PCS-12) and mental (MCS-12) health and the Maslach Burnout Inventory-General Survey (MBI-GS) to evaluate the three burnout syndrome dimensions: 'exhaustion', 'cynicism' and 'efficacy'.
Main results and the role of chance: The final response rate was 34.7% (254/731). The PCS-12 obtained for the Spanish embryologists was higher than that for the reference population (54.1 ± 6.0 vs. 52.6 ± 0.2, $p < 0.001$). However, the total MCS-12 was significantly lower than those of the reference group (43.0 ± 11.0 vs. 50.6 ± 0.2, $p < 0.001$). The linear regression model for the dependent variable PCS-12 included the variables number of hours worked per week, BMI, back pain, leg pain and visual discomfort. This model explained 27% of the variance in PCS-12. The linear regression model for the dependent variable MCS-12 included the gender (male reference; female

coefficient regression:-3.23), exhaustion and cynicism dimensions of the MBI-GS. These variables contributed significantly to explaining 30.4% of the variance in MCS-12.

Limitations, reason for caution: BMI was calculated from self-reported height and weight, and studies have suggested that some respondents tend to over or underestimate these data. These misclassifications will tend to bias findings toward detecting no difference and might underestimate potential differences across BMI.

Wider implications of the findings: Since this syndrome has been related with potentially substandard patient care and with errors, strategies for improving conditions in the workplace are of fundamental importance. Attention should be paid not only to working time but also to qualitative aspects of work. Finally, strategies to reduce occupational stress and problems should form part of the training provided for clinical embryologists.

Study funding/competing interest(s): Funding by hospital/clinic(s), Hospital Universitario Virgen de las Nieves.

Trial registration number: Not applicable.

P-357 Wellbeing and need for psychosocial support in cross-border oocyte recipients

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Study question: This study aims to evaluate the wellbeing status (quality-of-life[QoL], anxiety, and depression) and need for psychosocial support of women seeking oocyte donation treatment abroad. Do these vary according to country of origin?

Summary answer: Almost half of patients present anxiety symptoms. Most women consider psychological support helpful and would appreciate if the clinic would provide it. Comparing France and Italy, we observe that French patients have lower QoL and express a higher demand of psychological support than Italian ones.

What is known already: Assisted reproduction technologies treatment causes a psychological burden that impacts patients' wellbeing. Individuals vary in the level of burden experienced and support needed. Patients travelling abroad for their treatment may experience higher burden and need more support due to the increased practical demand (e.g., travelling, different language and culture) and lack of informal support networks.

Study design, size, duration: Survey of 224 women from 7 countries (Italy n = 80, France n = 80, Germany n = 27, England n = 13, The Netherlands n = 12), Switzerland n = 4, Ireland n = 2) attending a large fertility clinic for oocyte donation in March-November 2013.

Participants/materials, setting, methods: All patients completed validated questionnaires assessing their QoL (FertiQoL) and levels of anxiety and depression (HADS) immediately after the embryo transfer. Three additional questions were presented in order to evaluate their perception of psychological support: the need for psychological support, previous support requests, and expectation of support from the clinic.

Main results and the role of chance: Mean age was 40 (SD 4.8) with 3 (SD 2.8) previous IVF cycles. In line with reported studies, the overall FertiQoL core score was 69.4 (range 0–100). There were 43.9% and 9.9% of patients with HAD-A and HAD-D scores over 7 (threshold for borderline anxiety/depression), respectively. Psychological support was considered useful by 59.8% of patients, 23.2% had consulted a psychologist and 54.0% considered helpful for the support to be offered by the clinic. Subgroup comparisons by country showed that French women reported lower QoL than Italian in the core (p = 0.008), emotional (p < 0.001), mind-body (p = 0.011), and relational (p = 0.005) FertiQoL domains. No differences were observed for social QoL, anxiety and depression. Finally, 32.5% of French had accessed support for infertility problems before, against 12.5% of Italian (p = 0.005).

Limitations, reason for caution: Country representation is not homogeneous and for 2 countries we had <5 patients; therefore we compared directly only the 2 largest groups of patients. Patients in the study were women in a heterosexual relationship undergoing oocyte reception treatment; perceptions of women in a different situation could be different.

Wider implications of the findings: This study corroborates the psychological burden of ART, usually resulting in high anxiety during treatment. More than half of cross-border patients consider helpful to receive psychological support from the clinic. The impact of treatment on cross-border patients seems to vary according to their country of origin. Profiling this differential impact is useful for clinics to provide patient-centered care but more research needs to be done to understand the causes of such differences.

Study funding/competing interest(s): Funding by hospital/clinic(s), No competing interests are declared.

Trial registration number: NA.

P-358 Severe depressive symptoms at baseline predict individual and partner infertility distress at year-1 follow-up

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Study question: Are severe depressive symptoms at baseline a predictor of individual and partner infertility-related distress after 1-year of unsuccessful fertility treatments?

Summary answer: Yes, severe depressive symptoms at baseline significantly predict increased infertility-related distress at the individual and partner level in couples undergoing unsuccessful fertility treatments at 1-year follow-up.

What is known already: Cross-sectional studies have shown that severe depressive symptoms are significantly associated with increased infertility-related distress in individuals and their partners. Studies have also shown that a prior history of depression is a risk factor for future depression in those undergoing fertility treatments. Research studies using a longitudinal design to study the impact of severe depressive symptoms on infertility-related distress in couples are lacking.

Study design, size, duration: The study used a longitudinal design with men and women who were consecutively referred patients undergoing fertility treatments in Denmark in years 2000–2001. A total of 826 men and 923 women were included at baseline in the study, and 343 men and 402 women were included at the 1-year follow-up.

Participants/materials, setting, methods: Participants were consecutively referred patients undergoing fertility treatments at Danish fertility clinics (n = 5). The Mental Health Inventory-5 and COMPI Fertility Problem Stress Scale was used to measure depressive symptoms and infertility-related distress. Multilevel modeling using the Actor-Partner Interdependence Model was used to study the couple as the unit of analysis.

Main results and the role of chance: At baseline the average age of study participants was 34.2 years for males and 31.9 years for females. There were significant effects for males and female depression on all three levels of distress meaning that baseline severe depressive symptoms significantly predicted infertility-related personal, marital, and social distress in males and females at 1 year follow-up (p < 0.01). Significant partner effects also found (i.e., an individual's severe depressive symptoms predicted infertility-distress in their partner at 1-year). Female baseline depressive symptoms predicted male personal, social, and marital distress at 1-year follow-up. Male baseline depressive symptoms predicted female marital distress at 1 year follow-up.

Limitations, reason for caution: The limited sample size need to be taking into consideration when interpreting these findings. The Mental Health Inventory-5 was not specifically developed to measure depressive symptoms; however the scale has been used in many studies to measure depressive symptoms.

Wider implications of the findings: The findings from this study underscore the impact of depressive symptoms on both the individual and their partner, and add to the growing body of literature using the couple as the unit of analysis in men and women undergoing fertility treatments. Male distress appears especially impacted by female depressive symptoms at baseline. Health professionals can work together to educate couples about potential risk factors when one or both partners experience severe depressive symptoms during treatment.

Study funding/competing interest(s): Funding by national/international organization(s), This study is funded by the Danish Health Insurance Fund. The funds had no influence on the data collection or the data analyses. The authors have no conflicts of interest to declare.

Trial registration number: N/A.

P-359 Development and validation of the fertimed-questionnaire to assess hormonal fertility medication from the patient's viewpoint

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Study question: Which specific hormonal medication aspects are valued by patients and can patient experience with these aspects be assessed with a valid and reliable tool, discriminating between different medications used to achieve the same clinical objective?
Summary answer: Patients value various specific aspects of hormonal fertility medication besides their effectiveness and route of administration. These valued aspects can be assessed by patients with the aid of the FertiMed-questionnaire, which proved to be valid, reliable and capable of identifying differences between medications used to achieve the same clinical objective.
What is known already: Hormonal fertility medications cause emotional strain and differ in dosage regime and route of administration but often have comparable effectiveness. Choice of medication should take account of assessments by former patients. Rather than previous studies assessing patients' satisfaction with medication aspects specified by professionals, this requires insight into specific medication aspects valued by patients and development of a valid, reliable tool, capable of identifying differences in patients' experiences between various medications with identical clinical objectives.
Study design, size, duration: Developing the FertiMed-questionnaire for patients to assess medications (n = 8) used for ovarian stimulation, induction of pituitary down-regulation, ovulation triggering or luteal support regarding specific aspects, which patients value according to literature review and 23 patient interviews. Disseminating the FertiMed-questionnaire (Fall 2013) to 411 IVF-patients, each assessing one of eight medications.
Participants/materials, setting, methods: The FertiMed-questionnaire, disseminated in two university fertility clinics (Belgium, Netherlands), assessed patient characteristics, importance of and experience with specific medication aspects, two overall satisfaction questions and patient preference between used medications. The FertiMed-questionnaire's psychometric characteristics were tested and improved. Analysis focused on validity, reliability and discriminative potential of the FertiMed-questionnaire.
Main results and the role of chance: Fifty-one valued specific medication aspects were identified, through literature review (n = 31/51) and patient interviews reaching data saturation (n = 51/51). In total, 276 patients (on average 35 per medication), completed the FertiMed-questionnaire (response rate = 67%). Item-analysis deleted 10 medication aspects. Reliability, confirmatory and exploratory factor-analysis identified ten dimensions. The final model was valid (Adapted Goodness of Fit Index = 0.95) and all but one dimension ('ease of use: disturbance') could be assessed reliably. The two overall satisfaction questions had no discriminative potential. Overall patient-centeredness scores, combining importance and experience ratings of all specific medication aspects, adjusted for its determinants (i.e. age, fulltime employment and spontaneous pregnancy), discriminated between the three medications used for ovarian stimulation (p = 0.01). This difference was confirmed by preference of patients having used all ovarian stimulation medications.
Limitations, reason for caution: As all eight medications prescribed in the recruiting clinics were questioned, sample sizes per medication were rather small for testing the discriminative potential of the FertiMed-questionnaire. The validity and reliability of this questionnaire, proven in the two-country setting, needs to be confirmed for other countries.
Wider implications of the findings: Patients value various specific aspects of hormonal fertility medication, besides their effectiveness and route of administration, which could be important when developing new medications. Tailoring medication choice to individual patient preference, requires developing and using a decision aid for shared decision-making among patients and physicians for each clinical objective requiring use of medication. Information provided in this decision aid could be based on valid and reliable assessments made by patients using the FertiMed-questionnaire.
Study funding/competing interest(s): Funding by University(ies), Leuven University and Amsterdam University Medical Center.
Trial registration number: Not applicable (reference form Belgian ethical committee approval: s55156; ML9054).

P-360 There is not a day goes by that I don't think about it: The experience and meaning of male factor infertility to men

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Study question: What impact does the diagnosis of azoospermia have on men's psychological and social functioning?
Summary answer: Male infertility remains a taboo subject and most men are reluctant to discuss it. Receiving a diagnosis of azoospermia shocks most men. Investigation and treatment pose a substantial emotional burden on men and therefore a deeper understanding of these issues is important in providing patient-centred care, tailored to individual needs.
What is known already: Male factor is the main or a contributing cause in up to 50% of couples presenting with infertility. Unlike psycho-social aspects of female infertility, male attitudes to their own infertility are poorly understood. Male infertility can potentially have a significant impact on psychological and social aspects of men's lives, impacting negatively on self-image, relationships and psychological distress, but objective data in this area is lacking.
Study design, size, duration: A qualitative interview study with 15 participants. Inclusion criteria: Men attending a fertility clinic, over the age of 23, suffering from primary infertility for more than a year and diagnosed with azoospermia. Data were collected between June 2013 and November 2013.
Participants/materials, setting, methods: We conducted semi-structured, face-to-face interviews in the clinic or in patients' homes with/without partners present, with 15 men diagnosed with azoospermia. Participants gave their own account of how they perceived the experience of receiving a diagnosis, undergoing investigation and treatment. The interviews were fully transcribed and analysed thematically using NVivo.
Main results and the role of chance: Major interview themes included: 'Reaction to diagnosis', 'Lack of cause and explanation', 'Effect on interpersonal relationships', 'Disclosure of diagnosis', 'Support seeking' and 'Decisions regarding treatment'. Preliminary results show that the initial diagnosis of infertility mostly comes as a shock. Men find the lack of a precise aetiology frustrating/distressing. Most men are reluctant to share the diagnosis beyond close family members. The diagnosis brings partners closer together in most cases, but some couples became more emotionally distant. Men didn't feel the need to seek external psychological support and were satisfied with the support provided by clinic staff. A sperm retrieval operation was in most cases the only hope for establishing biological fatherhood. Decision-making with regards to this and donor sperm treatment took into account multiple factors.
Limitations, reason for caution: This was a qualitative, explorative study on a clinic-based sample of 15 men seeking treatment. It looks at the experience of male infertility in the short-term. It does not examine a broad population sample and does not include men that are not seeking treatment. The findings therefore cannot be generalised.
Wider implications of the findings: Male infertility impacts substantially on men's quality of life and healthcare professionals should be aware of this when investigating/treating patients with azoospermia. The development of information resources aimed at men diagnosed with azoospermia and their partners, is important in helping couples to understand male infertility, its impact, and to support them more effectively. Men find the lack of specific aetiology frustrating and therefore further research is required into the aetiology of male infertility.
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by commercial/corporate company(ies), 1. Newcastle Fertility Centre, International Centre for Life, Newcastle-upon-Tyne, UK, 2. ProStrakan Group plc, UK.
Trial registration number: National Institute for Health Research (NIHR) portfolio study number: 118390.

P-361 Male narratives on infertility and the couple relationship: a qualitative analysis of men weblogs

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Study question: What are the perceptions and experiences of men regarding the way infertility affects the couple and their role in the marital relationship?
Summary answer: Men experience distress in witnessing their wives suffer both physically and psychologically. These concerns are accompanied by a sense of powerlessness in protecting their partners, challenging their traditional models of male masculinity.
What is known already: While much is known about women's psychosocial adjustment to infertility, there is little evidence on male adaptation. Previous studies have suggested that men can reveal higher scores of adjustment to infertility than women because they tend to contain their own emotions in order to support their wives. However, there is no data supporting this hypothesis. There is a need to better understand how men dealing with infertility perceive their role within the relationship.
Study design, size, duration: This study used a qualitative design. The target population were weblogs written in English by men who self-identified as diagnosed with infertility. We used the search query "male infertility blog" to begin a modified snowball sampling method and identified 27 weblogs posting entries from July 2004 to October 2010.
Participants/materials, setting, methods: Each weblog was archived chronologically, with data analysis ending at the date of data collection or at a positive pregnancy test, adoption, or for no explicit reason. The final sample was composed of 22 weblogs. Two coders analyzed and coded the entire data set using a grounded theory approach.
Main results and the role of chance: Bloggers were from 6 countries on 4 continents. Participants had on average 32.7 years, were in a marital union for 4.5 years, and were trying to conceive for 2 years at the beginning of their blogs. Besides the impact of infertility in the relationship, 5 other main themes emerged from the data and were mentioned by all participants: involvement in treatments; hoping for a pregnancy; acceptance of infertility; feeling integrated in a trying-to-conceive community; and the others don't get it. Bloggers described their relationship as close, assuming the responsibility for support within the relationship. Supportive behaviors were accompanied by the perception of insufficiency in comforting their partners and a sense that infertility is not as central to them as it is to their partners.
Limitations, reason for caution: Even though weblog data is unbiased by the research process, this sample might not be representative of men diagnosed with infertility due to bloggers traits. Social endorsement can also compromise validity and reliability of data.
Wider implications of the findings: These findings shed light on the ambivalence men can experience as they navigate the unexpected stress of infertility and try to correspond to traditional gender-based expectations to solve the couple problems. Mental health professionals should work with the couple addressing each member perceptions of how they are being supported and support the other. Additionally, educational interventions can be implemented to normalize the varied reactions men are likely to experience in facing infertility.
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: Not applicable.

P-362 Presentation of the time-lapse video of the transferred embryo and changes of psychological status during the implantation period

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Study question: The aim of this study was to assess psychological changes of the women with time-lapse (TL) based single embryo transfer (SET) from the time of video presentation of the transferred embryo by an embryologist to the time just after knowing the results of pregnancy test (PT).
Summary answer: Showing the TL video helped patients to understand the embryo quality and thus allowed relief in the period between embryo transfer (ET) and PT. However, the result of negative PT significantly brought the women regret for having watched the video and grief as if she had experienced miscarriage.
What is known already: The days between ET and PT are considered to be one of the most stressful periods for the female assisted reproductive technology (ART) patients. However, to our knowledge, there is no literature on the psychological changes during the implantation period for ART patients including those with repeated implantation failure. Our preliminary questionnaire revealed that most of the women were favourable for the TL video of the transferred embryo.
Study design, size, duration: The questionnaires were administered after urine sample was taken for PT. Two hundred and seventy-six fresh or frozen/thaw SET cycles were included. Patients filled out the first part of the questionnaire before the PT results were informed. The latter part was filled out after the results were informed.
Participants/materials, setting, methods: All the women with ET during this study period were included because TL videos were provided and SET was done for all the women. The embryologists in charge presented the TL videos. Patient self-evaluation of satisfaction was based on a scale of one to five at ET, pre-PT and post-PT.
Main results and the role of chance: Satisfaction (as Rank-4 and -5) was attained by 246 patients (89%) at ET and was maintained during the implantation period. In the patients with negative (compared with positive) PT, the satisfaction rank just after they knew the results (at post-PT) was decreased from the time of ET (P = 0.0007; Wilcoxon signed-rank test). More women with negative PT answered that they did not want to watch the video again if there was a next chance (P = 0.0006; Mann-Whitney U test). Parous women tend to be satisfied with the video at pre-PT (P = 0.014) and post-PT (P = 0.003). Prior history of miscarriage was not associated with degree of satisfaction at pre-PT (P = 0.429) and post-PT (P = 0.501).
Limitations, reason for caution: Lack of a control group is a limitation of the current study because we inform all the patients in advance that the TL videos were taken for each embryo of all the patients.
Wider implications of the findings: TL video presentation provided the women affection for the transferred embryo from its developing images as if it were a fetus already. However, such affection might suddenly change to deep sadness if PT was negative. Our data suggest that parous women might have ability to regard the transferred embryo as her fetus. TL video presentation also should be promising for the patients with repeated failure of implantation.
Study funding/competing interest(s): Funding by hospital/clinic(s), Toyohashi Municipal Hospital. The authors have no competing interest to declare.
Trial registration number: Not applicable.

P-363 What about the donor after conception – a qualitative study on the meaning of the anonymous sperm donor in heterosexual families

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Study question: What meanings do heterosexual couples attach to their anonymous sperm donor?
Summary answer: In the participants' family narrative, the donor was constructed as an abstract person, partly because of his anonymity and partly because they wanted to keep the donor at a distance.
What is known already: So far, many researchers studied disclosure decisions of DI families. The context of this research focus can be seen within the ongoing debate about the right of the child to know his/her genetic roots. This research tradition is mainly dominated by studies from countries where donor anonymity has been abolished. Current study aims to gather in-depth

information on parents' constructions about their sperm donor in the Belgian context, where mainly anonymous sperm donation is practiced.
Study design, size, duration: Semi-structured interviews were conducted with nine heterosexual couples and one individual (19 participants), recruited via the Department of Reproductive Medicine of the Ghent University Hospital. All participants had at least one child conceived via anonymous donor insemination (DI), ranging from 7 to 10 years old.
Participants/materials, setting, methods: Thematic Analysis was used as method to perform the analysis. 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: Central to the analysis, was the message that the donor was not on the parents' minds. They started their treatment wondering about this third party, but as soon as their family was formed, thoughts about the donor faded into the background. Physical and non-physical resemblances with the children enabled parents to distance and even ignore the donor origin, as they felt (for themselves) and appeared (to others) connected with their children. For non-disclosing as well as disclosing parents the donor was constructed as an abstract, unknown figure. This view of the donor helped to protect themselves (and their children) from imagining and thinking about the unknown genetic link.
Limitations, reason for caution: Participants were interviewed as a couple which might have led them to present a joint narrative and limited them from talking freely about their personal views. Possibly, some mothers did not elaborate on the meaning of the donor, because they did not want to threaten their partner.
Wider implications of the findings: Findings of this study add to the debate on anonymity and openness of donors, by taking into account parents' experiences with anonymous donation. A more systemic approach is suggested for research as well as counseling practice, as it seems that the way parents relate to their donor may be influenced by hospital policy, the legal context, the family context and the social environment.
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.
Trial registration number: N/A.

P-364 Is infertility a taboo subject – attitudes towards infertility in different countries

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Study question: The objective of the study is to ascertain the level of taboo that exists in different European countries regarding infertility, and to find out whether patients confronting this issue openly discuss it with their friends and family.
Summary answer: There are significant differences between countries regarding infertility as a taboo subject. However, independent of their nationality, all patients discuss the issue with their closest family and friends. If asked to discuss the issue with the media or social networks then they are far less willing.
What is known already: Each country offers a different cultural context in which to view infertility, and legislation controlling fertility treatments also form opinion. In Europe the law differs very widely between countries, with some countries being extremely restrictive (such as Italy) and others, such as Great Britain or Spain, where infertility treatments are far more visible in society.
Study design, size, duration: Between June and December 2013 questionnaires were filled in by 369 patients from 27 countries. They were asked if infertility is a taboo subject in their country, whether or not they have explained to others that they are undergoing fertility treatment, and if they have told friends, family or both.
Participants/materials, setting, methods: Couples or single mothers undergoing fertility treatments at a clinic in Barcelona completed the questionnaire on the day of their embryo transfer. It covered both their attitudes towards the treatment process and the degree to which they discuss the issues of infertility with their family and friends.
Main results and the role of chance: Infertility is viewed quite differently by the different societies in Europe. The majority of Italian (74%), Irish (55%) and German (61%) patients felt that in their country the subject is taboo, whereas only 38% of Spanish patients and 36% of British patients felt similarly. In 75% of cases patients did share their experience of infertility with their closest family and friends. There was no difference in this respect between countries of origin. 40% of patients speak only to their family, 11% only to friends and 24% to both. Sharing the experience felt good only if shared privately. The majority of responders (60%) did not want to share their experience with media, and only 19% actively participated in internet boards and social networks that discuss infertility.
Limitations, reason for caution: The nature of the questions and the inclusion of personal values regarding the concept of a 'taboo' issue could give rise to ambiguity and therefore limit the validity of some replies to the questionnaire.
Wider implications of the findings: The results allow us to better define the psychological needs of infertile patients, and to see how the cultural context of their country may affect them. This may condition how they deal with the fertility treatment and time at the clinic.

The data shows the importance of sharing the experience of infertility with close family members. In clinical practice it is useful to know this in order to help the patient remain emotionally healthy throughout.
Study funding/competing interest(s): Funding by hospital/clinic(s), Instituto Marques.
Trial registration number: Nil.

P-365 More androgynous or less feminine is for better qol – a German-Hungarian comparison study in fertility specific quality of life and traditional gender-role attitudes

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Study question: Are there any differences in fertility specific quality of life and gender-role attitudes in German and Hungarian couples? Does it differ how gender-role attitudes correlate with better infertility-related quality of life in a Hungarian and a German sample?
Summary answer: Hungarians have better fertility specific quality of life and more "feminine" gender-role attitudes than Germans. In the total sample, "androgynous" and "masculine" attributes correlate with better quality of life than "feminine" and "undifferentiated" gender-role attitudes. In the Hungarian group, only "femininity" correlates with the poorest QoL-scores.
What is known already: Desire for a child and consequences of failure of conceiving are culturally and socially contingent, so in Germany and Hungary with different political and socioeconomic background, involuntary childlessness might be experienced in different ways. Realizing gender roles in a more traditional way brings more distress for a woman, but not for a man. In infertile groups, "femininity" correlated with less emotional stability, less marital satisfaction and more anxious symptoms.
Study design, size, duration: This cross-sectional study was conducted in one German and five Hungarian fertility clinics between February 2012 and March 2013. Couples attending the first medical consultation were enrolled.
Participants/materials, setting, methods: 270/473 couples (response rate: 57%) filled out our questionnaire package which contained International FertiQoL measuring fertility specific quality of life, Personal Attribute Questionnaire about gender roles (PAQ), and socio-demographic questions. *T*-tests and multivariate analyses of variance were used to compare groups according to countries and gender-role attitudes.
Main results and the role of chance: German couples were older aged ($t(482) = 5.67$, $P < 0.001$) and had lived for longer in a partnership ($t(496) = 2.76$, $P < 0.01$) than Hungarians. Hungarian couples scored higher on FertiQoL-subscales as well as on the expressive/"feminine" scale of PAQ

(all *P*s < 0.001). Sex and education had only a minimal effect on these cross-country differences. In the German and in the total sample, participants with "androgynous" or "masculine" attitudes scored significantly better than ones with "feminine" or "undifferentiated" attitudes in emotional, mind/body and global domains of FertiQoL (all *P*s < 0.05). Among Hungarians, only men and women with "feminine" attitudes reported worse quality of life than "androgynous" and "masculine" groups on the Emotional, Mind/body subscales and on the Global scale of FertiQoL (all Ps < 0.05).

Limitations, reason for caution: A relatively low response rate in the Hungarian group (43%) may have influenced the results through selection bias, whereas the data of German couples were collected only in one fertility centre which can lead to contra selected results.

Wider implications of the findings: This study using a cross-country study design gave a differentiated picture of influences of gender-role attitudes on one's infertility related quality of life. The results can give new information about effects of gender-role attitudes for reproductive medical staff and patients. Our findings regarding cross-country differences can provide new implications in planning international or national guidelines for psychological counselling with infertile individuals or couples (There is not a Hungarian guideline for psychosocial counselling in infertility).

Study funding/competing interest(s): Funding by national/international organization(s), European Union, State of Hungary, European Social Fund (TÁMOP-4.2.4.A/ 2-11/1-2012-0001 'National Excellence Program').

Trial registration number: Nil.

P-366 The influence of the polycystic ovary syndrome on couples' relational and sexual satisfaction

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Study question: What is the influence of objective and subjective characteristics of the polycystic ovary syndrome (PCOS) on the relational and sexual satisfaction of PCOS women and their partners?

Summary answer: Relational and sexual satisfaction levels were significantly higher in PCOS women than in their partners. Moreover, the influence of parity, women's body mass index (BMI), unfulfilled wish-to-conceive and women's subjective experience of fertility problems on relational and sexual satisfaction were significantly different between PCOS women and their partners.

What is known already: There is some evidence indicating an association between objective characteristics of PCOS and sexual satisfaction of PCOS women. However, this evidence is conflicting, scarce, and often no standardized questionnaires are used to evaluate sexual satisfaction. No evidence is available about the influence of subjective characteristics of PCOS on sexual and relational satisfaction, and about the influence of PCOS on relational and sexual satisfaction as experienced by partners of PCOS women.

Study design, size, duration: We set up a cross-sectional study from April 2007 till April 2009 including 31 obese (BMI > 25 kg/m^2) PCOS women at reproductive age having a relationship at the time of recruitment.

Participants/materials, setting, methods: The study was performed at the fertility center of the Ghent University Hospital. Objective PCOS-characteristics were registered and the subjective experience of PCOS was evaluated by the PCOS questionnaire. Relational (rs) and sexual satisfaction (ss) were measured by the Maudsley Marital Questionnaire. Data were analyzed using linear mixed models ($\alpha < 0.05$).

Main results and the role of chance: The response rate on the Maudsley Marital Questionnaire was 26/31 for PCOS women and 24/31 for their partners. Relational and sexual satisfaction were significantly higher in PCOS women than in their partners ($p_{rs} = 0.007$ and $p_{ss} = 0.017$). A higher parity tended to increase relational and sexual satisfaction, with a significantly stronger effect in PCOS women than in their partners ($p_{rs} = 0.009$ and $p_{ss} = 0.015$). A lower BMI tended to influence relational and sexual satisfaction of PCOS women negatively and of their partners positively, with a significantly stronger effect in the partners ($p_{rs} = 0.021$ and $p_{ss} = 0.029$). The presence of an unfulfilled wish-to-conceive and a bad subjective experience of fertility problems by the PCOS women had a significantly stronger negative effect on their relational satisfaction versus their partners ($p_{rs} = 0.021$ and $p_{rs} = 0.011$ respectively).

Limitations, reason for caution: The fact that this study was performed in a sample of PCOS women who were all obese and the small sample size are limitations of this study. Data were partially missing in some couples but this limitation was dealt with by using linear mixed models.

Wider implications of the findings: Our results suggest a differential influence of PCOS on relational and sexual satisfaction for PCOS women and their partners. This should be kept in mind during the psychological guidance of couples dealing with PCOS.

Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), Funding by commercial/corporate company(ies), Veerle De Frène is holder of a Special PhD Fellowship by the Flemish Foundation for Scientific Research; Belgium. Petra De Sutter is holder of a fundamental clinical research mandate by the Flemisch Foundation for Scientific Research; Belgium. This research also received financial support by Merck Serono; Belgium and the Artevelde University College Ghent; Belgium.

Trial registration number: Not applicable.

P-367 Relation of meta-cognitive beliefs and psychological disorders with success in assisted reproductive techniques

Abstract withdrawn by the author

P-368 Men, masculinity and infertility treatment

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Study question: How do aspects of masculinity affect men's experiences of infertility treatments and their relationships with health professionals working in the field of assisted conception?

Summary answer: Men's involvement within the consultation process appears guided by the notion that men prefer to discuss technical or informational aspects of their treatment rather than their well-being, which is reinforced by the fact that women experience invasive procedures that are a more legitimate cause for concern than men's emotional distress.

What is known already: Men account for approximately 45% of infertility problems experienced by couples. While much is known about the causes of male infertility relatively little is known about the ways in which masculinity shape men's understandings and experiences of their infertility treatment.

Study design, size, duration: This was a qualitative study that incorporated observation within the study setting, interviews with 22 men currently undergoing infertility treatment and interviews with 10 health professionals working with infertile men. Recruitment took place over a period of 12 months and all participants self-recruited.

Participants/materials, setting, methods: The study site was an assisted conception clinic in the UK. Data was collected via semi-structured interviews with 22 men currently undergoing infertility treatment and semi-structured interviews with ten health professionals working in the field. Observation took place within main reception/waiting area of study setting.

Main results and the role of chance: The process of providing a sperm sample could leave men feeling that they were on 'a conveyer belt' and the pressures on them to 'perform' meant that the provision of pornography in the sample room was seen as necessary. However, men highlighted the contradiction between the clinical nature of the room and the possibility that they could come into contact with other men's seminal fluid. The nature of how men were incorporated into the consultation process appeared to be guided by the notion that men prefer to discuss technical or informational aspects of the treatment rather than be asked about their well-being. However, the fact that women experience often painful invasive procedures was defined as a more legitimate cause for concern than men's emotional distress.

Limitations, reason for caution: This was a relatively small scale study and the sample was self-selected.

Wider implications of the findings: This study sheds light on how aspects of masculinity mediate men's experiences while undergoing infertility treatments and their relationships with health professionals working in the field. It also illustrates how health professionals' conceptualisations of masculinity have implications for their practice with men. Therefore, it can help to inform the planning, development and delivery of infertility treatments in relation to men.
Study funding/competing interest(s): Funding by national/international organization(s), Economic and Social Research Council – ES/I02834X/1.
Trial registration number: N/A.

P-369 The support and information currently provided by fertility counsellors to intended parents planning a child by embryo donation or double donation treatment

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Study question: What support and information regarding disclosure is currently provided by fertility counsellors to intended parents who are planning a child by embryo donation, or double donation treatment?
Summary answer: Findings will be presented on the extent of support and information that counsellors currently provide on disclosure, with intended parents who are planning a child by embryo donation or double donation treatment, and how this may vary between counsellors.
What is known already: Evidence suggests that being open about donor conception origins may be beneficial; however the majority of previous research focuses solely on egg or sperm donation. Little is known about the extent that counsellors encourage engagement with thoughts, feelings and concerns about disclosure in cases of embryo donation or double donation treatment.
Study design, size, duration: This study is a questionnaire design where fertility counsellors are invited by email to complete an online survey. All eligible counsellors in the UK have been approached. Data collection started on 25.11.2013 and will end on 25.05.2014.
Participants/materials, setting, methods: Participants will complete an online survey consisting of questions aiming to ascertain what is covered in counsellors' discussions with intended parents planning a child by embryo donation or double donation. Specific questions will address how counsellors explore the implications of treatment, particularly the consideration of thoughts and feelings towards disclosure.
Main results and the role of chance: Data will be analysed in terms of percentages and frequencies. Results will examine the extent to which counsellors address specific topics with intended parents, and how much this varies between counsellors. Particular focus will be on the support and information provided on the possibility of disclosing this information, and how such disclosure processes might be approached.
Limitations, reason for caution: Embryo donation and double donation treatments are relatively uncommon; therefore some fertility counsellors might have had only minimal experience working with intended parents planning a child this way. Findings will be limited to the current practice of counsellors in the UK only.
Wider implications of the findings: Findings will contribute to our understanding of this relatively unexplored type of family creation. Conclusions will summarise current practice by fertility counsellors working with intended parents planning a child by embryo donation or double donation treatment. Based on these findings, recommendations will be made aiming to maximise the efficacy of future practice.
Study funding/competing interest(s): Funding by University(ies), The University of Warwick.
Trial registration number: N/A.

P-370 Exploring involuntary childlessness in men: a qualitative study investigating men's experiences and perceptions in the process of diagnostic, fertility treatment and quality of life

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Study question: Main goal of the study is to explore the experience and perception of men in diagnostic and sustained process of fertility treatment as the impacts of role concepts, control beliefs and quality of life for men in coping with the undesired circumstances.
Summary answer: Participants experiences and perceptions differed in enormity according to the cause of infertility, period of dealing with the topic, previous lifetime challenges and perceived prospect of success. Notion of active role functions, perceived control and relevance of social support affected the treatment process.
What is known already: Contradicting to former research, involuntary childlessness is seen as a major life crisis also for men. Confrontation with diagnostic or treatment procedures is sensed as a high burden; however perceived quality of life seems not to alter significantly. Mixed findings can be found regarding the suffering of men due to the cause of infertility. A high importance of control beliefs and diverging role concepts in infertility process for men is suspected.
Study design, size, duration: A qualitative study was devised, approved by the Ethics Committee of the Heidelberg University Hospital. The study consisted of thirteen semi-structured individual interviews and took place from July to September 2013.
Participants/materials, setting, methods: Interviews were conducted with men being currently in or are about to start fertility treatment. Recruitment took place by direct approach through researcher or physician in the fertility clinic. Care was taken to obtain men from different stages in the treatment process. Data were analyzed using grounded theory approach.
Main results and the role of chance: Men were willing to talk openly about issues affecting them in treatment and their coping with undesired circumstances. Participants differed in perception of treatment process, in particular men knowing long-term about the risk of childlessness compared to men having recently found out that difficulties exist. Men indicated the importance of constructive disclosure of results and effectual information about the treatment process. Furthermore, they emphasized their struggling with possessing less to no control over the treatment process, especially regarding the uncertainty of outcome, and their attempts in trying to find an appropriate role in the whole process. Participants highlighted their wish for a more holistic approach in treatment and the importance of partnership, while their notion of social support was highly ambivalent.
Limitations, reason for caution: Generalization of findings is limited due to the small sample, above-average educational background of participants and the fact that not all involuntary childless couples seek infertility treatment in an university hospital. Furthermore, all interviews and data analysis were conducted by the same researcher, therefore potentially accounting for restricted reliability and validity.
Wider implications of the findings: The major differences in dealing with involuntary childlessness revealed by participants need to be identified and addressed by health professionals. The significance and impact of diverse infertility causes for disclosing results, adoption of roles and quality of life for males ought to be more valued and an enhanced holistic approach embraced. Conducting interviews provided distinct insight in the male perspective, which is highly needed in view of the great number of men attending fertility services.
Study funding/competing interest(s): Funding by hospital/clinic(s), Abteilung für Gynäkologische Endokrinologie und Fertilitätsstörungen, Universitäts-Frauenklinik Heidelberg.
Trial registration number: None.

P-371 Assisted Reproductive couples preferred twins: a questionnaire study

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Study question: To determine the proportion of patients who prefer a multiple birth over a singleton after an IVF/ICSI attempt.
Summary answer: A majority of infertile couples undergoing an IVF/ICSI cycle prefers a twin gestation as the treatment outcome. More carefully prepared information seems to be needed.

What is known already: Twin pregnancies carry risks for both mother and child. Compared with singleton pregnancies there is an increase in obstetric complications and in neonatal complications. The infant mortality rate in twins is about twice as high as in singleton pregnancies. Despite of this data, IVF/ICSI patients prefer twins over singletons.
Study design, size, duration: Prospective survey study including 390 couples undergoing embryo transfer during an IVF/ICSI cycle, during 2013, in both a public university hospital and a private practice reproductive clinic.
Participants/materials, setting, methods: Both the patient and her partner were asked to fill out a 10-question survey in order to determine demographic characteristics, past obstetric history, infertility history, study level and desire regarding multiple births, while waiting for embryo transfer before any clinical counseling about the number of embryos to be transferred had occurred. The questionnaire was collected 30 min later, and non-responders were excluded.
Main results and the role of chance: About 58.2% preferred having twins to having one child at a time (37.1%) and 4.8% preferred triplets. Primary reasons for preferring twins were "wish not having a new IVF attempt" (61.3%), a positive attitude towards twins (27.1%), "avoiding the waiting list" (5.8%), and "with the current advanced technology, complications in multiple pregnancies are low" (5.2%). Economic considerations were not important.

Primary reasons for preferring one child at a time were "lower risks for the mother" (36.8%), "this is my reproductive project" (30.9%), "economic considerations" (15.8%), "lower risks for the baby" (12.0%). Main secondary reasons were "lower risks for the baby" (52.2%) and "lower risks for the mother" (28.4%).

About 62.2% of couples from the public hospital preferred twins vs. 47.7% of the private center ($p < 0.05$).
Limitations, reason for caution: Different population size between both Centers.
Wider implications of the findings: Patient education may be an effective strategy to reduce the incidence of twin and higher-order multiple pregnancies.
Study funding/competing interest(s): Funding by hospital/clinic(s), I confirm that I have no conflict of interest in relation to this work.
Trial registration number: Unnecessary.

P-372 Fertility awareness and family intentions among Danish and British men and women – an internet-based survey

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Study question: To what extent are Danish and British men and women aware of the female age-related decline in fertility, and is fertility awareness associated with own family planning among those who do not have children?
Summary answer: Gaps in knowledge about female fertility decline and likelihood of pregnancy were present, particularly among men, those not living with a partner and among participants <24 or >45 years old. Low fertility knowledge did not show any significant association with preferred time to start a family.
What is known already: The majority of young people desires parenthood. However, across European countries men and women often postpone trying to conceive until an age where female fertility has declined. Knowledge about the age-related fertility decline has been shown to be insufficiently communicated to populations in many countries, not giving people a sufficient basis for informed decision-making regarding family planning. Further, it has been shown that people over-estimate the chances of achieving a live-birth after assisted reproduction treatment.
Study design, size, duration: The study included $n = 1237$, men ($n = 237$) and women ($n = 1000$), who answered an internet-based survey advertised through websites, libraries, and educational institutions from October 2012–September 2013. Advertising of the survey aimed at achieving representation from all adult age groups, men and women, and individuals with different educational backgrounds.
Participants/materials, setting, methods: Participants were from Denmark (55 %) and United Kingdom (45 %). Univariate and multivariate logistic regression analyses were used to identify predictors of low fertility awareness and intended time to start a family, and whether fertility awareness predicted the intended time of the first child.
Main results and the role of chance: In total, 46% of all participants (54% of men) responded that a woman's fertility begins to decline at age 30 years or later, and 21% (men 35%) indicated no difficulties for women aged 40–45 years in achieving a pregnancy. Further, 9% (men 15%) overestimated the chances of pregnancy for a 35-year-old woman during 1 year of unprotected sex, the corresponding number was 23% (men 35%) for a 40-year-old woman. In multivariate regression analyses, the lowest level of awareness was found among men, those not living with a partner, those without children and those <24 and >45 years of age. No significant associations were found among those without children between fertility awareness and the time they wished to have their first child.
Limitations, reason for caution: The generalizability of the results is limited due to the internet-based data collection. Respondents may be a selected group of the population.
Wider implications of the findings: Low levels of fertility awareness among men and youngest group points to a need for communicating fertility knowledge to the next generation of parents to enable them to make well-informed life choices. The missing link between fertility awareness and intended time to start a family could indicate that reproduction education is not the only answer to solving the issue of age-related infertility. Over-optimism regarding ART success could be part of the explanation.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), This study is part funded by an unrestricted grant from Merck Serono who had no influence on data collection or data analyses. The authors have no conflicts of interest to declare.
Trial registration number: None.

P-373 What do oocyte donors know about fertility – a survey about fertility knowledge, awareness, and attitude towards motherhood in candidates for oocyte donation in spain

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Study question: This study investigates childbearing intention in women candidate for oocyte donation in Spain, and what is their degree of fertility knowledge and awareness. Effect of age, education level and previous pregnancies is analyzed.
Summary answer: Candidates for oocyte donation in Spain have overall comparable attitudes towards motherhood. Fertility knowledge and awareness is poor, regardless of age, university education or having children. Among them, the most educated women are more likely to delay childbearing and at higher risk of childlessness by the age of 30.
What is known already: Most women want to have children some day, but the trend is to postpone motherhood, frequently after 30 years for the first child. A lack of an accurate knowledge about fertility lifespan is in part responsible of this phenomenon, in the general population as well as in highly educated women. However, no investigation has been performed in Spain – the European country with the highest proportion of first births above 30 years, or in oocyte donors.
Study design, size, duration: Prospective study consisting in an original survey administered to 229 oocyte donation candidate between March and October 2013 in a large private fertility center on the day of their first appointment.
Participants/materials, setting, methods: Participants were on average 24.6 years old; 19.0% of them attained university education and 36.7% had children. The intervention consisted in an interview using a questionnaire related to attitudes towards maternity, fertility awareness and knowledge about age limits for achieving a pregnancy.
Main results and the role of chance: The vast majority of participants in the study desired to have children some day (95.6%). However, the answers to the questionnaire indicate poor fertility knowledge: 49.3% failed at identifying the fertile window within the menstrual cycle; 48.5% at recognizing the most fertile age; 45.0% considered that women could get pregnant naturally and easily beyond 40 years old; and 27.9% overestimated age limits for assisted reproduction. University education does not improve global fertility knowledge ($p = 0.44$), and is associated to later intended ages for childbearing ($p = 0.001$).

This results in a twofold risk of unintended childlessness at age of 30 in university educated women compared to women without university education (RR = 1.95, 95% CI 1.11–3.43).
Limitations, reason for caution: The target population was women candidates for oocyte donation; these are healthy women with no reported fertility problems, so caution should be exerted when generalizing the results to young women seeking a treatment of assisted reproduction.
Wider implications of the findings: The sample included women between 18 and 35 years old from different cultural backgrounds and education levels, therefore, the results may be generalized to healthy women of reproductive age. On the basis of these results, the future fertility of young people should be protected through educational interventions which emphasize the increasing phenomena of age-related infertility. Research on the effect of specific interventions is needed.
Study funding/competing interest(s): Funding by hospital/clinic(s).
Trial registration number: NA.

P-374 Fertility knowledge among adults in Japan, measured with the Japanese version of Cardiff Fertility Knowledge Scale (CFKS-J)

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Study question: What factors are associated with fertility knowledge in Japan?
Summary answer: People with more interest in childbearing and with greater health literacy had greater fertility knowledge.
What is known already: Fertility knowledge is generally greater in women, in those of higher socio-economic status, and in those with a history of medical consultation for infertility. Japanese students perform very well in international surveys of scholastic ability, but a recent survey of 79 countries (the International Fertility Decision-Making Study (IFDMS)) showed that fertility knowledge was lower in Japan than in any other developed country.
Study design, size, duration: This cross-sectional survey used online social-research panels in Japan in October 2013. There were 2 groups: a representative sample of the general population, 18–59 years old, n = 4,328 (the 'General' group); and people who had been trying to conceive for at least 6 months, 18–50 years old, n = 618 (the 'Triers' group).
Participants/materials, setting, methods: Fertility knowledge was assessed using the Japanese version of the 13-item Cardiff Fertility Knowledge Scale (CFKS-J). All participants provided socio-demographic information (i.e., age, university education [yes/no]) and completed a 14-item health literacy scale and an 11-item health numeracy scale.
Main results and the role of chance: The response rate was 70.8%. Fertility knowledge was greater in those who had been trying to conceive. The average percentages of CFKS-J items answered correctly were 53.1% in the Triers group and 44.4% in the General group (difference between groups: $p < 0.001$). Multivariate regression: in the Triers group greater fertility knowledge was associated with greater health literacy and prior medical consultation regarding their fertility. In the General group greater fertility knowledge was associated with being female, younger, university educated, currently trying to conceive, non-smoking, higher health literacy and higher health numeracy. Among all participants, those who were aware that fertility declines with age said they had first learned that fact 'at school' (3.1%), 'through mass media' (55%), and 'ia the Internet' (11%).
Limitations, reason for caution: Reasons for caution include the possibility of selection bias; the use of social research panels with access to the internet and volunteer bias toward those who are more interested in fertility.
Wider implications of the findings: Comparing the Triers group to the Japanese participants in IFDMS in 2009–2010, fertility knowledge had improved, possibly due to recent media coverage of age-related infertility. Still, it was lower than the average score in developed countries. Educational interventions, perhaps in schools, may be needed to increase fertility knowledge in the general population because most people obtain fertility knowledge from mass media, which has been shown to often present distorted and inaccurate fertility information.
Study funding/competing interest(s): Funding by national/international organization(s), This study was funded by National Center for Child Health and Development, Seiiku Medical Study Grant (24-6).
Trial registration number: Not applicable.

P-375 Effect of failure in infertility treatment on anxiety and depression

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Study question: What are the effects of infertility treatment failure(s) on anxiety and depression?
Summary answer: Our data shows that infertile patients after the first treatment failure are prone to anxiety and depression.
What is known already: Infertility itself and also assisted reproductive techniques can cause anxiety and depression. Also treatment failure can worsen the situation. There is not any reports about the effect of treatment failure in Iran with its special culture and beliefs. Our study was to verify the effect of treatment failure and the number of failures on patients' anxiety and depression.
Study design, size, duration: This was a descriptive cross sectional study in 2013. Sample size was calculated 200 with 2 losses made 198 people. Sampling method was simple random.
Participants/materials, setting, methods: 198 infertile people referring to Royan Institute in Tehran (Capital of Iran). Royan Institute is a referral infertility clinic which has patients from all around the country. A demographic questionnaire and also Hospital Anxiety and Depression Scale (HADS) was used.
Main results and the role of chance: Patients were divided into 4 groups according to the number of their previous treatment failure: no failure (NF), one failure (1F), two failures (2F) and three and more failures (MF) (99, 43, 32 and 24 cases respectively). One-way ANOVA showed anxiety (mean ± SE) in NF (7.26 ± 0.43), 1F (9.2 ± 0.72), 2F (7.18 ± 0.93) and MF (6.25 ± 0.76) with significant difference between all groups (p = 0.043). But for depression: NF (6.93 ± 0.38), 1F (8.14 ± 0.5), 2F (6.64 ± 0.51) and MF (6.95 ± 0.53), the difference was not significant (p = 0.213). Maximum anxiety was seen in patients after one failure and the minimum was seen in multi-failure patients. Same maximum was seen in depression (1F), but multi-failure patients were more depressed.
Limitations, reason for caution: Data gathered from just one center and larger multi-center studies is suggested.
Wider implications of the findings: Failure should be considered as a difficult situation and psychological and maybe psychiatric interventions could be advised specially for the first failure.
Study funding/competing interest(s): Funding by national/international organization(s), Royan Institute.
Trial registration number: Nil.

P-376 Depression among women in successful assisted reproduction technology treatment – a national register-based cohort study

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Study question: Do women, after successful assisted reproduction technology (ART) treatment, have a higher risk of a depression diagnosis compared to women having unsuccessful ART treatment?

Summary answer: Women, who achieved a live birth after ART treatment, have an increased risk of a subsequent depression diagnosis compared to women not achieving a live birth after ART.
What is known already: Studies investigating the association between successful ART treatment and depression among women are ambiguous. Most studies find that women with unsuccessful fertility treatment have a higher risk of depression or depressive symptoms compared to women achieving a live birth. However, one study do find that women evaluated for infertility and not achieving a live birth have a lower risk of a depression compared to women achieving a live birth.
Study design, size, duration: A national, register-based cohort study, including all women treated with ART and recorded in the Danish IVF Register from 1994–2009 (N = 42,915). Data were linked to the Danish Medical Birth Register (1994–2009) and the Danish Psychiatric Central Research Register including depression diagnoses (1969–2009).
Participants/materials, setting, methods: Total population was 41,050 women without a depression prior to ART and with complete data on ART treatments. After ART treatment, 552 (1.34%) women received a depression diagnosis. Cox regression analysis was used to investigate the association between live birth and depression diagnosis subsequent to ART treatment.
Main results and the role of chance: Women with a live birth had an increased risk of having a depression diagnosis compared to women with no live birth (adjusted Hazard Ratio (aHR) = 1.36 (1.14–1.63), $p < 0.001$). In a more elaborated model including time after a live birth, we found the highest risk of a depression diagnosis in women with a live birth 0–42 days after the live birth compared to women with no live birth (aHR = 5.08 (3.11–8.29)). Women with a depression diagnosis had a lower educational level compared to women without a depression diagnosis. The descriptive analysis among women with and without a depression diagnosis showed the same proportion of women with a live birth in the two groups (46.38% vs 44.62%, $p < 0.409$).
Limitations, reason for caution: Only women with a depression diagnosis recorded in a psychiatric hospital setting were included; hence only the most severe cases of depression were included. Women with milder depression episodes treated at e.g. the general practitioner are not registered with depression in the national Psychiatric Register.
Wider implications of the findings: In contrast to previous studies, our findings show that women with successful ART treatment have an increased risk of depression compared to women with no live birth. Similar to women delivering after natural conception, women with a live-birth after ART are at the highest risk of a depression in the first months after delivery. This increased risk of depression is important to remember, in the post-natal of mother and child.
Study funding/competing interest(s): Funding by national/international organization(s), Funding by commercial/corporate company(ies), 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 conflict of interests to declare.
Trial registration number: N/A.

P-377 Is it really necessary to push patients to be more "optimistic" about their treatment

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Study question: The objective of the present study is to determine if there is an association in Spaniards between the single factor of pessimism/optimism and the outcome of an AR treatment.
Summary answer: Our study confirms in a Spanish population the results of Lancastle & Boivin (2005). On its own, optimism/pessimism is not a unique factor that is related with the final outcome of an AR cycle.
What is known already: The possible association between the result of an ART and the patients' attitude, i.e. optimism or pessimism, regarding the result, as well as their capacity to deal with their infertility have been studied (Bleil et al. 2012; Kirchner et al. 2011; Lancastle & Boivin, 2005). Some studies have analysed the possibility that only one factor, optimism/pessimism, could be associated with treatment result. Lancastle & Boivin found that a single factor was not enough to predict treatment success, while Bleil et al. found pessimism to be a risk factor for failed ART.
Study design, size, duration: Prospective, non-randomised cross-sectional study performed from mid-October 2013 to mid-January 2014 in FivMadrid Clinic. 98 patients answer the questionnaires.
Participants/materials, setting, methods: 154 consecutive AR women patients received questionnaires, and 98 (63.6%) responded. The self-administered questionnaire filled in by the women was specially designed for this study.

Results on the cycle and data on the treatment type and years of infertility were obtained from the patients' clinical history. Significance was set at $P < 0.05$.
Main results and the role of chance: ·There were no statistically significant differences between groups (optimistic/pessimistic) regarding age, years of infertility or use of their own or donated oocytes. 59.2% of patients believe they will achieve pregnancy in the current cycle. 61.7% of patients believe that positive thoughts will help achieve pregnancy. However, significant differences in actually achieving pregnancy ($p > 0.05$) were not found between patients reporting optimism and those reporting pessimism during treatment. No significant differences were found in the expression of optimism or pessimism on the part of the patient's partners regarding the outcome of treatment and the actual achievement of pregnancy ($p > 0.05$). No significant differences were found in the result of treatment between patients reporting not undergoing any relaxational therapy or psychological counseling and those who did ($P > 0.05$). No significant differences were found in the actual outcome of treatment between patients affirming that positive thinking would help achieve pregnancy and those who did not ($P > 0.05$).
Limitations, reason for caution: Study performed in a private Centre for Reproductive Medicine in Spain. Although more data from other clinics (private and public) would give a larger and more representational sample, these results may be taken into account, for the wellbeing of the patient.
Wider implications of the findings: Our results confirm those presented by Lancastle & Boivin. The results of this study may be taken into consideration by healthcare professionals in order to not "push" patients to be positive or, in case they are not, do not make them feel guilty with a negative pregnancy test result.
Study funding/competing interest(s): Funding by hospital/clinic(s), Fundación FivMadrid.
Trial registration number: No trial registration.

P-378 Social egg freezing: profile and motivations of Dutch candidate women

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Study question: (1) Their demographic and relationship features, (2) Their motives, (3) Characteristics of their child project, (4) Their Quality of life (QOL).
Summary answer: Women were heterosexual, highly educated with a mean age of 37 years. The majority was single and had a current child wish. Child bearing was postponed in order to find a male partner who could father their child. More than half considered to use their egg cells in future.
What is known already: An increasing number of (single) women use this technique to preserve their fertility. However, social egg freezing remains controversial. Little is known about the profile and motives of those involved.
Study design, size, duration: (1) *Explorative study*: 60 women applying for social egg freezing (from July 2011–July 2012). Written questionnaire covering the above mentioned study questions. Data were analyzed by SPSS 20.0, descriptives. (2) *Comparative study*: data with regard to their psychological functioning were compared with a control group of 60 single women applying for DI at the same clinic. Data were analyzed by SPSS 20.0, non parametric statistics.
Participants/materials, setting, methods: *All participants were clients* at the MCK fertility Centre in the Netherlands. *A self developed questionnaire* covering the above mentioned study questions was filled in during their first visit at the clinic, before the start of the treatment.
Main results and the role of chance: All women had meaningful partner relationships in the past. Of them 30% had already tried to conceive. For the 85% with a current child wish, egg freezing was a method of gaining time. The major reason for postponement was the absence of an appropriate partner. Only 2 women delayed childbearing because of professional considerations. The remaining women

without a current child wish wanted to preserve their fertility in order to decide later. Although the majority still hoped to conceive naturally, 65% estimated the chance to make use of the egg cells higher than 50%. Their maximum acceptable age for having a child varied between 40 and 50 years (mean 43 years). For 72%, single motherhood would be an option to consider in future. Compared with a control group of single women applying for DI, their QOL score was significantly lower.
Limitations, reason for caution: Findings remain preliminary because of the explorative nature of the study. Data were collected in only one fertility centre. Follow-up studies are needed.
Wider implications of the findings: The information gathered in this study may be of use for future guidelines concerning this topic.
Study funding/competing interest(s): Funding by hospital/clinic(s), MCK Fertility Centre.
Trial registration number: None.

P-379 What do couples think when deciding about freezing embryos?

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Study question: What do couples think when deciding about freezing embryos?
Summary answer: Desperation for a baby is the dominant drive when considering freezing embryos; but on reflection, couples' views were nuanced and complex. This study unravels the clarity, confusion, and conflicts when making the decision.
What is known already: Embryo freezing is a standard clinical practice in most fertility units. But there is little evidence about how couples make the decision to freeze their surplus embryos, or regarding their perceptions during the time of freezing. This study explores this neglected area. The aim of the study is to inform both clinicians and patients to be used to provide better support for patients, during their difficult decision making.
Study design, size, duration: This is a qualitative study of couples who have had ≥1 IVF treatment in a tertiary care centre in the north-east of England.
Participants/materials, setting, methods: 16 couples were interviewed using a semi-structured questionnaire, followed by thematic analysis and category mapping.
Main results and the role of chance: Couples expressed ethical conflicts about freezing 'babies'. Their ethical reservations were overtly revealed by the relief expressed by couples pregnant from the fresh cycle, at not having any embryos frozen. However, the overwhelming desire to have a baby influenced their decision favouring embryo freezing; and in seeing it as a 'part of a process'. Only on reflection were the issues fully considered. The vast majority of the couples were clear about wishing to maximise their chances towards the goal to have a baby, by freezing any surplus embryos. They perceived this as providing a 'back-up', although they disapproved of the term 'Insurance Policy'. They also favoured freezing to avoid a further full IVF cycle, and because freezing enhanced their sense of autonomy. Despite being given the relevant written information, couples were confused about the practical aspects of embryo freezing, namely: success rates, any funding available to freeze, freezing expenses. This confusion suggests that couples were preoccupied with the complexities of immediate treatment, and were less able to process any extra information at that point. There were also confusions associated with the 'freezing' term, related to concerns expressed about the safety of the procedure, and the interesting analogy drawn with freezing food. Nonetheless there was no regret expressed about the decisions they made.
Limitations, reason for caution: The views of certain categories of patients such as the ones already with children, or those who had strong reservation against embryo freezing, could not be captured in this study, which could have influenced the study outcome.
Wider implications of the findings: Although this study indicates that more detailed information may not have influenced their decision, it provides the basis for further study comparing the influence of more targeted information on freezing decisions.
Study funding/competing interest(s): Funding by hospital/clinic(s), This study was funded by the Fertility Research Fund of the NFCL.
Trial registration number: Not relevant.

POSTER VIEWING

SAFETY AND QUALITY OF ART

P-380 Does high dose hCG triggering bring favorable outcomes in IVF cycles with GnRH antagonist protocol

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Study question: To evaluate whether increased to double dose hCG injection at 36 h before ovum retrieval can brings more favorable outcomes compared with ordinary dose of hCG triggering in IVF cycles with GnRH antagonist protocol.
Summary answer: Increased dose of hCG for ovulation triggering in GnRH antagonist IVF cycle seems to have advantageous roles for achieve favorable outcomes. But, larger scaled randomized study is needed to generalize this strategy.
What is known already: None.
Study design, size, duration: 465 fresh IVF-ET cycles that undergoing fresh IVF-ET cycle with GnRH antagonist protocol were included prospectively, from January 2013 to November 2013.
Participants/materials, setting, methods: 253 cycles used ordinary dose of r-hCG injection (Group 1), 150 used double dose r-hCG (Group 2) and 62 used 10000 IU of u-hCG (Group 3). Serum hCG level of OPU day, oocyte maturation rate, fertilization rate, implantation rate, β-hCG positive and clinical pregnancy rate were compared between groups.
Main results and the role of chance: Serum hCG level of oocyte retrieval day (Group 1; 77.8 mIU/mL vs Group 2; 171.28 mIU/mL vs Group 3; 238.18 mIU/mL) and fertilization rate (Group 1; 68.9% vs Group 2; 74.1% vs Group 3; 77.4%) were significantly higher in double dose r-hCG and u-hCG group than that of *n* single dose r-hCG group. Oocyte maturation rate (Group 1; 74.0% vs Group 2; 76.9% vs Group 3; 76.7%) and β-hCG positive rate (Group 1; 45.5% vs Group 2; 52.0% vs Group 3; 58.5%) were seems to be higher in double dose r-hCG and u-hCG group too.
Limitations, reason for caution: there is a controversy about whether increased dose hCG injection and clinical outcomes of IVF cycle have correlation or not.
Wider implications of the findings: None.
Study funding/competing interest(s): Funding by hospital/clinic(s), no funding.
Trial registration number: None.

P-381 Prediction of preterm deliveries by cervical length measurement with catheter before embryo transfer in ICSI pregnancies

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Study question: Can cervical length measurement by catheter before embryo transfer predict preterm deliveries?
Summary answer: Cervical length measurement with embryo transfer catheter can identify the high-risk group for preterm delivery before conception, and this will expose which pregnancy outcome would improve with single embryo transfer by avoiding from multiple pregnancies that also increases preterm deliveries.
What is known already: Preterm delivery (PTD) defined as birth before the completion of 37 weeks' of gestation. Several studies suggest that cervical length (CL) measurement in mid-trimester (22–24 weeks) is a useful method for the prediction of PTD. Additionally, it has realized that the risk for PTD is inversely related to CL at first trimester (11–13 weeks) in high risk pregnancies. No study has performed to evaluate the prediction of PTD by analyzing measurements of CL, preconceptionally.
Study design, size, duration: We analyzed data of 494 pregnant women resulting from ICSI treatment during the years 2011–2013 at IVF center, Acibadem Kayseri Hospital, Turkey. Exclusions (multiple pregnancies, miscarriages, PTD history, progesterone use after 12 weeks, cerclage …) comprised 250 patients. This study is a retrospective case control study of 244 singleton pregnancies.

Participants/materials, setting, methods: Spontaneous preterm delivery occurred in 22 (9.1%) cases. Median cervical length of term delivery and preterm delivery groups were 4.0 mm and 3.3 mm, respectively. The difference between cervical length measurements is statistically significant ($p = 0.019$).
Main results and the role of chance: The median maternal age for the women involved in this study was 30.00 (range, 21–45) years. There was no statistically significant difference. ICSI cycle rank, previous parity and type of delivery were statistically similar between the group which delivered at term and preterm. Preterm delivery rate in the occurrence of first trimester bleeding is significantly higher than non-occurrence of first trimester bleeding ($p = 0.008$). Regression analyses demonstrated that, a 1 mm decrease in cervical length a 1.96 times increase in the probability of having a preterm delivery ($B = 0.51$, $p = 0.032$). In addition, there is a three-fold increased risk of preterm birth when first trimester bleeding occurs ($B = 3.35$, $p = 0.009$).
Limitations, reason for caution: Area under the ROC curve (AUC) was calculated as 0.65 (95% Confidence Interval; 0.59–0.71, $p = 0.012$) and sensitivity and specificity values were found as 73%, 52% respectively for the cut-off value of cervical length measurement at 3.5 mm.
Wider implications of the findings: There are two advantages of measuring cervical length, before conception in ICSI pregnancies. First of all, single embryo transfer should be considered as the preferred approach when a patient has short cervical length. Secondly effectiveness of prophylactic administrations (progesterone, cervical cerclage etc.) may be initiated at most effective time.
Study funding/competing interest(s): Funding by hospital/clinic(s), Acibadem Kayseri Hospital.
Trial registration number: The study is not a RCT.

P-382 Hospital costs from birth up to age five of multiples and singletons born by *in vitro* fertilization: a longitudinal 5-year follow-up study

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Study question: Do *in vitro* fertilization (IVF) multiples generate higher hospital costs than IVF singletons, from birth up to age five?
Summary answer: Although the gap between IVF multiples and singletons is by far the biggest during the first life year, hospital costs of multiples remain elevated at least until the age of 5 years.
What is known already: Concern has risen over the long-term outcome of children born after IVF. The increased incidence of multiple births in IVF as a result of double embryo transfer predisposes children to a poorer neonatal outcome such as preterm birth and low birth weight. As a consequence, IVF multiples require more medical care. Costs and consequences of poorer neonatal outcomes in multiples may also reveal later in life.
Study design, size, duration: All 5,497 children born from IVF in 2003–2005, whose parents received IVF treatment in one of five participating Dutch IVF centres were recruited for a retrospective cohort study. Based on gestational age, birth weight, Apgar and congenital malformation, children were assigned to one of three risk strata (low-, moderate- or high-risk).
Participants/materials, setting, methods: 816 multiples and 584 singletons were selected for 5-year follow-up based on stratified (risk)sampling. Parental informed consent was received of 322 multiples and 293 singletons. Individual level hospital resource use data (hospitalization, outpatient visits, diagnostic- and (non-)surgical interventions) were retrieved from hospital information systems of 302 multiples and 278 singletons.
Main results and the role of chance: Of the children who were considered to be low-risk, hospital costs were significantly higher for multiples compared with singletons (bootstrapped costs difference: 1,648, 95% CI 850 to 2,475). On the contrary, of the children who were moderate-risk, hospital costs were significantly lower for multiples compared with singletons (bootstrapped cost difference: −4,497, 95% CI −9,263 to −329). The vast majority of hospitals costs were incurred during the first life year (multiples: 90%; singletons: 74%) and were related to hospitalizations (multiples: 91%, singletons: 74%). The average hospital costs amount 12,193 and 3,410 up to age five for multiples and singletons, respectively. Thus, a multiple child is 4.4-times more costly than a singleton. Hospital costs from the second till the fifth life year were 1.3-fold higher for multiples than for singletons.
Limitations, reason for caution: Statistical tests have been performed to compare multiples and singletons within the risk strata. No statistical tests comparing (all) IVF multiples and singletons are performed because of the stratified sampling.
Wider implications of the findings: This study confirms that the increased utilization of health care by IVF multiples mainly occurs during the first life-year. Single embryo transfer may result in a substantial saving, particularly in the first life-year, although these savings need to be compared with the extra costs of additional embryo transfers needed to achieve successful pregnancy. Furthermore, both costs and outcomes (quality-adjusted life years) of IVF multiples and singletons should be considered in decisions regarding embryo transfer strategies.
Study funding/competing interest(s): Funding by national/international organization(s), The Netherlands Organisation for Health Research and Development.
Trial registration number: Not applicable.

P-383 Is the modified natural IVF cycle justified in patients with genuine poor response to controlled ovarian hyperstimulation

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Study question: To examine whether poor ovarian response (POR) patients during conventional IVF/ intracytoplasmic sperm injection (ICSI) cycle, may benefit from a modified natural cycle (MNC)-IVF.
Summary answer: MNC-IVF is of no benefit for "genuine" poor ovarian responders.
What is known already: Modified natural cycle-IVF (MNC-IVF) with minimal stimulation has been proposed and became popular among POR. It has been proposed that it may provide a single oocyte of better quality and thus allow the transfer of a healthier embryo into a more receptive endometrial environment. Although MNC-IVF has been suggested as a promising treatment option for younger normal responders, its potential in poor responders hasn't been clarified yet.
Study design, size, duration: In this Cohort historical study, we examined 380 genuine poor ovarian responders during 12 years period.
Intervention: Modified natural cycle IVF protocol with GnRH antagonist (ant) supplementation. GnRH-ant treatment was started when a follicle of 13 mm was present. Two to three ampoules of hMG were co-administered daily during the GnRH-ant treatment.

Participants/materials, setting, methods: "Genuine poor responders": Those fulfilling the Bologna criteria (2 out of 3), of whom we selected a sub-group of patients that also yielded up to 3 oocytes following a failed COH with a minimal gonadotropin daily dose of 300 IU.
Setting: Tertiary, University affiliated Medical Center.
Main results and the role of chance: One hundred and one genuine poor responders, who underwent a subsequent MNC-IVF within 3 months of the previous failed conventional IVF/ICSI cycle had <1% live birth rate. 269 genuine poor responders who underwent a subsequent IVF/ICSI within 3 months of the previous failed conventional IVF had 4.4% live birth rate ($P < 0.001$). Furthermore, in the subgroup of POR patients, who underwent a previous conventional IVF/ICSI cycle with a yield of only one oocyte, no pregnancies were achieved during the MNC-IVF.
Limitations, reason for caution: A limitation of our analysis is its retrospective design. However, based on our patients' selection process, only consecutive patients fulfilling the inclusion criteria were enrolled. Moreover, we couldn't demonstrate any significant differences in patients demographics or stimulation variables between those who underwent a subsequent MNC-IVF or conventional COH-IVF cycles.
Wider implications of the findings: The clinical implication of the present study is that the potential of MNC-IVF is limited for "genuine" poor ovarian responders, as described by the Bologna criteria with 3 or less oocytes following COH with high daily gonadotropin dose. According to the data presented, MNC-IVF shouldn't be offered to this subgroup of poor responders.
Study funding/competing interest(s): Funding by University(ies), Chaim Sheba Medical Center, Tel Hashomer, affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.
Trial registration number: 0899-13-SMC.

P-384 Effect of low oxygen tension of culture conditions on neonatal outcome

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Study question: Study question, To ascertain as whether the use of the low oxygen tension during *in vitro* embryo culture affect neonatal outcomes.
Summary answer: Neonatal outcome was no affected by the oxygen concentration used during the *in vitro* embryo culture.
What is known already: *In vitro* embryo culture conditions are important factors involved in the IVF clinical outcome and the also may have a longer term effect on the newborns. We have previously published that despite the use of low oxygen during *in vitro* embryo culture improves the morphological embryo quality, no benefits were seen on terms of pregnancy ongoing pregnancy rates per intention to treat patients in ovum donation cycles.
Study design, size, duration: Prospective, randomized, nonblind, single-center, parallel-group study on 203 single pregnancies from egg donation cycles (105 from low oxygen tension and 98 from atmospheric oxygen concentraiton) from 2010 to 2012.
Participants/materials, setting, methods: Gestational age, birth weight, tall, apgar and cranium perimeter and sex ratio was evaluated from ovum donation pregnancies from embryos cultured under low or air oxygen tension. χ^2 test and Student *t* test, were used for statistical evaluation.
Main results and the role of chance: Neonatal outcomes were not affected by the oxygen concentration used during the *in vitro* embryo culture. No differences were observed regarding gestational age: 38.7 ± 1.9 vs 38.5 ± 2.3, birth weight: 3.242 ± 447 vs 3.237 ± 603, tall: 50.3 ± 2.4 vs 50.3 ± 2.6, apgar: 9.8 ± 0.5 vs 9.7 ± 0.5 or sex ratio xx/xy: 0.78 vs 0.78.
Limitations, reason for caution: These results are obtained exclusively from oocyte donation cycles carrying singletons.
Wider implications of the findings: Oxygen concentration during the *in vitro* embryo culture does not have any impact of neonatal outcome parameters included in this study.
Study funding/competing interest(s): Funding by national/international organization(s), Generalitat Valenciana (Regional Valencian Goverment), project identification no. IMPIVA IMDTF/2011/214.
Trial registration number: None.

P-385 A morphologically poor blastocyst might affect the implantation rate for a good blastocyst during a double blastocyst transfer

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Study question: Double embryo transfers are sometimes selected with the expectation that poor embryos might help to associate with the implantation of a good embryo. When one blastocyst is morphologically good (MGB) and the other is poor (MPB), do we have to choose between a single or a double blastocyst transfer?
Summary answer: The implantation rate for a double blastocyst transfer with morphologically good and poor blastocysts was significantly lower than that for a single good blastocyst, and we concluded that the presence of a poor blastocyst interfere with the implantation of a good blastocyst.
What is known already: A double blastocyst transfer contributes to an increase in the rate of pregnancy compared with that for a single blastocyst transfer. Double blastocyst transfers also increase the risks for multiple pregnancies. It is suggested that one blastocyst might have a positive influence over the implantation of the other blastocyst, however, there is little evidence to support that the transfer of two blastocysts will have either a positive or a negative effect on each other.
Study design, size, duration: This is a retrospective study performed between April 2009 and April 2014. The study included 1,433 cycles for 768 patients who were recruited for this study after experiencing vitrified-warmed single or double blastocyst transfer (BT) in either the natural ovulatory cycle or hormone replacement cycle.
Participants/materials, setting, methods: MGB consisted of ICM and TE grade A or B with the exception of early blastocysts. The remainders were MPB. We compared the implantation rates among the groups that received single BT, double BT with two MBG, and double BT with a MGB and a MPB each.
Main results and the role of chance: The implantation rate in the group of double BT with a MGB and a MPB each was 31.3%, and it was significantly lower than that in the single BT group with a MGB (53.1%; $p < 005$), and it was comparable to that in the single BT group with a MPG (35.6%). This rate was 26.8% in the double BT group with two MPBs.

	Single BT		Double BT	
No. of transferred blastocysts	747	323	246	250
No. of MGB	747	0	123	125
No. of MPB	0	323	123	125
Implantation rate (%)	53.1	35.6	31.3*	26.8
Pregnancy rate (%)	53.1	35.6	57.7	48.8
Multiple pregnancy rate (%)	0	0	8.4	9.8

*$p < 0.05$; vs single BT with a MGB

Limitations, reason for caution: It was impossible to confirm which blastocysts from a double BT with a MGB and a MPB each were implanted. Therefore, it was not proven that a MPB interfered directly with the implantation of a MGB.
Wider implications of the findings: The implantation of a double BT with a MGB and a MPB were expected to be equal to that of a single BT with a MGB, or be improved. However the results proved to be significantly lower. This indicated that a MPB interfered with the implanting of a MGB. A double BT didn't decrease the pregnancy rate, but increased multiple pregnancies. Therefore, we should avoid double BTs despite the possible accrual of only MPGs.
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. And there are no competing interests.
Trial registration number: This study is not an RCT study, so no trial registration number was assigned.

P-386 Congenital anomalies after assisted reproduction techniques (ART) and their correlation with embryo quality

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Study question: The objective of the study is to establish the influence of the embryo quality transferred in *in vitro* fertilization cycles (IVF) and Intracytoplasmic Microinjection (ICSI), with the subsequent appearance of congenital anomalies.
Summary answer: We have found that the poorer the quality of embryos transferred, the risk of congenital anomalies increases significantly, both aneuploidy and structural malformations.
What is known already: ART could have a negative effect on the formation and development of the fetus, although most of the neonatal morbidity is due to the consequences of multiple pregnancies and prematurity associated. However recently it has been confirmed that even in singleton pregnancies, the risk is also increased.
Study design, size, duration: A retrospective case-control was performed to assess 76 cycles with congenital anomalies collected between 1999 and 2012. Control group was of 240 cycles with normal child born collected between 2008 and 2011.
Participants/materials, setting, methods: 76 cases resulting in 103 infants with congenital malformations, which were compared with 332 healthy children from 240 pregnancies resulting from assisted reproduction treatment. We divided the embryo quality in three categories: top quality embryos, medium quality (when you mix one top quality embryo with poor quality embryo) and poor quality.
Main results and the role of chance: Differences between groups were found in age (mean in control group 33.9 vs 35.5 in case group; $p = 0.004$) Although no statistical differences were found in technique of fertilization; IVF (25% vs 34.2%) or ICSI (75% vs 65.8%), multiple pregnancies (38.3% vs 34.2%) and sperm count (41.7 M/ml vs 40.8 M/ml) or motility (43% vs 43.8%). Logistical regression adjusted for age was performed to analyze the risk that the embryo quality may have on the occurrence of congenital anomalies. We observed an increased risk of poor quality embryos transferred compared with the top quality embryo transfer (OR 2.32; 95% CI 1.1 to 5.2; $p = 0.04$).
Limitations, reason for caution: Not all patients have reported the results of the treatments, so there could be uncontrolled biases.
Wider implications of the findings: We have demonstrated that the bad embryo quality transferred is strongly associated with the occurrence of anomalies. It must be seen as high risk pregnancies, and we must pay special attention in their respective monitoring and especially with the proper application of the various protocols and techniques of prenatal diagnosis, not forgetting the pediatric follow-up of these children.
Study funding/competing interest(s): Funding by hospital/clinic(s), Instituto Bernabeu.
Trial registration number: Nothing no disclose.

P-387 Should patients with at least a previous unsuccessful elective blastocyst transfer be offered an elective two blastocyst transfer in a subsequent FET treatment

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Study question: The aim of this study was to compare clinical pregnancy rates and multiple pregnancy rates in Frozen Embryo Transfer (FET) HRT treatments for patients where a double elective blastocyst transfer took place in comparison to a repeat elective single blastocyst transfer after previous failed treatments with elective single blastocyst transfer.
Summary answer: Regardless of the patient's age, repeat transfer of a single blastocyst achieves similar pregnancy rates (29.6% vs. 39.1%, $p = 0.13$) to elective double embryo transfers with a highly significantly reduced (4.3% vs. 39%, $p < 0.0001$) incidence of twin pregnancies.
What is known already: Couples with at least one or two previous unsuccessful D5/6 elective single embryo transfers (eSBT) from fresh and frozen cycles often request elective double blastocyst to be transferred (eDBT) in subsequent FET in the hope of improving success rates. Similarly, clinicians also desire to obtain high pregnancy rates in subsequent cycles but without an accompanying increased risk of multiple pregnancies. We are not aware of any publications answering this question.
Study design, size, duration: This is a retrospective cohort study analysing 764 FET-HRT treatments performed from 1/1/2008 to 31/12/2013. Patient's age was calculated on the day of embryo freeze. Data was collected from the Unit's electronic database and crosschecked. Age groups analysed were: <35 years, 35–39 years and ≥40 years.
Participants/materials, setting, methods: Academic ART centre. We measured clinical and multiple pregnancy rates after FET HRT in patients that had at least one unsuccessful previous elective single blastocyst transfer (fresh or frozen). We compared the rates among the ones that had a repeat eSBT and the ones that had a double blastocyst transfer.
Main results and the role of chance: In the <35 group ($n = 353$), 328 (93%) eSBT were performed, 99 (30.2%) resulted in a clinical pregnancy with a 6% twin rate ($n = 6$). Only 25 (7%) elective double blastocyst transfers (dEBT) occurred, 52% ($n = 13$) had a clinical pregnancy with a 38.4% ($n = 5$) twin rate. In the 35 to 39 group ($n = 331$), 294 (89%) transfers were eSBT resulting in 85 clinical pregnancies (28.9%) with a 2.35% twin rate ($n = 2$). dEBT were performed in 37 cases resulting in a 27% ($n = 10$) clinical pregnancy and a 33% twin rate ($n = 3$). In women ≥40 ($n = 80$), 73 (91%) eSBT occurred, 30.1% ($n = 22$) resulted in a clinical pregnancy and 4.5% twins ($n = 1$). An eDBT was performed in 7 cases (57%), a 57.14% ($n = 4$) clinical pregnancy and a 25% ($n = 1$) twin rate.
Limitations, reason for caution: The limitations of this study are the retrospective nature and the small sample in the elective double blastocyst transfer group.
Wider implications of the findings: The decision to effect an elective two versus repeat single blastocyst transfer should be balanced against the significantly increased risk of a twin pregnancy with a 2 blastocyst transfer. We found that repeat elective single blastocyst transfer offers an excellent pregnancy rate with a low multiple pregnancy rate and should be the first choice in patients returning after one or two failed previous transfers.
Study funding/competing interest(s): Funding by hospital/clinic(s), HARI, Dublin, Ireland.
Trial registration number: None.

P-388 Guideline and practical technique of handling transabdominal ultrasonography transducer during embryo transfer

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Study question: Is there an optimal technique of handling the transabdominal transducer during ultrasonography guided embryo transfer?
Summary answer: Practice of this technique will play important part in the skilled manipulation of the transducer to obtain accurate view of uterus and cervix during embryo transfer. The transducer handling technique is similar to the technique of obtaining cardiac views in fetal echocardiography. Ultrasound guidance allows the clinician to visualize the tip of the catheter as it is advanced along the cervical canal into the endometrial cavity.
What is known already: Rigorous and atraumatic embryo transfer (ET) procedure is essential for successful outcome in assisted reproduction technique. Transabdominal ultrasound-guided ET has been shown not only to improve the ease with which the procedure of ET is done but also to improve the pregnancy rate and delivery rate. So it is important to handle transabdominal transducer in an appropriate way.
Study design, size, duration: Here we will describe a guideline and practical technique of handling transabdominal transducer in order to make improve skill of transabdominal guided embryo transfer. The guideline of transabdominal guided ultrasonography during embryo transfer.
Participants/materials, setting, methods: 1 Bladder size should be optimized: Not over-distended and not under-distended. 2 Vagina and cervix can be distinguished by visualizing the echogenity of speculum. 3 Proper visualization of all structures that the embryo catheter passes. These structures are cervix, uterocervical angle, endometrium, uterine fundus which has to be on the same plane. This can be accomplished by handling the transducer in appropriate way. Skilled person handle the transabdominal transducer is in vertical position relative to the patient in order to visualize cervix, uterocervical angle, endometrium in the same plane. According to some of uterine positions or uterine anomalies, this vertical position can be oblique according to the patient. The mark points are echogenity of speculum cervix, uterocervical angle endometrium and fundus uteri. The relationship between movements of the transducer and image is obtained by movements of the transducer. The image alternates by these movements. As catheter moves, the skilled person uses these transducer movements

techniques. Transducer simultaneous movements ease to orientate the clinician to embryo transfer catheter by imitating effect of 4D real time ultrasonography. There are four main movements of transducer. One of the movement of transducer is transducer plane slide. The transducer slides over the patients skin vertically. Here the point of skin contact of transducer changes. The other movement is the rotation of the transducer on its cable axis. The point of contact of skin is unchanged. Transducer rotates on its cable axis. Another movement of transducer that helps to visualize the tip of catheter is the angulation movement. Here the point of contact with the skin remains constant but person bends the transducer. If the patient is obese, last movement of the transducer is to change the pressure of transducer over the skin. The person compresses the transducer in order to intervene the tissue.

Main results and the role of chance: Optimizing these four main movements of transducer during transfer will ease to visualize the tip of the catheter and make embryo transfer atraumatic and easy.

Limitations, reason for caution: No limitations.

Wider implications of the findings: This guideline and movement technique of transducer during transfer will ease to visualize the tip of the catheter and make embryo transfer atraumatic and easy.

Study funding/competing interest(s): Funding by University(ies), Gazi School of Medicine.

Trial registration number: No registration number.

P-389 Does ovarian stimulation or laboratory procedures of assisted reproductive technologies affect the birth weight of the neonate

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Study question: Is neonatal birth weight in ART affected by ovarian stimulation (by comparing *in vitro* fertilization with a single embryo transfer (IVF-SET) to IVF in a modified natural cycle (IVF-MNC)) or laboratory procedures (by comparing IVF-SET with intrauterine insemination with controlled ovarian hyperstimulation (IUI-COH)?

Summary answer: In this homogenous study population no large differences in birth weight could be found in the comparisons of IVF-SET to IVF-MNC or IVF-SET to IUI-COH.

What is known already: Several studies have shown that IVF babies have lower birth weights than naturally conceived babies. It is unclear, however, whether the smaller size at birth is due to the hormone stimulation, the lab procedures or the underlying sub-fertility. This study randomized couples to either IVF-SET, IVF-MNC or IUI-COH, therefore allowing us to disentangle the effects of hormone stimulation and lab procedures in an experimental setting. Here were report on the effects on birth weight.

Study design, size, duration: The INeS trial was a randomized controlled trial comparing IVF-SET and IVF in a modified natural cycle (IVF-MNC) to IUI-COH. Couples with poor fertility prospects and unexplained or mild male subfertility were eligible. The follow up period was 12 months or until 6 weeks after delivery.

Participants/materials, setting, methods: The study population consisted of 313 singletons born from couples participating in the INeS study. There were 86 singletons born after IVF-SET, 44 after IVF-MNC and 75 after IUI-COH.

Main results and the role of chance: In the group conceived after IVF-SET, mean birthweight was 3444 grams (SD = 449) in the IVF-MNC group it was 3520 g (SD = 491) and it was 3329 g (SD = 538) in the group conceived with IUI-COH. There were no significant differences in birth weights between each of the three groups.

Limitations, reason for caution: Our results are based on a relatively small study population limiting statistical power to detect relatively subtle effects on birth weight. Also, local variations between hormone stimulation schemes and lab procedures in the participating centres could have contributed to heterogeneity.

Wider implications of the findings: The absence of substantial differences in this study, suggest that the smaller size at birth of IVF babies may be due to the underlying subfertility. Follow up of these ART children is warranted since intrauterine programming is known to occur without affecting size at birth. Moreover, the randomized setting of this trial will uniquely allow us to dissect potential effects of hormone stimulation and lab procedures on later health.

Study funding/competing interest(s): Funding by national/international organization(s), The trial was supported by a grant from ZonMW, the Netherlands Organization for Health Research and Development, and a grant from Zorgverzekeraars Nederland, the Netherlands association of health care insurers.

Trial registration number: The INeS trial was registered at the Dutch trial registry (NTR 939).

P-390 Moving with the times: the changing landscape of IVF at New York University Langone Medical Center – NYU Fertility Center (NYUFC)

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Study question: Have IVF practice patterns changed over time?

Summary answer: A retrospective review evaluating the distribution of cycle treatment types at our center demonstrates that emerging technologies have had a significant impact on clinical demand and practice. What was considered standard IVF, i.e. IVF with fresh-embryo transfer (IVF-ET), is no longer the predominant treatment modality; others are becoming more prevalent.

What is known already: NYUFC strives to be at the forefront of assisted reproductive technologies (ART) by implementing up-and-coming technologies. For instance, in 1995 preimplantation genetic diagnosis for monogenic diseases was initiated. In 2000, blastocyst culture was instituted and soon thereafter limited-aneuploidy embryo screening. By the mid-2000s, oocyte cryopreservation using both slow freezing and vitrification was added. More recently, in 2009, blastocyst vitrification and trophectoderm (TE) biopsy with complete 24-chromosome analysis (CCS) became routine practice at our center.

Study design, size, duration: Retrospective review of 8459 ART treatment cycles completed in six representative years spanning from 1995 to 2013. Distributive results are shown in the Table below and analyzed across years using contingency χ^2.

Participants/materials, setting, methods: All ART treatment cycles involving IVF-ET, donor-egg-to-recipient fresh ET (DE-ET), preimplantation genetic diagnosis/screening (PGD/S), frozen embryo transfer (FET), oocyte cryopreservation (OC) and oocyte thaw (OT) at a major metropolitan university-based infertility center during the designated years shown were included.

Main results and the role of chance: See Table. Cycle distribution was noted to vary significantly over time ($p < 0.001$). In 1995, IVF-ET was most commonly performed (76%), followed by FET (16%), DE-ET (6%), and PGD (1%). Although cycle volume quadrupled by 2000, the distribution changed only slightly. However, by 2010, OC rose to contribute 16% of total ART cycles; more recently IVF-ET decreased to 25% of the total cycle volume while OC increased to 24% and PGD to 19%.

CYCLE TYPE *n*(%)	1995	2000	2005	2010	2012	2013
IVF-ET	278 (76)	967 (71)	1139 (70)	784 (53)	699 (38)	455 (25)
DE-ET	22 (6)	211 (15)	157 (10)	119 (8)	79 (4)	54 (3)
PGD/S	4 (1)	17 (1)	114 (7)	118 (8)	270 (15)	333 (19)
FET	60 (17)	171 (13)	196 (12)	217 (15)	398 (22)	453 (25)
OC	0 (0)	0 (0)	12 (1)	244 (16)	347 (19)	427 (24)
OT	0 (0)	0 (0)	11 (1)	12 (1)	15 (1)	76 (4)

Limitations, reason for caution: New technologies should be cautiously introduced and only with informed consent. Using this judicious approach, for

autologous cycles completed in women <38 years, we have been able to achieve clinical pregnancy (fetal heart) in 44% of IVF-ET, 36% of OT cycles, and significantly, 55% of FETs where CCS-euploid embryos were replaced.
Wider implications of the findings: As treatments and options improve/expand, if added with caution, state-of-the-art technologies can become clinically available. Importantly, newer treatments have afforded more-appropriate, individualized care. For instance, OC permits fertility preservation in women facing reproductive loss while PGD improves the efficacy of IVF. To that end, we constantly work to improve clinical modalities. Our ultimate goal is a single-embryo transfer using embryos most likely to produce livebirth in hopes of maximizing pregnancy potential while minimizing fetal/maternal risk.
Study funding/competing interest(s): Funding by hospital/clinic(s), NYU Fertility Center.
Trial registration number: N/A.

P-391 Congenital anomalies after a history of subfertility: a registry-based study in the Northern Netherlands

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Study question: Are there any specific congenital anomalies that occur more often after a history of parental subfertility and/or the application of IVF/ICSI in a pool of foetuses/children that all have a congenital anomaly?
Summary answer: In our registry-based study, parental subfertility was associated with an increase in epispadia, penoscrotal hypospadia and right ventricular outflow tract obstruction, and with a decrease in congenital hip dysplasia. IVF/ICSI was associated with an increase in polydactyly of the hands and a decrease in genital anomalies, including hypospadias.
What is known already: Children born following IVF/ICSI are at increased risk for congenital anomalies: a relative risk of 1.32 (95% CI: 1.24–1.42) was recently reported. The risk seems increased for cardiovascular malformations, gastrointestinal defects, musculoskeletal malformations and defects in the central nervous system. Recent studies suggest that not only the IVF/ICSI-procedure is responsible for the overall risk increase, but also the underlying subfertility. Knowledge on exactly which congenital anomalies occur more often after a history of subfertility is scarce.
Study design, size, duration: We performed a registry-based study using data from the birth defects registry Eurocat Northern Netherlands. We included 4525 malformed foetuses/children born between 1997–2010. Of those, 4185 were born to fertile couples and 340 to subfertile couples (139 conceived after IVF/ICSI, 201 conceived naturally after a time to pregnancy >12 months).
Participants/materials, setting, methods: Live births, still births and terminated pregnancies with congenital anomalies without a confirmed cause (such as chromosomal aberrations) were included. Within this dataset we analysed whether a history of subfertility (fertile vs. total subfertile group) or IVF/ICSI (subfertile but conceived naturally vs. IVF/ICSI) was associated with specific congenital anomalies.
Main results and the role of chance: After correction for maternal age and periconceptional use of folic acid, we found a history of subfertility to be associated with an increase in: epispadia (OR: 4.92, 95% CI: 1.16–20.87), penoscrotal hypospadia (OR: 8.64, 95% CI: 3.27–22.84) and right ventricular outflow tract obstruction (OR: 1.74, 95% CI: 1.05–2.90). Congenital hip dysplasia occurred less often after a history of subfertility (OR: 0.66, 95% CI: 0.45–0.96). IVF/ICSI was associated with an increase in polydactyly of the hands (OR: 4.25, 95% CI: 1.33–13.56) and a decrease in genital anomalies (OR: 0.23, 95% CI: 0.08–0.70), including hypospadia (OR: 0.23, 95% CI: 0.08–0.70). As we analysed several organ systems (n = 9) that include many specific congenital anomalies (n = 49), the above named associations could be chance findings.
Limitations, reason for caution: As Eurocat does not include healthy non-malformed controls, the risk increase for any congenital anomaly could not be analysed. This study can therefore not confirm the well-known risk increase for congenital anomalies after a history of subfertility and IVF/ICSI, that is described in the literature.
Wider implications of the findings: Our results suggest that some congenital anomalies occur more often, and others occur less often after a history of subfertility, and after IVF/ICSI. However, none of the associations seems strong enough to explain the well-known risk increase for congenital anomalies. Possibly, rather than particularly increasing the risk for some specific anomalies, a history of subfertility and IVF/ICSI may well increase the risk for most types of defects equally.
Study funding/competing interest(s): Funding by national/international organization(s), The Eurocat registry is financed by the Dutch Ministry of Public Health, Welfare and Sports (VWS). There are no competing interests.
Trial registration number: Not applicable.

P-392 Incidence of monozygotic twins with blastocyst single embryo transfer

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Study question: What are the incidence of monozygotic twins (MZT) in blastocyst stage embryo transfer?
Summary answer: Our data showed a higher incidence of monozygotic twins (MZT) in blastocyst single embryo transfer (SET) than the previous reports in the literature.
What is known already: The prevalence of monozygotic twins in the general population is 0.42%, and MZT are associated with significant obstetric and perinatal morbidity, as increased fetal loss, intrauterine growth restriction, preterm deliveries, and perinatal loss. The incidence of MZT after blastocyst transfer has been reported in the literature.
Study design, size, duration: Retrospective cohort study with 86 cycles with blastocyst single embryo transfer between 2011 and 2013.
Participants/materials, setting, methods: Eighty six women undergoing intracytoplasmic sperm injection (ICSI) cycles, with mean age 33.6 years, transferred blastocyst single embryo transfer. The incidence of MZT was observed on vaginal ultrasound at 6 to 8 weeks after embryo transfer.
Main results and the role of chance: The pregnancy rate was 50.0% (43/86), with a clinical pregnancy rate of 43.0% (37/86). Four patients who were pregnant resulted in monozygotic twin pregnancy (10.8%). Monozygotic twin pregnancy was also observed in patients that transferred 2 blastocysts. From the 150 pregnancies, 3 resulted in two gestational sacs with 3 fetuses (2%). It was not observed any MZT in transferred performed on day 3 (cleavage embryos) during the same period. All seven MZT pregnancies, 1 aborted, 4 deliveries and 2 on going gestation.
Limitations, reason for caution: The study was conducted with a small number of patients.
Wider implications of the findings: Currently, although there is a reduction in the number of embryos to be transferred in order to avoid multiple pregnancies, couples should be informed about the possibility of MZT.
Study funding/competing interest(s): Funding by hospital/clinic(s), Fertilitat – Centro de Medicina Reprodutiva.
Trial registration number: Not available.

P-393 The Groningen ART cohort study: does ovarian hyperstimulation, the *in vitro* procedure or a combination of both influence cognitive and behavioural development of 4-year-old IVF-offspring?

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Study question: Evaluating the effects and underlying causal relationships of ovarian hyperstimulation, the *in vitro* procedure and the combination of both on cognitive and behavioural development in 4-year-olds.

Summary answer: Our preliminary results suggest that ovarian hyperstimulation, the *in vitro* procedure and the combination of both are not associated with cognitive and behavioural development in 4-year-olds. The child's cognitive and behavioural development largely depend on parental characteristics such as level of education and maternal age.
What is known already: Long-term follow-up of health and development of IVF-offspring is important since the use of assisted reproduction techniques (ART) is steadily increasing. Results of long-term studies on cognitive and behavioural development in IVF-children vary, partly due to difficulties in interpretation of direct and indirect underlying causal relationships between ART, subfertility- and parental- and child aspects. The present study focuses on cognitive and behavioural development of 4-year-old IVF-offspring and underlying causal relationships.
Study design, size, duration: A prospective follow-up study, in which 195 4-year-old singletons were assessed. They were born to subfertile couples (n = 195) following IVF with controlled ovarian hyperstimulation (COH-IVF, n = 63), IVF in the modified natural cycle (MNC-IVF, n = 53) and natural conception (Sub-NC, n = 79). The attrition rate since birth was 9.3%.
Participants/materials, setting, methods: Cognitive development was evaluated with the Kaufman Assessment Battery for Children; behavioural development was evaluated with the Child Behavior Checklist. Primary cognitive outcome parameter was the total intelligence quotient (IQ); behavioural outcome parameter was the total problem *T*-score. Regression analyses, causal inference search algorithms and structural equation modelling were applied.
Main results and the role of chance: The total IQ score [mean (sd)] for COH-IVF, MNC-IVF and Sub-NC children was 106.1 (11.8), 105.2 (13.3) and 108.9 (10.7), respectively and did not differ significantly between the three groups. Similarly, behavioural scores did not differ between the three groups. The causal models suggested that ovarian hyperstimulation and the *in vitro* procedure did not affect cognitive and behavioural outcome. Rather, cognitive and behavioural outcome were associated with parental characteristics such as maternal age and educational level.
Limitations, reason for caution: The prospective design of our study and small post-natal attrition rate reduced potential selection bias based on the child's development or health. The assessors were blind to the mode of conception. However, our results cannot be generalized to multiples, as we studied singletons only.
Wider implications of the findings: Our study contributes to the understanding of the relation between ART-aspects and cognitive and behavioural development. Long-term monitoring of development and growth of children born after ART remains of importance, especially since in society maternal age at child birth, and with that subfertility and the use of ART are steadily increasing.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Funding by national/international organization(s), University Medical Center Groningen, Groningen, The Netherlands, Junior Scientific Master Class, Groningen, The Netherlands, Postgraduate School of Behavioural and Cognitive Neurosciences, Groningen, The Netherlands, Cornelia Foundation, Beetsterzwaag, The Netherlands.
Trial registration number: Inapplicable.

P-394 Intracytoplasmic sperm injection (ICSI) and *in vitro* embryo culture (IVC) does neither affect neonatal body weight nor adult behavior in mice

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Study question: Do ICSI and IVC influence birth weight and behavior in mice?
Summary answer: ICSI and embryo culture in ART media do not impact on neonatal body weight and there is no evidence for behavioral abnormalities in ART offspring in mice.
What is known already: There is increasing concern about long-term implications of ART for the health of offspring. In humans, IVC can lead to altered body weight at birth. In mice, IVC can result in offspring with altered anxiety-like and explorative behavior.
Study design, size, duration: Two ART IVC protocols were applied to B6C3F1 × B6 zygotes produced by ICSI. Controls: KSOM(aa); oviduct (no-culture). Resultant blastocysts were transferred to foster mothers, resulting in 37 mice. Birth weight, anxiety-like and explorative behavior was assessed using the light/dark, open field, and elevated plus-maze test.
Participants/materials, setting, methods: Mice from different culture conditions (HTF/MultiBlast, 9 mice; ISM1/ISM2, 8 mice) were compared to controls (KSOM(aa), 7 mice; oviduct, 13 mice). For an additional positive behavioral control KSOM(aa) was supplemented with serum. Body weight (at birth and at 96 days) and behavior (at 96 days) was analyzed using ANOVA.
Main results and the role of chance: For equally sized litters, neonatal as well as adult (at 96 days of age) body weights are similar according to the ART culture protocol the mice experienced during embryonic life (ANOVA, $p \geq 0.05$). Using the light/dark test, mice of the positive behavioral control recapitulated the published observations on the effect of serum at an age of 15 months (25% (+ serum) vs. 41% (− serum) of time spent in the illuminated area; *t*-test, $p \leq 0.05$). The light/dark test revealed similar behavior in mice from the ART culture conditions compared to oviduct (ANOVA, $p = 0.260$). Using the open field and the elevated plus-maze tests no differences could be detected between the culture conditions and the oviduct (open field test, ANOVA, $p = 0.597$; elevated plus maze test, ANOVA, $p = 0.765$).
Limitations, reason for caution: The number of ICSI mice could be increased so as to gain in statistical power. ICSI is one of the most common ARTs, though not the only one. Detection of behavioral effects of ART culture medium may be dependent on the assays used and can be influenced by age and genetic background.
Wider implications of the findings: Possible effects of ART on mouse body weight and adult behavior should not be overinterpreted. Neonatal care, mother-infant interactions and social interactions are superimposed with possible differences induced by the culture media. Human infants receive significantly more neonatal care when compared with mice, so that earlier deficits are most likely to be compensated and differences evened out.
Study funding/competing interest(s): Funding by national/international organization(s), Deutsche Forschungsgemeinschaft (DFG, BO 2540/4-1).
Trial registration number: Not applicable.

P-395 IVF conceived children Health: a retrospective growth study

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Study question: Gametes environment may alter epigenetic profile of growth gene with a possible impact on children growth curves. Is the growth curve from birth to 10 years in IVF conceived children different to that of spontaneously conceived children?
Summary answer: Hypotrophy of IVF children observed at birth is corrected during second year of life by minor lost of adiposity during locomotion acquisition. Other periods studied did not point out any slope difference in growth charts up to 10 years old between IVF and non IVF children.
What is known already: Although malformation is the first indicator of children' health in a given population, low birth weight is also admitted. Previous studies have identified catch-up growth in IVF children and found no difference at 2 years of age compared with naturally conceived children. This finding supports the idea that exaggerated growth of IVF children during infancy is a physiologic process that promotes the restoration of the infants' genetic growth trajectory after a period of prenatal restriction.
Study design, size, duration: Case control study of longitudinal growth data was conducted between 2009 and 2011, in 438 IVF children from 5 to 10 years old. This monocentric IVF cohort was compared to 320 primary school spontaneously conceived children of same age and living in same geographic area as IVF children.
Participants/materials, setting, methods: Parents answered a questionnaire about children' weight and height measurements available in the health report documented by a physician. The growth curves were established as for national references curves. A statistical mixed model was use to compare IVF and non IVF children growth curves from birth to 10 years.
Main results and the role of chance: A sample of 120 IVF participants, whose parents answered the questionnaire, was representative of 438 IVF children

in the same age range. Analysis did not point out any difference between the growth curves of IVF, ICSI and spontaneously conceived children except for fat lost during the second year of life. Mean decrease for classical IVF children is –1.2 ±0.2 ($p < 0.001$), –0.6 ±0.2 for ICSI children ($p = 0.0025$) and –0.1 ±0.1 for spontaneously conceived ($p < 0.0001$).

Cofounding factors showed that malformations, maternal Body Mass Index and neonate hospitalization influence weight gain during the first year ($p < 0.03$, 0.009 and 0.07, respectively). From birth to 2 years height gain is dependent to paternal age ($p = 0.06$). From 1 to 10 years, weight gain depends on of familial affluence, socioeconomic and education level ($p < 0.001$).

Limitations, reason for caution: Cofounding factors were not different except for paternal age which influences height up to 2 years and neonate pathology that influences weight gain during the first year. Tobacco and professional exposure during pregnancy, placental detachment which were different between groups, did not influence growth charts in any period.

Wider implications of the findings: This study updates data about IVF children follow-up. As previously reported, present data show that IVF children are healthy, suggesting low epigenetic impact of procedure on growth in infancy, if any. Effect of paternal age on height gain could be explained by recent result on biological molecular research. Although IVF was shown not to affect children growth from birth to 10 years, Teenagers health needs to be investigated.

Study funding/competing interest(s): Funding by hospital/clinic(s), Hôpital saint Joseph de Marseille France.

Trial registration number: None.

P-396 Family functioning and psychological well-being of 5- to 6-year-old singletons born after preimplantation genetic diagnosis; a prospective case-controlled matched study

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Study question: As preimplantation genetic diagnosis (PGD) is applied to families who might have suffered traumatic family histories, questions remain about the psychological development of their offspring. We investigated if PGD preschoolers differ in their social-emotional development from children born after intracytoplasmic sperm injection (ICSI) and spontaneous conception (SC).

Summary answer: The psychological development of PGD children was not different from those of children born after ICSI and SC, nor in the perceptions of the children themselves neither by the reports of their parents. The amount of parental stress concerning family life is more prominent by SC mothers than PGD/ICSI families.

What is known already: Literature about the broad psychological development of children at preschool age born after PGD is scarce. One study showed that, at toddler age PGD/PGS (preimplantation genetic screening) mothers reported less behavioral problems. Two other studies found that PGD/PGS parents experienced lower stress levels in comparison to controls. As PGD patients have a different indication than PGS couples, long term follow-up of their children is needed.

Study design, size, duration: Between April 2011 and May 2013, the psychological development of 47 5- to 6-year-old PGD singletons was assessed in a prospective case-controlled matched follow-up study. ICSI and SC children were matched according to age, gender, educational level of mother and birth order. ART group assessments were mostly blinded.

Participants/materials, setting, methods: 47 PGD singletons, 50 ICSI and 55 SC children were examined with the family relations test (FRT) measuring the perceptions of family interactions of the children themselves. PGD/ICSI children were tested in the UZ Brussel. Both parents completed the Child Behavior Checklist (CBCL) and the Parenting Stress Index (PSI).

Main results and the role of chance: No group differences were detected concerning children's positive, negative and global perceptions on their mothers and fathers using the FRT. Fathers of the three groups did not differ significantly in their stress experience (PSI/NOSI) as opposed to mothers ($p = .004$; $\eta^2 = .086$). *Post hoc* Tukey tests ($p = .001$) confirmed that mothers of SC children ($M = 313.37$, $SD = 10.16$) reported significantly more stress than PGD ($M = 265.31$, $SD = 10.32$) and ICSI mothers ($M = 275.71$, $SD = 9.98$). The CBCL data did not reveal any group differences regarding behavioral problems.

Limitations, reason for caution: Given the fact that we assessed a relatively small group of Caucasian Dutch speaking PGD singletons our data might not be generalizable. These are the first data of a broad evaluation of PGD preschoolers and their families; it will be interesting to report on the whole data set.

Wider implications of the findings: Our results confirm those of the few other studies. PGD families do not seem to differ in their daily interactions/perceptions from SC or ICSI families. Furthermore our findings suggest that at preschool age PGD mothers are rather more relaxed than SC mothers. On the long term, when PGD parents might develop their own diseases or deteriorations, it might be useful to comprehend how these families interact and how they perceive each other.

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 funded by the Onderzoeksraad (OZR) of the Vrije Universiteit Brussel and the FWO (Fonds Wetenschappelijk Onderzoek) and Wetenschappelijk Fonds Willy Gepts. The UZ Brussel and the Centre for Medical Genetics have received many educational grants for organizing the data collection from IBSA, Ferring, Organon, Shering-Plough, Merck, Merck Belgium. M. Bonduelle has received consultancy and speaker's fees from Organon, Serono Symposia, Merck.

Trial registration number: No clinical trial.

P-397 "UNE 179007": new quality standard for human art laboratories

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Study question: Human IVF units have been certified to ISO's management system standards, although this certification is not specific for the human ART labs. To ensure an efficient quality management in ART labs it's also necessary to define and standardise specific activity control, professional training and tasks and human and infrastructures resources.

Summary answer: The aim of this new quality standard, UNE 179007, was to define specific requirements for human ART labs to improve quality and welfare safety. It establishes common criteria regarding professional qualification, processes and responsibilities, characteristics and controls on infrastructure and equipment, product traceability and safety and quality lab indicators.

What is known already: Scientific associations have had different initiatives to unify human IVF labs standards and to improve the quality management. These include the Certification for embryologists (ASEBIR; ESHRE), Best Practice Guidelines, Guidelines for human and infrastructure resources, etc. Many of them have been useful to elaborate the Quality Standard UNE 179007 specific for human ART labs which base document is the ISO standard. It's a national norm developed by the Spanish Association of Normalisation and Certification (AENOR).

Study design, size, duration: The Spanish Society for Reproductive Biology (ASEBIR) requested AENOR the development of a Quality Standard for human ART labs. From November 2011 until October 2013, a committee met periodically to write the UNE 179007 based on ISO 9001. Before its final official publication, 27 drafts were performed.

Participants/materials, setting, methods: The committee was represented by members of scientific associations, public and private human IVF units, AENOR, professional associations and health administration. Each ISO 9001's chapter was analysed and adapted to specific requirements of human ART lab. Consensus for each chapter was mandatory. The final document was approved by AENOR.

Main results and the role of chance: The new quality standard for Human ART laboratories, 'UNE 179007', has increased ISO 9001 requirements by definition of human training (e.g., embryology lab's responsible must have a biomedical science degree, a PhD or Master degree and more than 5 years of work experience); professional tasks (e.g., responsible for the embryology, andrology and cryopreservation laboratory); minimum human and infrastructure resources plus environmental conditions needed (e.g., cleaning and disinfection, personnel clothing, air conditioning, air recycling and filters, positive pressure); labs equipments control (e.g., calibration and validation, control type, frequency, parameter, measurement range, acceptance criteria),

traceability (e.g., embryologist, culture media, material and equipments in each process); lab indicators (process, method, periodicity standard value); and product preservation (e.g., contingency and transport protocol, product data saved in 2 supports).
Limitations, reason for caution: Once the human ART labs begin getting certification by UNE 179007 (published on November 2013) the AENOR Committee and the Interest Group of Quality (ASEBIR) are permanently active and able to do a feedback work to keep the Norm up-to-date.
Wider implications of the findings: UNE 179007 was implemented to adapt ISO 9001 to the human ART labs. This new quality management system will allow the certificated labs to improve the monitoring and measuring process by the standardisation of the specific lab processes. All together it will improve the quality of their services, increase their results and will also benefit the comparison among certificated labs. UNE 179007 also intends to be an international standard for human ART labs.
Study funding/competing interest(s): Funding by national/international organization(s), Spanish Association of Normalisation and Certification (AENOR).
Trial registration number: None.

P-398 Single embryo transfer vs. double embryo transfer in oocyte donation: a pilot randomized clinical trial

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Study question: In an oocyte donation program (OD), is cumulative pregnancy rate of elective single embryo transfer (eSET) similar to elective double embryo transfer (eDET)?
Summary answer: Cumulative pregnancy rate and cumulative live birth rate are similar in both strategies. With eDET, pregnancy is achieved before it is with eSET, although it implies an increased multiple pregnancy rate.
What is known already: Multiple pregnancies involve high obstetric and perinatal risks that are enhanced by the advanced maternal age of the recipients, the only prevention strategy being single embryo transfer. In IVF/ICSI, several meta-analyses confirm that although cumulative pregnancy rate is higher with eDET, cumulative live birth rate is similar in both groups, with a clear reduction in multiple pregnancies in the eSET group. This is yet to be demonstrated in OD.
Study design, size, duration: Equivalence parallel group study with an equivalent randomization 1:1 performed between October 2010 and July 2012. A total of 65 patients were included, 34 (52.3%) were assigned to the eSET group and 31(47.7%) to the eDET group.
Participants/materials, setting, methods: Patients between 18 and 50 years old, first or second synchronous oocyte donation cycle, with ≥5 embryos available for transfer (≥2 good-quality). Variables examined: cumulative pregnancy and live birth rates, multiple pregnancy rate and the relationship between pregnancy rate and time needed to achieve pregnancy.
Main results and the role of chance: Cumulative pregnancy rates (eSET: 73.5% and eDET: 77.4%. OR: 0.8 95% CI: 0.3–2.5) and live birth rates (eSET: 58.8% and eDET: 61.3%. OR: 0.9 95% CI: 0.3–2.4) were similar with both strategies. Pregnancy rate in fresh embryo transfers (time zero) was higher in the eDET group (63.1%) than in the eSET group (47.1%) but the cumulative pregnancy rates were leveled at time t = 3.46 months. The recruitment of patients was interrupted due to the high twin pregnancy rate (47.7%) in the DET group.
Limitations, reason for caution: Although this is a prospective study with homogeneous groups, the equivalence of both strategies cannot be demonstrated due to the limited number of cases. The decision to discontinue the study was taken in view of the high multiple pregnancy rate, to avoid exposing patients to the unnecessary risks involved.
Wider implications of the findings: The clinical relevance of our study leads to the conclusion that transferring a single embryo in oocyte recipients with good prognosis is a valid option to achieve satisfactory pregnancy rates and avoid multiple pregnancies and their complications.
Study funding/competing interest(s): Funding by hospital/clinic(s), Hospital Universitari Quiron Dexeus.
Trial registration number: www.clinicaltrials.gov NCT01228474.

P-399 Comparison of the neonatal outcomes of children conceived from day 1 rescue ICSI and births from conventional ICSI cycles

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Study question: Are the neonatal outcomes of children conceived from day 1 rescue ICSI (rescue ICSI: R-ICSI) different from births generated conventional ICSI (conventional ICSI: C-ICSI) cycles?
Summary answer: The fetal outcomes of children conceived from day 1 R-ICSI are comparable with those of C-ICSI treatments, including the incidence of abnormalities.
What is known already: A small number of reports on the health of day 1 R-ICSI children are currently available.
Study design, size, duration: In cleavage-stage embryo transfers, there were 46 singletons of R-ICSI babies from fresh cycles and 28 singletons from frozen–thawed cycles. Data of 74 singletons were compared with those of 148 births from cleavage-stage embryo transfers conceived from C-ICSI cycles performed (92 From fresh cycles, 56 from frozen–thawed cycles).
Participants/materials, setting, methods: C-ICSI cycles were chosen as a control group to exclude possible influences of the insemination technique. To eliminate the effects of age, the female ages were matched between R-CSI cycles and C-ICSI cycles (one R-ICSI cycle were matched with two C-ICSI cycles.)
Main results and the role of chance: In single births from cleavage-stage embryo transfers, no significant differences were found between R-ICSI cycles and C-ICSI cycles in birth weight of singletons (3295 ± 339 g versus 3331 ± 512 g, respectively) and gestational age (39.1 ± 1.4 versus 38.6 ± 1.9 weeks, respectively) in fresh cycles, and there are no significant differences found between R-ICSI cycles and C-ICSI cycles in birth weight of singletons (3352 ± 599 g versus 3406 ± 474 g, respectively) and gestational age (38.7 ± 1.5 versus 39.1 ± 1.3 weeks, respectively) in frozen–thawed embryo transfers. In single births from cleavage-stage embryo transfers, abnormalities were 1 (2.2%) in R-ICSI group and 1 (1.1%) in C-ICSI cycles in fresh cycles, and no abnormality was found in frozen–thawed embryo transfers.
Limitations, reason for caution: A more comprehensive assessment of the health and development of day 1 R-ICSI children's will require larger prospective studies.
Wider implications of the findings: The current study showed that the neonatal outcomes of children conceived from day 1 R-ICSI are comparable with those of C-ICSI treatments. A more comprehensive assessment the health status of R-ICSI children needs to be studied further.
Study funding/competing interest(s): Funding by national/international organization(s), Natural Science Foundation of China (81070534).
Trial registration number: The study was approved by the Ethics Committee of Peking University Third Hospital (reference no. 20080612).

P-400 Despite significant financial incentives many couples in the usa still decline elective single embryo transfers (eset)

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Study question: Multiple pregnancies remain the most common cause of maternal, fetal, and perinatal morbidities. Strategies to increase eSET use in the USA (less than 15% of cycles in 2012) have been frustrating, and patient resistance remains a major barrier to significant implementation.
Summary answer: Despite significant financial incentives and high ongoing PR, nearly 44.1% of couples eligible declined to have an eSET, not out of fear of lower success rate but because they want twins. Many couples with insurance coverage declined to participate, providing yet another barrier for implementation of eSET.
What is known already: single embryo transfers have resulted in a significant drop in twin rates.
Study design, size, duration: Prospective pilot study.
Participants/materials, setting, methods: All women <38 proceeding with ART, regardless of ovarian reserve status, diagnosis, prior failed ART cycles, and insurance coverage, were consented for participation in a novel program to incentivize them to undergo eSET: couples were provided with

free gonadotropins (Menopur), free embryo freezing of all extra blastocysts, and free storage for the first year if they agreed to have an eSET.
Main results and the role of chance: Between October 2012 and January 2014, 56 couples were consented for their participation in this novel program. Twenty two couples (39.3%) declined to participate because they wanted to have a chance at conceiving with twins despite an extensive review of the increased morbidities associated with twins. To date, 34 women completed an eSET cycle, and the IR/clinical PR and ongoing/delivered PR were 73.5% (25/34) and 55.9% (19/34). Twenty two couples (39.3%) had insurance coverage for IVF, and despite having at least one other cycle covered by insurance, 7 (31.8%) declined to participate. In contrast, 34 couples were self-pay, and 15 (44.1%) declined eSET ($P = 0.52$). Two women in the DET changed their minds at the time of ET and had eSET, and both conceived with singletons. Of the 20 that received DET, 4 (20%) conceived with twins. No cases of MZT have been encountered to date.
Limitations, reason for caution: few number of cycles and, therefore, inadequate power to detect a small but clinically relevant difference.
Wider implications of the findings: Short of mandating eSET, it seems many couples in the USA will remain reluctant to have an eSET despite excellent pregnancy rates.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Partially funded by Ferring Pharmaceuticals.
Trial registration number: N/A.

P-401 Factors associated with ectopic pregnancy after *in vitro* fertilization and embryo transfer: an analysis of 23,317 transfer cycles

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Study question: What factors are associated with the incidence of ectopic pregnancy (EP) after *in vitro* fertilization/intracytoplasmic sperm injection and embryo transfer (IVF/ICSI-ET)?
Summary answer: Tubal factors (tubal blockage, salpingitis, salpingoplasty, hydrotubation, EP history) and pelvic inflammation, and frozen thawed-embryo transfer (FET) were associated with a significantly higher incidence of EP. However, the difference in the risk of EP between day-3 compared with day-5 blastocyst transfers was not significant.
What is known already: Some investigators advocate that the EP rate is significantly reduced in FET cycles compared with fresh ET cycles. Meanwhile, blastocyst transfers have also been shown to reduce the risk of EP compared with day-3 embryo transfers. However, since the sample sizes in most of these studies are relatively small, the effects of FET and blastocyst transfer on risk of EP are controversial.
Study design, size, duration: This retrospective cohort study included 23,317 ET cycles from August 2009 to January 2014, which resulted in 10,150 clinical pregnancies.
Participants/materials, setting, methods: Data were stratified by categorical variables. Univariable logistic regression analysis was firstly used to predict the odds ratio (OR) and 95% confidence interval (CI) for EP by single factor. Then, multivariable logistic regression analysis was used to association between factors and EP after controlling for other variables.
Main results and the role of chance: The total incidence of EP in IVF/ICSI-ET was 3.41% (346/10150). The incidence of EP was 3.85% (223/5793) in patients with tubal factor and pelvic inflammation, and was 2.82% (123/4357) in patients without tubal factor (Crude OR from tubal factor and pelvic inflammation: 1.38; 95% CI: 1.10–1.72; $P < 0.01$). The EP rate was 3.13 (212/6766) and 3.96 (134/3384) in fresh-ET and FET cycles, respectively (Crude OR from FET: 1.28; 95% CI: 1.02–1.59; $P = 0.03$). The EP rate in day-3 embryo transfers and blastocyst transfers was 3.43% (318/9278) and 3.21% (28/872), respectively (Crude OR from blastocyst transfer: 0.94; 95% CI: 0.63–1.38; $P = 0.93$). In multivariable logistic regression analysis, adjusted OR from tubal factor and pelvic inflammation, FET was 1.39 (95% CI: 1.11–1.74; $P < 0.01$) and 1.29 (95% CI: 1.04–1.61; $P = 0.02$), respectively.
Limitations, reason for caution: Since the incidence of EP was low, it had 81.33% power to detect the difference of EP rate between patients with and without tubal factor and pelvic inflammation infertility, with a two-sided significance level of 0.05. However, the power of blastocyst transfer was only 58.14% to detect the difference.
Wider implications of the findings: In this study, except for tubal factor and pelvic inflammation, we can control the other two factors (fresh ET/FET, cleavage stage embryo/blastocyst embryo transfer) to reduce the incidence of EP. However, due to the small power and the discrepancy between this study and others, prospective studies with larger sample size should be carried out to give us a firm conclusion.
Study funding/competing interest(s): Funding by national/international organization(s), This work was supported in part by the National Natural Science Foundation of China (Grant NO.31271605).
Trial registration number: N/A.

P-402 Culture media influence on birthweight of newborns following IVF

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Study question: Do certain culture media are associated with birthweight for singleton newborns?
Summary answer: Significant difference was observed in gestational age- and gender-adjusted birthweight (*Z* scores) of singletons between G1 (Vitrolife, Gottenburg, Sweden) and G1-PLUS (Vitrolife, Gottenburg, Sweden) medium.
What is known already: It was reported that the birthweight of singletons born from Vitrolife was significantly higher than Cook group. To investigate whether or not fetal overgrowth was attributed to certain culture media, G1-PLUS was included in this cohort study.
Study design, size, duration: This study was a retrospective analysis of newborns birthweight, including 1120 singletons born from fresh embryo transfer at the Center for Reproductive Medicine, Peking University Third Hospital from 2011 to 2012. The number of singletons born from G1 was 507, and the number of singletons from G1-PLUS was 613.
Participants/materials, setting, methods: Patients younger than 40 years of age with a BMI <30 kg/m^2 were analyzed. Only data from singleton pregnancies newborns born alive after the 28th week of gestation were included in the data analysis. Patients who received PGD and cycles with donor oocytes were excluded.
Main results and the role of chance: Multiple linear regression analysis suggested that female weight ($p = 0.00$), male height ($p = 0.05$), gestational age at birth ($p = 0.00$), infant gender ($p = 0.00$), pregnancy-related complications ($p = 0.01$) and type of culture media (G1 vs G1 PLUS) ($p = 0.03$) have significant effects on absolute birthweight. The absolute birthweight for singletons resulting from G1 PLUS was not different from singletons resulting from G1 (3371.78 ± 488.62 g versus 3323.85 ± 497.75 g respectively, $p = 0.11$). The *Z* scores for singletons from embryos cultured in G1 PLUS were significantly higher than singletons from cultured in G1 (0.28 ± 1.13 versus 0.04 ± 1.16 respectively, $p = 0.03$).
Limitations, reason for caution: This study was limited by retrospective design.
Wider implications of the findings: Our study suggests that certain culture media has a significant effect on the *Z* scores of singleton newborns. The effect of certain culture media on epigenetic changes in the embryo and placenta needs further study.
Study funding/competing interest(s): Funding by national/international organization(s), National Natural Science Foundation of China for Young Scholars (81300483).
Trial registration number: Not applicable.

P-403 Assisted reproductive technologies (ART) have influence on first trimester usually used factors for Down's syndrome screening

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Study question: The primary objective of our study was to show that Crown-Rump Length (CRL) measured in pregnancies obtained by ART (IVF, ICSI, and FET frozen embryos transfers) is longer than expected when comparing

to Robinson's curve, invaliding this curve for ART's pregnancies early growth follow-up.
Summary answer: Our study shows a significant difference between ART gestational age (defined from oocytes retrieval day or calculated from embryo's transfer day for frozen embryos) and the gestational age obtained by CRL measurement basing on Robinson curve.
What is known already: CRL is the first standardized measurement that can be made during the pregnancy. In our knowledge only two studies suggested there could be a difference between ART gestational age and gestational age obtained by sonographic measurement of the CRL, but there were no control group.
Study design, size, duration: We made a retrospective study including 6739 women (Group A: 6221 patient with natural pregnancies and group B: 528 pregnancies obtained by ART). All women had an ultrasound scan before 14 gestational weeks in our hospital, Strasbourg (France) between 2007 January the first and 2012 the 30th of June.
Participants/materials, setting, methods: Only regular cycles women were selected in group A: gestational dating was made with last period's first day. Group A and B gestational ages were compared to the one obtained by CRL measurement using a Robinson curve equation.
Main results and the role of chance: Mean difference between calculated gestational age and estimated gestational age by CRL measurement was of 0, 84 days for group A and 2, 3 for group B. The 1.46 difference was significant ($p < 0.001$). Difference was higher when antagonist were used in IVF procedure (2, 6 days in antagonist group and 2.1 days in the agonist group $p = 0.025$). Pregnancy associated plasma protein A (MoM PAPP-A) was significantly lower in group B ($p < 0.001$) and human chorionic gonadotropin (MoM HCG) level was significantly increased ($p = 0.013$). Estradiol level at ovulation had a significant correlation with MoM PAPP-A decreasing in group B ($p = 0.23$). Nuchal translucency was thinner in the agonist group than in the antagonist group (respectively MoM NT were 0.90 and 0.98 $p = 0.042$).
Limitations, reason for caution: Systematic error that can be made by performing an ultrasound examination has been corrected by comparing to a natural pregnancies patients group.
Wider implications of the findings: Although the difference is tiny, this study shows that Robinson curve is not strictly applicable to pregnancies obtained by ART. Therefore CRL curves created by using ART pregnancies cohorts wouldn't be strictly applicable for natural pregnancies. This work also makes the confirmation that PAPP-A and HCG levels are different in ART pregnancies and so Down's syndrome risk evaluation can be perturbed. Estradiol level at ovulation triggering and IVF procedure could have an influence.
Study funding/competing interest(s): Funding by hospital/clinic(s), Centre Medical Chirurgical et Obstétricale (CMCO), Strasbourg, France.
Trial registration number: No.

P-404 Cardiovascular changes in children born to women with ovarian hyperstimulation syndrome: follow-up study and proteomic analysis

Abstract withdrawn by the author

POSTER VIEWING

REPRODUCTIVE (EPI)GENETICS

P-405 Polymorphism R72 of P53 gene is associated with lower pregnancy and implantation rates in recipients undergoing IVF with donated oocytes

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Study question: P53 gene activity has been observed to influence embryo implantation. In the present study we assessed how the p53 codon 72 functional polymorphisms could influence the outcome of recipients undergoing IVF with donated oocytes in an endometrial receptivity model.
Summary answer: The frequency of the allele Proline 72 (P72) at P53 gene is enriched in the whole population of women receiving and IVF with donated eggs. However decreased pregnancies and implantation rates (IR) were observed in recipient homozygous for Arginine 72 (R72) allele being associated negatively with the outcome.
What is known already: P53 has been shown to participate in embryo implantation by regulating leukemia inhibitory factor (LIF) levels. The p53 codon 72 functional polymorphism modifies p53 activity and its role in reproduction has been previously reported. Furthermore P72 allele is enriched in population of infertile women and has been proposed as a risk factor for implantation failure in IVF, new findings could help to clarify its function in the reproductive system, which is not fully understood.
Study design, size, duration: The present study was based on a nested case control study involving a total of $N = 207$ recipients undergoing IVF treatments between July 2009 and February 2011 at the Instituto de Fertilidad Clinica Rincon. Groups were established according to the final results obtained after the treatments.
Participants/materials, setting, methods: Genomic DNA was extracted from buccal swaps and genotyping was performed under TaqMan® OpenArray® Genotyping System (Applied Biosystems). Chi-squared test were performed to determine differences in implantation rates and logistic regression model to compute for odd ratio (OR), 95% confidence interval (CI) for assess possible genotype association with pregnancy outcome.
Main results and the role of chance: Distribution of genotypes frequencies between different groups of recipients, accomplished with Hardy-Weinberg equilibrium ($p > 0.05$). Frequency of R72 allele was higher in recipients that had unsuccessful outcome after receiving embryo transfer (ET) (34%) compared to recipient that get pregnant (22%) (*p value = 0.01*). The assessment of IR showed a decrease in homozygous recipients carrying R72/R72 genotype (21.95%) compared to P72 carriers (40.80%) (*p value = 0,0282*). The recessive inheritance model showed that homozygous for R72 was associated negatively with pregnancy compared to R72/P72 + P72/P72 [2.86 (1.06–7.73) OR (95% CI)] (*p* value = *0.035*).
Limitations, reason for caution: The present study focused in recipients undergoing IVF with donated oocytes from donors and focused in TP53 gene polymorphisms. P53 is a highly connected protein and new findings are needed to assess more accurately functional relationship with reproductive system.
Wider implications of the findings: Negative association of P72 allele with embryo implantation has been previously reported in homologous IVF patients. The enrichment of allele P72 in our infertile recipient population correlates with previous observations, by contrast the allele R72 is associated with the poorer pregnancy and implantation rates in recipients using donated oocytes. These results could hypothetically imply that oocytes are influenced by changes on p53 activity, which finally would be related with pregnancy success.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Institutional fundings. University of Malaga (SAF2008-03314). Instituto de Fertilidad Clínica Rincón (PTQ 09-01-00496).
Trial registration number: None.

P-406 IMSI selection in normozoospermic patients does not improve preimplantation genetic screening outcome

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Study question: This study aims to examine the effect of high magnification sperm selection (IMSI) on the rate of euploid blastocysts formation assessed by array Comparative Genomic Hybridization (aCGH) in Preimplantation Genetic Screening (PGS) cycles with normal semen (WHO, 2010).
Summary answer: In presence of normal semen, classical ICSI (400×) equals the injection of top quality sperm selected at 6600× magnification (IMSI) in terms of production of euploid blastocysts
What is known already: Morphological abnormalities of the sperm head are associated with low ICSI outcomes. Severe oligozoospermia was also shown

to be frequently related with structural and numerical chromosomal alterations in the sperm. Although normozoospermia is always associated with good prognosis, a semen sample defined as normal could still be the origin of negative outcome. Recently several articles have investigated the outcome of IMSI on normozoospermic patients and the results indicated no differences as compared to classical ICSI.

Study design, size, duration: Couples undergoing PGS (March-October 2013) were randomly allocated into ICSI-group (N = 25) or IMSI-group (N = 25). Only normozoospermic patients (WHO, 2010) were included in the study. Indication for PGS were evenly distributed between the two groups: repeated implantation failures (4%), advanced maternal age (46%), repeated miscarriages (4%), genetic disorders (4%), no reason (42%).

Participants/materials, setting, methods: IMSI was performed with high resolution Nomarski optics (100×), whereas classical ICSI was carried out at 400× magnification. Only spermatozoa without vacuoles or with 1 small vacuole (Type I) and spermatozoa with maximum two small vacuoles (Type II) were selected for injection (small vacuole: <4% of the sperm head).

Main results and the role of chance: Respectively in the IMSI-group and in the ICSI-group average maternal age was 37.28 ± 3.74 and 37.60 ± 3.70 (NS). A total of 72 (51 day-5, 19 day-6, 2 day-7) and 62 (41 day-5, 19 day-6, 2 day-7) blastocysts were biopsied in each group. One blastocyst in IMSI-group and two in ICSI-group did not give PGS results. Non-affected blastocysts were 25 (34.7%) in the IMSI-group and 25 (39.7%) in the ICSI-group (NS). Cumulative (fresh/frozen) embryo transferred were 16 and 11 and implantation rates were 11/16 (68.7%) and 5/11 (45.5%) in each group, respectively.

Limitations, reason for caution: Our study is still ongoing and our sample size is going to be improved to make our conclusion stronger.

Wider implications of the findings: In presence of normal sperm, IMSI selection of top-quality spermatozoa produces a rate of normal blastocysts equal to that obtained with classical ICSI. Routine application of IMSI is not necessary in these cases. The use of IMSI on normozoospermic samples has shown a high percentage (around 75%) of type-I and II spermatozoa. Therefore, in this cases, the probability to select the right sperm is high also with classical 400× selection.

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 declared.

Trial registration number: Not applicable.

P-407 The application of post-light semiconductor-based next-generation sequencing in clinical cases of preimplantation aneuploidy screening (PGS) with fresh embryo transfer

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Study question: To report the application of post-light semiconductor-based next-generation sequencing (PLS-NGS) in clinical cases of preimplantation genetic diagnosis processed with innovative protocol.

Summary answer: Application of our innovative approach designed for PLS-NGS platform allowed to determine successfully the status of aneuploidy and sex with day 3 biopsy and day 5 embryo transfer.

What is known already: Screening of all chromosomes is now a gold standard in PGS. Although NGS techniques are today best method of choice they require more than 24 h to perform, consequently blastocyst vitrification is needed.

Study design, size, duration: 8 couples with the average maternal age of 34.4 was referred to PGS procedure from 08/2013 to 12/2013. All together 28 blastomeres were biopsied. The short duration of the procedure allowed fresh embryo transfer without need of blastocyst vitrification.

Participants/materials, setting, methods: Oocyte retrived, blastomeres, embryo evaluation, sequence depth of coverage, total number of reads, mapped number of reads, z-score.

Main results and the role of chance: We demonstrate 8 cases of application NGS in IVF-PGS clinical procedure. All IVF were performed in fresh cycles. 7 out of 8 cases resulted in pregnancy in first cycle giving pregnancies rate of 87.5%. 3.5 blastomers on average per cycle were biopsied, resulted in 1.25 blastomers on average per cycle with no aneuploidy detected. 35.7% of embryos were euploid and on other side, chromosome 5 and chromosome 15 aneuploidies were the most frequent, as we found 4 and 3 aneuploid blastomeres, respectively. We not only used cutting-edge technology in the field of PGD but we went further and designed and preformed in clinical IVF-PGD procedure innovative protocol adjusted to single blastomere biopsy and fresh transfer. The additional benefit is the cost 5 times lower in comparison to aCGH.

Limitations, reason for caution: Sequence composition, such as: nucleotide homopolymers, high GC content, may influence the accuracy of reads. Developing PLS-NGS technology require use of the latest chemistry and software updates to improve analysis quality. The highest standards and stringency in quality system of results is required to avoid diagnosis failure.

Wider implications of the findings: New technology of PLS-NGS possess strong research potential allowing for generation of large amount of data in scale of hours. Hereby, we report successful application in clinical preimplantation diagnosis. In addition, our innovative single-plot short-time protocol require only single blastomere biopsy and is adjusted to fresh embryo transfer. We put efforts in increase of reproduction success rate by increase implantation and decrease miscarriages rates and this aspect need to be followed up.

Study funding/competing interest(s): Funding by commercial/corporate company(ies), Invicta Sp. z o.o.

Trial registration number: Not applicable.

P-408 A deep RNA sequencing study of mammalian sperm RNA: identifying common cross-species expression motifs indicating functionality

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Study question: What are the similarities and differences between bovine, ovine, porcine and human sperm RNA expression profiles?

Summary answer: We believe comparison of sperm RNAs from several distinct species will identify common gene 'expression' networks indicative of functional requirements in both spermatogenesis and fertilisation. Furthermore, such a comparison will help us define networks that are deregulated in the infertile genotype and the genes involved.

What is known already: A complex population of large and small RNAs exists in human ejaculate spermatozoa

Study design, size, duration: A laboratory based investigation using molecular biological techniques and a combination of online GALAXY tools and Bioconductor for *in silico* data analysis.

Participants/materials, setting, methods: Bovine, ovine, porcine and human spermatozoa samples were purified using density gradient centrifugation. Total sperm RNA was isolated via a modified Trizol procedure and the rRNA was removed, before using the SMARTer system. These RNAs were processed for next generation sequencing.

Main results and the role of chance: We were able to generate long RNA (mRNA and lncRNA) cDNA libraries from as little as 2 ng input each of human, porcine, bovine and ovine ds cDNA followed by next generation sequencing on our Illumina HiSeq instrument. The data were mapped on to the human genome and subsequently analysed using the GALAXY online toolset and Bioconductor tools. A summary of our findings will be presented.

Limitations, reason for caution: Considering that spermatozoa do not contain a large amount of RNA compared to other cell types, the commercially available kits which are available for RNA extraction and NGS library creation are not optimised for our purposes and needed to be optimised.

Wider implications of the findings: We are interested in the underlying causes of male factor infertility and reproductive dysfunction. As a proxy for the testis, spermatozoal RNA may be useful for distinguishing and understanding the differences between fertile and infertile phenotypes and exploring the differences between fertility-associated gene expression networks in several species (*H. sapiens, B. taurus, O. aries* and *S. scrofa).*

Study funding/competing interest(s): Funding by national/international organization(s), Marie Curie ITN Network.

Trial registration number: Not known.

P-409 Polar body analysis by array comparative genomic hybridisation reveals a distinct aneuploidy pattern in women of advanced maternal age

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Study question: Do polar bodies from women with advanced maternal age (AMA) show a discriminatory aneuploidy signature after array comparative genomic hybridization (aCGH) based analysis?
Summary answer: Polar body results of that cohort showed aneuploidies of almost all chromosomes with a small bias towards chromosomal gains, no numeral differences in the meiotic origin of the errors and a trend towards more complex aneuploidies (aneuploidies ≥3).
What is known already: Advanced maternal age is positively correlated with an increased risk for aneuploidy in the oocyte being a major cause for implantation failure or pregnancy loss. aCGH based aneuploidy testing, compared to 5-colour fluorescence *in-situ* hybridization (FISH), offers a more quantitative and comprehensive view on the oocyte's chromosomal constitution, revealing premature sister chromatid separation as the dominating meiotic error (Handyside et al., 2012).
Study design, size, duration: In a retrospective cohort study lasting for 2 years, polar bodies from 21 IVF-patients (24 cycles) of advanced maternal age (≥38 years) were simultaneously biopsied. aCGH test results were analyzed in terms of segregation error-frequency, -kind and meiotic origin.
Participants/materials, setting, methods: In two participating IVF-centers[2,3], both polar bodies from *in vitro* fertilized oocytes were biopsied and sent to the diagnostic laboratory. The bioptats were analyzed for aneuploidies by aCGH (Bluegnome, UK). Results were reported in 24 h. The average maternal age of the patient cohort was 42.0 years.
Main results and the role of chance: Of 236 first and second polar bodies, 68 (29%) were found to be euploid. We observed segregation errors of all chromosomes with chromosomes 15, 16, 19, 21 and 22 showing the highest error frequency. Overall, a trend towards chromosomal gains could be observed. The frequency of errors occurring at meiosis 1 didn't differ significantly from meiosis 2 errors except for chromosomes 16, 18 and 22: mainly losses of these chromosomes were detected in second polar bodies. Chromatid gains and losses in first polar bodies were corrected in second polar bodies mostly for chromosomes 14, 15, 16 and 21 correlating with the overall error frequency of these chromosomes. Complex aneuploidy patterns were observed in both polar bodies with a weak correlation to maternal age.
Limitations, reason for caution: The sample size of the present study is limited and states preliminary results. The observed aneuploidy pattern in AMA-patients does not include post-zygotic errors, which can only be tested by trophectoderm testing.
Wider implications of the findings: aCGH based polar body analysis identifies far more aneuploidies than FISH testing. The similar incidences of meiosis 1 and 2 errors imply that both polar bodies should be tested to accurately predict the chromosomal constitution of the corresponding oocyte.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Center for Human Genetics and Laboratory Diagnostics, Dr. Klein, Dr. Rost and Colleagues.
Trial registration number: None.

P-410 Comparative genomic hybridization selection of blastocysts for repeated implantation failure treatment: a pilot study

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Study question: The aim of this study was to determine if the use of preimplantation genetic screening (PGS) by comparative genomic hybridization array (aCGH) and the transfer of a single eupolid blastocyst in patients with repeated implantation failure (RIF) can improve clinical results.
Summary answer: It was found that embryo selection by aCGH performed after biopsy at the blastocyst stage, together with single euploid blastocyst transfer appears to be a successful strategy for patients with multiple failed IVF attempts.
What is known already: RIF refers to a situation when good quality embryos fail to implant. Such mechanism is still poorly understood, but it is clear that it can involve both maternal and embryonic factors. A high incidence of complex chromosome abnormalities has been discovered in cleaving embryos from patient with RIF.
Study design, size, duration: This observational, case control study was performed between March 2012 and March 2013. A total of 121 couples were involved. All females patient were less than 36 years old.
Participants/materials, setting, methods: Three patient groups are compared: 43 couples with RIF for whom embryos were selected by array-CGH (group A), 33 couples with the same history for whom array-CGH was not performed (group B), and 45 good-prognosis infertile couples with array-CGH-selected embryos (group C).
Main results and the role of chance: A total of 190, 171 and 257 blastocyst were obtained in group A, B and C, respectively. A single euploid blastocyst was transferred in 41 and 44 patients in groups A and C, respectively. Array-CGH was not performed in group B, in which 41 blastocysts were transferred in 33 patients (25 single embryo transfer an 8 double embryo transfer). One monoembryonic sac with heartbeat was found in 28 patients of group A and 31 patients of group C showing similar clinical pregnancy and implantation rates (68.3% and 70.5%, respectively). In contrast, an embryonic sac with heartbeat was only detected in 7 patients of group B leading to clinical pregnancy and implantation rates (21.2% and 22.0%, respectively) lower than groups A and C ($p < 0.001$).
Limitations, reason for caution: Expenses for suitable embryo culture system. Time-spending resources to gain laboratory knowledge and to set up genetic protocols. Risks of misdiagnosis within chromosomal mosaicism affected embryos.
Wider implications of the findings: The success rate of IVF after single euploid blastocyst transfer gives similar results for couples with RIF and for good-prognosis couples in their first attempt. This comparison demonstrates that embryo aneuploidies are by far the main cause of RIF as compared with other possible etiologies. The identification of the most viable embryos within a cohort is one of the main goals in IVF in order to perform a single embryo transfer and avoid multiple pregnancies.
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 declared.
Trial registration number: Not applicable.

P-411 The first clinical use of the next generation sequencing in preimplantation genetic diagnosis of Robertsonian translocation – case report

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Study question: To report the normal pregnancy following preimplantation genetic diagnosis (PGD) with use of next-generation sequencing (NGS) as a screening method for 24 chromosome aneuploidy in case of Robertsonian translocation.
Summary answer: This NGS-based PGD provides the first successful clinical application allowing to improve outcome through comprehensive identification of euploid embryos from Robertsonian translocation carrier couple.
What is known already: The Robertsonian translocation carrier-state is connected with high risk during reproduction for unbalanced conceptuses with complete aneuploidy. The standard multicolor FISH analysis for translocation chromosomes is not capable to eliminate risk connected with interchromosomal effect compared with new screening methods detecting all chromosomes number abnormalities.
Study design, size, duration: Day-3 embryos screening for chromosomal aneuploidy was performed in two consecutive IVF cycles firstly with FISH and then with NGS-based protocol. In each IVF attempt three embryos were biopsied.

The short duration of procedures allowed fresh embryo transfer without need for vitrification.

Participants/materials, setting, methods: The PGD for rob (14;15) was performed with FISH for translocation chromosomes and search for aneuploidy with set of probes for five chromosomes. In the next IVF and PGD trial the genome material from single biopsy blastomere was analysed with use of the Ion Torrent Personal Genome Machine (Life Tech).

Main results and the role of chance: The first IVF cycle with PGD analysis using FISH method finished with 8 HBD pregnancy loss of selected embryo. The second attempt with NGS-based aneuploidy screening helped to exclude one embryo with 22 monosomy and one with multiple aneuploidies: −7, −11, −17, +21. No allele drop out (ADO) or contamination were detected in analysed samples. The transfer of the only euploid blastocyst resulted in successful pregnancy outcome.

Limitations, reason for caution: Case report.

Wider implications of the findings: The application of NGS to preimplantation genetic screening of embryos to predict chromosome copy number for diagnosis of aneuploidy gives alternative to FISH and CGH-microarray new approach without limitations of those methods. In case of Robertsonian translocation carriers it allows to evaluate simultaneously results of abnormal segregation of translocation chromosomes both with interchromosomal effect products.

Study funding/competing interest(s): Funding by commercial/corporate company(ies), 1. INVICTA Fertility and Reproductive Centre, Gdansk, Poland, 2. INVICTA Fertility and Reproductive Centre, Warsaw, Poland, 3. Department of Gynaecology and Obstetrics, Varmia and Masuria University, Olsztyn, Poland, 4. Department of Nursing, Medical University, Gdansk, Poland.

Trial registration number: Not applicable.

P-412 Preovulatory aging of murine oocytes affects transcript levels and poly(A) tail length of maternal effect genes

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Study question: Does preovulatory oocyte over ripeness influence transcript levels and poly(A) tail dynamics of maternal effect (ME) genes?

Summary answer: Oocyte over ripeness induced by preovulatory aging *in vivo* led to decreased transcript levels and increased poly(A) tail length of certain ME genes, while poly(A) tails of ME genes of *in vitro* matured (IVM) oocytes became deadenylated.

What is known already: Transcription in oocytes ceases at the onset of maturation and is resumed after zygotic genome activation (ZGA). Between fertilization and ZGA, embryonic development is regulated by ME gene mRNAs, which are stored in the oocyte. Protein synthesis during this period is regulated at the posttranscriptional level, for example by poly(A) tail length of mRNAs. We could show previously that preovulatory aging, caused by delayed ovulation, is associated with developmental defects in mice.

Study design, size, duration: Oocytes were obtained after superovulation (*in vivo* maturation) or from preantral follicles isolated and grown for 12 days *in vitro*. Preovulatory *in vivo* aged oocytes were generated by delaying ovulation for 3 days using the GnRH antagonist cetrorelix. *In vitro* follicles were cultured for 14 days to induce aging.

Participants/materials, setting, methods: Transcript levels and poly(A) content of 10 ME genes in preovulatory aged oocytes were compared to controls in an *in vivo* and an *in vitro* mouse model. qRT-PCR was used with either random hexamer-primed cDNA to determine transcript levels or oligo$(dT)_{16}$-primed cDNA as indicator of poly(A) tail length.

Main results and the role of chance: Random hexamer priming showed significantly decreased transcript levels of *Brg1* (*Smarca4*) and *Tet3* for *in vivo* aged oocytes. No or only small effects were found for *Trim28* (*Kap1*/*Tif1*β), *Zfp57*, *Dnmt1*, *Nlrp2*, *Nlrp5* (*Mater*), *Nlrp14*, *Oct4* (*Pou5f1*) and *Zar1*. Using oligo$(dT)_{16}$ priming, we observed a tendency towards increased poly(A) mRNAs for *Brg1*, *Tet3*, *Trim28*, *Zfp57*, *Dnmt1*, *Nlrp2* and *Nlrp5*. This could indicate continuing polyadenylation and precocious recruitment of maternal mRNA during preovulatory *in vivo* aging, while overall transcript levels decline. During preovulatory aging of IVM oocytes, transcript levels did not change significantly, but we did observe a decrease of poly(A) content for most of the transcripts.

Limitations, reason for caution: Only a selection of 10 ME genes was investigated. To make a more general statements considering poly(A) tail dynamics during preovulatory aging, a transcriptome-wide analysis would be needed.

Wider implications of the findings: The data argue that preovulatory aging affects transcript levels and poly(A) tail length of selected ME genes. Also, IVM oocytes respond differently to preovulatory aging than *in vivo* matured oocytes. Since *in vivo* preovulatory aging can occur during prolonged hormonal treatment in the course of assisted reproduction, and cryopreservation of follicles followed by *in vitro* culture is handled as option for fertility preservation in women diagnosed with cancer, our results may be of clinical relevance.

Study funding/competing interest(s): Funding by national/international organization(s), Deutsche Forschungsgemeinschaft.

Trial registration number: Not required.

P-413 Is embryo score correlated with aneuploidy type

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Study question: Is there a relationship between embryo development and the type of aneuploidy?

Summary answer: Embryo morphology is influenced by the chromosomal status.

What is known already: It is now that the morphology and the viability of the embryos are influenced by the chromosomal status but all the studies published up to now have been performed by FISH. However, it is reported that some aneuploidies such as trisomies reach blastocyst of very good quality that are able to implant and become a newborn.

Study design, size, duration: Retrospective and observational study including 2428 embryos from our CCS program conducted from September 2012 to January 2014.

Embryos were grouped according to their chromosome status: euploid 342), aneuploid (1866). The group of aneuploid embryos was divided into 4 groups; trisomies (263), monosomies (346), complex (739) and chaotics (469).

Participants/materials, setting, methods: Cell number, fragmentation and symmetry on day 2 and 3 were evaluated in all biopsied embryos. Byopsed was performed on day 3 and genetic analysis was carried out by using Array-CGH. Embryos were cultured until day 5 when morphological status was evaluated again. Euploid and viable embryos were transferred.

Main results and the role of chance: We found significant differences for different chromosomal abnormalities in the following variables: number of cells on day 2 ($p < 0.0419$), symmetry on day 2 ($p < 0.0007$), number of cells on day 3 ($p < 0.001$) and symmetry on day 3 ($p < 0.001$). On the contrary we didn't find statistical difference in fragmentation both at day 2 and day 3. Also we saw statistically significant differences between the chromosomal status and quality of the blastocyst on day 5 ($p < 0.001$), noticing a clear relationship between the quality of the blastocyst and the aneuploidy.

Limitations, reason for caution: A deeper analysis taking into account the etiology should be done.

Wider implications of the findings: Embryo development is affected by its chromosomal endowment but not always is toward a poor morphology unable to implant therefore it is recommended to perform biopsy and Array CGH to analyze.

Study funding/competing interest(s): Funding by hospital/clinic(s), IVI.

Trial registration number: None.

P-414 Functional analysis of a novel preimplantation-specific gene Zfp371

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Study question: We have identified a novel mouse gene '*Zfp371*' that likely encodes a SCAN zinc finger domain and is expressed specifically in preimplantation embryos and embryonic stem (ES) cells. The aim of this study was to perform a detailed analysis of its expression pattern and investigate its function in early development.
Summary answer: This study showed that *Zfp371* is expressed exclusively in preimplantation embryos and ES cells. *Zfp371* knockout mice and ES cells from *Zfp371* knockout blastocysts were phenotypically normal, but demonstrated numerous random chromosomal gap. These results indicate that *Zfp371* may play a role in genome stability.
What is known already: After fertilization, zygotic genome activation (ZGA) occurs leading to totipotency in preimplantation embryos. Genes such as *Zscan4* that are specifically expressed during ZGA, are likely to have important roles in establishing pluripotency or in preimplantation development. In this study, we found that the novel mouse zinc-finger gene *Zfp371* is expressed in preimplantation embryos and embryonic stem cells from the ZGA phase to the blastocyst stage.
Study design, size, duration: *In silico* analysis using public database and DNA microarray studies identified novel genes that are exclusively expressed in preimplantation embryos. We focused on *Zfp371* and verified its specific expression pattern. Intercrosses between heterozygote transgenic mice harboring a loss-of-function allele of *Zfp371* generated viable homozygotes.
Participants/materials, setting, methods: RT-PCR analysis of adult mouse and preimplantation embryo cDNAs was used to confirm the exclusive zygotic expression of *Zfp371*. Immunocytochemical staining using a polyclonal antibody to ZFP371 was also employed. Transgenic *Zfp371* knockout mice and ES cell lines with a targeted disruption of the *Zfp371* gene were also generated.
Main results and the role of chance: *Zfp371* expression in ES cells and in 2-cell to blastocyst stage embryos was detected by RT-PCR. Immunostaining showed nuclear localization of the ZFP371 protein at the 8-cell stage. Almost all *Oct4*-positive undifferentiated ES cells also expressed *Zfp371*. To investigate the *in vivo* functions of *Zfp371*, we generated *Zfp371* knockout mice. Viable homozygous (*Zfp371*i–/–) offspring were generated from intercrosses between heterozygous (*Zfp371*+/–) mice, implying that *Zfp371* is not essential for embryonic development. Second-generation homozygotes were also viable, indicating that *Zfp371* is not essential for fertility. Furthermore, homozygote litter sizes were not significantly lower than those of wild-type (*Zfp371*+/+) mice. However, analysis of mouse karyotypes revealed high rates of random chromosome gap in *Zfp371*i–/– mice and *Zfp371*i–/– ES cells.
Limitations, reason for caution: We have not yet elucidated a molecular mechanism which would explain the role of *Zfp371* in the generation of random chromosome gap in both *Zfp371*i–/– ES cells and somatic cells from *Zfp371*i–/– mice.
Wider implications of the findings: It is possible that UV or ionizing radiation might enhance the phenotype of *Zfp371*i–/– mice or *Zfp371*i–/– ES cells and this will be investigated in our next study. Our current results suggest that it may be worthwhile investigating the potential role of the human ortholog of mouse ZFP371 in the generation of embryonic chromosomal aberrations.
Study funding/competing interest(s): Funding by University(ies), Keio University.
Trial registration number: None.

P-415 The clinical value for re-biopsy, frozen and thaw of test-failure blastocysts in preimplantation genetic diagnosis cycle

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Study question: Is there a clinical value to do the second round of biopsy, vitrification and thawing for test-failure blastocyst from first biopsy in preimplantation genetic diagnosis/screening (PGD/PGS) cycle? Does the extra manipulation have detrimental effect on the implantation potential of blastocysts?
Summary answer: Test-failure blastocysts have high survival ability and implantation potential after second round of biopsy, vitrification and thawing. This provide more chance for pregnancy in PGD/PGS practice.
What is known already: Blastocyst biopsy combined with frozen embryo transfer is more adopted in PGD/PGS program, and about 2 ~ 7% of the biopsied blastocysts were reported to be test failure due to various reason and were excluded for transfer. Previously, there was only case report of successful pregnancy after re-biopsy of blastocysts following allele dropout after day 3 biopsy, but there is no clinical data for the value of blastocyst re-biopsy up to date.
Study design, size, duration: This was a retrospective study of the clinical outcomes of 77 re-biopsy cycles for 106 blastocysts from October 2011 to September 2013.
Participants/materials, setting, methods: From 77 re-biopsy cycles, 106 blastocysts were thawed and those survived blastocysts underwent re-biopsy. The blastocysts were then vitrified again and stored. The blastocysts diagnosed to be normal by SNP array were thawed and only re-expanded blastocysts were transferred either alone or with other non-re-biopsied blastocysts in natural cycle.
Main results and the role of chance: In total of 1855 blastocysts from 403 PGD/PGS cycles, 106 blastocysts (5.7%) failed in genetic test due to various reasons. After thawing, 73 blastocysts survived and underwent successful re-biopsy. Three re-biopsied samples had unsuccessful amplification. In the other 70 samples, 31 were diagnosed to be chromosomal normal (44.3%) and 39 were abnormal. The 31 normal blastocysts came from 29 cycles, among which 18 frozen embryo transfers were carried out. 19 re-biopsied blastocysts had been warmed and 18 survived (surviving rate 94.7%). Ten cycles has single blastocyst transfer and resulted in 50% implantation rate, and no early miscarriages were observed to date.
Limitations, reason for caution: The long-term effects of second round biopsy, vitrification and thawing on late pregnancy and the health of offspring need to be further monitored to evaluate the safety of this manipulation.
Wider implications of the findings: For test-failure blastocysts, it could be a routine practice to do the biopsy again to increase the chance for a normal blastocyst for transfer.
Study funding/competing interest(s): Funding by national/international organization(s), This work was supported by the Major State Basic Research Development Program of China (No. 2012CB944901) and National Science foundation of China 81222007.
Trial registration number: None.

P-416 Rapid sequential aneuploidy screening in oocytes with digital PCR

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Study question: Identification of euploid oocytes should allow to transfer good embryos and improve pregnancy and take home baby rates.
Summary answer: This strategy is fast and reduces costs to the minimum necessary to identify euploid oocytes and exclude aneuploid oocytes. A small preclinical trial will test the usefulness of this approach.
What is known already: Oocyte testing requires analysis of two single cells per oocyte – polar body (PB) I and II, which is time consuming and expensive with established methods. Moreover resolution at the chromatid level is difficult and not always possible with microarray formats. We have established a simple PCR method to count chromatids directly with DNA in limiting dilution and digital readout.
Study design, size, duration: 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 oocytes can be diagnosed as aneuploid at that stage and are excluded from further analysis. All oocytes, euploid for these 8 chromosomes, undergo a second round of single marker PCR to analyse the 8 next most aneuploid chromosomes. The few remaining oocytes are analysed with the remaining 7 chromosomes and chromosome Y as a control.
Participants/materials, setting, methods: retrospective analysis of polar bodies with single cell molecular copy number counting (scMCC).
Main results and the role of chance: A small preclinical trial will test the usefulness of this approach.
Limitations, reason for caution: pregnancy outcome and take home baby rate will test the usefulness of aneuploidy screening.

Wider implications of the findings: method can be used for analysis of somatic single cells.
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by commercial/corporate company(ies), Fertility Center Wiesbaden, Germany, GFI grant for fertility innovation – Merck Serono method can be used for somatic single cells.
Trial registration number: None.

P-417 Detection of Y-chromosome in human embryonal culture medium before embryo transfer: a non-invasive method to predict gender, the prerequisit of further investigating gender-specific abnormalities

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Study question: If the amount and quality of fragmented cell-free DNA, released by male human embryos into day 3 and day 5 embryonal culture medium permit the detection of the presence of the Y-chromosome?
Summary answer: Y-chromosome-specific DYS14 gene could be detected in the medium of the male embryos, both at days 3 and 5 of culturing.
What is known already: Fragmentation is a common feature of human embryos during culture, which processes may also imply the release of genomic DNA (gDNA) into culture medium. However, the DNA content of the culture medium has not been used before for the purpose of non-invasive prenatal diagnostics.
Study design, size, duration: Human fertilized oocytes were individually cultured from days 1 to 5. After embryo transfer, spent mediums were stored at –70°C. A total of 25 samples (16 male: 11 at day 3, 5 at day 5 and 9 female: 5 at day 3, 4 at day 5) were enrolled in our retrospective study.
Participants/materials, setting, methods: Total gDNA was purified from 25 embryo culture medium. The male-specific Y-chromosomal DYS14 gene and the gender-independent control gene beta globin were detected by sequence-specific RT PCR TaqMan assay. Results were compared according to the gender of the newborns.
Main results and the role of chance: Beta globin and DYS14 gene-specific RT PCR TaqMan assay confirmed the presence of cell free DNA in day 3, as well in day 5 spent mediums. Beta globin as control gene was detected in all cases. Out of the 16 male samples, 11 (6 from day 3 and 5 from day 5) gave positive DYS14 gene result. It is possible that in case of the 5 false negative male mediums, DNA quality and quantity did not allow the detection of DYS14 gene. In case of female spent mediums (5 at day 3 and 4 at day 5) DYS14 gene false-positivity did not occur.
Limitations, reason for caution: Sample size need to be increase in order to establish the results of the current study whether spent medium's DNA content based method could be applied to predict male specific mutations.
Wider implications of the findings: The results of the current study may provide a novel, non-invasive tool to predict gender specific chromosomal abnormalities before embryo transfer; in order to select viable embryo in cases where sex chromosome abnormalities occur in the family history.
Study funding/competing interest(s): Funding by national/international organization(s), SROP-4.2.2.A-11/1/KONV-2012-0053 Investigation of biomarkers in culture medium for the success rate of *in vitro* fertilization and Hungarian Academy of Sciences-University of Pécs Human Reproduction Section – 14013.
Trial registration number: The study was approved by the Medical Research Council – Human Reproduction Committee, approval number: 5273-2/2012/EHK.

P-418 Preimplantation genetic diagnosis of single-gene disorders: experience for 74 different monogenic conditions

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Study question: What are the clinical outcomes of cycles performed for preimplatation genetic diagnosis (PGD) for single-gene disorders (SGD) by one of the largest single *in vitro* fertilization (IVF) and Reproductive Genetics Center and has the technique been successful in terms of both diagnostic efficiency and clinical safety?
Summary answer: In this study we present 11 years of experience on clinical and technical aspects of PGD for 74 different monogenetic disorders, showing that this technique is successful and effective tool of disease prevention for couples at risk of transmitting genetic disorders.
What is known already: PGD aims to detect any previously known SGD and avoid passing those genetic diseases onto next generation for couples at risk. Therefore, this effective method with high accuracy is being used more commonly for prevention of genetic disorders before establishing pregnancy. Since its first application in 1990, PGD has been performed for more than 300 different monogenetic diseases resulting in births of thousands of healthy children.
Study design, size, duration: This study consists of retrospective analysis of PGD cycles performed for monogenic disorders between years 2003–2013. Until 2011 pre-clinical work-up studies were performed with collaborator centers and after were in house performed; where all procedures related with genetic testing and analysis of PGD results have been performed since 2003.
Participants/materials, setting, methods: In total, 287 PGD cycles were performed for 208 couples carrying monogenetic disorders. PGD set up procedure was performed initially on the parents' peripheral blood DNA samples using 10–12 polymorphic STR markers. PGD was performed on cleavage stage. Biopsied samples were analyzed with single-cell multiplex PCR techniques.
Main results and the role of chance: The mean patient age was 32.7. In those 287 PGD cycles, 4036 oocytes were retrieved (mean 14.1), 3081 mature oocytes were injected (mean 10.7) and 2645 oocytes were fertilized (mean 9.2). 1827 embryos were diagnosed (85%) out of 2150 biopsied embryos (mean 7.5). Embryo transfer was performed in 264 cycles (92%) with a clinical pregnancy rate (CPR) of 46.6% and implantation rate of 32%. 114 babies were born and 13 more ongoing pregnancies have not yet reached to term during preparation of this report.
Limitations, reason for caution: The high rate (25%) of consanguineous marriages in Turkey creates technical limitations in set-up studies by decreasing the number of informative STRs. Diagnosis of *de novo* mutations is challenging due to unavailable inherited linkage information. Allele drop-out (ADO) and recombination are the main factors influencing the accuracy of the test.
Wider implications of the findings: A prominent feature of our patient group was the high diversity of genetic conditions which could be attributable to the presence of consanguineous marriages in the Anatolian region. In the Mediterranean region, especially heterozygosity for beta-thalassemia may reach to 14%. Although the clinical outcomes are affected by limitations such as maternal age, the results showed that PGD for single-gene disorders is an effective reproductive strategy with high diagnostic efficiency and successful clinic outcomes.
Study funding/competing interest(s): Funding by hospital/clinic(s), Istanbul Memorial Hospital.
Trial registration number: None.

P-419 Children born after biopsy of morula-stage embryos

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Study question: Does biopsy of human morula-stage embryos for preimplantation genetic diagnosis (PGD) affect the health of newborns?
Summary answer: Biopsy of morula-stage embryos poses no risk to the health of newborns.
What is known already: PGD and preimplantation genetic screening (PGS) are commonly performed on biopsies from cleavage-stage embryos or blastocyst trophectoderm obtained on day 3 or 5, respectively. We have reported that biopsies can be performed on day 4. This finding was first presented at the 28th Annual Meeting of ESHRE, 2012 (P-480). There is no published data on children born after embryo biopsy on day 4.

Study design, size, duration: To evaluate the safety of PGS with morula-stage embryos (day-4), this study compared children who were conceived via intracytoplasmic sperm injection (ICSI) with PGD/PGS and children who were conceived via standard ICSI treatment. Patients who underwent IVF treatment between September 2011 and December 2012 were included.
Participants/materials, setting, methods: A prospective cohort study was undertaken using the approach employed to follow-up IVF and ICSI children conceived in the same centre. Questionnaires were sent to physicians and parents at conception and at delivery. Groups were compared by examining the delivery date, birth height, birth weight, and rates of major malformations.
Main results and the role of chance: Data collected on 54 children born following PGD/PGS (36 singletons, 18 twins) showed that the delivery date, birth height, birth weight, and rates of major malformations were not statistically different from those of 54 children conceived via standard ICSI (38 singletons, 16 twins).
Limitations, reason for caution: None.
Wider implications of the findings: Embryo biopsy on day 4 poses no health risk to singleton or twin children born after PGD/PGS. Biopsy of morulae does not adversely affect embryo development *in vitro*; rather, it has advantages. First, the increased number of cells in such biopsies might facilitate diagnostic screening. Second, the PGD results can be obtained prior to embryo transfer on day 5–6 in the current IVF cycle. Thus, biopsy of morula-stage embryos is safe and clinically useful.
Study funding/competing interest(s): Funding by hospital/clinic(s), Center for reproductive medicine MAMA.
Trial registration number: None.

P-420 Chromosome segregation analysis of day-3 embryos from carriers of structural chromosome abnormalities in PGD cycles using arrayCGH

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Study question: Whether some characteristics of chromosome translocation and inversion carriers, including terminal breakpoints, acrocentric chromosome or carrier gender, are related to meiotic segregation patterns indicating success in an Artificial Reproductive Technology (ART) cycle.
Summary answer: This study suggested that reciprocal translocation involving acrocentric chromosomes resulted in a lower rate of normal/balanced karyotype in preimplantation embryos. Some characteristics of reciprocal translocations, such as terminal breakpoints, acrocentric chromosome and carrier gender, are related to the segregation patterns. The percentage of embryos consisted with normal/balanced segregation prevailed in carriers with pericentric inversion and Robertsonian rather than reciprocal translocations.
What is known already: Most translocation/inversion carriers are phenotypically normal because there is no net loss or gain of genetic information. However, chromosome segregation during gametogenesis in these individuals may lead to production of gametes/embryos with lack or excess of genetic material resulting in infertility, first trimester miscarriage and/or pregnancy or birth of an affected child. Preimplantation Genetic Diagnosis (PGD) provides benefit for these couples by avoiding the transfer of the unbalanced forms before implantation.
Study design, size, duration: Cleavage stage biopsy and arrayCGH analysis of a hundred and twenty-one embryos from carriers with structural chromosome abnormalities undergoing preimplantation genetic diagnosis (PGD) to identify different types of chromosome segregation patterns. The study was conducted for 2 years (2011 to 2013). All couples signed consent forms for participating in the study at 'Embryogenesis'.
Participants/materials, setting, methods: Seventeen couples underwent twenty-one IVF cycles with PGD (n = 2 for pericentric inversion, n = 15 for reciprocal and n = 4 for Robertsonian translocation chromosome imbalance). Array CGH was used to detect chromosome copy number and segmental changes in blastomeres and to predict aneuploidy and modes of segregation in the corresponding embryos.
Main results and the role of chance: The overall incidence of 2:2 alternate segregation was 54.5% (66/121); 2:2 alternate segregation (14/21 = 66%) was the most frequent type in case of Robertsonian translocation carriers. 90% (17/19) of embryos were normal/ balanced in inversion carriers. In both male and female heterozygotes with reciprocal translocation 2:2 alternate was more frequent (35/81 = 43%) versus 2:2 adjacent-1 (28/21 = 34.6%) segregation. 2:2 adjacent-2 (7/81 = 8.6%) and 3:1 disjunction (2/81 = 2.4%) with uneven number of crossovers were less frequent. Adjacent-1 (19% versus 28%), adjacent-2 (15.1% versus 12%) and 3:1 (39.8% versus 30.5%) segregation were not significantly different in translocations with terminal breakpoints versus those without. Translocation with acrocentric chromosomes showed significantly lower rate of 2:2 segregation (38% versus 59.1%,p = 0.001) and a higher rate of 3:1 segregation (42.7% versus 26.3%,p = 0.005) than those without acrocentric chromosomes.
Limitations, reason for caution: Single cell chromosome analysis might not be indicative of the chromosome complement for the corresponding embryo due to high rate of mosaicism at cleavage stage. Ideally, higher accuracy in estimating the segregation patterns in translocation carriers will require the follow-up of whole euploid and aneuploid surplus embryos at cleavage.
Wider implications of the findings: In this study we conclude that chromosome segregation patterns in embryos obtained from translocation reciprocal and Robertsonian carriers depend on the type of translocation and the involvement of acrocentric chromosome(s) but not the carrier gender and the terminal breakpoint(s). These results should be helpful in counseling the carriers with structural chromosome abnormalities by estimating their reproductive success undergoing PGD.
Study funding/competing interest(s): Funding by hospital/clinic(s), Private self-funding/no competing interests.
Trial registration number: This is not a clinical trial.

P-421 DNA copy number variations in women with premature ovarian insufficiency

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Study question: Whether screening of premature ovarian insufficiency (POI) patients for genome-wide copy number variations (CNV) and runs of heterozygosity (ROH) with high-resolution DNA microarrays will help to identify novel regions and candidate genes related to POI?
Summary answer: Single-nucleotide polymorphism (SNP) arrays are useful in identifying single genes and genome regions responsible for the onset of POI.
What is known already: The cause of POI remains unknown in many cases. In recent years studies have highlighted novel genetic changes responsible for POI. Conventional karyotyping has identified several X-chromosome regions that harbour genes critically required for normal functioning of the ovaries. High-resolution DNA microarray techniques even further improved our knowledge about the genetic causes of POI. Indeed, those techniques have revealed that in addition to X-chromosome rearrangements, POI is associated with aberrations in autosomal chromosomes.
Study design, size, duration: Case-control genetic association study of 709 POI cases, using the Database of Genomic Variants as control population.
Participants/materials, setting, methods: The study included women with idiopathic POI with the cessation of ovarian function before 40 years of age. Women with history of gynaecological surgery, cancer treatment and genetic syndromes were excluded. Genomic DNA samples were obtained from Tartu University Hospital and Estonian Genome Center and analysed using high-resolution SNP-arrays.
Main results and the role of chance: A number of CNVs were detected on both X-chromosomes and on autosomes. The majority of CNVs fall within the common polymorphic regions, while the others have the clinical significance and harbour previously reported and novel POI candidate regions and genes. For example, identified aberrations include a 24Mb Xp22.33-p21.3 hemizygous deletion and a novel microdeletion in 15q21.3 region very close to aromatase gene (CYP19A1). In addition to CNVs, numerous ROH regions were detected, which may possibly contain homozygous mutations in POI-associated genes. Most of the ROH regions were found on X-chromosomes and contained known POI genes.

Limitations, reason for caution: Although SNP-arrays provide higher resolution in genomic studies, some CNVs may still remain undetected. Furthermore, in order to understand the role of genes located in the ROH regions, it is necessary to perform mutational analysis by sequencing the candidate genes.
Wider implications of the findings: DNA microarrays are a suitable tool for evaluating genomic imbalances in POI patients when compared to the conventional cytogenetic methods. The present study, with high number of POI cases, provides novel data on associations between the genomic variants and aberrations and POI phenotype.
Study funding/competing interest(s): Funding by national/international organization(s), Estonian Research Council, European Community 7th FP.
Trial registration number: Not required.

P-422 Identification of non-parthenogenetic and euploid blastocysts from one pronuclear oocyte for embryo transfer by single nucleotide polymorphism (SNP) array

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Study question: One pronuclear (1PN) oocyte has high developmental potential but is routinely excluded for embryo transfer due to its possible parthenogenetic origin and increased risk for aneuploidy. SNP array is now widely used for aneuploidy screening. Could SNP array also help to distinguish parthenogenetic origin of 1PN oocyte?
Summary answer: The blastocysts from parthenogenesis (either with or without extrusion of second polar body) exhibit low degree of heterozygosity (below 2%) when compared to blastocysts from normal fertilized oocyte (range from 3.0%–9.3%) after SNP analysis. This characteristic is efficient to select non-parthenogentic embryo for transfer and resulted in normal live birth.
What is known already: Parthenogenetic stem cell (pSC) lines have unique SNP signature (highly homozygous or peri-centromeric homozygous) when compared to stem cell lines derived from normal fertilized zygote (highly heterozygous). But currently no data indicate whether this signature could be translate into preimplantation genetic screen to distinguish parthenogenetic origin for 1PN embryos.
Study design, size, duration: This was an experimental study to compare the SNP signature and the degree of heterozygosity of 4 pSC lines and 5 blastocysts from parthenogenesis with 200 normal fertilized embryos, followed by cases report.
Participants/materials, setting, methods: Small cell samples from 4 pSC lines and 5 parthenogenetic blastocysts were processed for whole genome amplification (WGA) and SNP array analysis. The SNP signature and heterozygosity were analyzed by software (Affymetrix CNAT4.0 edition) and compared to previously generated SNP data of 200 blastocysts. The established criteria were utilized for 8 1PN blastocysts for selection.
Main results and the role of chance: The typical homozygous distribution patterns were not observed in pSC lines and parthenogenetic blastocysts under the detection of low density SNP array after WGA, but the samples from parthenogenesis have reduced rate of heterozygosity, range from 0.5 ~ 1.5%, when compared to 3.3 ~ 9.3% of 200 normal fertilized blastocysts. Thereafter, 8 1PN-derived blastocysts were analyzed by SNP array to determine the chromosomal constitution and the rate of heterozygosity for transfer. Two highly homozygous and diploid blastocysts were identified and further validated by imprinted gene expression to be parthenogenesis, which indicates that 1PN oocytes may originated from parthenogenetic activation with the extrusion of second polar body and undergo diploidization in subsequent cleavages. To date, one blastocyst confirmed to be normal were transferred and resulted in a healthy birth.
Limitations, reason for caution: The clinical value for identification of 1PN-derived embryos resulted from parthenogenesis by SNP array needs to be further validated by larger sample size.
Wider implications of the findings: Our result suggested a new application of SNP array for preimplantation genetic screen.
Study funding/competing interest(s): Funding by national/international organization(s), The national Science foundation of China: 81222007.
Trial registration number: Not applicable.

P-423 The segregation pattern study of heterologous Robertsonian translocations

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Study question: Do the different heterologous Robertsonian translocations have the similar segregation patterns?
Summary answer: The different heterologous Robertsonian translocations have the similar frequency of unbalanced sperms.
What is known already: Robertsonian translocations are among the most chromosome structural rearrangements, including 5 types of homologous and 10 types of heterologous Robertsonian translocation. Heterologous Robertsonian translocations are more common and could be benefit from preimplantation genetic diagnosis (PGD). However, whether the different heterologous Robertsonian translocations have the same reproductive risk is unknown. Sperm fluorescence in situ hybridization (FISH) could be used to study the meiosis patterns and further evaluate the reproductive risk.
Study design, size, duration: Totally 46 male patients with Robertsonian translocation were performed sperm FISH analysis from September 2010 to December 2013, including 35 patients with rob(13;14), 4 rob(14;21), 2 rob(13;15), 2 rob(13;22), 2 rob(13;21), 1 rob(15;21).
Participants/materials, setting, methods: The study was set at the Reproductive and Genetic Hospital of CITIC-Xiangya, China. The frequency of normal/balanced sperm was analyzed by FISH.
Main results and the role of chance: At least 2000 sperm nuclei from each Robertsonian translocation carrier were analyzed by FISH. The normal/balanced sperms resulting from alternate segregation varied from 58.34% to 89.92%. The frequency of disomic and nullisomic sperms due to adjacent segregation varied from 41.66% to 10.08%. No 3:0 segregation pattern was tested. Our results showed the rare Robertsonian translocation such as rob (15q21q) had the similar reproductive risk as the common Robertsonian translocations such as Rob (13q14q) and Rob(14q21q).
Limitations, reason for caution: Only 9 rare Robertsonian translocations were included in the study. Only two related subtelomeric probes involved in the translocations were used, and interchromosomal effect (ICE) could not be excluded.
Wider implications of the findings: Our study may help to improve the genetic counseling for Robertsonian translocations carriers, especially for those rare types of Robertsonian translocations.
Study funding/competing interest(s): Funding by national/international organization(s), the Major State Basic Research Development Program of China (No.2012CB944901).
Trial registration number: Not applicable.

P-424 Predictive value of sperm FISH analysis on the outcome of preimplantation genetic diagnosis for pericentric inversion carriers

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Study question: Does sperm fluorescence in situ hybridization (FISH) analysis for pericentric inversions(PIs) have any predictive value of on the outcome of preimplantation genetic diagnosis (PGD)?
Summary answer: Sperm FISH analysis could predict the risk of unbalanced progeny for PI carriers. It is a better choice to perform PGD for high-risk carriers. For those low-risk carriers, natural pregnancy or ordinary assisted reproductive technology should be recommended.
What is known already: Each individual inversion carries has its own individual risk. Many PIs may produce chromosomally unbalanced gametes and thus

be associated with reproductive problem, and many other PIs are frequently innocuous or only associated with low risk. FISH has been used to study sperm chromosomal abnormalities for decades. However, a systematic evaluation of the relationship between the risk of PIs by FISH testing and the outcome of PGD has not been reported.

Study design, size, duration: Totally 24 male patients with autosomal PIs were performed dual-color sperm FISH analysis for risk evaluation. Among them, 10 couples with a risk greater than 10% were accepted PGD, and 8 low risk couples with a recombinant rate close to zero were recommended to routine assisted reproductive technology (ART) treatment.

Participants/materials, setting, methods: The study was set at the Reproductive and Genetic Hospital of CITIC-Xiangya, China. All the couples were infertile and seek for ART treatment. The frequency of recombinant sperm (dup(p)/del(q) and dup(q)/del(p)) was analyzed by FISH. PGD was carried out by D3 FISH or D5 SNP array.

Main results and the role of chance: A total of 934 and 1894 sperms from 24 PIs were analyzed by FISH, with a recombinant varied from 0 to 31.75%. Based on the sperm FISH results, we classify the genetic risk as three types: high risk with a recombinant sperm rate >5%; medium risk with a recombinant rate between 1 ~ 5%, and low risk with a recombinant rate close to zero. The risk was related to the size and ratio of the inverted segment of the chromosome. A total of 47 embryos were tested by PGD. Among them 30 were normal/balanced, 10 were recombinant and 7 carried other chromosomal abnormality. The 8 low risk couples accepting routine ART treatment resulted in the similar clinical pregnancies and no recombinant child was delivered.

Limitations, reason for caution: Due to the small number of inversion heterozygotes and the nature of specimens varies widely and might have an impact, there may exist a bias between the predictive value and actual reproductive risk.

Wider implications of the findings: Individual risk evaluation is important for the PI carriers. For those patients with high risk, PGD is a better choice; and for those low risk carriers, natural pregnancy or ordinary assisted reproductive technology might be acceptable.

Study funding/competing interest(s): Funding by national/international organization(s), the Major State Basic Research Development Program of China (No.2012CB944901).

Trial registration number: Not applicable.

P-425 FISH is equivalent to SNP array for preimplantation genetic diagnosis on young translocation carriers undergoing day 5 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 biopsy?

Summary answer: For young translocation carriers (age ≤ 37), D5 FISH-PGD generated higher clinical pregnancy and lower spontaneous miscarriage rate 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 634 chromosome translocation carriers with the female age under 37 years old, who were treated by D5 FISH-PGD between November 2012 and August 2013, D3 FISH-PGD between January 2005 and October 2011, and D5 SNP array-PGD between November 2011 and May 2013.

Participants/materials, setting, methods: The study was set at the Reproductive and Genetic Hospital of CITIC-Xiangya, China. Totally 634 couples were recruited, including 113 couples treated by D5 FISH-PGD, 406 by D3 FISH-PGD and 115 by D5 SNP array-PGD. 3968 8-cell embryos and 1160 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 97.5% (593/608), 87.0%(3452/3968) and 93.8%(1088/1160), respectively. In D5 FISH-PGD group, the proportions of normal/balanced embryos, clinical pregnancy rate and early miscarriage rate were 64%, 84% and 7.4% respectively for Robertsonian translocation carriers (ROBs), and 42%, 65% and 9.2% respectively for reciprocal translocation carriers (RECs). In D3 FISH-PGD group, 36%, 38% and 17% respectively were for ROBs, and 20%, 39% and 16% respectively were for RECs. In D5 SNP-PGD group, were 58%, 63% and 15% respectively were for ROBs, and 36%, 63% and 8% respectively were for RECs. D5 FISH-PGD was significantly better than D3 FISH-PGD in clinical pregnancy rate and spontaneous miscarriage; and no significant difference exists compared with D5 SNP-PGD.

Limitations, reason for caution: There is a chance of sample 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 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). The authors have no competing interests to declare.

Trial registration number: Not applicable.

P-426 Pregnancy rates after preimplantation genetic diagnoses on polar bodies using CGH

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Study question: Does CGH-based preimplantation genetic screening for aneuploidies on polar bodies improve reproductive outcome?

Summary answer: In an advanced maternal age collective (>38 years) women receiving CGH-polar body analysis before ET showed significant better implantation and pregnancy rates.

What is known already: Patients over 35 years show a rapid increase in aneuploidy rates and a decrease in pregnancy rates. Large RCTs investigating FISH-based PGS on Blastomeres and Blastocysts failed on showing a significant effect on reproductive outcome.

Study design, size, duration: Retrospective study on consecutive patients being treated between March 2012 and October 2013. 353 patients have been included. 113 patients received polar body analyses before ET and 240 did not.

Participants/materials, setting, methods: Indications for polar body analyses were advanced maternal age (AMA) or repeated implantation failure. Polar body analyses was performed using comparative genomic hybridization on all chromosomes. Primary outcome measure: pregnancy rates in the CGH vs. the control group. Secondary outcome measures: pregnancy and implantation rates in an AMA subgroup.

Main results and the role of chance: There was no significant difference in pregnancy rates in the polar-body versus the control group (42.9% vs. 36.7%, with, respectively without PGS $p = 0.352$). In the polar body group significantly less oocytes have been transferred than in the control group (1.57 vs. 1.76; $p = 0.027$).

Women of advanced maternal age (>38 years) receiving polar body analyses before ET showed significantly improved pregnancy (39.5% vs. 21.9%; OR 2.34; $p = 0.033$) and implantation rates (20.93% vs. 7.55%; $p = 0.031$).

In the control group pregnancy rates decreased significantly with age (47.4% vs. 21.9%, $p < 0.001$). In the polar body group there was no significant decrease in pregnancy rates between younger patients and patients of advanced maternal age (48.2% vs. 39.5%; $p = 0.479$).

Limitations, reason for caution: Retrospective study.

Wider implications of the findings: By applying CGH-polar body analyses in women of advanced maternal age, pregnancy rates could be increased. At the same time the risk of multiple pregnancies might be minimized in the CGH-group by transferring fewer embryos. Not least polar body analyses could offer

an alternative in countries with rigid law restrictions on PGS and patients opposing diagnoses on blastomeres and blastocysts out of religious reasons.
Study funding/competing interest(s): Funding by hospital/clinic(s). None.
Trial registration number: None.

P-427 Meiotic chromosome behaviors and sperm aneuploidy in an infertile man with 46, XY/45, X (50/50) mosaic karyotype

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Study question: What is the pattern of meiotic segregation, meiotic recombination, and sperm aneuploidy in an infertile patient with 46, XY/45, X mosaicism?
Summary answer: Testicular sperm of the patient showed higher rates of sex nullisomy, XY disomy and trisomy 18 compared to control fertile men, whereas spermatocytes did not display increased rates of meiotic recombination and synaptic defects.
What is known already: The formation of the synaptonemal complex (SC) between sister chromatids aids in crossing over and ensures proper chromatid segregation at the first meiotic division. Loss of recombination events is thought to lead to errors in segregation and thus sperm aneuploidy. Our previous studies demonstrated that meiotic recombination defects increase the risk of sex chromosomal aneuploid sperm in infertile men with normal karyotype (Ferguson et al., 2007).
Study design, size, duration: A 27 years old infertile man (proband) with azoospermia and 45X/46XY (50/50) mosaic karyotype participated in this study. Testicular tissues from the proband and five fertile men (controls) were obtained. Global recombination, sex body recombination, discontinuities in SC synapsis and sperm aneuploidy rates were analyzed.
Participants/materials, setting, methods: Spermatocytes from the proband and controls were immunostained with antibodies against MLH1, SCP3, SCP1, and CREST to characterize recombination sites, SC and centromeres respectively. Fluorescent in situ hybridization (FISH) for chromosomes 18, X and Y in sperm was used to determine aneuploidy rates in the patient and control men.
Main results and the role of chance: The proband's sperm (n = 1702) showed increased levels of sex nullisomy (1.7% vs. 0.24%, $P < 0.001$), XY disomy (2.0% vs. 0.21%, $P < 0.001$), and disomy 18 (0.18% vs. 0.085%, $P < 0.05$) compared to control men (n = 5831) (Chi squared test). The ratio of X:Y sperm in the patient was significantly different from the controls ($P < 0.001$), indicating that some 45, X germ cells completed the first meiotic division and the sex nullisomic cells were arrested during the second meiotic division. Spermatocytes of the patient (n = 101) and controls (n = 414) displayed no significant difference in the frequency of crossovers per cell. Sex chromosome recombination rates and frequency of SC asynapsis between the controls and patient also did not differ significantly, suggesting that some 46, XY cell lines have the capacity of normal meiotic division.
Limitations, reason for caution: Testicular tissues from men undergoing vasectomy reversals were used as controls for this study. Although the men were proven to be fertile prior to the vasectomy procedure, the quality of the testicular sperm and pattern of spermatogenesis might have been impacted after the procedure with time.
Wider implications of the findings: Despite a high level of sex chromosomal mosaicism in the patient's somatic cells, the majority of sperm contain normal chromosomal complements. The important clinical implication from this unique case is that the testicular sperm from this man were predominantly normal in chromosome constitution. This suggests that men with high levels of mosaicism and azoospermia can produce normal sperm, which can be retrieved from the testis for use in ICSI.
Study funding/competing interest(s): Funding by national/international organization(s), Canadian Institutes of Health Research.
Trial registration number: Not applicable.

P-428 The applicability of targeted capture and massively parallel sequencing in preimplantation genetic diagnosis for monogenic diseases

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Study question: Can targeted capture and massively parallel sequencing MPS) be applied for preimplantation genetic diagnosis (PGD) of monogenic diseases?
Summary answer: Targeted MPS can be used for PGD of monogenic disease. It can provide an accurate and cost-effective PGD method with short turnaround time.
What is known already: PGD are available for over one hundred of monogenic diseases. Many methods have been developed, but most of them needs a long procedure of designing and optimizing for the family-specific detection, which is a labour-intensive and time-consuming process.
Study design, size, duration: For this proof-of-concept study, a preliminary experiment was carried out to evaluate the accuracy of target-MPS at single-cell level at first. Then targeted-MPS was performed on excess PGD for monogenic disease samples provided by the collaborating IVF center. The accuracy, cost, turnaround time and limitation of the targeted MPS based family pedigree method for PGD was studied.
Participants/materials, setting, methods: Genomic DNA and 4 whole genome amplification (WGA) product of single lymphocyte from the same healthy woman were used in the preliminary experiment. Excess samples from PGD for monogenic disease, including genomic DNA from father, mother and affected daughter, as well as single blastomere cell WGA products from eleven blastomeres were used for the study. Double-blind design was taken in the study. Targeted MPS and family pedigree haplotype analysis was adopted in the method.
Main results and the role of chance: Despite the influence of PA and ADO for the WGA samples, an average of 104 informative SNPs flanking both sides of the disease causing mutation were detected for each embryonic haplotype. The detected informative SNPs and gene mutation were closely linked, the average interval between them was within 10 kb. The whole test was finished within a week, with the final result 100% consistent with the result provided by the reference laboratory, at a reagent cost of about 100 US dollars per sample in our study.
Limitations, reason for caution: The whole process of testing will require about a week, so it cannot meet the requirement of fresh embryo transfer to finish the test within 2 days at the present.
Wider implications of the findings: The new targeted MPS based method will make the PGD for monogenic disease more accurate, faster and more affordable, providing 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).
Trial registration number: Not applicable.

P-429 Association between embryonic genotype for the TP53 codon 72 polymorphism (rs1042522) and clinical outcomes after ART

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Study question: Is there an association between embryonic genotype for the TP53 codon 72 polymorphism (encoding Arginine (Arg) or Proline (Pro)) and clinical outcomes after IVF/ICSI?
Summary answer: Couples with embryos carrying only the Arg/Arg genotype at codon 72 of TP53 exhibited increased implantation after IVF/ICSI.
What is known already: The TP53 protein plays a critical role in maintaining genomic stability and mediates pregnancy by regulating the activation of steroid hormones and functionally related genes essential for fetal-maternal interactions during implantation. The literature provides evidence that maternal

genotype for the TP53 codon 72 polymorphism is associated with repeated implantation failure after IVF/ICSI or recurrent miscarriages (best prognosis with Arg allele). However, no studies have yet determined the relevance of paternal and embryonic genotypes.
Study design, size, duration: A prospective cohort study was performed on 354 couples submitted to IVF/ICSI and recruited from 03/2012 to 07/2013. The couples were divided into two groups according to their TP53 genotype combinations: Arg/ArgxArg/Arg (because the Arg allele is correlated with the best fertility prognosis) and all of the other genotypes combined.
Participants/materials, setting, methods: DNA was extracted from peripheral blood samples taken from each participant. The The TP53 codon 72 SNP (rs1042522) (Arg/Pro) was genotyped by real-time PCR. Cumulative results (including fresh and frozen cycles) were analyzed. Hardy-Weinberg genotype distributions of the entire sample indicated concordance between the observed and expected frequencies.
Main results and the role of chance: Characteristics such as age, infertility etiology, number of transfers and number of transferred embryos were not significantly different ($P > 0.05$) between the groups.

Couples with embryos that only carried the TP53 codon 72 Arg/Arg genotype experienced increased implantation rates compared with couples with embryos with other possible genotypes (Table 1).
Limitations, reason for caution: Additional validation of the analyzed SNP (increasing the number of cases) will be important to provide more information about the potential use of this polymorphism. Differences in the genetic backgrounds of various ethnic populations should also be considered.
Wider implications of the findings: The ability to predict implantation using genetic markers during IVF/ICSI treatment can encourage patients to undergo additional cycles of ART. In the group with the best prognosis (all embryos with the Arg/Arg genotype), the number of transferred embryos could be reduced to avoid complications associated with multiple pregnancies.

Table 1 | Results.

General features and clinical outcomes	Couples' genotype combinations		
	ARG/ARGxARG/ARG	PRO/PROxPRO/PRO, ARG/PROxPRO/PRO, ARG/ARGxPRO/PRO, ARG/PROxARG/PRO, ARG/ARGxARG/PRO	*P*
N	87	267	
Embryo genotype	ARG/ARG	ARG/ARG, ARG/PRO, PRO/PRO	
Implantation rate	23.8%(57/239)	17.1% (142/829)	0.02
Clinical pregnancy rate/patient	51.7% (45/87)	42.7% (114/267)	0.17
Clinical pregnancy rate/transfer	36.9%(45/122)	30% (114/380)	0.17

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 authorized by the local ethics committee.

P-430 Preimplantation genetic screening is not necessary for blastocysts resulting from vitrified donor eggs

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Study question: Prevalence of aneuploidy in human embryos derived from frozen donor eggs is unknown.
Summary answer: Aneuploid rate was low in human blastocysts derived from frozen donor eggs. However, reduced pregnancy and implantation were obtained if the blastocysts were biopsied for aneuploidy screening and then either fresh or frozen transfer was performed.
What is known already: Increased embryonic aneuploid rate was found in the patients with advanced maternal ages and increased embryo implantation was reported after transfer of euploid embryos selected by preimplantation genetic screening (PGS). Development and implantation of human embryos derived from frozen donor eggs are comparative to fresh eggs.
Study design, size, duration: Total 764 frozen eggs from 75 egg thawing cycles were warmed from September of 2012 to August of 2013 and 37 blastocysts from 8 cycles were biopsied for PGS before embryo transfer.
Participants/materials, setting, methods: Infertile patients with advanced maternal ages chose frozen eggs from anonymous donors in the egg bank at Houston Fertility Institute. Eggs were cryopreserved by vitrification and PGS was performed on 8 cycles in which patients requested aneuploid screening by DNA microarray.
Main results and the role of chance: A 97.1% of egg survival rate was obtained in the study period and 59.1% of embryos developed to blastocyst stage. After biopsy and PGS in 37 blastocysts from 8 cycles, 86.5% were euploid and transfer of euploid blastocysts either fresh or frozen resulted in low pregnancy and implantation rates as compared with blastocyst transfer without biopsy and PGS.
Limitations, reason for caution: This study was carried out on limited case numbers and further large number of cycles is necessary to investigate the reason in which reduced embryo implantation being observed after transfer of biopsied embryos from frozen eggs.
Wider implications of the findings: Aneuploidy rate is low in the blastocysts derived from frozen donor eggs and PGS may not be necessary for embryos derived from donor eggs.
Study funding/competing interest(s): Funding by hospital/clinic(s), there was no external funding for this study and all authors have nothing to disclose.
Trial registration number: NA.

P-431 The selection of cryopreserved euploid blastocysts with array comparative genomic hybridization (ACGH) increases pregnancy outcome across all age groups

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Study question: How does aCGH affect the efficacy of selecting euploid blastocysts and pregnancy rate across all age groups?
Summary answer: aCGH is able to select the euploid blastocyst efficiently and increases pregnancy rate significantly once the euploid embryo is transferred. The effect of aCGH on pregnancy rate is markedly significant in patient age group of 38 years old or older.
What is known already: Numerous studies on human oocytes and embryos derived from IVF cycles have shown that aneuploidy is the most common abnormality which contributes to poor reproductive outcomes and miscarriages in IVF treatment. aCGH was introduced to screen all 23 pairs of human chromosomes in order to improve the efficiency of IVF treatments. This study is to investigate the impact of aCGH on clinical outcome across all age groups.
Study design, size, duration: A retrospective analysis of clinical and embryological database.
Participants/materials, setting, methods: A total of 821 blastocysts were biopsied for aCGH testing among 134 patients with their ages ranging between 29 to 47 years old.
Main results and the role of chance: A total of infertile women were included in the study. In the age group less than 35 years old and the age group between 35 and 37 years old, 55.65% ($n = 115$) and 50.33% ($n = 153$) euploid embryos were identified respectively. On the other hand, in the age group between 41 and 42 years old and the age group older than 42 years of age, 17.77% ($n = 197$) and 14.56% ($n = 158$) euploid embryos were identified respectively. The pregnancy rate of transferring an euploid embryo is 70.6% (48/68) Per transfer across all age groups.
Limitations, reason for caution: The study is limited by the number of patients in each age group. Among 115 blastocysts and 197 blastocysts that were analyzed respectively.
Wider implications of the findings: The study demonstrates that aCGH in combination with embryo banking can significantly improve the implantation rate in age group that are 40 years old and older age group.
Study funding/competing interest(s): Funding by hospital/clinic(s), New Hope Fertility Center.
Trial registration number: None.

P-432 Are female carriers of inversions at risk for accelerated ovarian aging

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Study question: What is the clinical relevance of chromosomal inversions among female carriers regarding ovarian reserve?
Summary answer: Diminished ovarian reserve (DOR) among female carriers of inversion (9) (41%) is significantly higher when compared to DOR in infertile patient population having no risk factor for DOR (25.5%).
What is known already: Inversions are structural chromosomal abnormalities, which result from breakage and rotation before reunion of the inverted segment. One of the most common structural balanced chromosome rearrangements is inv (9) (p11q12). Pericentric inversions result from a two-break event in which there is a break in each arm including the centromere. An inversion does not usually have phenotypic effect when it is a balanced rearrangement. However, infertility, miscarriages and/or chromosomally unbalanced offspring are reported in inversions carriers.
Study design, size, duration: A total of 16119 patients undergoing 23673 IVF-ICSI cycles between years 2001–2014 were analyzed. Our primary aim was to compare the rate of diminished ovarian reserve (DOR) in inversion carriers to the rate among all infertile patients.
Participants/materials, setting, methods: We excluded patients having risk factors for DOR such as age 40 and above, history of gonadotoxic therapy or ovarian/tubal surgery, and abnormal karyotype. After exclusions the ratio of DOR in 10973 and 54 patients from infertile patient population and inversion carriers were compared, respectively. For statistical comparison chi square test was used.
Main results and the role of chance: Among infertile couples with a known female karyotype, 1.6% (54/3299) was inversion carriers. Of 54 patients 47 patients were inversion 9 carriers and included in comparison analysis except 2 patients who had additional chromosomal abnormalities and thus excluded. After excluding other risk factors for DOR 16 of 39 patients (41%) and none of 5 patients of inv (9) and other inversion carriers had DOR, respectively. Because of the small sample size we did not include other inversion carriers for comparison. DOR was significantly higher among female inv (9) carriers when compared to patients with DOR within our infertile patient population (41% vs. 25.5%, $p = 0.027$).
Limitations, reason for caution: The main limitation of our study is the small sample size of female inversion carriers. However this is the largest group of patients with female inversion carriers reported to date investigating the clinical relevance of this abnormal karyotype regarding ovarian reserve.
Wider implications of the findings: Any microduplication/deletion involving the inverted segment and/or in the break points may result in disrupt oogenesis depending on the size and the distribution of euchromatin regions, leading to accelerated ovarian aging and infertility. This position effect may cause a change in the expression of the genes related with the sexual development and oogenesis.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), none.
Trial registration number: None.

P-433 First study to review the outcome of preimplantation genetic diagnosis (PGD) for fragile X syndrome, using pre preimplantation genetic haplotyping (PGH)

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Study question: We sought the outcome of PGD in fragile X syndrome. We compared the outcome in Permutations carriers with full mutations.
Summary answer: PGD for fragile X syndrome is feasible for a number of couples. The outcome is worse in PGD cycles with permutation carriers compared to full mutations. Cycles with FRAX Permutations carriers was associated with nearly 2 folds decrease in the odds of the clinical pregnancy compared to with full mutation.
What is known already: Fragile X syndrome is the most common single cause of severe mental retardation after Down's syndrome. The disorder is inherited as an X-linked trait**.** Fragile X mutations can be divided into permutations and full mutations. Permutations involve a limited increase in the number of CGG repeats ranging between 50–60 and 200 repeats. Full mutations involve larger expansions of 200 CGG repeats up to even several thousand repeats. Permutations are not associated with obvious clinical manifestations; they are found in unaffected carrier females and normal transmitting males. Women with a permutation are at increased risk of premature ovarian failure and linked with reduced ovarian reserve. Permutations are unstable when transmitted by a carrier mother, and can become full mutations in both male and female offspring.
Study design, size, duration: Prospective study of 40 cycles PGD fragile X syndrome between January 2008 and December 2013. 29 cycles permutations and 11 full mutations.
Participants/materials, setting, methods: We analyzed the outcome of 40 PGD cycles for Fragile X syndrome performed. 29 cycles permutations and 11 full mutations. We used pre preimplantation genetic haplotyping (PGH), as it is a rapid way of developing indirect test of X-linked disease**.**

Regression analysis was performed to study the impact of different factors (AMH, age, number of oocytes retrieved, no of biopsied and replaced embryos) on CPR. Statview software was used for statistical analysis.
Main results and the role of chance: In PGD cycles with Fragile X syndrome permutations ($n = 29$) the mean number of oocytes retrieved and embryos biopsies were significantly lower 8.4 and 4.4 respectively, compared with a mean of 18.5 and 7.8 respectively in cycles with Fragile X syndrome full permutations ($n = 11$).

The overall CPR/ PGD cycle started was 20%. CPR: 7.5 % in cycles with Fragile X syndrome permutations, compared to 12.5% in cycles with Fragile X syndrome full permutations.
Limitations, reason for caution: Further large prospective studies are needed to confirm our study findings. Pregnancy could still be achieved in cycles with Fragile X syndrome permutations.
Wider implications of the findings: This is first study to compare the Outcome between the FRAX Permutations carriers with full mutations. Cycles with FRAX Permutations carriers was associated with nearly 2 folds decrease in the odds of the clinical pregnancy compared to with full mutation. The clinical pregnancy rate wan not compromised in the full mutations group. Fragile Xa premutation carriers should be advised not to postpone reproduction for too long, and should be counselled about poor outcome.
Study funding/competing interest(s): Funding by hospital/clinic(s), GSTT.
Trial registration number: None.

P-434 The detection of single aneuploidies by Polar Body (PB) analysis is predictive of the euploid/aneuploid condition for that chromosome in the corresponding embryo

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Study question: Is the testing of Polar Bodies predictive of the embryonic chromosome status when single aneuploidies are detected?
Summary answer: When single aneuploidies are detected by testing PBs, the corresponding abnormality is found in the resulting embryos. In addition, in case of single anomaly compensation, the resulting embryos were confirmed to be euploid for that chromosome.
What is known already: The removal of PBs from fertilized oocytes is the less invasive form of biopsy for PGS purposes. However, beside being informative for the maternal counterpart only, the accuracy of PB testing has been reported to be low due to the high incidence of post-zygotic events. Based on these considerations, discarding the biopsy at the cleavage stage because of mosaicism, the biopsy of trophectoderm cells is considered to provide the most reliable results for PGS.
Study design, size, duration: This is a prospective study performed between October 2012 and December 2013 including 20 infertile couples (38.9 ± 2.50) undergoing PGS by array comparative genomic hybridization (aCGH) on PBs. In case of single or compensated aneuploidies, the corresponding embryo was biopsied and analyzed by fluorescence in situ hybridization (FISH) or aCGH.
Participants/materials, setting, methods: A total of 35 fertilized oocytes were diagnosed aneuploid for one single chromosome ($n = 29$) or euploid after single aneuploidy compensation ($n = 6$). The corresponding embryos

were biopsied at the cleavage (n = 10) or blastocyst stage (n = 25) and analyzed by FISH or aCGH to verify the condition of aneuploidy predicted by PBs' testing.
Main results and the role of chance: The chromosome abnormality identified by the aCGH on PBs from 29 oocytes was detected in the corresponding embryo. More specifically, in 22 cases the embryo carried that single aneuploidy, while in the remaining 7 cases beside that single aneuploidy, an additional chromosome was found to be aneuploid. The 6 oocytes with a single aneuploidy in the first PB compensated by the opposite aneuploidy in the second PB generated embryos, which were found to be euploid for that chromosome. The analysis of the embryos was performed by FISH in 31 cases and by aCGH in the remaining 4 cases. According to these results, the chromosome status predicted by PB analysis was confirmed in all corresponding embryos, both at the cleavage and blastocyst stage.
Limitations, reason for caution: The testing of PBs can only predict the maternal contribution to aneuploidy. In addition, the majority of the embryos were analyzed by FISH to verify the status of the chromosomes found to be aneuploid. This does not exclude that aneuploidies for other chromosomes could appear in the developing embryo.
Wider implications of the findings: The data reported here demonstrated that the aneuploid condition for single chromosomes predicted by PB analysis was always reflected in the corresponding embryos. Although this does not prevent the appearance of other abnormalities contributed by the sperm or first mitoses, it is clear that the oocyte has a predominant role in determining the embryo chromosome status.
Study funding/competing interest(s): Funding by hospital/clinic(s), Crivello, Magli, Pomante, Crippa, Valerio, Ferarretti, Gianaroli.
Trial registration number: Not applicable.

P-435 Establishment of a combined day 5/day 6 trofectoderm biopsy strategy 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 is similar for both day 5 and day 6.
What is known already: Contemporary preimplantation genetic screening (PGS) requires a trophectoderm biopsy on developing good quality blastocysts. However, not all fertilized embryos develop blastocyst stage at similar rates. If fresh transfer is planned on day 6, only the embryos that are available for biopsy on day 5 are taken into account for analysis and embryo transfer (ET). 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.
Study design, size, duration: This retrospective comparative study has been performed in Bahceci Fulya Assisted Reproductive Technology Centre between January 2013 – January 2014. It includes 134 consecutive CCS cyles in which 349 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 was planned for the next suitable time frame with hormone replacement therapy. Within the study period, 275 embryos were biopsied on day 5 (group I) and 88 on day 6 (Group II) and vitrified individually.
Main results and the role of chance: Mean female age in the study group was 36.1 ± 5.1. No normal embryo was found and the ET was cancelled in 45 cycles (33.6%). Transfer of 24 normal blastocysts have so far resulted in 14 clinically confirmed pregnancies (63.6%). Results of the CCS analysis as well as the distribution of chromosomal abnormalities for each biopsy day are shown in table.

Day of biopsy	Day 5	Day 6
N	261	88
Not analyzed	2.7%	3.4%
Normal	28.7%	31.8%
Abnormal	71.3%	68.2%
Complex abnormalities	45.3%	34.5%
Monosomies	17.7%	25.9%
Trisomies	29.3%	29.3%
Deletions/duplications	7.7%	10.3%

Limitations, reason for caution: The number of cases and the embryos analyzed are the main 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 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: None.

P-436 Re-defining the concept of advanced maternal age in Indian patients with implantation failure

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Study question: Is embryo aneuploidy, as the main parameter of advanced maternal age, advanced in Indian population compared to Caucasian patients?
Summary answer: Indian patients with implantation failure (IF) have a significant increase in embryo aneuploidy rates after 33 years old (62.2% vs. 87%, p = 0.0028) suggesting a premature reproductive advancement in aging due to their racial origin as previously suggested.(1)
What is known already: Chromosome aneuploidies in preimplantation embryos can be the cause of infertility in patients with IF. Higher aneuploidy rates are found with the increase in maternal age. In Caucasian population, different age ranges have been established for the advanced maternal age (AMA) indication, nowadays it is mainly accepted above 38–40 years old. Regarding ovarian reserve, Indian infertile women seems to age 6 years before Caucasians (1), but embryo aneuploidies have not been tested in the Indian population.
Study design, size, duration: Retrospective analysis of the Comprehensive Chromosomal Screening (CCS) cycles performed in Indian patients with implantation failure from September 2013 to January 2014. Twenty-two patients with ≥3 implantation failures were divided according to their age in: ≤33 years old (group A = 8 patients, range 27–33), and ≥34 years old (group B = 14 patients, range 34–44).
Participants/materials, setting, methods: Day-3 embryo blastomere biopsy was done in 122 embryos. DNA was amplified and arrayCGH protocol performed (BlueGnome Ltd., Cambridge, UK). Euploid day-5 blastocysts were transferred in the same cycle. Results in both groups of patients were compared in terms of embryo aneuploidy rate, pregnancy rate per transfer (PR/ET) and pregnancy rate per cycle (PR/cycle).
Main results and the role of chance: A total of 27 out of 122 embryos analyzed were chromosomally normal (22.1%). In 63.6% (14/22) of the cases at least one chromosomally normal embryo was available for transfer, with a PR/ET of 64.3% (9/14), and PR/cycle of 40.9% (9/22).

Interestingly, the cut-off of maternal age at 34 years identified a statistically significant threshold in embryo chromosomal abnormalities detected: 62.2%

(28/45) versus 87% (67/77), $p = 0.0028$. At the clinical level the results were embryo transfer rate of 87.5% (7/8), PR/ET 71.4% (5/7) and PR/cycle 62.5% (5/8) in group A, while in group B embryo transfer rate was 50.0% (7/14), PR/ET 57.1% (4/7) and PR/cycle 28.6% (4/14).

Limitations, reason for caution: This is a retrospective study including a small sample size. Pregnancy rates are very promising, but live birth rate and miscarriage need to be added in the coming months.

Wider implications of the findings: This is the first experience of CCS using arrayCGH in Indian patients with IF and results are encouraging. Most importantly, we reconfirmed previous preliminary data(1) suggesting a 6-years difference in the ovarian reserve lifespan between Caucasian and Indian women that is reconfirmed in this report by the significant increase in the embryo aneuploidy rate in Indians patients over 33 years.

References

(1) Iglesias, C. et al. "Ethnic differences in ovarian aging between Caucasian and Indian women". Fertility and Sterility, Volume 100, Issue 3, Supplement, September 2013, Pages S152–S153.

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

Trial registration number: None.

P-437 Preimplantation genetic diagnosis, prenatal diagnosis and other reproductive options for couple carriers of genetic diseases: comparison of the efficacy and acceptance

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Study question: We examine reproduction patterns in carriers of genetic diseases who are offered the possibility of preimplantation genetic diagnosis.

Summary answer: Our data suggest that most pregnancies among carriers of genetic diseases occur as a result of natural conception with prenatal diagnosis, followed by preimplantation genetic diagnosis (PGD) and, less frequently, gamete donation and natural conception without prenatal diagnosis.

What is known already: There are several reproductive options available to couples who are carriers for genetic diseases: prenatal diagnosis, natural conception without prenatal diagnosis, preimplantation genetic diagnosis, gamete donation and adoption.

Study design, size, duration: We have examined reproduction patterns in carriers seen at Hospital Virgen del Rocio from 2005 to 2012. This public hospital is the Andalucia (Spain) referral hospital for preimplantation genetic diagnosis. At hospital we give genetic counseling and free preimplantation genetic diagnosis is offered for carriers without unaffected children.

Participants/materials, setting, methods: The study group included 279 couples who came to the hospital Virgen del Rocio for genetic counseling and they were offered free preimplantation genetic diagnosis. Between June 2013 and December 2013 they were telephoned and were asked about their reproductive choices.

Main results and the role of chance: Of the 279 couples who were involved in the study, 41 got pregnant as a result of PGD cycles at our hospital. Of the 238 couples who did not become pregnant, 57% did not have any children. Of those couples who had children, 76% elected prenatal diagnosis, 10% preimplantational genetic diagnosis in other hospital, 8% gamete donation and 6% natural conception without prenatal diagnosis. Of those pregnancies with prenatal diagnosis, 23.6% ended in pregnancy termination. Of those pregnancies without any prenatal o preimplantation diagnosis, 50% resulted in affected children (haemophilia children). Of those 41 couples who got pregnant as a result of PGD cycles, three couples had children later: they chose PGD, prenatal diagnosis and natural conception without prenatal diagnosis, respectively.

Limitations, reason for caution: There were neither limitations nor reasons for caution.

Wider implications of the findings: Most of the couples who are carriers of genetic diseases opt for conventional prenatal diagnosis. Preimplantational genetic diagnosis is acceptable to patients and remove the difficult decision on whether to terminate an affected pregnancy. The main disadvantage is the low success rate. But this technique is still in the development process.

Study funding/competing interest(s): Funding by hospital/clinic(s), Hospital Universitario Virgen del Rocío (Sevilla).

Trial registration number: My study is no a RCT.

P-438 Fetal fraction of cell free fetal DNA from maternal plasma is higher for in vitro fertilization (IVF) pregnancies then in natural conceived ones

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Study question: Are fetal fraction of cell free fetal DNA (cff DNA) levels from maternal plasma different in IVF pregnancies compared with natural conceived pregnancies?

Summary answer: Levels of fetal fraction of cff DNA in maternal plasma is increased in pregnancies obtained by IVF in comparison with natural conceived pregnancies.

What is known already: In IVF pregnancies the serum level of free beta HCG in first trimester is increased in comparison with natural conceived pregnancies and should be taken into account when conventional methods (biochemical screening) for fetal aneuploidy screening are applied. Fetal fraction of cff DNA is a parameter used to evaluate the predictive value of fetal DNA noninvasive prenatal test (NIPT), but the modification in relation to IVF was not established yet.

Study design, size, duration: We performed a cross-sectional case control study including pregnant women consecutively evaluated in Medlife MMF Department between March and November 2013. Patients were divided in two groups: group A 30 patients with IVF pregnancy and group B (154 patients) with natural conceived pregnancy.

Participants/materials, setting, methods: Patients were referred to our Department for prenatal screening. Blood samples were collected between 9 and 13 weeks + 6 days of gestation in all subjects for measurement of plasma cff DNA concentrations by RT-PCR amplification and for maternal biochemical serum test by standardized clinical protocol, part of first trimester screening.

Main results and the role of chance: The two groups were similar in terms of age, body mass index and gestational age. Samples from pregnant women who used egg donors and surrogates, were not accepted. The fetal fraction of cff DNA in samples were determined by Natera laboratory as a part of NIPT for detection of fetal aneuploidy. Only in 4 (2%) of cases the detection of cff DNA in maternal plasma were not possible. The fetal fraction of cff DNA concentration was significantly higher in IVF cases comparing to natural conceptions (11.2% ± 5.14 vs 7.88% ± 5.78, $p < 0.05$). The sensibility of the fetal DNA NIPT for detection of trisomy 13, 18 and 21 was 100%. There were 2 cases of trisomy 21 confirmed after abortion.

Limitations, reason for caution: There is a relatively small sample size of patients with IVF obtained pregnancies. The results of our study should be confirmed by larger studies.

Wider implications of the findings: Since cff DNA appears to be an independent maternal serum marker and its positive predictive value is higher when the fetal fraction is higher, it can be used as an additional marker for Down syndrome screening without adjustment for IVF pregnancy.

Study funding/competing interest(s): Funding by hospital/clinic(s), MEDLIFE S.A. Bucharest.

Trial registration number: The study is not a RCT.

P-439 Morphokinetics and aneuploidy risk on human embryos: are they really correlated?

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Study question: Can embryo morphokinetic help in creating new models of aneuploidy risk selection?

Summary answer: Chromosomally normal and abnormal blastocysts seem not to have statistically significant different kinetic behaviour.

What is known already: Chromosomally normal and abnormal embryos have different kinetic behaviour and algorithms have been proposed to increase the probability of selecting chromosomally normal embryos. T5-t2, cc3 and delayed blastulation result the most relevant variables. But a very recent debate was opened to put a cautionary note on this assessment.
Study design, size, duration: Retrospective study from may to december 2013 of 89 blastocysts biopsied on Day 5–6, analyzed for aneuploidies with Array-CGH technique and cultured in a time-lapse imaging technology incubator. Blastocyst morphokinetic was evaluated by a unique operator.
Participants/materials, setting, methods: Thirty couples of patients enrolled for progressed recurrent implantation failure, recurrent miscarriage, severe male factor infertility, previous aneuploidy or advanced maternal age (>39). Female age mean ± standard deviation was 38.3 ±3.6. Embryos were recorded in Embryoscope; Unisense Fertilitech, since immediately after insemination. Student's *t*-test was used for *P*-values.
Main results and the role of chance: Morphokinetic and chromosomal assessment of 89 biopsied blastocysts were analyzed. Of the 56 day-5 biopsied blastocysts, 50% were aneuploids. Of the 33 day-6 biopsied blastocysts, 51.5% were aneuploids. Blastocysts were then divided into two groups: group A (32 blastocysts achieved by 9 women <36 aged) and group B (57 blastocysts achieved by 21 women ≥36 aged). As expected, percentage of aneuploids was lower in group A (34.3% vs. 59.6%). All morphokinetic variables were evaluated since timing of second polar body extrusion (tPB2) to blastocyst formation (tB). Also cell cycles were considered: cc2, cc3, s2, s3, t5-t2, tB-tM. No statistical difference was found comparing euploid to aneuploid embryos either within and between the two age groups.
Limitations, reason for caution: Limited number of the study group. Further studies are needed.
Wider implications of the findings: To create new non-invasive models of selecting chromosomally normal embryos.
Study funding/competing interest(s): Funding by hospital/clinic(s), Tecnobios Procreazione clinic, Bologna, Italy.
Trial registration number: Not applicable.

P-440 What to expect if you are a chromosomal rearrangement carrier: prognostic values after Preimplantation Genetic Diagnosis (PGD)

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Study question: To give a prognostic value in reproductive terms for chromosomal rearrangement carriers (reciprocal translocation, RECT, Robertsonian translocation, ROBT, and inversion, INV, carriers).
Summary answer: RECT carriers have worst prognostic than ROBT and INV carriers in terms of euploid (transferable) embryos.
What is known already: Carriers of chromosomal rearrangements (RECT, ROBT, INV) are at risk of implantation failure, repeated abortion and the pregnancy with an affected child. They produce a high number of unbalanced gametes due to the different segregation patterns. They are also at risk of aneuploidy embryos due to maternal age effect and interchromosomal effect.
Study design, size, duration: A retrospective study was performed in 122 PGD cases: 63 of RECT carriers, 40 of ROBT carriers and 19 of INV carriers. Cases were performed between 2010 and 2013. They belong to a 103 different patients. Overall, 692 blastomeres (366 from RECT, 223 from ROBT, 103 from INV) were analyzed.
Participants/materials, setting, methods: One blastomere of each embryo was biopsied on Day +3 of development and processed for Whole Genome Amplification (WGA). The amplification product was analyzed by means of arrays of Comparative Genome Hybridization (aCGH) according to manufacturer's protocol (24Sure, BlueGnome®). Maternal age mean for each group was: 33.68, 33.41, 35.59, respectively.
Main results and the role of chance: 13.02% of RECT, 20% of ROBT and 34.44% of INV carriers' embryos were euploid (transferable). There are statistically significant differences between the 3 groups (RECT vs ROBT, $p = 0.0379$; RECT vs INV, $p < 0.0001$; ROBT vs INV, $p = 0.0119$). Aneuploidy rate was: 45.56% for RECT, 58.54% for ROBT and 56.67% for INV. There are statistically significant differences between RECT and ROBT groups ($p = 0.0035$). The percentage of unbalanced embryos was: 67.16% for RECT, 30.24% for ROBT and 10% for INV. There are statistically significant differences between the 3 groups (RECT vs ROBT, $p < 0.0001$; RECT vs INV, $p < 0.0001$; ROBT vs INV, $p = 0.0001$). Most of the abnormal embryos in RECT carriers are due to unbalances in the rearranged chromosomes, while in ROBT and INV are due to aneuploidies in non-rearranged chromosomes.
Limitations, reason for caution: Although the cohort size is acceptable, more studies are needed to confirm these data.
Wider implications of the findings: This study gives a prognostic value for patients carriers of a chromosomal rearrangement in terms of reproductive chances. It also explores the type and causes of the high percentage of abnormal embryos usually found between these groups of patients.
Study funding/competing interest(s): Funding by hospital/clinic(s), own funding (Reprogenetics Spain). Retrospective study.
Trial registration number: None.

P-441 Identifying the key regulating genes in DOR by studying interaction and enriched sub-networks pathways

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Study question: Are there key regulating genes which are effective in Diminished Ovarian Reserve (DOR) and infertility?
Summary answer: Effective genes in DOR may provide more insight into the etiology of DOR and infertility through network interactions.
What is known already: Diminished ovarian reserve (DOR) as one of infertility reason not only affects old ages as expected but also involves young women as well. Ovarian reserve (OR) assessment can be used as a new prognostic tool for infertile treatment decision.
Study design, size, duration: Constructing interaction networks based on the biological processes.
Participants/materials, setting, methods: up and down regulated gene expression profiles of granulosa cells were analyzed to generate a putative interaction map of these genes including proteome map based on biological processes and molecular functions of proteins.
Main results and the role of chance: Eleven up-regulated genes as well as nine down-regulated genes were identified and assessed by constructing interaction networks based on the biological processes. PTGS2, CTGF, LHCGR, CITED, SOCS2, STAR and FSTL3 were the key nodes in up-regulated networks while IGF2, AMH, GREM, FOXC1 proteins did the same job in down regulated networks. MIRN101-1, MIRN153-1 and MIRN194-1 inhibited the expression of SOCS2, while CSH1 and BMP2 positively regulated IGF1 and IGF2.
Limitations, reason for caution: Interaction network prediction.
Wider implications of the findings: Large scale data production from experimental studies such as genome sequencing, microarray and next generation sequencing have produced large quantity of data which are very useful materials for bioinformatics analysis. Therefore various computational strategies have been employed to discover genes and proteins structures through their expression and function. Dynamic protein interaction network prediction at the molecular (Molecular function) or cellular levels (biological process) to identify domain interactions, co-expression, phenotypes, sequence and protein structure have already been reported (Shoemaker and Panchenko, 2007).

Herein, protein interaction networks of up- or down-regulated genes drawn to investigate the underlying biological processes governing these genes' functions and operations and also to predict any novel interactions there.
Study funding/competing interest(s): Funding by University(ies), Tabriz University od Medical university.
Trial registration number: Nil.

P-442 The role of septin 12 codon 474 G > A polymorphism in male infertility

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Study question: Does SEPTIN12 codon 474 G > A polymorphism have a role in with male infertility?
Summary answer: In the teratozoospermia group, significant neck anomalies are detected in patients with GG genotype, compared to those with GA genotype. In the unexplained infertility group, the presence of the A allele is found to be correlated with decreased sperm concentration and motility.
What is known already: 15–20% of the male infertility cases have a genetic basis. Some single nucleotide polymorphisms (SNP) in genes involved in sperm production and testicular function are found to be risk factors for infertility and cause lower sperm counts and decreased motility. Therefore, SNPs should be studied in various populations to resolve its genetic basis. In one study (Lin et al. 2012), SEPTIN12 codon 474G > A polymorphism was shown to be a male infertility risk factor.
Study design, size, duration: Association of SEPTIN12 codon 474 G > A polymorphism with male infertility was investigated in 164 fertile and 115 infertile men. The patient group is classified according to sperm defects and the effects of the genotypes on sperm parameters were also examined.
Participants/materials, setting, methods: Cheek epithelial cells were collected upon informed consent of the individuals. Following DNA isolation, SEPTIN12 gene was amplified, codon 474 G > A polymorphism was detected by restriction fragment length polymorphism (RFLP) and sequencing.
Main results and the role of chance: Significant neck anomalies are detected in patients with GG genotype, compared to those with GA genotype. In the teratozoospermia group, means of the neck defects; in unexplained infertility group, means of sperm concentration, total sperm count, total motile and progressive motile sperm count are found statistically significant using the codominant model group. Using the dominant model, statistically significant results are found in all infertile and teratozoospermia groups in terms of neck defects, in unexplained infertility group in terms of sperm concentration, total sperm count, total motile and progressive motile sperm count. The presence of the A allele in the unexplained infertility group is found to have a negative effect on sperm concentration and motility.
Limitations, reason for caution: Population size of the study can be increased to get more significant correlations.
Wider implications of the findings: Upon repeated analyses with wider populations, more distinct and significant results can be obtained, and the studied polymorphism can be included in male infertility screens in the future.
Study funding/competing interest(s): Funding by University(ies), Haliç University.
Trial registration number: None.

P-443 What is the minimal number of MII oocytes or day-3 embryos for a successful comprehensive chromosomal Screening (CCS) cycle

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Study question: To know what is the minimum number of metaphase II (MII) oocytes or cleavage stage embryos biopsied on day-3 to ensure the best reproductive clinical outcome in terms of percentage of cycles with euploid embryos for transfer (TR), ongoing pregnancy rate per transfer (OPRT) and ongoing pregnancy rate per stimulated cycle (OPRC).
Summary answer: Both TR and OPRC were significantly better when ≥8 MII or when ≥5 day-3 embryos were available for biopsy. Oocyte or embryo vitrification and accumulation is a recommendable strategy to this minimal outcome in low responder patients to optimize outcome in CCS cycles.
What is known already: In patients with advanced maternal age (41–44 years), our group has previously described a better prognosis in terms of OPRC in normal responders when compared to low responders.
Study design, size, duration: Retrospective cohort study including 1,154 fresh cycles from our CCS program, since January 2011 to December 2013. Patients between 25 and 46 years were included under indications such as advanced maternal age, recurrent miscarriage, repetitive implantation failure, increased aneuploidy rate on sperm, male factor, and previous aneuploid conception.
Participants/materials, setting, methods: Chromosomal analysis for all 24-chromosomes was performed on single blastomere obtained at day 3 using aCGH (BlueGnome, Cambridge, UK). Embryo transfer of euploid embryos was performed on day-5. Threshold values, sensitivity and specificity were calculated using receiver operating characteristic (ROC) curves. Study groups were analyzed by Chi-square test. Statistical significance was considered with $p < 0.05$.
Main results and the role of chance: The ROC curve analysis revealed a cut-off value of 7.5 MII oocytes for TR (70.9% sensitivity and 55.7% specificity) and 8.5 MII oocytes for OPRC (62.1% and 57.9%). Regarding day-3 biopsied embryos, cut-off values were 3.5 embryos both for TR (78.2% and 59.8%) and OPRC (80.1% and 48.0%). According to this, the clinical outcome of patients with <8 or ≥8 MII oocytes; and ≤4 or >4 biopsied embryos was performed. Significantly higher TR and OPRC were observed when ≥8MII (32.3% vs. 59.3% in TR; 15.1% vs. 29.2% in OPCR; $p < 0.0001$) or >4 biopsied embryos (33.5% vs. 68.5% in TR; 15.2% vs. 34.7% in OPRC; $p < 0.0001$) were obtained. OPRT remain comparable between groups ($p = 0.58$ and $p = 0.26$ in MII oocytes and biopsied embryos, respectively).
Limitations, reason for caution: Retrospective study and heterogeneity of patients included.
Wider implications of the findings: To establish the minimal number of MII oocytes or day-3 biopsied embryos that could be accumulated through several stimulation and vitrification cycles to improve clinical outcome in CCS cycles.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), IVIOMICS S.L.
Trial registration number: None.

P-444 Copy number changes in normal human fetuses could be residues from genome instabilities in preimplantation development

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Study question: Origin of copy number variations (CNVs) between tissues in non-genetic diseases is unknown. Those might be either hotspot for genetic variation which led to shared CNVs by separate events during the life or residues from events in common lineages of early embryos. We aim exploring fetal mosaicism and its origins.
Summary answer: The frequency of reciprocal CNVs was varied from 2 to 18. According to distribution pattern of frequent CNVs, their origin should be early development. Genes involved in fetal CNVs mostly have developmental role. Shared CNVs between fetuses are mostly known hotspots; those occurred in same tissues might have functional role.
What is known already: Intra-individual genetic variation has been reported in diseases without and with genetic component. Such genomic differences caused by post-zygotic events. Mosaicism is prevalent in preimplantation stage. Mutations during first post-zygotic cell divisions lead to beginning the life with many mutated stem cell lineages. Differentiation is known as the barrier for eliminating mosaic embryos. Mosaicism is highly reported in miscarriages; however some mosaicisms, such as CNVs could be compatible with live birth.
Study design, size, duration: Two apparently normal fetuses were achieved after informed consent of the parents following legal dispositions for therapeutic

abortion due to maternal indications on 5th month of pregnancy. Dissections of 21 tissues from the fetus no. 1 and 22 tissues of the fetus no. 2 were studied.
Participants/materials, setting, methods: Tissues' DNA was studied by array Comparative Genomic Hybridization (array CGH) using 195K probe slides in simple loops separately designed for each fetus. Copy number calling was performed using Circular Binary Segmentation (CBS) method. Reciprocal CNVs as high confidence CNVs validated by qPCR. Functional analysis was performed by Gene Ontology (GO).
Main results and the role of chance: Sixty two CNVs were observed 187 times in the fetus 1; of those 67 were reciprocal happened in 13 locations. In the fetus 2, explored CNVs were 56 which observed 108 times; of those 45 were related to 14 reciprocal events. Some of CNVs were shared between both fetuses, some were found in the same tissues and some in different tissues. GO showed that altered genes are mostly involved in embryonic development pathways. Tissues clustering according to CNVs revealed those from the same embryonic origin in some cases are close together in a cluster; however, there were large disagreements with clustering of embryonic layers derivatives. Analysis of the CNVs by array CGH and qPCR showed that quantity of their change were not mostly integer multiples.
Limitations, reason for caution: We studied more than 20 tissues using array CGH. Whole genome study of more derivatives by SNP array and/or next generation sequencing certainly in single cell level could answer more questions. Since our finding is preliminary, its generalization as a reason for preimplantation genetic screening must be with caution.
Wider implications of the findings: Shared variations seem to be hotspots for CNV events while those occur in the same tissues might be functional. Disagreements with clustering of embryonic layer derivatives could arises from multi-layer origin, high ratio of cells from layers except original layer, and extensive cell mixing /migration in embryonic development. All biopsied cells from each tissue have had not the same change because of either different origins or occurrence after tissue differentiation.
Study funding/competing interest(s): Funding by University(ies), 1. Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran, 2. KU Leuven, Leuven, Belgium.
Trial registration number: N/A.

P-445 Is it obligatory to apply trophectoderm biopsy on the fifth day

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Study question: Is it obligatory to wait for the fifth day and for hatching of blastocysts to be able to apply trophectoderm biopsy?
Summary answer: Trophectoderm biopsy on the fourth day is easy to apply and advantageous in preventing prolonged culture which may disturb the synchrony between embryo development and endometrium. Also, cryopreservation of many blastocyst which brings increased labor to the laboratory and increased expenses to the patient can be prevented.
What is known already: Trophectoderm biopsy is an increasingly used method with several advantages over blastomer biopsy for preimplantation genetical diagnosis. But the necessity for waiting embryos to reach blastocyst stage and herniation brings some disadvantages as extended culture to day 6 for embryo transfer or cancellation of transfer and cryopreservation of all blastocyst for a future thawing cycle, which is very laborious and expensive.
Study design, size, duration: This is a retrospective cohort study. 20 single gene disorder cases between November 2012 and December 2013 to whom embryo transfers applied were included in the study. A total of 134 embryos were biopsied. Average female age was 29.8 and average number of embryos transferred was 1.3.
Participants/materials, setting, methods: All of the cases were incubated in time-lapse incubators and timing of biopsies were decided accordingly. Biopsies were applied between 100–104 h post-ICSI for embryos with explicit ICM and TE cells (1 or 2 expansion acc. to Gardner's classification). At least 3 trophectoderm cells were taken per each suitable embryo. Embryo transfers were applied on the fifth day between 123–126 h post-ICSI.
Main results and the role of chance: Genetic diagnosis were obtained for all biopsied embryos, which shows also the safety of the strategy. Biochemical, clinical and ongoing pregnancy rates were 70%, 60% and 55% respectively which is comparable to the average results of day 5 biopsies in the same group. Our main purpose for this strategy was to eliminate late day 5 or day 6 embryo transfers which seems to effect embryo-endometrium synchrony but it may also be useful for the laboratories cryopreserving all biopsied embryos to have time for diagnosis and decreases the cost caused by freezing-thawing procedure.
Limitations, reason for caution: This strategy is possible for the cases having at least early blastocyst stage embryos on the fourth day evening which may also be related with the choice of culture medium. On the other hand, it may still be necessary to biopsy remaining available embryos on day 5 and cryopreserve.
Wider implications of the findings: We selected only single gene disorder cases for this study since they were fertile couples with similar demographic properties. This strategy may also be used for other PGD indications and already being used in our laboratory based on the availability of embryos.
Study funding/competing interest(s): Funding by hospital/clinic(s), Authors have nothing to disclose.
Trial registration number: Nil.

P-446 Mutation analysis of Phospholipase C Zeta (PLC?) in patients with low fertilisation rate

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Study question: PLCζ, sperm soluble factor, has crucial roles in oocyte activation reported by various studies. Our objective was to investigate *PLC*ζ mutations in patients who were globozoospermic, total-fertilization-failure, nuclear anomally, total immotility to delineate if *PLC*ζ mutations have the potential to alter gene function and lead to low fertilization.
Summary answer: Investigation of 15 *PLC*ζ exons with sequence analysis highlighted various variants (p.Q94X, p.R197H, p.L440R, p.T303A, p.S500L). Some of these variants were not reported in the literature before and were only detected in patients with low fertilization rate so they may have a significant effect on *PLC*ζ function.
What is known already: PLCζ is a known regulator of intracellular Ca2+ oscillations. In cases of male infertility, disruption of PLCζ function or expression leading to oocyte activation deficiency was reported. Studies have focused on expression profiling of the protein but there are limited number of studies investigating mutations in the gene itself. Detection of p.H398P and p.H233L mutations which modify structure of the protein and cause altered Ca2+ oscillations suggested a correlation between *PLC*ζ mutations and infertility.
Study design, size, duration: 19 patients, globozoospermic (7), total-fertilization-failure (8), Total pinhead (2), total immotility (2), that had fertilization failure or low fertilization rate were included in the study.
Participants/materials, setting, methods: DNA was extracted from peripheral blood and primers were designed to encompass all 15 exons of *PLC*ζ gene. Sequence analysis was performed using mutation surveyor. Sperm samples were collected from patient for future expression profiling studies.
Main results and the role of chance: Sequence analysis revealed two variants that were already reported: p.Q94X (rs138801851), p.S500L (rs1050530) and three variants that were not reported before: p.R197H, p.L440R, p.T303A. p.Q94X causes an early termination of the transcription and can lead to a truncated protein and abolish the function of protein. Other missense variants can alter 3D structure of the protein. Investigation of population frequency of these variants is required in order to provide further support for this study and exclude role of chance. Family studies of these patients should also be designed to make sure that the variants are inherited.
Limitations, reason for caution: TFF patients are key patients in correlating PLCζ variants with infertility. We had limited number of TFF patients so sequence analysis of additional TFF patients should be performed. This was a preliminary study and future studies will focus on these detected variants to perform population screening and family studies.
Wider implications of the findings: There is only one reported PLCζ mutation in the Human Gene Mutation Database. PLCζ function is crucial for oocyte activation and delineating key variants in PLCζ gene will help us to correlate in PLCζ gene with male infertility. This will enable us to perform molecular screening of patients with TFF or low fertilization rate. Further investigation of

detected variants will also enlighten us about the effects of variants on PLCζ structure and function.
Study funding/competing interest(s): Funding by University(ies), Istanbul Bilim University.
Trial registration number: Istanbul Bilim University.

P-447 Preimplantation genetic screening as a useful tool to lower the risk of adverse reproductive outcomes in good-prognosis patients undergoing in vitro fertilization

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Study question: Can preimplantation genetic screening (PGS) using array comparative genomic hybridization (aCGH) be adopted as a tool to prevent useless transfers, early abortion and the birth of children with an aneuploidy condition, in groups of infertile patients undergoing IVF with a priori low risk for detection of chromosome abnormalities in embryos?
Summary answer: The unexpected high incidence of aneuploidy detected in embryos derived from good-prognosis patients suggests that chromosomal embryo assessment may also represent a useful tool to avoid an adverse reproductive outcome, regardless of risk factors, over the usual controversial PGS purpose to improve the clinical success of the IVF techniques.
What is known already: Historically, the main indications of PGS included advanced maternal age, repeated implantation failure and recurrent pregnancy loss, with the purpose of improving the clinical outcome of the IVF treatments. To date, the technique is currently not suggested for so called 'good prognosis' patients, i.e. infertile patients <35 years undergoing IVF, with no history of miscarriages or repeated IVF failures, or patients at risk for monogenic diseases undergoing preimplantation genetic diagnosis (PGD).
Study design, size, duration: PGS by aCGH analysis was offered to 'good prognosis' patients undergoing IVF treatments in the period between January-December 2013. Embryo biopsy was performed on day-5/day-6, followed by embryo transfer on a fresh or frozen cycle. The incidence and type of aneuploidy, miscarriage, clinical pregnancy and implantation rates were also determined.
Participants/materials, setting, methods: 147 patients (mean age 34.1 years) undergoing IVF were enrolled in the study: 108 (mean age 34.1 years) were simply infertile patients and 39 (mean age 34.1 years) were PGD patients. Biopsied cells were first lysed, DNA amplified by whole genome amplification and analyzed by aCGH.
Main results and the role of chance: A total of 560 blastocysts from 159 PGS cycles were diagnosed, 300 (53.6%) of which resulted aneuploid: 231/420 (55.0%) involved infertile patients and 69/140 (49.3 %) were from PGD patients. The majority (61.3%) of aneuploidy detected in both groups resulted with low implantation potential, including single (22.7%) or double (5.3%) monosomy or complex aneuploidy (33.3%). Notably, 23.7% of blastocysts presented aneuploidies with high implantation potential, including 7.3% of common aneuploidy (e.g. trisomy 21, 18, 13, sex chromosomes aneuploidy), single (14.0%) or double (2.3%) trisomy involving other chromosomes. Finally, in 15% of embryos the presence of mosaic aneuploidy was detected. Following transfer of 108 embryos, 56 women achieved a sustained pregnancy (57.1% clinical pregnancy rate/ET; 57.4% implantation rate).
Limitations, reason for caution: Although PGS results have demonstrated a high incidence of aneuploidy also in embryos derived from good-prognosis patients, further evidence is required from large-scale prospective clinical trials before its clinical use on routine basis. Data for the above studies will help to define the clinical utility of chromosome screening in the IVF setting.
Wider implications of the findings: The use of comprehensive chromosome screening technologies for selecting the most competent embryo(s) for transfer holds great promise. PGS provides an important contribution to the prediction of the reproductive competence of embryos, regardless of indication for testing or risk factors. The enhanced selection empowered by PGS may provide a practical way to substantially lower the risk of an adverse reproductive outcome related with the transfer of chromosomally abnormal embryos, without compromising clinical outcomes.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), "genoma."
Trial registration number: None.

P-448 Aneuploidy screening of human blastocysts derived from PGS patients using next-generation sequencing

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Study question: Does next-generation sequencing (NGS) detect all types of aneuploidies of human blastocysts derived from IVF patients undergoing preimplantation genetic screening (PGS) efficiently?
Summary answer: NGS detects all types of aneuploidies of human blastocysts accurately and provides PGS results with a high level of consistency compared with array CGH (aCGH). Moreover, NGS screening identifies euploid blastocysts for transfer and may improve pregnancy and implantation rates for PGS patients.
What is known already: Recent advances in next-generation sequencing have provided new tools for detecting DNA mutations and chromosome aberrations for clinical diagnosis purposes. However, there is still very limited information about clinical application of NGS in IVF and PGS treatment cycles. Accordingly, the current study aims at evaluating the efficiency of aneuploidy screening using NGS for patients with unknown recurrent pregnancy loss and previous aneuploid conceptions.
Study design, size, duration: Blinded DNA samples from blastocytsts derived from PGS patients with unknown recurrent pregnancy loss and previous aneuploid conceptions were analyzed with NGS and aCGH (BlueGnome/Illumina, Cambridge, UK) individually. Results obtained from the same embryos were compared and the two methods were evaluated for consistency.
Participants/materials, setting, methods: 56 blastocysts from 15 PGS patients at a mean age of 36.4 ± 3.6 years were biopsied and vitrified on day 5. Whole genomic amplification was performed and analyzed with NGS and aCGH. Based on the NGS results, one to two euploid blastocysts were thawed and transferred to individual patients.
Main results and the role of chance: NGS detected all types of aneuploidies including monosomy, trisomy, dual and complex chromosomal abnormalities and provided 100% equivalent PGS diagnoses compared with aCGH. Moreover, NGS screening identified euploid blastocysts for transfer in 93.3% (14/15) of the PGS patients. Following the NGS screening, one to two euploid blastocysts were thawed and transferred to individual patients. 71.4% (10/14) of the patients became clinically pregnant with gestation sac(s) and fetal heart beat(s) and the implantation rate per embryo transfer was 72.2% (13/18). A 64.3% (9/14) of ongoing pregnancy rate was achieved while a 10% (1/10) of miscarriage rate was observed in the current study.
Limitations, reason for caution: Results are based on observations with blastocysts derived from PGS patients with unknown recurrent pregnancy loss and previous aneuploidy conceptions and may not fully generalize to all IVF and PGS patients with different clinical indications.
Wider implications of the findings: Our data demonstrate that NGS provides accurate diagnoses for PGS patients with unknown recurrent pregnancy loss and previous aneuploidy conceptions compared with the established methodology. With increasing sensitivity for aneuploidy screening and reducing the overall cost of sample testing, NGS may offer additional advantages over aCGH. Further randomized clinical trials are planned to verify these preliminary findings.
Study funding/competing interest(s): Funding by hospital/clinic(s), this study is supported by internal funding. All authors report no competing interests.
Trial registration number: Not applicable.

P-449 No difference in embryo development and aneuploidy rates between successful and unsuccessful cycles following embryo transfer of euploid embryo(s) for patients with advanced maternal age

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Study question: Is there any characteristics of patients or embryo developmental parameters that would relate to the success of an in vitro fertilization (IVF)

cycle following the transfer of euploid embryos for patients with advances maternal age (AMA)?
Summary answer: The outcome of an IVF treatment combined with preimplantation genetic screening (PGS) seems to be independent of the number of oocytes, fertilization and euploidy rates, the number of embryo transferred and their morphology when they have at least one euploid embryo.
What is known already: Historically, PGS were developed to patients having a risk of producing aneuploid embryos with a higher frequency. It is a well known phenomena that numerical errors of chromosomes increases with advancing age in women. PGS is proven to be an effective tool reducing miscarriage and facilitates single embryo transfer as well. For proper consultation of patients with AMA, it has a great importance to determine characteristics that exclude patients from PGS.
Study design, size, duration: Data from fifty-one patients with AMA (≥35 years of age) having embryo transfer following IVF treatment with preimplantation genetic screening between September of 2012 and December of 2013 was retrospectively analyzed.
Participants/materials, setting, methods: Fifty-one patients with AMA with at least one euploid embryo were included in the study. Comparison of patients average age, the number of oocytes and embryos, fertilization, euploidy and blastulation rates and transfer score was carried out between successful and unsuccessful cycles. Student *t*-test and chi square analysis were performed.
Main results and the role of chance: Chemical and clinical pregnancy rates of 56.86% (29/51) and 45.10% (23/51) were achieved with patients aged 35 and older with IVF combined with PGS, respectively. After adjusting *p* value for multiple comparisons ($n = 10$; $p < 0.005$), no difference was found between unsuccessful and successful PGS cycles in age (37.43 ± 0.079 vs. 37.69 ± 0.084), previously failed cycles (1.75 ± 0.076 vs. 1.70 ± 0.084), number of oocytes (11.75 ± 0.205 vs. 9.96 ± 0.217), number of mature oocytes (9.79 ± 0.161 vs. 7.65 ± 0.177), fertilization rate (70.85 ± 0.624 vs. 68.17 ± 0.866), euploidy rate (42.34 ± 0.857 vs. 54.68 ± 1.359), blastulation rate (29.62 ± 0.979 vs. 34.80 ± 1.176), transfer score (4.84 ± 0.088 vs. 5.37 ± 0.098) and the number of embryos transferred (1.36 ± 0.020 vs. 1.48 ± 0.022). Also, the frequency of euploid embryo transfers did not reach blastocyst stage after day 3 biopsy did not differ between the two groups (8/28 (28.57%) vs. 5/23 (21.74%)).
Limitations, reason for caution: The sample size of these types of retrospective studies always plays a crucial role and with a higher number of participants stronger conclusions can be drawn. Results need to be periodically re-evaluated when more data is available for AMA patient for proper consultation.
Wider implications of the findings: For a proper consultation of patients with AMA often experienced multiple failed cycles already, clinics need to continuously evaluate their results in their own settings in order to be able to safely offer these state-of-the-art treatment options like PGS. It is reassuring that patients seeking PGS treatment with AMA even with a lower number of embryos achieve pregnancy with the same chance as with those with high number of embryos.
Study funding/competing interest(s): Funding by hospital/clinic(s), Versys Clinics Human Reproduction Institute.
Trial registration number: None.

P-450 Relevance of single nucleotide polymorphism on p53, IL-11, IL-10 and VEGF in patients with repeated implantation failure and pregnancy loss

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Study question: Investigate whether single nucleotide polymorphism on p53, IL-11, IL-10 and VEGF has a higher prevalence among women with a history of recurrent implantation failure (RIF) and pregnancy loss (RPL).
Summary answer: In a population of RIF and RPL patients a high incidence on p53 gene polymorphism PP at position 72 was observed. As for IL-10 the genotype AA at position −1082 was more prevalent in RIF patients.
What is known already: Recurrent pregnancy loss (RPL) and implantation failure (RIF) are the most common cause of lack of unsuccessful pregnancy after IVF. Underlying causes such as genetic, endocrine, anatomical or autoimmune was found in more than 50% couples. Several factors such as a variety of environmental as well as lifestyle factors have been considered for idiopathic pathogenesis. Moreover, gene polymorphism may predispose to an increased risk and less attention has been paid to gene polymorphisms.
Study design, size, duration: SNPs genotyping has been studied in 266 women. The control group included 81 oocyte donors. In the study group 185 women were included: 94 with recurrent implantation failure (RIF) and 91 with recurrent pregnancy loss (RPL).
Participants/materials, setting, methods: RIF was defined as a cumulative total of four cleaved good quality embryos with negative hCG serum levels. RPL was defined as two or more miscarriages. Oocyte donors were selected according to Instituto Bernabeu egg donation program requirements. The main outcome measures were biochemical pregnancy, implantation rate and ongoing pregnancy.
Main results and the role of chance: The frequency of P72/P72 genotypes on p53 gene among women experiencing RIF was 9.5% compared with 9.9% for those with a history of RPL and 6% in controls ($p < 0.01$). The frequency of AA genotypes on IL-10 gene among women experiencing RIF was 43.2% compared with 33.7% for those with a history of RPL and 22.9% in controls ($p < 0.01$). There were no significant differences with respect to VEGF and IL-11.
Limitations, reason for caution: This study adds data suggesting genetic factors may increase susceptibility to RIF and RPL. However an individual is embedded with the context of that individual's entire genome and environment. In fact, some others genes related to implantation could also play an important role in determining the causes of implantation failure.
Wider implications of the findings: This investigation reveals that in RIF and RPL patients P72 on p53 genes and −1082 AA on IL-11 are more prevalent than fertile population. This information together with some additional markers will allow developments of diagnostic tests for detect risk for RIF and RPL before infertility treatment is initiated.
Study funding/competing interest(s): Funding by hospital/clinic(s), Conflicts of interest and source of funding none declared.
Trial registration number: The study is not an RCT.

P-451 A comparison of aneuploidy rates between Asian and Caucasian patients

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Study question: Does a difference in aneuploidy rates explain the observed differences in pregnancy and live birth rates between Caucasian and Asian patients undergoing assisted reproduction?
Summary answer: There is no significant difference in aneuploidy rates between embryos from Asian and Caucasian patients. Therefore aneuploidy cannot be the etiology of the observed difference in IVF outcomes between these two patient populations.
What is known already: Aneuploidy contributes to the majority of in vitro fertilization (IVF) failures. Previous observational studies have shown that Asian women undergoing IVF without pre-implantation genetic screening (PGS) have lower pregnancy and live birth rates compared to Caucasian counterparts, but have failed to identify an etiology for this discrepancy. No prior studies have investigated if aneuploidy rates vary between different ethnicities. Different rates of aneuploidy between different ethnicities would explain the observed difference in IVF outcomes.
Study design, size, duration: This was a retrospective cohort study. Three-hundred and eight IVF cycles using PGS with 24-chromosome analysis between 2012 and 2013 were included.
Participants/materials, setting, methods: The 308 patients, or oocyte donors when applicable, who underwent IVF with PGS with a day 5 biopsy and day 6 transfer, who identified as Caucasian or Asian ethnicity were included for analysis. Charts were reviewed for patient age, oocyte yield, and PGS results. Chi-squared analysis was used for analysis.
Main results and the role of chance: PGS was performed on 2,439 embryos, including 736 from oocytes of patients or donors identifying themselves as Asian ethnicity and 1,703 from oocytes of patients or donors identifying themselves as Caucasian ethnicity. The overall aneuploidy rate was 58.2%. After stratifying by age, there was no significant difference in euploid rates among embryos of Asian patients compared to embryos of Caucasian patients (Age ≤25 years: 44% vs. 51%, $p = 0.10$, Age 26 to 29: 50% vs. 48%, $p = 0.71$, Age

30 to 35: 39% vs. 40%, $p = 0.84$, Age 35 to 39: 16% vs. 23%, $p = 0.20$, Age 40 to 45: 7% vs. 15%, p = 0.07), and in fact Asians >40 years were significantly more likely to have at least one euploid embryo available for transfer (27% vs. 21%, $p = 0.002$).

Limitations, reason for caution: This study was limited by its retrospective nature and limited sample size. With greater numbers, some of the trends may reach significance. Additionally, ethnicity was self-reported and mixed ethnicity (i.e., Caucasian-American) was unaccounted for. Paternal contributions to aneuploidy were not accounted for, and may also confound these results.

Wider implications of the findings: There was no observed difference in aneuploidy rates between similarly aged patients who identify as Asian and those who identify as Caucasian, therefore aneuploidy does not explain the observed difference in IVF outcomes between these patient populations. Future research should investigate other etiologies that might explain this difference, including endometrial receptivity or non-ploidy related embryo viability.

Study funding/competing interest(s): Funding by hospital/clinic(s), La Jolla IVF.

Trial registration number: Not applicable.

P-452 The FSH receptor genotype affects responsiveness to controlled ovarian stimulation in normogonadotropic women treated with GnRH-a long protocol plus recombinant FSH

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Study question: Could FSH receptor (FSH-R) polymorphsim (Thr307/Asn680 and Ala307/Ser680) influence controlled ovarian stimulation in IVF/ICSI candidates?

Summary answer: This study confirms that the FSH-R genotype may interfere with physiological responsiveness of the target organ to human recombinant FSH (r-hFSH) stimulation.

What is known already: Early studies identified a subgroup of normogonadotropic patients who have normal estimated ovarian reserves but resistance to FSH stimulation during controlled ovarian stimulation. Carriers of FSH-R Thr307/Asn680 and Ala307/Ser680 polymorphisms seems to have the same "hypo-response" profile showing higher requirement of exogenous gonadotrophins during COS.

Study design, size, duration: A retrospective study on 42 normogonadotropic patients undergoing a standard IVF/ICSI cycle in our Department (Federico II University), from October 2011 to April 2012, has been carried out.

Participants/materials, setting, methods: Based on r-hFSH consumption, the study population was divided into 2 groups: hypo-responders with cumulative r-hFSH >2500 IU (Group A; $n = 17$) and a control group (Group B; $n = 25$) with cumulative r-hFSH ≤2500 IU. The FSH genotype was assessed by polymerase chain reaction and restriction fragment length polymorphism analysis.

Main results and the role of chance: Demographic and anthropometric characteristics did not differ significantly between the two groups. FSH basal levels ($p = 0.02$), the mean number of r-hFSH vials ($p = 0.0001$) and days of stimulation ($p = 0.03$) were significantly higher in group A. The number of oocytes retrieved ($p = 0.0003$) and embryos transferred ($p = 0.001$) was significantly lower in group A. No statistically significant differences regarding cumulative pregnancy rates, abortion rates and rates of ongoing pregnancy was observed. Serum levels of estradiol, measured on the day of hCG administration, were significantly lower in group A ($p = 0.0001$).

The incidence of Ser/Ser genotype was higher in Group A ($p = 0.02$) while the Asn/Ser genotype was more frequent in the control group ($p = 0.04$).

Limitations, reason for caution: Considering the small study population size, these observations needs to be confirmed by larger well-conducted studies.

Wider implications of the findings: On the basis of patient genetic profile, a "tailored" FSH therapy could be opted giving the chance to customize the dosage and the timing of stimulation. The immediate implications would be a saving in costs and an increase in treatment acceptance.

Study funding/competing interest(s): Funding by University(ies), Dipartimento di Neuroscienze, Scienze Riproduttive ed Odontostomatologiche – Università degli Studi di Napoli 'Federico II', Via Sergio Pansini, 5 – 80131, Naples, Italy.

Trial registration number: Not available.

P-453 Development of an NGS-based solution for the identification of individuals carrying recessive genetic mutations in reproductive medicine

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Study question: The identification of couples at risk of transmitting a recessive genetic disorder allows them to take informed and responsible actions regarding their reproductive plans. NGS is revolutionizing genetic research and diagnosis in reproductive medicine. Can NGS technologies efficiently evaluate the carrier status for most commonly genes mutated in recessive disorders?

Summary answer: We developed an NGS-based approach targeting genes causing prevalent and severe recessive diseases for testing healthy, IVF couples and donors/recipients in donation programs in order to reduce the odds of passing a recessive or X-linked disorder to the offspring.

What is known already: Screening tests for carriers of recessive disorders have been developed using SNP-genotyping approaches. SNP-based arrays interrogate specific mutations within selected genes but they do not explore all known and unidentified disease-causing mutations, and only interrogate point mutations or small in/dels present in a given gene. By contrast, NGS technologies are capable of detecting a wider range of disease-causing mutations in a cost-efficient manner, and are likely to replace genotyping methods in the near future.

Study design, size, duration: We developed the NGS assay to measure the analytical validity of our test by analyzing positive and negative control DNA samples. We measured overall sensitivity and specificity of different known genetic mutations present in a blinded training set. Clinical validity study is ongoing.

Participants/materials, setting, methods: The qCarrier test is based in sequence capture, followed by high-throughput sequencing of the captured genome using a personal genome analyzer. Bioinformatic analysis is keystone in the process, as it combines several algorithms optimized for the identification and annotation of different types of mutations (point mutations, indels, copy-number and rearrangements).

Main results and the role of chance: Our experience with NGS gene panels showed extremely high sensitivities (>99%) for all kinds of mutations [1–2]. For qCarrier validation, we obtained DNA from 57 unrelated individuals: 39 patients and 18 previously genotyped controls. The validation set was composed of 49 different known mutations (67 in the patients), including 29 SNVs, 13 indels and 25 CNVs causing different diseases: cystic fibrosis, phenylketonuria, spinal muscular atrophy, hypothyroidism, thalassemia, factor V deficiency and Duchenne muscular dystrophy. All but one (48/49) different mutations were correctly scored in the blinded study and only one deletion-type mutation remained undetected. This information allowed us to finely tune the algorithm to reach maximum sensitivity. All single nucleotide changes were validated and no known recessive mutations were called in the control samples.

Limitations, reason for caution: A major limitation is the current knowledge of the real extent of human genetic variation. The identification of previously unknown variations challenges the communication of results. This emphasizes the absolute need for an adequate pre and post-test genetic counselling that clearly states the advantages and limitations of the current knowledge.

Wider implications of the findings: After demonstrating the technical and clinical validity of the approach for donor/receptor matching, we plan to offer the test to couples with immediate reproductive plans, as we envision

that they might also benefit from knowing their carrier status. Regarding technical improvements, it is crucial to have an unbiased representation of all genes included in the test, to expand the target genes to additional disorders (mainly X-linked) and to precisely measure nucleotide-repeat expansions from NGS data.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), qGenomics and Hospital Universitari Quirón Dexeus.
Trial registration number: N/A.

P-454 Validation of various embryo morphokinetic models for assessing aneuploidy risk in day 3 and day 5/6 PGS biopsies

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Study question: To validate our own and other previously published predictive models of embryo ploidy in day 3 and day 5/6 PGS biopsies.
Summary answer: Our aneuploidy risk model, which integrated three early kinetic parameters and maternal age, demonstrates higher sensitivity and specificity for embryo ploidy assessment compared to previously published models.
What is known already: An aneuploidy risk model was developed for day 3 biopsy embryos (Basile et al., 2014) using two early morphokinetic parameters for embryo euploid prediction. Another risk model was published for day 5 biopsied embryos (Campbell et al., 2013) indicating correlation of aneuploidy risk and the time of blastulation.
Study design, size, duration: A retrospective analysis of 133 PGS cycles including 66 day 3 and 67 day 5/6 biopsies. Correlation among embryo ploidy and morphokinetic parameters were performed separately for day 3 and day 5/6 biopsies. Multivariate statistical analysis was used to develop our model and to validate published models.
Participants/materials, setting, methods: Embryos (n = 852) were cultured in time-lapse incubators (EmbryoScope®, Unisense Fertilitech, Denmark) and embryo kinetic times were annotated blindly to the PGS results. Chromosomal analyses were done by FISH, CGH or SNP. Embryo ploidy was classified as euploid, aneuploid (single chromosome abnormalities) or complex abnormalities (two or more abnormalities).
Main results and the role of chance: When the Basile model using two parameters was applied to our day 3 biopsy data, respectable sensitivity (80%, true positive) and specificity (46%, true negative) was found. Adding another embryo synchronization parameter and maternal age strengthened the predictive power of our model. This resulted in comparable sensitivity (77%) and an increase in specificity (57%). We were unable to validate the Campbell model using our day 5 biopsy data, even after lowering blastulation time (<118 h) as suggested by the authors. When our day 3 biopsy model was applied to day 5 biopsy data, similar sensitivity (79%) and specificity (60%) were observed. This indicates that our model is valid regardless of the biopsy day.
Limitations, reason for caution: When FISH was used for day 3 biopsies, only 9 of 23 chromosomes were analyzed.
Wider implications of the findings: Validation of aneuploidy risk models need to be performed in each laboratory on their own data set. Differences in patient population, genetics, maternal and male age, day and methods of biopsy as well as method of chromosomal analysis can be responsible for model differences. Our model could be applied universally as an additional patient counseling tool prior to day 3 or day 5/6 biopsy if validated by other laboratories.
Study funding/competing interest(s): Funding by University(ies), CRM, Weill Cornell Medical College.
Trial registration number: None.

P-455 A model to assess anuploidy risk based on early morphokinetic parameters and female age for both day 3 and day 5 biopsied embryos

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Study question: To develop and validate an early morphokinetic aneuploidy risk model as a tool for prognosticating ploidy in day 3 or day 5/6 embryos.
Summary answer: Three early embryo morphokinetic parameters assessed in the interval between 2 and 5 cell stage along with maternal age, positively correlates with ploidy regardless of embryo biopsy stage.
What is known already: An aneuploidy risk model has been developed for day 3 biopsy embryos (Basile et al., 2014) where early morphokinetic parameters were associated with euploidy prediction. Similarly, an aneuploidy risk model for day 5 biopsy embryos indicated a correlation between aneuploidy and blastulation time (Campbell et al., 2013). Both models require individual laboratory validation and improvements.
Study design, size, duration: An aneuploidy risk model was developed and retrospectively validated using multivariate statistical analysis on 852 embryos from 133 PGS cycles (66 day 3 and 67 day 5/6). Although it was a retrospective analysis, embryo morphokinetic parameters were assessed blindly to the PGS results. maternal age was included in the model.
Participants/materials, setting, methods: Embryo morphokinetic parameters were assessed by time-lapse incubators (EmbryoScope®, Unisense Fertilitech, Denmark) through day 6. Embryos were analyzed by FISH, aCGH or SNP and classified as euploid, aneuploid (ANU, single chromosomal abnormality) or complex abnormalities (CxA, two or more abnormalities). Multivariate statistical analysis were used for model building and validation.
Main results and the role of chance: Blastocyst rate is negatively correlated with advancing maternal age (49.7% < 40, 36.7% > 40). This negative trend was also correlated with euploidy within blastocysts (63.9% to 25.9%), indicating maternal age as the principal determinant of aneuploidy. Higher incidence of complex abnormalities was observed in day 6 compared to day 5 blastocysts. Embryos with CxA presented different morphokinetic behaviors compared to other ploidy groups. Our model, using early morphokinetic parameters and maternal age, positively estimated ploidy regardless of embryo biopsy stage (day 3, 5, 6). In patients <40, the model had higher efficiency in detecting complex abnormalities (AUC = 0.69) vs. aneuploidies (AUC = 0.58). In patients >40, differentiation of various ploidies was comparable but with higher incidence of abnormalities, indicating requirement for PGS.
Limitations, reason for caution: All embryos, even those not reaching blastocyst stage, should be chromosomally analyzed. Other than age, patient specific inherited factors that might influence ploidy should be explored and integrated.
Wider implications of the findings: Our model is based on early kinetic parameters (between 2–5 cells) and maternal age. This model can be used as an additional patient counseling tool prior to day 3 or day 5/6 biopsies. It can be particularly effective for younger patients presenting suboptimal morphokinetic parameters.
Study funding/competing interest(s): Funding by University(ies), CRM, Weill Cornell Medical College.
Trial registration number: None.

P-456 Differences in ploidy do not affect morphokinetic characteristics of embryos time-lapse-imaged up to the 8-cell stage after polar body diagnosis

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Study question: Can morphogenetic characteristics 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)? Preliminary data from our ongoing study will be presented.
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 compaction 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 have a delayed tSC, tSB and tB. However, until the 8-cell stage no morphokinetic differences were identified. (Campbell et al., 2013, RBM Online, 26, 477–485).
Study design, size, duration: This retrospective study analyzed 14 cycles with 13 patients undergoing ICSI and PBD in our clinic between 09/2013

and 01/2014. All women had a normal karyogram except for three (mosaic 47XXX/46XX, translocations *t*(1,18) or *t*(18,20)). ICSI was performed on $n = 95$ oocytes with $n = 67$ being fertilized and $n = 55$ included for analysis.
Participants/materials, setting, methods: PBD was done using FISH against chromosomes 13, 16, 18, 21, 22 ($n = 11$), *X* ($n = 1$) or translocations ($n = 2$). 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 20).
Main results and the role of chance: To identify morphokinetic differences among eE and aE we compared several parameters. The median time when the second PB was extruded was tPB2(eE) = 2.92(2.04–4.44) and tPB2(aE) = 3.50(2.66–4.44) ($p > 0.05$). The time when a pronucleus appeared was tPNa(eE) = 6.85(5.96–7.67) and tPNa(aE) = 7.22(6.58–8.40) ($p > 0.05$). Both pronuclei had faded at tPNf(eE) = 26.85(24.15–28.60) and tPNf(aE) = 25.05(23.04–29.55) ($p > 0.05$). The time from insemination to completion of division to 2–8 cells (t*n*) did not differ between euploid and aneuploid embryos. When comparing developmental periods, the time of pronuclear stage was cPN(eE) = 20.00(17.06–21.13) and cPN(aE) = 18.40(15.27–20.28) ($p > 0.05$), cell cycle times (cc*n*) did also not differ between euploid and aneuploid embryos. The synchrony of the *n*th cell cycle (s*n*) was s2(eE) = 0.67(0.17–1.17) and s2(aE) = 0.75(0.19–3.13), s3(eE) = 2.99(1.95–6.34) and s3(aE) = 4.16(2.25–6.33) ($p > 0.05$).
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 preliminary results are in agreement with previous findings by Campbell et al., 2013 and suggest that the analyzed morphokinetic parameters up to the 8-cell stage cannot be used to identify aneuploidy and recommend testing for ploidy by other means, i.e. FISH or ArrayCGH.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Heidelberg University Hospital, Heidelberg, Germany. The authors declare no competing interests.
Trial registration number: A trial registration number was not required due to the retrospective study design.

POSTER VIEWING

REPRODUCTIVE ENDOCRINOLOGY

P-457 Comparison of efficacy and safety of hCG trigger and a "dual trigger" in high responders undergoing fresh embryo transfer

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Study question: Is the "dual trigger" (GnRH agonist combined with low-dose hCG) associated with comparable live birth rate and risk of ovarian hyperstimulation syndrome (OHSS) in high responders receiving fresh embryo transfer when compared with a trigger of hCG alone?
Summary answer: After controlling for significant confounders, the dual trigger was associated with significantly greater live birth rate and reduced OHSS incidence.
What is known already: There is no prior published comparison of the dual trigger to an hCG trigger in high responders. Previous studies compared the dual trigger to agonist trigger in high responders or to hCG trigger in normal responders.
Study design, size, duration: This retrospective cohort study included 420 high responders with ≥20 follicles and fresh blastocyst transfer, 238 of which received dual triggers and 182 received hCG-only trigger in the period 2001–2012.
Participants/materials, setting, methods: Patients were stimulated with gonadotropins under a GnRH-antagonist protocol and "triggered" with hCG-only or else GnRH-agonist combined with low-dose hCG ("dual trigger"). Blastocyst transfer was the standard of care. Multiple logistic regression assessed the effect of trigger type on birth and OHSS rates while controlling for confounding variables.
Main results and the role of chance: After considering 14 potential confounding variables (maternal age, weight, BMI, stimulation duration, follicle count, pre-ovulatory estradiol and progesterone levels, oocyte number, mature oocyte number, fertilized oocytes, number blastocysts transferred, day of blastulation, presence of frozen supernumerary blastocysts, and endometrial thickness), stepwise logistic regression found the significant predictors of live birth were trigger type ($P < 0.0001$), day of transfer ($P < 0.0001$), duration of stimulation ($P = 0.0012$), and number of transferred blastocysts ($P = 0.0049$). The odds ratio for live birth with the dual trigger relative to hCG trigger was 2.7 (95% CI 1.7–4.4). Stepwise logistic regression found the significant predictors of OHSS were pre-trigger estradiol level ($P < 0.0001$) and trigger type ($P = 0.0001$). The odds ratio for OHSS with an hCG trigger relative to the dual trigger was 94 (95% CI 15–2101).
Limitations, reason for caution: This retrospective study was not randomized, and only considered cycles of embryo transfer.
Wider implications of the findings: While OHSS remains possible with the dual trigger, the risk appears to be substantially reduced when compared to hCG alone, and the greater live birth rate with the dual trigger suggests a reasonably safe and efficacious method for fresh transfer in high responders. The observed differences should be confirmed by further study and the cause(s) should be clarified, as these may have implications for fresh transfer and oocyte/embryo banking.
Study funding/competing interest(s): Funding by hospital/clinic(s), Fertility Center of Las Vegas.
Trial registration number: None.

P-458 Estrogen receptor gene variants affect the follicular outcome of standard gonadotropin stimulation

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Study question: Do the *ERα c.454-397T* > C, *ERα c.454-351A* > G and *ERβ 1730G* > A polymorphisms influence the controlled ovarian stimulation (COS) outcome?
Summary answer: The *ERα c.454-397T* > C, *c.454-351A* > G genotypes and diplotypes as well as the *ERβ 1730G* > A genotypes were associated with the ovarian response to standard gonadotropin stimulation of women undergoing in vitro fertilization.
What is known already: Estrogen, stimulating the FSH action on granulosa cells, plays a primary role in the development of follicles capable of responding to the appropriate hormonal stimulation to grow in size, produce a mature oocyte and develop into a corpus luteum. Estrogenic action in target tissues is mediated by estrogen receptors, which are present in the ovary, the uterus, the mammary glands, the thecal layer and the pituitary gland, showing their primary role in reproduction.
Study design, size, duration: Three hundred women with tubal or male-factor infertility, undergoing *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) in a period of 2 years constituted the study population. Furthermore, 300 women with at least one spontaneous pregnancy participated in this prospective study as the control group.
Participants/materials, setting, methods: The FSH, LH and E_2 levels were determined at the third day of the menstrual cycle, while the follicular size, the follicle and oocyte numbers were recorded during oocyte retrieval. *ERα* c.454-397T > C (*PvuII, rs2234693*) and c.*454-351A* > G (*XbaI, rs9340799*) polymorphisms as well as *ERβ 1730G* > A (*AluI, rs4986938*) polymorphism were genotyped.
Main results and the role of chance: Significant differences in the number of large follicles were observed among *ER* genotypes. Specifically, women with *PvuII CC* genotype presented with 7.64 ± 5.23 large follicles, whereas those with *PvuII TT* genotype with 6.07 ± 3.83 large follicles ($p = 0.045$, CI:0.599–3.442). On the other hand, *XbaI GG* women had higher large follicle numbers compared to *XbaI AA* women (8.5 ± 4.96 vs. 5.96 ± 3.66, $p = 0.01$, CI:0.293–4.559). The diplotype analysis showed that women with *PvuII CC/XbaI GG* presented with 8.5 ± 4.96 large follicles, while *PvuII TT/XbaI AA* women with 6.07 ± 3.83 ($p = 0.024$, CI:0.062–4.538). As concerns *ERβ* AluI polymorphism, *AluI GG* women presented with increased large follicle numbers compared to *AluI AA* women (6.62 ± 4.47 vs. 4.43 ± 2.94, $p = 0.033$, CI:0.345–3.78). No significant associations of *ER* genotypes with the hormonal profile, the follicle/oocyte numbers and the pregnancy rates were observed.

Limitations, reason for caution: Our study population was limited in Greek Caucasian women.
Wider implications of the findings: The current study, enrolling the highest to date number of patients for the simultaneous analysis of *ER*α and *ER*β gene polymorphisms, pointed out the significance of *ER*α and *ER*β genotypes/diplotypes for the follicular growth of women undergoing COS for IVF/ICSI. After the verification of our results in other ethnic groups and multicenter studies, *ERs* genotype analysis could help in the selection of the proper COS treatment so as to achieve sufficient mature follicle numbers.
Study funding/competing interest(s): Funding by University(ies), Ioannina University, Ioannina, Greece.
Trial registration number: Non applicable.

P-459 Supplementation of luteinizing hormone LH to follicle stimulating hormone fsh during ovarian stimulation is beneficial for poor ovarian responder – a matched pair controlled multicentre study

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Study question: Has LH supplementation with FSH during ovarian stimulation in IVF/ICSI a beneficial effect on Live birth occurrence, both for Normal (NOR) or Poor (POR) Ovarian responders?
Summary answer: Supplementing LH to FSH significantly increases the Live birth rate on Poor ovarian responders, and was not observed to have significant effect on Normal Responders.
What is known already: Recent trials report conflicting results on supplementing LH to FSH compared with FSH alone. A recent meta-analysis (Mochtar) provided some evidence that the benefits might be limited to Poor Ovarian Responders (POR). However, these results remain debatable as the studies are based on non comparable POR definition, and most of the studies were underpowered.
Study design, size, duration: Two-center Randomized sampling matched case study randomized from a large retrospective patient data base (2010–2013). Live Birth Rate (LB) was the primary and the number of Oocytes and Mature oocytes were the secondary endpoints. Following this model, an interaction between Treatment and ovaria response on LB characterized by a relative risk RR > 1.3 should be detected with power 0.8 at two-sided confidence 0.95 and a 1:2 ratio patient when at least 325 and 650 patient in FL and F groups, respectively.
Participants/materials, setting, methods: The screening and matching were organized in the two centres from 12841 patients. In each center, patients treated with FSH + LH (group FL) were randomly matched for blocking covariates (age, FSH, LH, AMH) with patients previously treated with FSH alone (Group F) following the optimal matching allocation method. The evaluability of patients was conducted on an Intent to treat basis. Generalized logit and Poisson Mixed model were used by considering Matched block and study as random factor, and matching variables used as covariates. Through ESHRE Bologna standard definition, we classified patients as POR or Normal Responders (NOR).
Main results and the role of chance: 975 patients were selected (median age of 36.5 years, interquartile range 33–40, AMH = 2.24 ± 2.4 pmol/L, BMI = 22.96 ± 3.94 k/m^2). A significant interaction effect (p = .031) was found between treatment and ovarian response with a Risk Ratio RR = 1.77 [1.04,3.03] in favor of FL in POR patients, whereas no significant differences was found in NOR patients (Table 1). We identified the same significant interaction effect for the number of oocytes and mature oocytes. No significant main or interaction center effect was found.

Table 1: Live Birth rate (%), Mean (SD) Number of total oocytes and mature oocytes between F and FL groups on POR and NOR subsets.

	Sample size		Life birth (%)		Oocyte number		Mature oocytes	
	F	FL	F	FL	F	FL	F	FL
NOR	376	188	23.7	21.8	9.5 (5.9)	8.7 (5.3)	6.7(4.6)	5.9(3.9)
POR	274	137	10.6	17.5	5.6 (4.1)	6.8 (4.1)	3.7(3.5)	5.1(3.5)

Limitations, reason for caution: This multi-center controlled study was adequately powered to identify a clinically relevant benefit LH supplementation on LB, and POR was homogeneously defined by the current consensus. Unknown confounding remains possible due to absence of a true randomization controlled group.
Wider implications of the findings: This observational study is based on real cases, thus our results are generalizable to routine medical practice. The assessment of LH supplementation on Life Birth occurrence was not as yet assessed on trials with a needed sample size to provide a sufficient power. Our main results confirms the suggestion of Mochtar meta-analysis. Although we confirm the benefit of LH supplementation on Life birth for POR patients, we also derived a significant increase of oocytes and mature oocytes.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Our retrospective data bases and extraction was free of any funding source. An unrestricted grant was provided by Merck-Serono for the logistic of the study, including travel costs.
Trial registration number: No trial registration number.

P-460 Oxidative stress affects FSH response in human granulosa-lutein cells

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Study question: Does a potent oxidative agent such as peroxinitrite reduce the granulosa cell response to FSH by a direct effect on FSHR expression?
Summary answer: Oxidative stress induced by addition of peroxinitrite reduces FSHR expression in cultured human granulosa cells from IVF patients.
What is known already: We have previously shown that glucose-induced oxidative stress alters the FSH response of cultured human granulosa-lutein cells by modifying the expression of the FSH-regulated genes CYP19A1 and PAPP and their correlation (ASRM 2013). FSHR levels were not modified suggesting a post-receptor mechanism.

ALDH3A2 is a known marker of intracellular oxidative stress. Peroxinitrite is a potent oxidative agent that is generated in vivo in mitochondria and rapidly degraded causing protein and lipid peroxidation.
Study design, size, duration: In vitro experiments using human granulosa cells collected from 10 oocyte donors attending our IVF program from September to December 2013.
Participants/materials, setting, methods: Granulosa-lutein cells from 10 oocyte donors attending a Private-University affiliated IVF center, were purified and cultured in the presence or absence of peroxynitrite (0.1 mM). Relative gene expression levels of ALDH3A2 and FSHR were determined by qRT-PCR. Statistical analysis was performed using the SPSS software.
Main results and the role of chance: The addition of peroxynitrite induces oxidative stress as reflected by a 1.9-fold increase of ALDH3A2, a known gene of oxidative stress response. These conditions induce a 2.7-fold reduction of the expression of FSHR. Differences were statistically significant (Student *t* test).
Limitations, reason for caution: The in vitro nature of this study limits the extrapolation of results to in vivo conditions.
Wider implications of the findings: FSHR expression, and consequently the granulosa cell response to FSH stimulation is reduced significamtly by oxidative stress. This may be one of the mechanisms involved in poor response and subfertility associated with age. Further clarification of these cellular mechanisms may lead to the development of adjuvant therapies for fertility improvement.
Study funding/competing interest(s): Funding by national/international organization(s), Fondo de Investigaciones Sanitarias (PI12/0729), Spain.
Trial registration number: Not applicable.

P-461 Importance of IL-18 in serum and follicle fluid in the context of fertility treatment

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Study question: Cytokines are key modulators of the immune system and also contribute to regulation of the ovarian cycle. In this study we analyzed

the importance of IL-18 levels in serum and follicle fluid in response to ovarian stimulation with gonadotropins, in correlation to pregnancy rate and also to clinical parameters.
Summary answer: Follicular fluid and serum collected from 90 IVF patients on the day of follicle puncture (FP) contain detectable levels of IL-18 concentration. IL-18 levels showed a good correlation in response to ovarian stimulation, pregnancy rate and to clinical parameters such as BMI.
What is known already: IL-18 is a pro-inflammatory cytokine which is produced primarily by haemopoietic cells and by several non-haemopoietic cell types, such as human ovary granulosa cells. Some authors detected significantly higher IL-18 levels in serum, peritoneal, and pleural fluids of patients with severe OHSS as compared with control groups and suggest a role of IL-18 as a marker of OHSS. Lower levels of IL-18 have been found to characterize unexplained infertility.
Study design, size, duration: From a total sample of 90 patients (2011–2013), we analysed the level of IL-18 in serum and FF on the day of FP. Furthermore, in response to ovarian stimulation, IL-18 cut-off levels were evaluated in serum between patients with poor and good response and in pregnancy rates.
Participants/materials, setting, methods: IL-18 mean and cut-off levels were evaluated in serum and FF on the day of FP by the ELISA method:

a) in response to ovarian stimulation with gonadotropins, between patients with a poor and a good response.
b) to the resulting pregnancy rate.
c) by comparing BMI.

Main results and the role of chance: IL-18 levels in serum were significantly higher than in FF ($p < 0.001$) revealing a positive and significant correlation ($r = 0.84$, $p < 0.001$). IL-18 levels in serum and in FF of patients with a BMI lower than 21 were significantly lower than in patients with a BMI higher than 22 ($p = 0.015$). Patients with a good response (E2 ≥ 2500 pg/ml, number of follicles ≥7 on the day of hCG injection) showed significantly higher IL-18 mean levels on the day of FP than patients with a poor response (E2 ≤ 2500 pg/ml, follicles ≤6, $p < 0.001$). We found a cut-off level of 158.6 (ng/ml) in serum with a sensitivity of 61% and a specificity of 64.5%. The pregnancy rates were 39% below and 32% above this cut-off level.
Limitations, reason for caution: None.
Wider implications of the findings: Our data suggest that IL-18 exerts an influence on ovarian function and thus on the result in hyper stimulated IVF/ICSI patients. Cut-off level for IL-18 is clinically relevant.
Study funding/competing interest(s): Funding by University(ies), University of Kiel, Department of Ob/Gyn Reproductive Medicine Campus Kiel, Kiel, Germany.
Trial registration number: No trial registration was required.

P-462 Antral follicle count on any day of the menstrual cycle strongly predicts ovarian response in IVF

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Study question: Antral follicle count (AFC) can be used on any day of the cycle as a marker of ovarian response to gonadotropins?
Summary answer: The evaluation of the AFC on any day of the cycle has a diagnostic accuracy similar to AMH levels in terms of ovarian response and pregnancy.
What is known already: AMH has shown to be comparable to AFC (follicles 2–10 mm during the early follicular phase) in the prediction of ovarian response and the stability of AMH during the cycle has been demonstrated. However studies where the intracycle variation of AFC has been analyzed are limited.
Study design, size, duration: Prospective cohort study performed between January 2011 and February 2012. We included 60 women attending the IVF/ICSI programme of our center.
Participants/materials, setting, methods: A transvaginal ultrasound and blood samples for AMH determination were performed independently of the day of the menstrual cycle (31 during follicular phase (5 in days 2–4); 29 during luteal phase). Poor ovarian response was defined as fewer than four oocytes retrieved or cycle cancellation.
Main results and the role of chance: Of 60 patients studied 13 were poor responders. AFC and AMH levels were significantly lower in poor responders than normal responders. AFC and AMH levels significantly correlated with number of follicles >16 mm on the day HCG and number of oocytes retrieved. The AUC_{roc} for AFC and AMH levels in predicting the likehood of poor or normal response was similar (0.85, 95% CI 0.74–0.93 and 0.76, 95% CI 0.64–0.86 respectively) however was higher than age (0.69, 95% CI 0.56–0.80). When the likehood of pregnancy was analyzed the AUC_{roc} for AMH and AFC was similar (0.78, 95% CI 0.66–0.88 and 0.80, 95% CI 0.67–0.89) but higher than age (0.56, 95% CI 0.43–0.69).
Limitations, reason for caution: AFC was not made with 3D ultrasound nor distinguished between different sizes of follicles less than 10 mm.
Wider implications of the findings: Our findings are consistent with the previous studies about the prognostic value of the AMH measured on any day of the menstrual cycle in terms of ovarian response. AFC measured independently of the day of the cycle has similar value that AMH levels being a cheap, feasible and available marker.
Study funding/competing interest(s): Funding by University(ies), "Agència de Gestió d´Ajuts Universitaris i de Recerca-Generalitat de Catalunya" (2009SGR1099).
Trial registration number: The study is not an RCT.

P-463 The ovarian stimulation method can cause oxidative stress in the follicular fluid

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Study question: Follicular fluid (FF) reflects its environment during follicular growth, and, therefore, the evaluation of oxidative stress in the FF can be used to predict oocyte quality. Therefore, could the measurement of oxidative stress in the FF be used as a biomarker to select an optimal stimulation protocol in ART treatment?
Summary answer: Ovarian stimulation increased the oxidative stress in the FF. The monitoring of both oxidative stress and antioxidant power in the FF might become a useful biomarker for selecting ovarian stimulation in ART treatment.
What is known already: FF reflects its environment during oocyte growth, and, therefore, the evaluation of oxidative stress in the FF could be used to predict oocyte quality. Total hydroperoxidase (TH) represents a group of reactive oxygen species (ROS). Thus, the determination of TH provides information about the fundamental mechanisms of oxidative stress. Conversely, the biological antioxidant potential (BAP) is used to represent overall antioxidant activity. Serum BAP provides a reliable measure of the power of an antioxidant barrier.
Study design, size, duration: Between December 2011 and November 2013, 89 samples from 76 patients were used in this study. The FF was obtained during the first puncture of follicular aspiration, and was stored at −30? until it could be assayed. Oxidative stress was measured using a Free Radical Elective Evaluator (WISMERLL, USA).
Participants/materials, setting, methods: d-ROM and BAP tests were used to measure oxidative stress (U.CARR) and anti-oxidant power (μmol/L), respectively. Twenty-six samples were obtained from the patients using clomiphene citrate (CC groups), 29 s were obtained following stimulation with CC and gonadotropins (CC-FSH group), and 34 were obtained in a natural cycle (natural group).
Main results and the role of chance: The mean d-ROM values in the CC, CC-FSH and natural groups were 358.0, 414.9, and 371.4, respectively, which showed a value for the CC group that was significantly lower than that for the CC-FSH group ($p < 0.05$). The mean BAP values for the CC, CC-FSH and natural groups were 2323.6, 2552.8, and 2354.8, respectively, which indicated a value for the natural group that was significantly lower than that for the CC-FSH group ($p < 0.05$). 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.

	CC group	CC-FSH group	Natural group
d-ROM (U.CARR)[#]	358.0[*]	414.9	371.4
BAP (μmol/L)[#]	2323.6	2552.8	2354.8[**]

[#]Values are reported as the means. [*]$p < 0.05$; vs. CC-FSH, [**]$p < 0.05$; vs. CC-FSH.

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 of the FF taken from the right and left follicles for any single patient. The measurements of oxidative stress in the FF for a single follicle might reflect the complete amount of oxidative stress in an ovary. The values found in the FF represented different levels depending on the stimulation protocol. Therefore, the values might be possible to become markers for the selection of a suitable stimulation protocol.
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: None.

P-464 Elevated early follicular progesterone levels and IVF outcomes: a prospective intervention study and meta-analysis

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Study question: What is the impact of elevated early follicular phase progesterone (P) levels in GnRH antagonist cycles on in vitro fertilization (IVF) outcome?
Summary answer: Elevated P levels on cycle day (CD) 2 affect ongoing pregnancy rates in GnRH antagonist cycles.
What is known already: Elevated P levels on CD 2 in GnRH antagonist cycles have previously been associated with a decreased chance of pregnancy.
Study design, size, duration: Open-label randomized controlled trial, conducted between September 2009 and July 2011 using a web-based program for randomization. 158 IVF/ICSI patients were included. As the incidence of elevated *P* levels is low and a large number of patients is needed to detect a significant difference, a meta-analysis was performed to assess its impact on ongoing pregnancy rates in GnRH antagonist cycles.
Participants/materials, setting, methods: Recombinant FSH (150-225 IU) was administered daily from CD 2 onward. GnRH antagonist treatment was started on CD 2 in the study group (CD2, $n = 81$), and on CD 6 in the control group (CD6, $n = 77$). These women were divided into two groups according to their P level on CD 2; normal ($n = 137$) or elevated P ($n = 21$). For the meta-analysis, a systematic search of MEDLINE and EMBASE from 1972–2013 was performed to identify relevant studies comparing elevated early follicular *P* levels in GnRH antagonist cycles ($n = 2$).
Main results and the role of chance: Elevated P levels were present in 13.3% of patients. There was a non-significant difference between the normal and elevated P group with regard to ongoing pregnancy rate per started cycle (27.0% vs. 19.0%, $p = 0.4$). Logistic regression demonstrated no differential impact of early or late GnRH antagonist initiation on the effect of high or normal *P* on ongoing pregnancy rates. These data and two eligible studies were included in a meta-analysis ($n = 1052$). Pregnancy rates were significantly affected in case of elevated *P* levels [absolute risk difference of 15% (95% confidence interval 7–23%, $p = 0.0003$)].
Limitations, reason for caution: The small sample size and low incidence of elevated *P* made it difficult to find a significant difference. Pooling the prospective data with existing data made it possible to detect a difference. The results showed substantial heterogeneity which may be caused by the different treatment regimens applied.
Wider implications of the findings: It is unknown how women with elevated *P* levels should be managed in order to optimize or normalize their prospects for successful IVF outcome. Different GnRH antagonist treatment regimens have been proposed, but so far none have demonstrated improved pregnancy rates. In view of the low incidence of this condition, routine screening for *P* is at present only justified for the purpose of research into solution management strategies.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), this study was partially supported by a grant from Merck Serono. O.H., M.J.C.E, A.V., P.A.D., R.E.B., G.J.E.O., C.A.G.H., G.C.D.M., H.J.V., P.F.M.H. and A.B. have nothing to declare. F.J.B. has received fees and grant support from the following companies; Ferring, Gedeon Richter, Merck Serono, MSD and Roche. B.J.C. has received fees and grant support from the following companies; Ferring, Merck Serono and MSD. C.B.L has received fees and grant support from the following companies; Auxogen, Ferring, Merck Serono and MSD. B.C.J.M.F. has received fees and grant support from the following companies; Andromed, Ardana, Ferring, Genovum, Merck Serono, MSD, Organon, Pantharei Bioscience, PregLem, Schering, Schering Plough, Serono and Wyeth. J.S.E.L. has received fees and grant support from the following companies; Ferring, Gennovum, MSD, Merck Serono, Organon and Serono. N.S.M. has received fees and grant support from the following companies: Anecova, Ferring, Merck Serono, MSD, Organon, Serono.
Trial registration number: www.clinicaltrials.gov, no. NCT00866034.

P-465 Comparisons of adiponectin and adiponectin receptors in human granulosa cells in obese and non-obese patients with poly cystic ovary syndrome

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Study question: What is the mRNA expression level of Adiponectin system in human granulosa cells and follicular level of high molecular weight adiponectin in obese and non-obese women with polycystic ovary syndrome?
Summary answer: Our data confirm that obese women, with similar pattern, have shown significantly a lower level of adiponectin, adipoR1 and adipoR2 mRNA than non-obese subjects. In addition, gene expression levels of adiponectin system were inversely with BMI both in PCOS and control women. Lastly *HMW* adiponectin in follicular fluid is present in women with *PCOS* at lower concentration of those found in controls.
What is known already: Adiponectin has become widely accepted as a key regulator of insulin sensitivity and metabolism but the physiological regulation

and role of adiponectin receptors, AdipoR1and AdipoR2 and glucose intolerance, in PCOS remain undefined. Some investigators showed that circulating adiponectin levels were not different between subjects with PCOS and BMI matched controls. However many investigators have reported a significant decrease in adiponectin level in obese women with PCOS compared to normal weight subjects with and without PCOS.

Study design, size, duration: This was a cross-sectional prospective study on 80 patients, conducted over a 18-month period. After approval of Iran University of medical sciences ethics committee and written informed consent of the patient, Human granulosa cells were obtained from women undergoing oocyte retrieval and were separated from follicular fluid. Patients were divided into four groups, according to Rotterdam criteria and BMI.

Participants/materials, setting, methods: A series of isolation and purification methods was performed, including density gradient centrifugation, MACS (use of antibody bead complexes) and RNA extraction. RT–PCR was applied to show the existence of adiponectin gene in granulosa cell. Quantitative real-time PCR analysis was applied to investigate the relative expression of this gene in purified granulosa cells. Obtained follicular fluid from each patient was used to determination of concentration of HMW adiponectin with ELISA technique (R&D system)

Main results and the role of chance: Our data revealed that Adiponectin and receptors are expressed in human mural granulosa cells. In Quantitative real-time PCR Adiponectin, AdipoR1, and AdipoR2 gene showed a significantly lower expression in PCOS patients compared with the control ($P < 0.05$). Also our results showed obese women with BMI ≥30 kg/m^2 especially in control groups have a lower gene expression of Adiponectin system than non-obese subjects with BMI <30 kg/m^2 ($P < 0.01$). The mean HMW adiponectin values were significantly lower in the serum (30.19 ± 20.8 vs. 48.47 ± 33.3 ng/ml, $P = 0.025$) and FF (7.86 ± 6.78 vs. 14.22 ± 11.55 ng/ml, $P = 0.025$) in the women with PCOS undergoing controlled ovarian hyper stimulation.

Limitations, reason for caution: Various phenotypes of PCOS (in accordance Rotterdam criteria) can effect adiponectin level.

Wider implications of the findings: The current results indicate that adiponectin with direct effect on granulosa cells significantly decreases in PCOS patient especially in obese subjects. This phenomenon can influence physiologic adiponectin roles such as interaction with insulin and LH, and cogonadotropins in induction of granulosa cell gene expression. These findings have been confirmed by the those of numerous studies. However, these have not been accepted in several studies.

Study funding/competing interest(s): Funding by University(ies), Iran University of Medical Sciences, Tehran, Iran.

Trial registration number: N/A.

P-466 Cryopreserved / warmed blastocyst transfer: comparison among hormonally controlled cycles without pituitary desensitization, natural cycles after spontaneous ovulation and hCG-primed natural cycles

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Study question: To assess the outcome of vitrified/warmed blastocyst transfer cycles using different endometrial preparation protocols. Three protocols were evaluated: natural cycles timed with urinary LH surge (group A), natural cycles but hCG-induced ovulation (group B) and hormonally controlled cycles (group C) with oral or transdermal estradiol without pituitary suppression.

Summary answer: Our data showed a statistically significant difference in clinical pregnancy rate between hCG-primed natural cycles and no pituitary-desensitized hormonally controlled cycles.

What is known already: To optimize pregnancy rates during frozen embryo transfer (FET) cycles, the embryo stage and the endometrium development must be properly synchronized. Timing of embryo transfer is determined by detecting the spontaneous LH surge or by administering hCG or by exogenous estrogens and progesterone administration. A recent meta-analysis of 43 publications and 20 articles found no differences in the clinical pregnancy rate among transfer protocols.

Study design, size, duration: We retrospectively analyzed 3 different endometrial preparations in 658 blastocyst frozen embryo transfers (FET) cycles performed between January 2012 and December 2013. 105 cycles were performed in group A, 138 cycles in group B and 415 cycles in group C.

Participants/materials, setting, methods: In Group A, FET was scheduled 7 days after urinary LH surge; in Group B, 7 days after hCG administered with a follicle size of 16 mm, and absent LH surge; in Group C, FET was scheduled after 5 days of 600 mg/day of vaginal progesterone supplementation.

Main results and the role of chance: The mean female age of the three groups was not different (group A: 36.6 + 3.6; group B 36.7 + 3.6; group C 36.7 + 3.8). Embryo's survival rate (group A: 98.1%; group B: 95.5%; group C: 96.8%) and number of embryos transferred (group A: 1.4 + 0.5; group B: 1.4 + 0.5 group C 1.4 + 0.5) were not significantly different. The pregnancy rate was 37.1% in group A; 43.5% in group B and 28.7 % in group C. The number of monitoring visits was 3 + 1.2 in group A, 2.7 + 1 in group B and 1.3 + 0.6 in group C. Our data showed a significant difference in clinical pregnancy rate between group B and C ($p < 0.001$). The differences between group C and A, and between group A and B were not significant.

Limitations, reason for caution: This study is a retrospective analysis and despite a large sample size, it should be confirmed by a RCT.

Wider implications of the findings: Contrary to what is purported in literature our data showed that programming FET using hCG to trigger ovulation during natural cycles is superior to hormonally-prepared cycles.

Study funding/competing interest(s): Funding by hospital/clinic(s), Humanitas Research Hospital.

Trial registration number: No.

P-467 Follicular fluid leptin help to predict in vitro fertilization outcomes

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Study question: A growing body of evidence suggests that leptin may have an important role in regulating ovarian function, so could follicular fluid leptin (FFL) alterations be related to IVF outcomes?

Summary answer: High FFL levels are related to a higher possibility of IVF failure. A diagnosis model is proposed including serum progesterone level the previous day at the hCG administration, follicle number and FFL at the time of oocyte retrieval.

What is known already: Some observational studies have found a possible negative relationship between FFL and negative IVF outcomes. Only one study found a positive correlation between FFL and oocyte quality. Other studies did not find any influence of FFL on pregnancy possibilities after IVF.

Study design, size, duration: Longitudinal, case-control study in a sample of women with diagnosis of infertility during their first, second or third cycle between September 2011 and February 2013

Participants/materials, setting, methods: A sample of 140 women, aged 26–40 years old. Forty per cent of patients had abdominal circumference (AC) over 80 cm and 22.9% were diagnosed with insulin resistance. Follicular fluid was collected from the first dominant follicle for leptin determination using ELISA (EZHL-80SK, Linco, Billerica, MA, USA).

Main results and the role of chance: FFL mean values were significantly lower in ongoing pregnancy, even after adjust for BMI, CA ≥80 cm or insulin resistance (IR). A multivariable logistic binary regression analysis showed that AC or IR have not influence in IVF outcomes, but FFL levels were significantly associated to probability of ongoing pregnancy. A lineal model with Forward LR method included serum progesterone level, follicle number and FFL and ROC analysis showed an area under the curve of 0.82. This model reaches a sensibility of 87% and a specificity of 71% for predicting the possibility of ongoing pregnancy.

Limitations, reason for caution: Our study has several limitations. First, FFL was obtained only of one selected follicle. Second, the study does not explain the mechanism of leptin action; in this sense, other serum or follicular fluid measurements such as androgens, oestrogens and growth factors, could help to know how leptin may operate.
Wider implications of the findings: FFL levels determination could play a prognostic role in human reproduction.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Oviedo University, CEFIVA.
Trial registration number: N/A.

P-468 Effect of PCOS on omentin-1, androgens, biochemical glycemic and lipid profile in pubertal girls with normal body mass index

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Study question: What is the role of Omentin-1 and the serum levels of androgens, glycemic-lipidemic markers in normal body mass index (BMI) pubertal girls with polycystic ovary syndrome (PCOS) compared with matched controls?
Summary answer: The negative cardiovascular effects of PCOS most likely begin as early as puberty, even in the presence of a normal BMI. Additionally, the association of PCOS and omentin-1 is more complex than previously thought and appears to regulate metabolism differently during puberty.
What is known already: Omentin-1 levels may be predictive of the metabolic consequences associated with PCOS patients. However, plasma levels of omentin-1 in patients with normal BMI are not as consistent as has been demonstrated in overweight or obese PCOS patients. To our knowledge; metabolic parameters, the relationship between omentin-1 and PCOS in pubertal girls has not been previously reported. Understanding the pathophysiological triggers that occur during puberty will benefit the future health of pubertal girls with PCOS.
Study design, size, duration: In this cross-sectional study we studied with 63 pubertal girls with PCOS and 159 matched controls. In all of the participants BMI was less than 25 kg/m^2.
Participants/materials, setting, methods: The diagnosis of PCOS was based on the recent ESHRE/ASRM proposal and required that all three of the Rotterdam criteria for diagnosing PCOS in adolescents be met. Indices of insulin sensitivity, metabolic variables, circulating androgen levels, lipidemic markers and serum omentin-1 levels were measured.
Main results and the role of chance: Blood pressure parameters (systolic/diastolic) were significantly higher in girls with PCOS (112. 44 mmHg vs. 104. 49 mmHg; $p < 0{,}001$ for systolic BP/74. 12 mmHg vs. 69. 01 mmHg; $p < 0{,}007$ for diastolic BP). There was no significant difference in the plasma levels of fasting glucose, total cholesterol, HDL, apo-A1 and SHBG in girls with PCOS compared to the control group.

Conversely, girls with PCOS had higher fasting insulin ($p = 0.007$), LDL ($p = 0.017$), triglyceride ($p = 0.045$), apoB ($p < 0.001$), total ($p < 0.001$) and free testosterone ($p = 0.001$) and omentin-1 ($p = 0.018$) levels compared to control group. Additionally apoB/apoA1 ($p = 0.021$) and FAI ($p = 0.004$) values were significantly higher in girls with PCOS compared to the control group.
Limitations, reason for caution: Limitations, reasons for caution: Cross sectional design of our study is main limitation. Long-term studies of girls with PCOS that begin during puberty will give us more knowledge about the regulation and effect of omentin-1 in PCOS.
Wider implications of the findings: Even in the presence of a normal BMI, decreased ApoB and increased ApoA1 levels in girls with PCOS may be an early sign of impending metabolic dysfunction and increased cardiovascular risk. Additionally, Omentin-1 regulation is multifactorial and dependent on mechanisms other than BMI and IR that have yet to be identified, at least in pubertal girls with normal BMI.
Study funding/competing interest(s): Funding by University(ies), Eskisehir Osmangazi University, Scientific Investigations Department.
Trial registration number: None.

P-469 Women diagnosed with Polycystic Ovary Syndrome with or without hyperandrogenemia; what are the true differences

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Study question: What are the known markers for cardiometabolic disease and cardiovascular risk factor distribution amongst women with a hyperandrogenic form of PCOS, compared to those presenting with ovulatory dysfunction and polycystic ovarian morphology (PCOM)?
Summary answer: Women with hyperandrogenic PCOS have a more unfavourable cardiometabolic profile and higher prevalence of cardiovascular risk factors compared to women with ovulatory dysfunction and PCOM.
What is known already: PCOS is a heterogeneous syndrome, in which different sub-phenotypes influence cardiometabolic presentation. **: Study design, size, duration:** A cross-sectional multi-centre study analysing 2288 well-phenotyped women with PCOS.
Participants/materials, setting, methods: Women of reproductive age (18–45 years) suffering from PCOS according to the Rotterdam criteria who underwent a standardized screening in one of our specialized reproductive outpatient clinics, enabling detailed comparison of the cardiometabolic profile in various PCOS sub-phenotypes.
Main results and the role of chance: Women with hyperandrogenic PCOS (n = 1219, 53.3% of total) presented with a less favourable cardiometabolic profile upon screening, resulting in a higher prevalence of cardiovascular risk factors such as obesity and overweight ($p < 0.001$), insulin resistance ($p < 0.001$) and the metabolic syndrome ($p < 0.001$), compared to women with non-hyperandrogenic PCOS. No significant differences in cardiometabolic risk factors, except for overweight or obesity, were observed amongst the different hyperandrogenic PCOS sub-phenotypes.
Limitations, reason for caution: This is a cross-sectional study assessing the cardiometabolic profile and cardiovascular risk factor distribution of women with different PCOS sub-phenotypes. Whether these risk factors result in cardiovascular disease and cardiovascular events remains to be determined in prospective follow-up studies.
Wider implications of the findings: Patients with hyperandrogenic PCOS might have an elevated risk of developing cardiovascular disease later in life and should be screened accordingly.
Study funding/competing interest(s): Funding by University(ies), University Medical Centre Utrecht, the Netherlands, Erasmus Medical Centre Rotterdam, the Netherlands.
Trial registration number: Not applicable.

P-470 Antral follicle counts: the application of 3D ultrasound in childhood cancer survivors

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Study question: How well do antral follicle counts (AFCs) of childhood cancer survivors (CCSs) assessed in real-time 2D agree with those obtained from stored 3D transvaginal ultrasound data and to what extent is the 3D technique subject to inter- or intra-observer reproducibility?
Summary answer: A high agreement between 2D and 3D measurements of AFCs was observed as indicated by ICCs, both for intra- and inter-observer reproducibility for the 3D technique. However, obesity and a high number of antral follicles seemed to weaken the reproducibility of 3D measured AFCs.
What is known already: Chemo- and radiotherapy may deplete the follicle pool, possibly leading to premature menopause in CCSs. AFCs may provide specialists with a tool to better estimate the age that menopause may occur. This could allow CCSs to make more informed choices about when to try to conceive. Both 2D and 3D measurements have demonstrated a high intra- and inter-observer reproducibility in healthy or subfertile subjects, but so far these findings have not been validated in CCSs.
Study design, size, duration: A cross-sectional study was performed in which AFCs of CCSs and controls were assessed. A random selection of 50 CCSs and 50 controls was taken. Intra-observer reproducibility was assessed in 27 subjects randomly selected from three groups of AFCs (low (<5) medium (5–11) and high (≥12)) measured in 2D.
Participants/materials, setting, methods: Subjects participated in the DCOG-LATER/VEVO-study, a cohort study evaluating reproductive outcomes after childhood cancer. AFCs measured in 3D by two different observers as well as AFCs measured in 2D and 3D by a single observer were compared. We analysed to what degree image quality or subject characteristics influenced reproducibility measures.
Main results and the role of chance: We found that ICCs were high for intra-observer, inter-observer and between-method reproducibility ((0.87, 0.85, and 0.80, respectively), indicating good agreement. However, when classifying subjects in three categories based on 2D measured AFC we observed only low to moderate agreement (0.40, 0.55, and 0.52 for intra-observer, inter-observer, and between-method reproducibility, respectively) using weighted Kappa values. This indicates that AFCs estimated with the 3D technique often classified women more than one category higher or lower than when using the 2D technique. Agreement was lower when AFCs were higher and in overweight subjects. Image quality was better in controls than in survivors ($p = 0.02$) who were more frequently overweight than controls ($p < 0.001$).
Limitations, reason for caution: Preferably, 3D measured AFCs should be compared with the gold standard, which is histology of the ovary. As this requires oophorectomy, this was not an option for this study. Previous studies have shown that 2D correlates well with histology, however, it is unknown if 3D AFCs shows similar agreement.
Wider implications of the findings: High ICCs validate the use of the 3D method for research purposes. This may have advantages such as blinding of data and using post-processing software and image enhancement. For counselling individual patients, caution should be taken when interpreting AFCs measured in 3D. Weighted Cohen's Kappa values showed that the agreement between 2D and 3D was low to moderate, making it plausible that individual patients are wrongly classified into a category with better or worse prognosis.
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.

P-471 Significant differences in gene expression and ontology profile exist between human granulosa and cumulus cells surrounding mature oocytes

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Study question: The main objective of the study was to analyse and compare genome wide gene expression profiles of individual mural granulosa (MGC) and cumulus (CC) cells samples derived from human ovarian follicles containing mature, metaphase II (MII), oocytes.
Summary answer: There was a clear separation pattern between MGC and CC after the principal component analysis (PCA) caught well by the first two most important principle components of variance. Furthermore, there were striking differences in gene expression between the two tissue types, with highly significant values of expression differences for individual genes.
What is known already: During human oocyte maturation granulosa cells differentiate into 2 populations: MGC and CC cells. The MGC form the follicular wall whereas CC are in direct contact with the oocyte. Communication between MGC, CC and oocyte is essential for the development of mature, competent oocytes.
Study design, size, duration: Twenty-three (23) infertile women were included in this prospective study. The study was approved by National Ethics Committee and all patients signed informed consent. Genome wide gene expression analysis was performed using microarrays on 64 individual MGC and CC samples.
Participants/materials, setting, methods:
Materials and methods: GnRH antagonist protocol was used for ovarian stimulation and ovarian follicles were aspirated individually. After that, MGC and CC were stored separately and oocytes cultured individually. Granulosa cells were isolated from follicular fluid using Dynabeads® CD45 Magnetic Beads. Agilent microarrays were used for analysis of differential gene expression.
Main results and the role of chance:
Results: There were 3310 genes significantly differentially expressed (q-value $< 10^{-4}$). Out of these, 356 showed ≥ 2-fold expression difference (logFC) with 288 up-regulated in MGC and 68 up-regulated in CC. Functions as immune response, defense response and lymphocyte activation were represented in the GSEA list for MGC. In CC RNA processing, cytoplasm organisation and biogenesis and glutamate signaling pathway were among enriched GSEA pathways.
Limitations, reason for caution: Limitations: Relatively small number of patients included in the study.
Wider implications of the findings: Unravelling of specific functions of MGC and CC would helps us understand the process of human ovarian folliculogenesis. A detailed understanding of this process could help optimize the processes of *in vitro* maturation of oocytes.
Study funding/competing interest(s): Funding by national/international organization(s), Public Research Agency of Slovenia.
Trial registration number: None.

P-472 The effects of folate supplementation on inflammatory factors and biomarkers of oxidative stress in overweight and obese women with polycystic ovary syndrome

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Study question: This study was conducted to determine the effects of folate supplementation on inflammatory factors and biomarkers of oxidative stress among women with PCOS.
Summary answer: In conclusion, folate supplementation (5 mg/day) among women with PCOS had beneficial effects on inflammatory factors and biomarkers of oxidative stress.
What is known already: Available evidences indicate that folate supplementation might help function of the vascular endothelium and reduced serum homocysteine (Hcy) levels among PCOS women.
Study design, size, duration: This randomized double-blind placebo-controlled clinical trial was conducted among 69 women diagnosed with PCOS, aged 18–40 years old. Participants were randomly assigned to three groups receiving: (1) Folate-1: 1 mg/day folate supplements (n = 23); (2) Folate-5: 5 mg/day folate supplements (n = 23) and (3) placebo (n = 23) for 8 weeks.
Participants/materials, setting, methods: Methods: This randomized double-blind placebo-controlled clinical trial was conducted among 69 women diagnosed with PCOS, aged 18–40 year old. Participants were randomly assigned to three groups receiving: (1) Folate-1: 1 mg/day folate supplements (n = 23); (2) Folate-5: 5 mg/day folate supplements (n = 23) and (3) placebo (n = 23) for 8 weeks. Fasting blood samples were taken at baseline and after 8 weeks' intervention to measure inflammatory factors and biomarkers of oxidative stress.
Main results and the role of chance: Results: Supplementation with 5 mg/day folate resulted in reduced plasma Hcy (–2.23 vs. –1.86 and 1.16 µmol/L, respectively, P-interaction = 0.01), HOMA-B (–7.63 vs. 1.43 and 13.66, respectively, P-interaction = 0.03), serum hs-CRP (–212.2 vs. –262.4 and 729.8 ng/mL, respectively, P-interaction = 0.04) and plasma MDA concentrations (–0.48 vs. –0.24 and 0.69 µmol/L, respectively, P-interaction = 0.008) compared with folate-1 and placebo groups. Furthermore, a significant rise in plasma TAC (0.64 vs. –3.53 and –215.47 mmol/L, respectively, P-interaction = 0.005) and GSH levels (162.13 vs. 195.80 and –158.24 µmol/L, respectively, P-interaction = 0.002) was also observed following the administration of 5 mg/day folate supplements compared with folate-1 and placebo groups. Taking folate supplements had no significant effects on plasma NO and catalase levels. When we adjusted the analysis for baseline values, the above-mentioned findings remained significant, except for plasma catalase ($P < 0.001$).
Limitations, reason for caution: We were unable to assess the effect of folate supplementations on other inflammatory factors and biomarkers of oxidative stress.
Wider implications of the findings: We are aware of no study examining the effects of folate supplementation on inflammatory factors and biomarkers of oxidative stress in patients with PCOS. The current study was, therefore, conducted to investigate the effects of the folate supplementation on inflammatory factors and biomarkers of oxidative stress in overweight and obese women with PCOS.
Study funding/competing interest(s): Funding by University(ies), The study was supported by a grant (no. 92109) from Kashan University of Medical Sciences.
Trial registration number: www.irct.ir: **IRCT201306085623N8**.

P-473 Levels of oxidative stress markers in follicular fluid of women undergoing IVF

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Study question: To compare the levels of oxidative stress markers in follicular fluid (FF) from infertility patients with polycystic ovary syndrome (PCOS) and tubal factors or male factors.
Summary answer: The lower levels of GSH-Px and SOD in FF, as effective oxidative stress markers, tend to decrease the clinical pregnancy rate.
What is known already: To date, the association of oxidative stress markers in the follicular fluid and IVF outcomes has been investigated in some studies. In these studies, the effect of oxidative stress on IVF patients outcomes have coflict results.
Study design, size, duration: A total of 138 infertile women from our center were enrolled in this study, including 85 patients due to tubal factors or male factors as the control group and 53 patients with PCOS (the PCOS group).
Participants/materials, setting, methods: Follicular fluid samples from patients undergoing controlled ovarian stimulation were collected on the day of oocyte collection. The pooled FF sample from each patient was centrifuged and frozen at −70°C until analysis. Hydrogen peroxide (H_2O_2), malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and glutathione (GSH) concentrations were detected. Patients were also further divided into the pregnant subgroup and the non-pregnant subgroup according to the clinical pregnancy outcome.
Main results and the role of chance: Serum levels of LH, LH/FSH and testosterone in the PCOS group were significantly higher than those in the control group ($p = 0.003$; $p = 0.008$; $p < 0.001$). There were no significant differences in six oxidative stress makers between the control group and the PCOS group. In the control group, the GSH-Px level of 24 pregnant women was 125.47 ± 52.08 U/ml, while it was 97.85 ± 30.73 U/ml in 43 non-pregnant patients ($p = 0.04$); the SOD level was 50.70 ± 12.83 U/ml in the pregnant subgroup and 42.65 ± 8.78 U/ml in the non-pregnant subgroup ($p = 0.02$). In the PCOS group, the GSH-Px level in the pregnant subgroup was significantly elevated than that in the non-pregnant subgroup (139.14 ± 40.46 U/ml vs. 102.18 ± 19.55 U/ml; $p = 0.019$), while the SOD level was also elevated (54.06 ± 11.70 U/ml vs. 44.18 ± 6.29 U/ml; $p = 0.033$). There were no significant differences in H_2O_2, MDA, CAT and GSH concentrations between the pregnant subgroup and the non-pregnant subgroup, in both control and PCOS groups.
Limitations, reason for caution: First, it was an observational study; hence, confounding variables such as age that may influence the results could not be avoided during the recruitment of patients. Then the current debate could possibly be attributed to the different assay methods and end point outcomes employed by each research group, along with the noted limited number of the relevant studies published on the subject.
Wider implications of the findings: Roles of ROS and antioxidants in female reproduction need to be further investigated, including potential relationship with PCOS follicle maturation.
Study funding/competing interest(s): Funding by national/international organization(s), This project was supported by China 973 Program (2012CB944703, 2012CB944702), National Science Foundation (81200439).
Trial registration number: No.

P-474 Benefit of dual trigger with GnRH agonist and hCG in women with high percentage of immature oocytes retrieved

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Study question: Is FSH rise induced with agonist trigger beneficial for final oocyte maturation in women with high percentage of immature oocyte retrieved in a previous cycle?
Summary answer: Double triggering with hCG and GnRH agonist trigger significantly increases the number of MII oocytes obtained in women with previous cycles with more than 50% immature oocytes.
What is known already: Some women show an unusual number of immature oocytes after inducing final oocyte maturation with hCG. Agonist trigger induces not only an LH rise but also an FSH rise that exists in natural cycles but not in hCG triggered cycles. The significance of this FSH rise is still not known.
Study design, size, duration: Retrospective cohort study of all cycles performed from January to December 2013 in which a prior cycle showed >50% immature oocytes at retrieval, that subsequently were dual triggered (GnRHa + hCG).
Participants/materials, setting, methods: 28 cycles were identified that met the required criteria: previous cycle with more than 50% of the retrieved oocytes were immature after rhCG (250 mg, Ovitrelle) triggering; subsequent cycle was performed with rhCG (250 mg) plus GnRHa (0.2 ml triptoreline, Decapeptyl), and ovum pick up was scheduled always at 36 h after triggering. Mean age of patients was 38.1 ± 3.1 years. The main outcome measure was percentage of MII oocytes retrieved. Paired analysis was performed with their previous cycle to compare outcomes. Paired t-test and Fisher's exact test were used were appropriate.

Main results and the role of chance:

	Prior cycle (hCG), n = 19	Dual trigger (hCG + GnRHa) n = 9	p-value
Peak E2 (pg/mL)	1088 ± 522	1157 ± 524	n.s.
Peak P4 (ng/mL)	0.51 ± 0.25	0.44 ± 0.29	n.s.
% of MII oocytes	24.11%	79.36%	<0.05
Fertilization rate (%)	25.65%	86.36%	<0.05

Limitations, reason for caution: Being a retrospective study with a limited sample size, results should be taken with caution until proper randomized trials are designed and presented.
Wider implications of the findings: If FSH rise exists in natural cycles, it may be relevant when inducing final oocyte maturation by improving oocyte competence.
Study funding/competing interest(s): Funding by hospital/clinic(s), IVI Madrid.
Trial registration number: NA.

P-475 CAG and GGN repeats in androgen receptor gene and its potential epigenetic effects in polycystic ovary syndrome (PCOS)

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Study question: The human androgen receptor (*AR*) gene contains two polymorphic trinucleotide (CAG and GGN) repeats, that of specific ranges of variants have been correlated with androgen-sensitive disease processes. The aim of our investigation is to identify the differences of CAG/GGN repeat numbers and expression levels between PCOS patients and control group.
Summary answer: The longer GGN repeat length of the *AR* gene is associated with PCOS occurrence. There were a significant relationship between mRNA expression and trinucleotide repeat length polymorphism, which may influence the disease process leading to PCOS.
What is known already: The human androgen receptor (*AR*) gene contains two polymorphic trinucleotide (CAG and GGN) repeats in exon 1, which can transcript into Glutamine (Gln) and Glycine (Gly) respectively. The number of CAG and GGN repeats may confer differential receptor activity, and specific ranges of variants have been correlated with androgen-sensitive disease processes.
Study design, size, duration: We compared frequency distributions of CAG and GGN repeat alleles and their pattern of expression via X-inactivation analysis among 76 age matched women with regular menstruation and 80 infertile women with PCOS in 2013, all of Han Chinese.
Participants/materials, setting, methods: HpaII tiny fragment enrichment by ligation-mediated polymerase chain reaction (HELP) was used to analysis the polymorphic trinucleotide (CAG and GGN) repeats sequence for finding the differences between PCOS patients and controls excluding the factor of X chromosome inactivity. Ovary tissues were used by real-time PCR for evaluate the relationship of CAG/GGN repeats numbers and expression. AR protein was found generally expressed on ovary section from PCOS patients by immunohistochemistry staining.
Main results and the role of chance: There were no significant difference of CAG repeat number between patients with PCOS and controls, but after X chromosome inactivity evaluation, the repeats in patients with PCOS were significantly longer than that in controls, and the number of homozygote in control group was significantly more than PCOS group ($p < 0.05$). The short number of GGN repeats and homozygote in control group were significantly more than that in PCOS group ($p < 0.05$). Moreover, our data have proved that expression levels of GGN repeats in *AR* gene were significantly increased than controls. However, a remarkable difference of *AR* activity was not found.
Limitations, reason for caution: Our study also suffers from limitations, such small samples, technology and artificial interference. All participants were chances Han people, which may cause some bias.
Wider implications of the findings: Our study offers evidence that the GGN repeat length of the *AR* gene is associated with PCOS occurrence, in a sample of Chinese women in reproductive age. Moreover, we also provided an evidence that there were a significant relationship between mRNA expression and trinucleotide repeat length polymorphism.
Study funding/competing interest(s): Funding by hospital/clinic(s), Center of Clinical Reproductive Medicine, The First Affiliated Hospital, Nanjing Medical University.
Trial registration number: This study is not RCT.

P-476 The effects of DASH diet on lipid profiles and biomarkers of oxidative stress in overweight and obese women with polycystic ovary syndrome: a randomised clinical trial

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Study question: We are aware of no study examining the effects of Dietary Approaches to Stop Hypertension (DASH) diet on metabolic profiles and biomarkers of oxidative stress in patients with PCOS. Summary answer: In conclusion, consumption of DASH eating pattern for 8 weeks among overweight and obese women with PCOS resulted in a significant decrease in weight and BMI. It led to a significant reduction in serum insulin, triglycerides and VLDL-C levels and a significant increase in plasma TAC and total GSH levels.
What is known already: The dietary approaches to stop hypertension (DASH) eating plan is a low-glycemic-index low energy-dense diet that has firstly been suggested for lowering blood pressure; however, its beneficial effects have also been reported in type 2 diabetes, gestational diabetes and metabolic syndrome. Although the influence of some dietary components of DASH diet like antioxidants, magnesium, dietary fiber, fruit and vegetables in PCOS has been assessed in previous studies, we are aware of no study examining the effects of DASH diet on metabolic profiles and biomarkers of oxidative stress in patients with PCOS. High contents of dietary fiber, antioxidants, phytoestrogens and iso flavones along with its low glycemic index might help PCOS patients to control their increased levels of lipid profile and oxidative stress.
Study design, size, duration: This randomised controlled clinical trial was done among 48women diagnosed with PCOS. Subjects were randomly assigned to consume either the control (n = 24) or the DASH eating pattern (n = 24) for 8 weeks.
Participants/materials, setting, methods: Methods: This randomised controlled clinical trial was done among 48 women diagnosed with PCOS. Subjects were randomly assigned to consume either the control (n = 24) or the DASH eating pattern (n = 24) for 8 weeks. Both diets were designed to be calorie-restricted. The DASH diet was consisted of 52% carbohydrates, 18% proteins, 30% total fats. It was designed to be rich in fruits, vegetables, whole grains, and low-fat dairy products and low in saturated fats, cholesterol, refined grains, and sweets. Prescribed sodium in the DASH diet was less than 2,400 mg/day. The control diet was also designed to contain 52% carbohydrates, 18% protein and 30% total fat; however, the two diets were different in terms of food groups contained. Fasting blood samples were taken at baseline and after 8-wk intervention to measure lipid profiles and biomarkers of oxidative stress including plasma total antioxidant capacity (TAC) and total glutathione (GSH).
Main results and the role of chance:
Results: Adherence to the DASH eating pattern, compared to the control diet, resulted in a significant decrease in weight (−4.4 vs. −1.5 kg; $P < 0.001$) and BMI (−1.7 vs. −0.6 kg/m^2; $P < 0.001$). Compared to the control diet, consumption of DASH eating pattern also led to decreased serum triglycerides (−10.0 vs. + 19.2 mg/dL; P-interaction = 0.005) and VLDL-C levels (−2.0 vs. + 3.9 mg/dL; P-interaction = 0.005). Individuals in the DASH group, compared to the control group, had reduced levels of serum insulin (−1.88 vs. + 2.89 μIU/mL, P-interaction = 0.03), but not fasting plasma glucose levels (−0.5 vs. + 5.7 mg/dL; P-interaction = 0.20), after intervention. Increased concentrations of plasma TAC (+98.6 vs. −174.8 mmol/L; P-interaction <0.001) and total GSH (+66.4 vs. −155.6 μmol/L; P-interaction = 0.005) were also found in the DASH group compared with the control group. We failed to find significant differences in mean changes of serum total cholesterol, HDL- and LDL-C levels between the two diets.
Limitations, reason for caution: The first limitation is the relatively short duration of intervention. Long-term interventions might result in greater changes in lipid profiles. Second, we could not assess the effects of DASH eating plan

on other biochemical indicators of oxidative stress and factors of peroxidation. Further studies are required to examine the effects of DASH diet on other measures of oxidative stress.
Wider implications of the findings: High contents of dietary fiber, antioxidants, phytoestrogens and iso flavones along with its low glycemic index might help PCOS patients to control their increased levels of lipid profile and oxidative stress.
Study funding/competing interest(s): Funding by University(ies), Funding: The study was supported by a grant (no. 9213) from Kashan University of Medical Sciences.
Trial registration number: www.irct.ir:IRCT201304235623N6.

P-477 DASH diet, insulin resistance and inflammation in polycystic ovary syndrome: a randomized controlled clinical trial

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Study question: Insulin resistance and increased inflammatory markers is one of the most common features of polycystic ovary syndrome (PCOS).
Summary answer: In conclusion, consumption of the DASH eating pattern for 8 weeks in overweight and obese women with PCOS resulted in an improvement in insulin resistance, serum hs-CRP levels and abdominal fat accumulation.
What is known already: Recently, it has been postulated that consumption of low-carbohydrate, high-protein diet as well as reduced glycemic load diets might help patients with PCOS to prevent complications. The dietary approaches to stop hypertension (DASH) eating plan is a low-glycemic-index low energy-dense diet that has firstly been suggested for lowering blood pressure; however, its beneficial effects have also been reported in insulin resistance, increased inflammation, T2D, gestational diabetes mellitus (GDM) and metabolic syndrome.
Study design, size, duration: This randomized controlled clinical trial was done among 48 women diagnosed with PCOS. Subjects were randomly assigned to consume either the control (n = 24) or the DASH eating pattern (n = 24) for 8 weeks.
Participants/materials, setting, methods: Methods: This randomized controlled clinical trial was done among 48 women diagnosed with PCOS. Subjects were randomly assigned to consume either the control (n = 24) or the DASH eating pattern (n = 24) for 8 weeks. The DASH diet was consisted of 52% carbohydrates, 18% proteins and 30% total fats. It was designed to be rich in fruits, vegetables, whole grains, and low-fat dairy products and low in saturated fats, cholesterol, refined grains, and sweets. Prescribed sodium in the DASH diet was less than 2,400 mg/day. The control diet was also designed to contain 52% carbohydrates, 18% protein and 30% total fat; however, the two diets were different in terms of food groups contained. Fasting blood samples were taken at baseline and after 8-wk intervention to measure insulin resistance and serum hs-CRP levels.
Main results and the role of chance: None.
Results: Adherence to DASH eating pattern, compared to the control diet, resulted in a significant reduction of serum insulin levels (−1.88 vs. 2.89 μIU/mL, P = 0.03), HOMA-IR score (−0.45 vs. 0.80; P = 0.01) and serum hs-CRP levels (−763.29 vs. 665.95 ng/mL, P = 0.009). Additionally, a significant decrease in waist (−5.2 vs. −2.1 cm; P = 0.003) and hip circumference (−5.9 vs. −1 cm; P < 0.0001) was also seen in the DASH group compared with the control group. QUICKI index tended to increase (0.02 vs. −0.01, P = 0.08) after the DASH diet than the control diet. We did not find significant differences in mean changes of fasting plasma glucose and HOMA-B index between the two diets.
Limitations, reason for caution: Our study was a relatively small randomized trial and larger trials are needed to confirm the findings. We were unable to assess the favorable effects of DASH eating pattern on other biomarkers of systemic inflammation. Furthermore, due to budget limitations we could not examine the effects of DASH diet on anti-mullerian hormone and endothelium-derived (nitric oxide) NO levels.
Wider implications of the findings: We are aware of no study examining the effect of DASH diet on insulin resistance and inflammation in patients with PCOS. As this condition is associated with hormonal imbalance, it is unknown if the DASH diet works in these women. High contents of dietary fiber, phytoestrogens and iso flavones as well as its low glycemic index might help PCOS patients to decrease insulin resistance and inflammatory factors.
Study funding/competing interest(s): Funding by University(ies), The study was supported by a grant (no. 9213) from Kashan University of Medical Sciences.
Trial registration number: www. IRCT: IRCT201304235623N6.

P-478 The use of a P73 gene polymorphism as a biomarker for the ovarian response to recombinant FSH stimulation

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Study question: Can a *P*73 gene polymorphism (G/A, rs4648551) be used as a biomarker for the ovarian response to recombinant FSH (rFSH) stimulation?
Summary answer: The *P*73 gene polymorphism was associated with the rFSH dosage used during IVF/ICSI treatment. The AA genotype was correlated with the use of a higher dosage of hormone. Genotyping of this polymorphism prior to stimulation might help physicians to personalize the treatment for each patient.
What is known already: Based on animals studies it is known that *P*73 (a member of P53 family) plays a role in primordial follicle pool, ovulation rate and maintaining genomic stability through the spindle assembly checkpoint. P73 null female mice have oocytes of reduced developmental competence and increased frequency of spindle defects that may also contribute to the sterility. These findings seem to be correlated with human infertility since an association of P73 polymorphism and woman age has been reported.
Study design, size, duration: This prospective cohort study included 153 infertile women subjected to IVF/ICSI ovarian stimulation from 01/2013 to 08/2013.
Participants/materials, setting, methods: All 153 patients were genotyped for polymorphisms in the *P*73 (G/A, rs4648551) gene using DNA extracted from peripheral blood and a TaqMan SNP genotyping assay. The results were correlated with the following parameters: age, AMH levels, antral follicular count, dosage of gonadotropins, follicle size and numbers of total and MII oocytes.
Main results and the role of chance: This analysis of the *P*73 gene polymorphism revealed an association between the AA genotype and higher dosages of rFSH (Gonal-F, Merck Serono) used during treatment. The mean dosage of rFSH was 2292 ± 769 IU in the AA group (n = 39) but only 2015 ± 902 IU in the group with other genotypes (AG + GG; n = 114) (P = 0.025). This association was confirmed by a positive Spearman's correlation between the AA genotype and rFSH dosage (P = 0.012). Hardy-Weinberg genotype distributions of the entire sample indicated concordance between the observed and expected frequencies. The other parameters analyzed in this study were not significantly different between the two groups.
Limitations, reason for caution: Because this was a preliminary study, additional validation of the analyzed SNP (i.e., increasing the number of cases) is needed to provide more information regarding the potential use of this polymorphism as an ovarian response biomarker. Differences in the genetic backgrounds of various ethnic populations should also be considered.
Wider implications of the findings: The ability to predict gonadotropin dosage using genetic markers can encourage patients to undergo additional cycles of ART with fewer side effects and low cost.
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. All patients provided written consent, and the local Research Ethics Committee approved the study.

P-479 Clomiphene citrate at low dosages reduces the rate of premature ovulation and increases the transfer rate per cycle in natural cycle IVF

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Study question: Can low dosages of clomiphene citrate reduce the rate of premature ovulation and thereby increase the transfer rate per initiated cycle in Natural Cycle IVF (NC-IVF) and is this effect age dependent?
Summary answer: Clomiphene significantly reduces the rate of premature ovulation and increases the transfer rate per initiated cycle, an effect which is not age dependent.
What is known already: NC-IVF has been suggested as an alternative to conventional gonadotropin-stimulated IVF in certain cases. However, even though implantation rates have been shown to be higher in NC-IVF than in conventional IVF, efficacy of this treatment is still limited due to a high rate of premature ovulation, resulting in low transfer rates per initiated cycle.
Study design, size, duration: Cross sectional study, involving 112 female IVF patients undergoing 211 IVF-cycles, 108 cycles without and 103 with clomiphene citrate from 2012 until July 2013 at a university based women's hospital.
Participants/materials, setting, methods: Women (35 ± 14.5 y) underwent one NC-IVF cycle and one NC-IVF cycle with clomiphene citrate 25 mg/day per day from day 6 or day 7 until the day of HCG application. On cycle day 10 or 11 ± 1, follicle diameter and concentration of 17β-estradiol and luteinizing hormone were analysed and follicle aspiration scheduled.
Main results and the role of chance: Clomiphene citrate reduced the rate of premature ovulation from 27.8% without clomiphene citrate to 6.8% ($p < 0.001$) and increased the transfer rate from 39.8% to 54.4% ($p = 0.039$) respectively. Pregnancy rates per transfer were 27.9% with and 25.0% without clomiphene citrate and per initiated cycle 11.1% and 13.6% respectively. The effect of clomiphene citrate was not age dependant. Use of clomiphene citrate resulted in mild flushes and headache in 5% of patients and nausea in none. Repetitive monthly treatment cycles were possible as low dosages of clomiphene citrate did not result in persisting ovarian cyst formation. On average of only 1.2 monitoring consultations per cycle were required before aspiration.
Limitations, reason for caution: Type of initial treatment cycle (with or without clomiphene citrate) was not randomized. 98 patients started NC-IVF cycle without clomiphene citrate and 14 patients NC-IVF with clomiphene citrate due to personal preferences. The dropout rate of supposedly very fertile patients, who became pregnant during the first treatment cycle, might have reduced the pregnancy rate in the second treatment cycle with clomiphene citrate.
Wider implications of the findings: The premature ovulation rate is one of the main draw backs of NC-IVF, resulting in lower treatment efficacy. As the use of clomiphene citrate increases treatment efficacy, allows monthly repetitive treatment cycles without relevant increase in costs and no additional injections and as only very low numbers of monitoring consultations before aspiration are required, NC-IVF using clomiphene citrate can become an alternative IVF treatment in certain cases.
Study funding/competing interest(s): Funding by University(ies), University womens hospital.
Trial registration number: DRKS00005137.

P-480 Analysis of ovarian function following chemotherapy for early breast cancer using an ultra-sensitive AMH assay

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Study question: What is the value of ultra-sensitive AMH assay (Ansh picoAMH) in the evaluation of ovarian activity in women with very low ovarian reserve after chemotherapy, in comparison with other current markers of the ovarian reserve?
Summary answer: The Ansh picoAMH assay showed a greater than 10 fold increase in sensitivity compared to previous assays. Post-chemotherapy AMH showed excellent prediction of future menses or amenorrhoea, although also revealed the extent of loss of the ovarian reserve in women with continuing ovarian activity.
What is known already: AMH serves as an early indicator of ovarian ageing including assessment of chemotherapy-induced ovarian follicle loss. AMH concentrations fall markedly to undetectable concentrations during/after chemotherapy and current assays are unable to detect AMH in women with very low ovarian reserve. However, the value of the new ultra-sensitive AMH assay is unknown.
Study design, size, duration: We performed a 5-year prospective cohort analysis of 56 premenopausal women with early breast cancer treated with surgery and adjuvant chemotherapy in most, with 14 not treated with chemotherapy. A similar but independent cohort of 42 women with early breast cancer treated with chemotherapy was also analysed to validate the data.
Participants/materials, setting, methods: We determined serum AMH levels using the Ansh picoAMH assay and other ovarian markers (menses, FSH, inhibin B, estradiol concentrations) at yearly interval for the 56 women described above. Comparison of hormone concentrations with menstrual function, and discriminatory value of ovarian markers in predicting menstrual pattern were made.
Main results and the role of chance: The assay limit of detection was 7.5 pg/ml (0.05 pmol/l). In premenopausal women with early breast cancer following chemotherapy, AMH was found to have high predictive value for menses for the subsequent 3 years. Undetectable AMH at 2 years followup suggested persistent amenorrhoea whereas detectable AMH concentrations suggested that ongoing menstruation was very likely at least 3 years thereafter, with a sensitivity of 96% and specificity of 90%; other markers of ovarian reserve (FSH, inhibit B) were also of value but to a lesser extent. AMH concentrations in women with menses after chemotherapy were not different to the older, no chemotherapy group (522 ± 169 vs. 275 ± 81 pg/ml; 35 ± 1.4 vs. 45.8 ± 1.4 years). These findings were validated in a second, independent cohort of women treated for early breast cancer.
Limitations, reason for caution: The cohorts of women are relatively small and further validation is required. Information on fertility potential or time to menopause in this group of women is not assessed.
Wider implications of the findings: This study shows that the Ansh picoAMH assay is more sensitive than the current assay and other ovarian markers in discriminating between women who retained ovarian function after chemotherapy from those without and it has high predictive value for subsequent menstrual pattern/ovarian function. This may provide valuable information to clinicians in deciding choice of subsequent treatment e.g. tamoxifen vs. aromatase inhibitor.
Study funding/competing interest(s): Funding by University(ies), MRC grant, University of Edinburgh.
Trial registration number: Nil as observation study.

P-481 Oocyte triggering in overweight and obese patients who undergo ICSI: 250 µg versus 500 µg recombinant hCG

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Study question: What is the impact of recombinant-human chorionic gonadotropin (r-hCG) dose; 250 µg or 500 µg, on intra cytoplasmic sperm injection (ICSI) outcomes in overweight and obese patients?
Summary answer: 500 µg r-hCG for oocyte triggering in ICSI cycles produced better oocytes and embryos and consecutively better pregnancy rates in overweight and obese patients when compared to 250 µg r-hCG.
What is known already: Although the follicular fluid hCG quantity was previously detected higher for 500 µg r-hCG users, the researchers did not find a significance in clinical pregnancy rates between the groups.
Study design, size, duration: Fifty-eight consecutive infertile patients (76 cycles) with a body mass index (BMI) ≥25 kg/m^2 who underwent ICSI between 2002 and 2012 were retrospectively evaluated according to the r-hCG dosage used for triggering ovulation.
Participants/materials, setting, methods: We included patients between 20–38 years of age and who has a BMI ≥ 25 kg/m^2, without polycystic ovarian syndrome (PCOS) and poor ovarian reserve. We used only fresh cycles. We stratified these cycles into two groups according to the method we used to trigger ovulation. Group I was constituted of 16 patients (21 cycles) who get 250 µg subcutan (s.c.) r-hCG for oocyte triggering whereas Group II was

constituted of 42 patients (55 cycles) who get 500 µg (s.c.) r-hCG for oocyte triggering.
Main results and the role of chance: The mean age (30.8 ± 4.7, 30.2 ± 4.1), BMI (28.6 ± 3.7, 28.4 ± 3.2) and infertility period in months (97.7 ± 51.1, 76.3 ± 55.1) were similar between the two groups. The numbers of retrieved oocyte-cumulus complexes (11.8 ± 6.8 vs. 11.6 ± 6.7), metaphase II oocytes (9.1 ± 5.0 vs. 9.6 ± 5.5), two pronucleated (2PN) oocytes (7.1 ± 4.1 vs. 7.7 ± 4.6) and the number of embryos transferred (1.5 ± 0.5 vs. 1.3 ± 0.4) were similar between groups. Of interest, Germinal vesicle (GV) oocytes/oocyte-cumulus complexes ratio (14.6% vs. 8.9%), rate of arrested embryos (18.0% vs. 7.5%) and rate of embryos with multinucleation (14.2% vs. 7.2%) were significantly higher in Group I when compared to Group II. The metaphase II oocytes/oocyte-cumulus complexes ratio (76.9% vs. 82.9%), the rate of embryos with ≥7 blastomeres on day 3 (61.4% vs. 79.4%) and rate of blastocyst transfer (11.8% vs. 51.8%) were higher in Group II when compared to Group I. Rates of implantation (12.9% vs. 32.4%) and clinical pregnancy per embryo transfer (19.0% vs. 41.8%, $P = 0.06$) were lower in Group I.
Limitations, reason for caution: This study was retrospectively designed and if more patients were evaluated; this would increase the power of this study.
Wider implications of the findings: This study will change the attitude towards the ICSI procedures on overweight and obese patients with a wide acceptability to other populations.
Study funding/competing interest(s): Funding by University(ies), Hacettepe University Hospital.
Trial registration number: GO 13/175, Hacettepe University Faculty of Medicine, Ethical Committee.

P-482 Effects of cabergoline administration for the prevention of OHSS upon the levels of VEGF, VEGFR-1 and VEGFR-2 in high-risk IVF/ICSI patients

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Study question: Which effects of cabergoline upon the vascular endothelial growth factor (VEGF) and its receptors (VEGFR-1/VEGFR-2) account for its preventive action in IVF/ICSI patients with high risk of ovarian hyperstimulation syndrome (OHSS)?
Summary answer: Protective effects of cabergoline against OHSS are mediated primarily through the system of VEGF receptors. Cabergoline increases the serum levels of VEGFR-1 and VEGFR-2 from the day of oocyte collection (OC) until the day of embryo transfer (ET), and has no effect upon the VEGF concentrations, which remain steadily elevated.
What is known already: OHSS is a potentially lethal iatrogenic complication of controlled ovarian stimulation. The main pathophysiological mechanism underlying OHSS is a dramatic increase in vascular permeability and angiogenesis, caused by the activation of VEGF system. Recently, an antagonist of D2-receptors with anti-angiogenic activity, cabergoline, started to be successfully used for the prevention of OHSS in high-risk IVF/ICSI patients. However its effects upon the balance between VEGF and its highly specialised and functionally diverse receptors (VEGFR-1/VEGFR-2) in this subgroup of patients remain unknown.
Study design, size, duration: An open, prospective, randomised controlled trial of 168 patients undergoing IVF/ICSI. Inclusion criteria: age <38 yrs, basal FSH <12 mIU/l, regular menstrual cycle, BMI:18–29 kg/m², ≤2 previous unsuccessful IVF/ICSI cycles. Randomisation to 'cabergoline' or 'no treatment' group was carried out only in a cohort of patients with high risk of OHSS (>15 oocytes retrieved, $n = 128$).
Participants/materials, setting, methods: Sixty-three patients were allocated to receive cabergoline (0.5 mg/day for 5 days from the day after OC until the day of ET at the blastocyst stage), and 65 – no treatment. Forty patients comprised a control group. All women received stimulation with rFSH + GnRH antagonists. Serum levels of oestradiol, progesterone, VEGF, VEGFR-1 and VEGFR-2 were assessed on the day of OC and ET.
Main results and the role of chance: Administration of cabergoline in high-risk IVF/ICSI patients from the day of OC until the day of ET resulted in a 19% reduction of absolute risk of early and moderate forms of OHSS. Serum VEGF levels in cabergoline and no treatment group were significantly elevated compared to controls throughout the observation period (OC: 534.8 ± 138.1 pg/ml, 529.9 ± 164.1 pg/ml and 389.8 ± 182.1 pg/ml; ET: 531.3 ± 176.9 pg/ml, 595.7 ± 182.9 pg/ml and 416.6 ± 180.8 pg/ml, $p < 0.05$ for each group vs. controls). Cabergoline significantly increased the serum levels of VEGFR-1 and VEGFR-2 from the day of OC to the day of ET ($p < 0.05$), replicating physiological changes in controls. In the no treatment group, no significant changes in the levels of VEGFR-1 and VEGFR-2 were detected. The use of cabergoline did not affect a pregnancy rate per cycle (32.3% vs. 36.5%, $p > 0.05$ for cabergoline vs. no treatment).
Limitations, reason for caution: This study did not include patients with polycystic ovary syndrome, and therefore, cannot provide answers whether cabergoline has similar effects in this subgroup of patients. Efficacy of cabergoline for the prevention of late and severe forms of OHSS was limited, resulting only in a 5% absolute risk reduction.
Wider implications of the findings: Cabergoline is effective for secondary medical prevention of early non-severe OHSS, and does not affect treatment success. However, careful selection of patients eligible for this strategy is mandatory to avoid undesirable complications. As an additional outcome of this study, we have identified a combination of threshold values for oestradiol and VEGF levels, and the number of retrieved oocytes, which can be used to select a specific subgroup of high-risk IVF/ICSI patients, eligible for the preventive administration of cabergoline.
Study funding/competing interest(s): Funding by hospital/clinic(s), This study received institutional support. The authors report no conflict of interest and have nothing to disclose.
Trial registration number: N/A.

P-483 Prostaglandin therapy during the proliferative phase improves pregnancy rates following frozen embryo transfer in a hormone replacement cycle

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Study question: To determine the efficacy of prostaglandin (PG) administration during the proliferative phase in order to improve pregnancy rates following thawed embryo transfer during a hormone replacement cycle (HRC).
Summary answer: Among patients who avoided fresh ET due to a thin endometrium, the pregnancy rate following a thawed cycle was low despite in hormone replacement cycle that used the graduated administration of estrogen. However, it was improved, when prostaglandin derivatives were used during the proliferative phase.
What is known already: When patients show a thin uterine endometrium (EM), they are forced to defer fresh embryo transfer (ET), and can undergo thawed ET in the following cycle. The EM is known to thicken with graduated administrations of estrogen, however a graduated estrogen administration protocol could not always improve pregnancy rate for women showing a thin EM. Therefore, an improvement is need in both the thickness and implantation ability of the EM.
Study design, size, duration: From September 2010 through March 2012, we randomly assigned 135 patients to two groups on the beginning day of their HRC; 67 patients underwent thawed ET using prostaglandin-HRC (PG group) and 68 used conventional HRC (conventional group).
Participants/materials, setting, methods: All patients were less than 40 years of age, and were forced to defer fresh ET due to a thin EM. Patients in prostaglandin group started to receive a prostaglandin E1 derivative (400 µg of misoprostol from D3 to D13). Pregnancy and implantation rates were compared between the two groups.
Main results and the role of chance: The endometrial thicknesses on the day of ET for the prostaglandin and conventional groups were 10.0 and 9.8 mm, respectively, and the changes in endometrial thicknesses between the ET cancellation day and the day of ET in the prostaglandin and conventional groups were +3.0 and +2.8 mm, respectively, and there were no significant differences

between the groups. The pregnancy rate in the prostaglandin group was 40.0% which was significantly higher than that in the conventional group (25.0%, $p < 0.01$). The implantation rate in the prostaglandin group (22.0%) was also significantly higher than that in the conventional group (13.0%, $p < 0.01$). The miscarriage rates for the prostaglandin and conventional groups were 25.1 and 28.6%, respectively, and there were no significant differences.
Limitations, reason for caution: We did not evaluated PG activity and several enzymes associated with PG synthesis in the myometrium among patients who were forced to defer fresh ET due to thin EM.
Wider implications of the findings: These data show that the condition of the EM might be easily reversed by HRC. However, the pregnancy rate in the conventional group was significantly lower, which raised the question of why this low pregnancy rate occurred despite an improvement in endometrial thickness by HRC. PG synthesis appears to be disrupted in patients with repeated IVF failure and they suggested that reduced PG synthesis in the human endometrium may lead to poor endometrial receptivity.
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 does not have RCT status, and, therefore, it did not receive a trial registration number.

P-484 Circulating irisin underscores the development of polycystic ovary syndrome

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Study question: Polycystic ovary syndrome (PCOS) is characterized by oligo/anovulation, polycystic ovary, and hyperandrogenism. Because many patients also present with dyslipidemia and insulin resistance, the causes of PCOS remain to be identified. We hypothesized that the newly identified brown adipose-differentiation hormone, irisin, may play a role in the development of PCOS.
Summary answer: PCOS patients have an overtly elevated fasting irisin level. This alteration is concurrent with the central presentation of PCOS (i.e., hyperandrogenism), and is significantly associated with a high risk of having PCOS even after controlling for confounding factors such as dyslipidemia and insulin resistance.
What is known already: The muscle-derived irisin is a newly identified myokine capable of promoting brown and beige adipose tissue differentiation, and thermogenesis in animals. Serum level of irisin is positively associated with body mass index (BMI), and is abnormally regulated in patients with type 2 diabetes or metabolic syndromes.
Study design, size, duration: A total of 202 patients diagnosed with PCOS according to the Rotterdam criteria and 47 healthy women were recruited over a 2-year period. Patients were subdivided based on the presence/absence of metabolic syndrome risk factors, as defined by the National Cholesterol Education Program's Adult Treatment Panel III report, or BMI.
Participants/materials, setting, methods: Healthy women and PCOS patients were recruited for a metabolic syndrome test at a teaching hospital. Levels of irisin, androgens, insulin, and adiponectin were measured by specific ELISAs. HOMA-IR, beta cell function, QUICKI, and $ISI_{Matsuda}$ were calculated to measure insulin sensitivity and insulin resistance.
Main results and the role of chance: As a group, PCOS patients exhibited hyperandrogenism, glucose intolerance, hyperinsulinism, and dyslipidemia. In addition, fasting irisin level was significantly elevated in PCOS patients as compared to control women ($p < 0.001$). Importantly, we found that fasting irisin level remains significantly elevated even after controlling for metabolic syndrome risk factors, or BMI. Analysis of the Likelihood Ratios, Predictive Values, and the effect size indicated that fasting irisin level is a significant risk factor for PCOS with an odds ratio of 6.6. Furthermore, our study showed that serum adiponectin levels in PCOS patients with metabolic syndrome risk factors are significantly reduced as compared with PCOS patients without the risk factors.
Limitations, reason for caution: Because the receptor(s) for irisin remain to be identified, it is not clear how irisin signaling is related to the central presentation of PCOS, hyperandrogenism, during the development of PCOS.
Wider implications of the findings: Aberrant regulation of the brown adipose tissue-differentiation factor irisin could represent an early event during the development of PCOS, and occur prior to the emergence of metabolic syndrome-like phenotypes in patients. This finding not only hinted at how endocrine factors from muscle and ovarian tissues interact to maintain normal ovarian physiology, but also revealed novel means to improve the detection of PCOS.
Study funding/competing interest(s): Funding by hospital/clinic(s), The study was supported by Chang Gung Memorial Hospital (CMRPG391151-3, CLC).
Trial registration number: Not applicable.

P-485 Thyroid axis dysregulation during in vitro fertilization in hypothyroid-treated patients

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Study question: How does thyroid-stimulating hormone (TSH) serum concentration change during In Vitro Fertilization (IVF) in hypothyroid-treated women?
Summary answer: Serum TSH significantly increases during IVF cycles in adequately treated hypothyroid women and exceeds the recommended threshold of 2.5 mIU/L in two thirds of cases.
What is known already: While there is a large body of evidence showing a significant impact of controlled ovarian hyperstimulation (COH) on thyroid function in euthyroid women undergoing IVF cycles, information on the effect of this treatment on thyroid axis equilibrium in hypothyroid-treated patients is insufficient.
Study design, size, duration: Prospective study including 72 hypothyroid-treated women selected for IVF between November 2010 and December 2011. Serum TSH was tested the month preceding the start of COH, at the time of hCG administration and at +16 days after hCG administration.
Participants/materials, setting, methods: Women were eligible if serum TSH tested the month preceding the IVF cycle was 0.4–2.5 mIU/L. Additional inclusion criteria were as follows: 1) a certified diagnosis of clinical or subclinical hypothyroidism; 2) assumption of at least 25 µg daily of levothyroxine; 3) Serum FT3 and FT4 tested the month preceding the IVF cycle within the reference range; 4) no previous IVF cycles; 5) regular menstrual cycles; and 6) day 3 serum follicle-stimulating hormone (FSH) <12 IU/ml and anti-Müllerian hormone (AMH) >0.5 ng/ml. Women who did not complete the treatment cycle and those failing to refer for the three assessments were subsequently excluded.
Main results and the role of chance: Serum levels of TSH at basal assessment, at the time of hCG administration and at +16 days after hCG administration were 1.7 ± 0.7, 2.9 ± 1.3 and 3.2 ± 1.7 mIU/L, respectively. All resulting comparisons were statistically significant. Serum TSH exceeded the threshold of 2.5 mIU/L in 46 subjects (64%, 95% CI: 53–75%) at the time of hCG administration and in 49 subjects (68%, 95% CI: 57–79%) at +16 days after hCG administration.
Limitations, reason for caution: We did not attempt to address the causes of these modifications and we exclusively monitored serum TSH without assessing serum-free T4 modifications. Further evidence is required to clarify these points. Moreover, based on the available evidence, we assumed that exceeding the threshold of 2.5 mIU/L may be detrimental but we do not have data to demonstrate that this may be true also in the particular population of hypothyroid-treated women undergoing IVF.
Wider implications of the findings: Based on our findings, we suggest to strictly monitor serum TSH in hypothyroid treated women during IVF cycles and, if necessary, to promptly adjust the levothyroxine dose. This is the most pragmatic approach but, to date, it is not supported by clinical evidence. Further studies aimed to clarify the most suitable therapeutic strategy are thus warranted.
Study funding/competing interest(s): Funding by hospital/clinic(s), Fondazione Ca' Granda Ospedale Maggiore Policlinico - Milan - Italy.
Trial registration number: Not applicable.

P-486 Phase I dose-ranging study of a recombinant human follicle-stimulating hormone (rhFSH) in healthy female volunteers

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Study question: This study was conducted to assess the safety, tolerability, pharmacokinetics, and dose-proportionality of 3 single subcutaneous (sc) doses (75, 150, and 300 IU) of a new rhFSH (XM17) in healthy female volunteers following a pilot-dosing phase in which the safety, tolerability, and pharmacokinetics of 37.5 IU sc were assessed.
Summary answer: In healthy female volunteers, XM17 was safe and well-tolerated at single doses up to 300 IU and exhibited dose-proportional pharmacokinetics at the 3 higher doses.
What is known already: XM17, manufactured using mammalian (Chinese hamster ovary) cells transfected with the human FSH gene, is being developed for use in women undergoing controlled ovarian hyperstimulation for assisted reproductive technologies (ART) and treatment of anovulation. The structure of XM17 is similar to the registered follitropin alpha (Gonal-f®) and its amino acid sequence is identical compared to follitropin alfa and follitropin beta (Puregon®).
Study design, size, duration: This was a single-center, open-label, dose-ranging, parallel group pharmacokinetic study of single sc XM17 doses in 40 healthy women whose endogenous FSH was downregulated using 3.6 mg goserelin. Blood samples for evaluation of standard pharmacokinetic parameters were drawn from 0–168 hours post-dose of XM17 (total per-subject study duration ~22–28 days).
Participants/materials, setting, methods: On Day 14 after FSH-downregulation, healthy women aged 29 ± 5.4 (SD) with mean body-mass index of 23.4 ± 2.9 received stepwise sc doses of XM17 beginning with 37.5 IU (pilot dose; *n* = 4), then 75, 150, or 300 IU (*n* = 12 each). Safety, tolerability, and maximal XM17 concentrations (C_{max}) and other pharmacokinetic parameters were determined.
Main results and the role of chance: Pharmacokinetic endpoints using individual baseline (pre-dose)-corrected XM17 serum concentrations were calculated using non-compartmental parameters based on actual times and summarized with descriptive statistics. Mean serum concentration-time profiles of XM17 demonstrated dose-related increases in C_{max} within 24 hours, followed by mono-exponential decay for the 3 higher dose levels. C_{max} rose by 0.032 IU/L per IU, and $AUC_{0\text{-}168h}$ rose 2.6 IU h/L per IU. Slopes estimated by linear regression for C_{max} and $AUC_{0\text{-}168h}$ were approximately equal to 1 (0.905 IU/L and 1.096 IU h/L, respectively). Geometric mean elimination half-life ranged from 54–90 hours. No antibodies to XM17 were detected. Headache was the most common adverse event, occurring in 11 subjects; dizziness occurred in 4 subjects, and 2 subjects reported mild injection site pain-to-touch.
Limitations, reason for caution: This open-label, single-center study of single doses of XM17 was conducted in a limited sample of healthy volunteers. It was not designed to infer efficacy, nor was it designed to infer safety following more than single doses. Results are not necessarily generalizable to infertile women undergoing ART.
Wider implications of the findings: Doses of 75, 150, and 300 IU of XM17 exhibited dose-proportional pharmacokinetics, and the anticipated clinical single sc doses of XM17 up to 300 IU per patient appear to be safe, with no evidence of antibody development or clinically relevant adverse events.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), This study was sponsored by BioGeneriX GmbH. Funding for editorial support was provided by Teva Women's Health, Inc., to MedVal Scientific Information Services, LLC, Skillman NJ. BG was an employee of BioGeneriX GmbH (organization responsible for conducting the study) and is now an employee of Merckle GmbH. AL and PB are employees of Merckle GmbH. BioGeneriX GmbH and Merckle GmbH are members of the Teva Group.
Trial registration number: This is a Phase I study and therefore exempt from the requirement to register with ClinicalTrials.gov.

P-487 Changes of PCOS (polycystic ovary syndrome)-related symptoms in mothers vs. non-mothers during long-term follow-up: the LIPCOS-Trial

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Study question: Do pregnancy and subsequent parenthood, as a major lifestyle intervention, influence the long-term course of PCOS-related symptoms?
Summary answer: By comparing a group of PCOS patients consisting of mothers and non-mothers, this study generated systematic information about the impact of pregnancy and parenthood on PCOS related symptoms, possibly mediated through the lifestyle changes associated with parenthood.
What is known already: Young women affected by PCOS often show clinical signs of hyperandrogenemia, hypertestosteronemia and insulin resistance. In addition, oligomenorrhea and infertility are common. Due to increasing endogenous FSH, advancing age has been shown to positively influence both cycle length and fertility. Whether the changes in lifestyle associated with motherhood can influence the course of PCOS-related symptoms has not been evaluated so far.
Study design, size, duration: This study recruited a cohort of 75 oligomenorrhoeic patients who underwent in-depth laboratory and sonographic evaluation for PCOS as well as interviews on symptom severity, lifestyle and nutrition, comparing baseline information with respective values for the preceding 10 years. Subsequently, participants were followed prospectively for 4 years.
Participants/materials, setting, methods: Intra-individual change of cycle length, clinical signs of hyperandrogenemia, serum testosterone, BMI and changes in activity and nutrition, were compared between childless PCOS-patients and PCOS-patients with preceding pregnancy and parenthood in the last 10 years at baseline. 15 women have completed the 4-year follow-up examination.
Main results and the role of chance: In 53 evaluable participants (Ø 38.8 years) at baseline, BMI had increased from 23.2 to 25.3, and total testosterone (TT) had decreased (0.75 ng/ml to 0.5 ng/ml), while average cycle length had shortened in the preceding 10 years (61.4 to 53.3 days). While 56.6% (*n* = 13) of mothers (*n* = 23, Ø 38.4 years, BMI 24.7) reported cycle length shortening (average from 55.3 to 47.6 days), only 43.3% (*n* = 13) of childless women (*n* = 30, Ø 32.1 years, BMI 25.3) reported shortening of cycle length (66.31 to 57.5 days). Baseline TT levels were lower in mothers than in non-mothers (0.45 vs. 0.54 ng/dl). Compared with their earlier years, 87% of mothers reported healthier nutrition vs. 73.3% of childless patients and 91.3% vs. 66.6% increased regularity of everyday life.
Limitations, reason for caution: Women with more severe PCOS may be less likely to achieve motherhood. Sample size may prevent subtle differences from reaching significance.
Wider implications of the findings: Should the lifestyle changes associated with parenthood prove to be beneficial in the course of PCOS, this could motivate PCOS patients for their active pursuit of fertility-enhancing strategies.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Technische Universität München, Klinikum rechts der Isar München.
Trial registration number: Non existent.

P-488 Impact of an oestradiol drop prior to human chorionic gonadotrophin administration on live-birth rates following gonadotrophin-releasing hormone antagonist co-treated cycles

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Study question: Do spontaneous oestradiol (E_2) serum level falls prior to final oocyte maturation and ovulation triggering with human chorionic gonadotrophin (hCG) affect in vitro fertilisation/intra-cytoplasmic sperm injection (IVF/ICSI) outcomes when ovarian stimulation (OS) is down-regulated with gonadotrophin-releasing hormone (GnRH) antagonists?
Summary answer: Spontaneous E_2 drops prior to hCG triggering do not affect IVF/ICSI outcomes in GnRH antagonist down-regulated cycles.
What is known already: Previous researchers have found that spontaneous (without gonadotropin step-down or "coasting") E_2 decreases prior to hCG administration may reduce fertilisation, implantation and pregnancy rates. However, these studies only accounted for age as a potential confounder and have only been performed in IVF/ICSI cycles with GnRH agonist co-treatment. Despite the lack of evidence of its deleterious effect, similar drops are still considered as a predictor for inferior IVF outcomes in GnRH antagonist co-treated cycles.
Study design, size, duration: This retrospective case-control study (between January-2006 and March-2012) involved all patients aged between 18–36 undergoing their first or second IVF/ICSI attempt in our centre with serum-E_2 assessments on both the day/day-before hCG (1149 cycles). Eighty-three

cycles with spontaneous E_2 drops were matched with another 83 control-cycles in which E_2 increased.
Participants/materials, setting, methods: Patients underwent OS with exogenous gonadotrophin/GnRH antagonist co-treatment. We administered hCG when ≥3 follicles of ≥17 mm were observed, followed 36 h later by oocyte retrieval. Computer-randomised control-cycle matching was performed using these variables grouped in regular intervals: age, number of oocytes retrieved, hCG-day E_2 levels and day of embryo transfer (ET).
Main results and the role of chance: We found no statistically significant differences between the study and matched-control groups in terms of clinical pregnancy (28.9% *versus* 31.3%, $p = 0.74$) and live-birth (16.9% *versus* 20.5%, $p = 0.55$) rates. Furthermore, maturation (82.7% *versus* 82.7%, $p = 0.51$), fertilisation (75.3% *versus* 71.2%, $p = 0.12$), cycles without ET (9.6% *versus* 9.6%, $p = 1.00$), single/multiple ET (84.0% *versus* 73.3%, $p = 0.58$) and miscarriage (21.3% *versus* 17.3%, $p = 0.54$) rates were similar between both groups. The mean hCG-day E_2 level, patient age and oocyte retrieval count were 1235.9 pg/mL, 30.3 years old and 10.7 oocytes, respectively. Sixty-three percent of all ETs were performed with a day-3 embryo. The type of exogenous gonadotropins used (recombinant follicle-stimulating hormone *versus* highly purified human menopausal gonadotrophin) and their start and total doses were similar.
Limitations, reason for caution: Although this is, to our knowledge, the largest study to ever assess the effect of spontaneous hCG-day E_2 drops, it may require further confirmation by a larger series. Furthermore, these results should not be extrapolated to patients with long-lasting follicular-phase E_2 drops far from the day of hCG administration.
Wider implications of the findings: Contrary to prior evidence, spontaneous E_2 decreases immediately before hCG administration do not seem to hinder IVF outcomes when GnRH antagonists are used for pituitary down-regulation. Hence, unexpected E_2 drops should not prompt hasty medical interventions or be considered as a predictor for poorer IVF/ICSI outcomes in a GnRH antagonist setting. Rather, this study points out a need for further evaluation of the effect and physiologic mechanisms behind both subtle and drastic spontaneous E_2 decreases.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Centre for Reproductive Medicine, Dutch-speaking University Hospital of Brussels.
Trial registration number: Not applicable.

P-489 PCOS in Asian vs. Caucasian women and in first vs. second generation Asian women; a comparative analysis

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Study question: What are the phenotypical differences between Asian and Caucasian women suffering from Polycystic Ovarian Syndrome (PCOS) and between first and second generations Asian women with PCOS?
Summary answer: Asian and Caucasian PCOS patients are phenotypically different with regards to PCOS. Further, first and second generation Asian women differ with regards to age of onset of symptoms, age of presenting complaint and SHBG levels.
What is known already: The available evidence has shown that Asian women from the Indian Subcontinent have a higher incidence of PCOS. Other studies have shown that the two ethnic groups have some phenotypical differences. There have been no published studies assessing if differences exist between the first and second generations of Asian women in the UK.
Study design, size, duration: This is a retrospective nested case control cohort study of 432 patients to examine the differences between Caucasian (288) and Asian (144) women from the Indian subcontinent suffering from PCOS (Rotterdam Criteria) and between first (102) and second (42) generations of Asian PCOS women in the UK.
Participants/materials, setting, methods: This is a nested case control study where for every Asian woman 2 Caucasian women matching for age were selected at a University Teaching Hospital. Several parameters were compared between the two groups. The first and second generation Asian women were identified and compared with each other.
Main results and the role of chance: The mean age was 26 years. Caucasians presented significantly more frequently with bleeding problems ($p < 0.001$) and Asians presented with infertility ($p < 0.001$). Age of menarche is significantly lower in Caucasian women ($p = 0.023$). Asians experienced significantly more irregular periods than Caucasians ($p = 0.001$). Asians had significantly higher family history of diabetes mellitus than Caucasians ($p < 0.001$). SHBG levels are significantly higher in Caucasians than Asians ($p < 0.001$). TSH levels ($p = 0.023$), BMI ($p = 0.024$) and Waist-hip Ratio ($p = 0.006$) are significantly higher in Asians than Caucasians. In the Asian population; the second generation patients presented at an earlier age ($p = 0.027$). Significantly more first generation patients presented with infertility ($p = 0.001$) while more second generation patients presented with PCOS related symptoms ($p = 0.001$). There are significantly higher levels of SHBG in second generation patients ($p = 0.022$).
Limitations, reason for caution: The retrospective study and the relatively small number for an epidemiological study have their inherent limitations on the study findings.
Wider implications of the findings: This study confirms the effect of ethnicity on the PCOS phenotypes and also suggests that migration of the Asian women to the UK have affected their phenotypes. This should inform the management of PCOS and health policy in an increasingly multi-ethnic society.
Study funding/competing interest(s): Funding by University(ies), University Teaching Hospital.
Trial registration number: Not Applicable.

P-490 Effect of AMH on granulosa-lutein cells response to gonadotropins

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Study question: The objective of this study was to assess the effect of increasing doses of Anti-Mullerian hormone (AMH) on basal and stimulated steroids production by granulosa-lutein (hGL) cells.
Summary answer: The expression of aromatase CYP19A and P450 side chain cleavage (P450scc) genes in hGL cells was significantly increased by luteinizing hormone (LH) and follicle-stimulating hormone (FSH) or combined. Although AMH was unable to affect the basal expression of aromatase genes, it completely prevented the stimulatory effect of gonadotropins.
What is known already: AMH has a negative and inhibitory role in many functions of hGL cells including notoriously the reduction of the aromatase CYP19A expression induced by FSH. No data have been provided on the possible role of AMH in modulating the response to LH (alone or combined with FSH) as well as its effect on other enzymes involved in steroidogenesis including P450scc.
Study design, size, duration: mRNA expression of CYP19A and P450scc modulated by AMH was evaluated in a dose/response study in primary hGL cell culture. Set of hGL cells were also tested after LH and FSH stimulation at 50 ng/ml dosage for 24 hours alone or combined and then treated with 10 ng/ml AMH.
Participants/materials, setting, methods: The CYP19 and P450scc mRNA expression, normalized by housekeeping ribosomal protein subunit 7 (RpS7) gene, was evaluated by RT-qPCR. Each reaction was repeated in triplicate. Negative controls using corresponding amount of vehicle control for each hormone treatment were performed.
Main results and the role of chance: Our results showed that AMH did not modulate the basal mRNA expression of both aromatases genes at any of the concentrations tested. Meanwhile the strong mRNA induction of CYP19A and P450scc generated by 24 hours gonadotropins treatment (alone and combined) was suppressed by 10 ng/ml AMH added to culture medium.
Limitations, reason for caution: This preliminary study was conducted only *in vitro* on primary hGL cell culture recovered from patients who underwent *in vitro* fertilization protocol. The individual variability of patients tested was statistically not significant and did not affect the consistency of the results on aromatase genes expression.
Wider implications of the findings: Our results contribute in clarifying the relationship between hormones regulating the early phase of steroidogenesis confirming, in agreement with literature, that AMH is playing a suppressive role on CYP19A expression stimulated by gonadotropin in hGL cells. Furthermore, a similar inhibitory effect for AMH was observed on P450scc gene expression when activated by gonadotropin treatment.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), University Hospital Policlinico of Modena.
Trial registration number: None.

P-491 Relation between serum progesterone at the end of controlled ovarian stimulation and treatment outcome across type of gonadotropin preparations

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Study question: How does serum progesterone at the end of stimulation affect the ongoing pregnancy rate in IVF/ICSI patients according to the type of gonadotropin preparation (HP-hMG versus recombinant FSH) used for controlled ovarian stimulation? At which progesterone level can a decline in ongoing pregnancy rate be predicted?
Summary answer: Overall, ongoing pregnancy rate decreased with increasing serum progesterone at the end of stimulation in IVF/ICSI patients. The relation between end-of-stimulation progesterone concentration and ongoing pregnancy rate appears to be influenced by the type of gonadotropin preparation.
What is known already: Elevated progesterone levels at the end of stimulation have been shown to have a detrimental effect on treatment outcome in IVF/ICSI patients. Different threshold values have been suggested, depending on patient characteristics and treatment protocol.
Study design, size, duration: Analysis of two large randomised controlled trials comparing HP-hMG versus recombinant FSH with respect to ongoing pregnancy rate in controlled ovarian stimulation cycles for IVF/ICSI. One trial included 731 patients in a GnRH agonist protocol (MERIT; Nyboe Andersen et al. 2006) and the other 749 patients in a GnRH antagonist protocol (MEGASET; Devroey et al. 2012). The gonadotropin starting dose was 225 IU/day and 150 IU/day, respectively, and was fixed for the first 5 days.
Participants/materials, setting, methods: A total of 1,419 IVF/ICSI patients contributed with data to this analysis; 710 were treated with HP-hMG (Menopur, Ferring Pharmaceuticals) and 709 were treated with recombinant FSH (352 with follitropin alfa and 357 with follitropin beta). Serum progesterone at the end of stimulation was analysed centrally using the same assay for both trials (ECLIA assay, Elecsys platform, Roche). The median (interquartile range) progesterone concentration at the end of stimulation was 2.9 (2.2; 3.7) and 2.5 (1.8; 3.3) nmol/L for rFSH and HP-hMG respectively. Ongoing pregnancy rate was defined as a viable fetus 10–11 weeks after transfer. Logistic regression was used to analyse the impact on ongoing pregnancy rate of serum progesterone at end of stimulation and of the exposure to progesterone (i.e. AUC from stimulation day 6 to the end of stimulation). The results are presented as odds ratios (OR) with 95% confidence intervals. Based on the logistic regression models, the progesterone concentration above which the predicted ongoing pregnancy rate declined to below average was identified for each type of gonadotropin.
Main results and the role of chance: For the overall population (N = 1.419), the ongoing pregnancy rate decreased with increasing end-of-stimulation progesterone with an OR of 0.87 [0.80; 0.95] (p = 0.003) per started cycle and 0.87 [0.79; 0.95] (p = 0.003) per cycle with embryo transfer. Among the patients treated with recombinant FSH, the impact of increasing end-of-stimulation progesterone was reflected by ORs of 0.83 [0.73; 0.95] (p = 0.005) and 0.84 [0.73; 0.96] (p = 0.01) for the two analysis population, respectively. For patients treated with HP-hMG, the OR for ongoing pregnancy by increasing progesterone at the end of stimulation was 0.93 [0.82; 1.05] (not significant) and 0.91 [0.80; 1.03] (not significant) per started cycle and per cycle with embryo transfer, respectively. Similarly, for the overall population the impact of progesterone AUC was also significantly (p < 0.01) inversely associated with ongoing pregnancy rate. The findings were consistently observed in both individual trials. Among the patients with embryo transfer, the predicted ongoing pregnancy rate declined to below the average observed in the trials when the end-of-stimulation progesterone levels reached above 2.4 nmol/L for patients treated with rFSH (2.4 nmol/L in the agonist protocol and 2.3 nmol/L in the antagonist protocol) and above 3.7 nmol/L for patients treated with HP-hMG (4.0 nmol/L in the agonist protocol and 3.5 nmol/L in the antagonist protocol).
Limitations, reason for caution: The trial populations were primarily good-prognosis patients.
Wider implications of the findings: Progesterone levels at end of stimulation should be interpreted in the context of the type of gonadotropin preparation used.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Ferring Pharmaceuticals.
Trial registration number: MERIT: not applicable. MEGASET: NCT00884221.

P-492 Relation between dose of FE 999049, a recombinant FSH derived from a human cell-line, used for controlled ovarian stimulation and number of oocytes, embryos and blastocysts

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Study question: What is the relationship between FE 999049 dose used for stimulation and number of oocytes retrieved as well as the subsequent development of embryos and blastocysts?
Summary answer: The number of oocytes retrieved and the number of embryos on day 3 increased significantly with increasing FE 999049 dose. A similar relationship was not observed between FE 999049 dose and number of blastocysts on day 5. Consequently, the blastocyst to oocyte ratio decreased significantly with increasing FE 999049 dose.
What is known already: It has been proposed that the use of mild or moderate doses of gonadotropins results in a sufficient number of good-quality embryos/blastocysts, even though fewer oocytes are obtained compared with higher doses.
Study design, size, duration: Pre-defined analysis of 222 women in a randomised, assessor-blind, multicentre trial investigating FE 999049 (Ferring Pharmaceuticals), a recombinant FSH expressed from a cell-line of human fetal retinal origin (PER.C6®), for controlled ovarian stimulation for IVF/ICSI. Patients were randomised to fixed doses of 5.2, 6.9, 8.6, 10.3 or 12.1 µg/day FE 999049 throughout stimulation. Randomisation was stratified by serum AMH.
Participants/materials, setting, methods: IVF/ICSI patients aged 18–37 years with a BMI of 18.5–32.0 kg/m^2 underwent stimulation in a GnRH antagonist cycle (ganirelix 0.25 mg/day from day 6). Triggering was done as soon as ≥3 follicles ≥17 mm were observed. Oocytes were cultured for 5 days and were assessed daily. Embryo morphology (number of blastomeres, blastomere uniformity, cell size classification, degree of fragmentation and multinucleation) was assessed on day 3, and good-quality embryos were defined as ≥6 blastomeres and ≤20% fragmentation. Blastocyst quality on day 5 was graded using the Gardner & Schoolcraft classification system (1999), i.e. blastocyst expansion and hatching status 1–6, inner cell mass grading A-C and trophectoderm grading A-C, and good-quality blastocysts were defined as at least 3BB. Continuous data were analysed using ANCOVA models and proportions were analysed using logistic regression models. The models included centre, AMH and log(dose) as explanatory variables.
Main results and the role of chance: The number of oocytes increased significantly (p < 0.001) from an average of 5.2 to 12.2 by increasing FE 999049 dose with a linear dose-response. The fertilisation rate, however, decreased significantly (p < 0.001) with increasing FE 999049 dose. On day 3, the total number of embryos and the number of good-quality embryos increased significantly (p < 0.001 and p = 0.01, respectively) by increasing FE 999049 dose. This was not accompanied by a significant relationship between FE 999049 dose and the number of blastocysts on day 5, neither the total number nor those of good-quality. Consequently, the proportion of oocytes that developed into blastocysts decreased significantly (p < 0.001) with increasing FE 999049 dose. The live birth rate in the fresh cycle and cumulatively after 6-months cryopreserved cycles was unrelated to FE 999049 dose.
Limitations, reason for caution: Extrapolation to FE 999049 doses outside those included in this investigation should be done with caution.
Wider implications of the findings: Although higher gonadotropin doses lead to more oocytes, this may not result in more blastocysts available for

transfer / cryopreservation once a certain threshold of oocytes is reached. In addition to safety considerations, a suitable target downstream from oocytes could be taken into account when establishing gonadotropin regimens for controlled ovarian stimulation.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Ferring Pharmaceuticals.
Trial registration number: NCT01426386.

P-493 Impact of body weight of IVF/ICSI patients on the pharmacokinetics and pharmacodynamic responses to FE 999049, a recombinant FSH derived from a human cell-line

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Study question: How does female body weight influence the pharmacokinetic profile during controlled ovarian stimulation with FE 999049? What are the implications for the pharmacodynamic response to FE 999049?
Summary answer: During stimulation with FE 999049, circulating levels of FSH were significantly negatively influenced by body weight, with patients at a lower body weight having higher serum FSH than those with higher body weight. Body weight also had a significant inverse relationship with estradiol, inhibin B, inhibin A and follicular development during stimulation with FE 999049, with lower weight patients having greater responses than higher weight patients.
What is known already: Body weight / BMI has been hypothesised to be a predictor of ovarian response to gonadotropin therapy.
Study design, size, duration: Explorative analysis of 222 women in a randomised, assessor-blind, multicentre trial investigating FE 999049 (Ferring Pharmaceuticals), a recombinant FSH expressed from a cell-line of human fetal retinal origin (PER.C6®), for controlled ovarian stimulation for IVF/ICSI. Patients were randomised to fixed doses of 5.2, 6.9, 8.6, 10.3 or 12.1 µg/day FE 999049 throughout stimulation. Randomisation was stratified by serum AMH. The body weight ranged from 46 to 95 kg, with an average of 62 ± 9 kg and a median of 61 kg.
Participants/materials, setting, methods: IVF/ICSI patients aged 18–37 years with a BMI of 18.5–32.0 kg/m^2 underwent stimulation in a GnRH antagonist cycle (ganirelix 0.25 mg/day from day 6). Serum samples for endocrine profile were analysed centrally and follicular development was assessed by ultrasound on stimulation day 4 (i.e. after 3 days of treatment), stimulation day 6 (i.e. after 5 days of treatment and before start of GnRH antagonist) and at the end of stimulation. Data were analysed on the log-scale using a linear model with body weight and log-transformed baseline observation as covariates. For illustration of the quantitative impact, patients were grouped above and below the median body weight. As the duration of stimulation was significantly ($p = 0.002$) different between body weight groups, focus is on the early stimulation period (i.e. stimulation days 4 and 6) rather than at the end of stimulation.
Main results and the role of chance: With increasing body weight, serum concentrations of FSH, estradiol, inhibin B and inhibin A on stimulation days 4 and 6 decreased significantly ($p < 0.001$ for all parameters at both time points). On stimulation day 6, the effect of body weight was also observed for follicular development as shown by significantly lower average follicle size and follicular volume ($p < 0.001$ for both parameters) with increasing body weight. On stimulation day 6, the estradiol, inhibin B and inhibin A levels and follicular volume were 41%, 12%, 42% and 20%, respectively, greater in women with lower body weight compared to those with higher body weight.
Limitations, reason for caution: Extrapolation to extremely high body weights beyond those tested in this investigation should consider that the relation between body weight and ovarian response (endocrine response and follicular development) may not be linear.
Wider implications of the findings: As body weight has a significant impact on serum FSH concentrations and consequently on follicular development and endocrine profile after treatment with FE 999049, considerations should be given to gonadotropin dosing regimens taking the patient's body weight into account in order to optimise ovarian response.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Ferring Pharmaceuticals.
Trial registration number: NCT01426386.

P-494 The influence of patient-specific variables and dose-response modelling in controlled ovarian stimulation with FE 999049, a human recombinant FSH derived from a human cell-line

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Study question: Model-based dose-response characterisation for oocytes retrieved with FE 999049 and quantitative assessment of selected predictors obtained at baseline.
Summary answer: A patient-specific dose-response model was identified. The expected number of oocytes retrieved was modelled as a function of serum AMH, FE 999049 dose and body weight.
What is known already: The ovarian response to controlled ovarian stimulation varies substantially across patients. The variation can partly be explained by baseline characteristics like serum AMH, basal FSH, basal inhibin B, antral follicle count, body weight and age.
Study design, size, duration: Pre-defined analysis of 222 women in a randomised, assessor-blind, multicentre trial investigating FE 999049 (Ferring Pharmaceuticals), a recombinant FSH expressed from a cell-line of human fetal retinal origin (PER.C6®), for controlled ovarian stimulation for IVF/ICSI. Patients were randomised to fixed doses of 5.2, 6.9, 8.6, 10.3 or 12.1 µg/day FE 999049 throughout stimulation. Randomisation was stratified by serum AMH.
Participants/materials, setting, methods: IVF/ICSI patients aged 18–37 years with a BMI 18.5 to 32.0 kg/m^2 underwent stimulation in a GnRH antagonist cycle (ganirelix 0.25 mg/day from day 6). Triggering was done as soon as ≥3 follicles ≥17 mm were observed. Data were analysed using pharmacokinetic and dose-exposure-response non-linear mixed effects models.
Main results and the role of chance: The pharmacokinetics of FE 999049 were significantly ($p < 0.001$) impacted by body weight. A significantly ($p < 0.001$) impact of body weight was also observed on follicular development and endocrine profile during stimulation. The relation between body weight adjusted FE 999049 dose and the expected number of oocytes retrieved was described using a sigmoidal model with patient-specific maximal drug response (Emax). The error terms were assumed to follow a negative binomial distribution. Step-wise forward selection identified serum AMH as the best parameter for predicting the patient-specific Emax. Introducing additional parameters (basal FSH, basal inhibin B, antral follicle count and age) did not result in a substantial improvement of the model fit. An exposure-response model did not provide a better fit to the data than the body weight adjusted dose-response model, and did furthermore confirm AMH to be the best predictor of the patient-specific Emax.
Limitations, reason for caution: The dose-response model needs to be confirmed prospectively.
Wider implications of the findings: Patient-specific dose-response models based on body weight and serum AMH can be used to propose an individualised dose of FE 999049 aiming at a desired target of ovarian stimulation.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Ferring Pharmaceuticals.
Trial registration number: NCT01426386.

P-495 Anti-Mullerian hormone in female hypogonadotrophic-hypogonadism

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Study question: What are the anti-Mullerian hormone (AMH) levels in female hypogonadotrophic-hypogonadism (HH) and what can we learn from them regarding pathophysiology?
Summary answer: AMH levels show positive correlation with number of antral follicles within the ovary and no correlation with gonadotrophin levels. These results suggest that follicles are capable of intrinsically producing AMH independently and are not influenced by gonadotrophin levels.
What is known already: AMH is produced by small antral follicles in the ovaries. Previous studies demonstrate correlations between serum AMH concentrations

and LH (positive) and FSH (negative). Little is known about AMH in HH. There is a subgroup of HH cases who respond to controlled ovarian hyperstimulation similarly to women with polycystic ovarian syndrome. Serum AMH has been demonstrated to be similar in HH compared with ovulating controls in one small study. The subject is far from clear.

Study design, size, duration: Retrospective observational study of 11 consecutive cases of HH (amenorrhoea, FSH and LH levels <2 mmol/l, hypo-oestrogenic). Ultrasound findings and AMH levels were collected and compared over a 3-year period.

Participants/materials, setting, methods: Patients recruited to the study were from those attending the fertility clinic at a tertiary referral university hospital. AMH was measured using the Beckman-Coulter Gen II assay.

Main results and the role of chance: 11 patients were recruited. The relationship between astral follicle count (AFC) and AMH was investigated using Spearman's rho. There was a large, positive correlation between the two variables, rho = 0.79, $n = 11$, $p = 0.004$, with high levels of AFC associated with high levels of AMH. The same test was conducted removing the one clear outlier. There was a larger, positive correlation between the two variables, rho = 0.85, $n = 10$, $p = 0.002$. All cases, by definition, had a very low FSH and LH levels and there was no relationship between AMH and gonadotrophin levels.

Limitations, reason for caution: The number of cases analysed is small but HH is relatively rare. Despite this the results show a strong and significant trend. A more detailed hormone profile including testosterone levels and further ultrasound details such as ovarian and stroma volume was not available which limited the scope of this study.

Wider implications of the findings: These results contrast with those previously documented suggesting that gonadotrophins drive AMH production or vice-versa. In this study of HH patients this was shown not to be the case as all subjects had very low gonadotrophin levels with wide ranging AMH levels. The results suggest that small ovarian follicles are intrinsic producers of AMH and not driven by gonadotrophins. The study also delineates two distinct types of HH, with or without multifollicular ovaries.

Study funding/competing interest(s): Funding by hospital/clinic(s), Fertility Unit, Homerton University Hospital.

Trial registration number: Nil.

P-496 Beneficial role of ethinyl estradiol in prevention of anti-estrogenic effects of clomiphene citrate in polycystic ovary syndrome patients undergoing intrauterine insemination: a randomized clinical trial

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Study question: This study was designed to assess if low-dose ethinyl estradiol (EE) can prevent anti-estrogenic effects of clomiphene citrate (CC) on endometrial thickness and uterine perfusion in polycystic ovary syndrome (PCOS) patients undergoing intrauterine insemination (IUI)?

Summary answer: CC used in combination with EE is likely has the potential to produce favorable outcomes in PCOS women undergoing IUI by improving endometrial thickness and uterine artery pulsatility index (PI).

What is known already: There is some evidence to suggest that use of estrogens as an adjuvant treatment may have a beneficial impacts in prevention of detrimental effects of CC.

Study design, size, duration: In this study, which was conducted at Royan Institute Research Center as a randomized, double blind, placebo controlled and parallel group design, a total of 154 eligible patients (77 in each group) were enrolled.

Participants/materials, setting, methods: During the recruitment period of 2009–2013 a total of 154 Patients were randomly divided in a double blind approach by computer-generated random numbers to receive the treatment of either study (CC plus EE) or control groups (CC plus placebo). The drugs similarly packaged in serially numbered, closed envelopes.

Main results and the role of chance: Of the 154 enrolled patients, a total of 59 patients' treatment cycles were cancelled before human chorionic gonadotropin (hCG) is given and these patients were excluded. Both groups were comparable in regards to mean age, body mass index, infertility duration and basal hormone levels. The women used EE and CC combination, had significantly thicker endometrium (9.7 ± 2.1 vs. 8.9 ± 1.6 mm, $P = 0.036$) and lower values for the PI of both uterine arteries compared with placebo-CC controls ($P = 0.027$; $P = 0.001$). Furthermore, statistically significant differences were found between the groups with respect to clinical pregnancy (28.9% vs. 10%, $P = 0.019$). The miscarriage rate was not statistically significant between groups.

Limitations, reason for caution: -

Wider implications of the findings: This finding is close to the estimate of one study, reported a higher ongoing pregnancy rate for patients with unexplained infertility when CC is used in combination of EE. Similarly, another study reported that adding EE creates a desirable endometrial response even with very low doses. In these two studies no significant differences in PI values were noted. There is little empirical evidence regarding the relationship between endometrial and uterine blood flow and pregnancy after IUI.

Study funding/competing interest(s): Funding by national/international organization(s), This study was funded by Reproductive Biomedicine Research Center, Royan Institute. This study has no conflict of interest.

Trial registration number: The trial registration number is NCT01219101.

P-497 Live birth rates in women with recurrent miscarriage and thyroid peroxidase antibodies

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Study question: How high are live birth rates in euthyroid women with unexplained recurrent miscarriage with thyroid peroxidase antibodies (TPO-Ab) compared to euthyroid women with unexplained recurrent miscarriage (RM) without these thyroid peroxidase antibodies?

Summary answer: In euthyroid women with unexplained recurrent miscarriage, the presence of thyroid peroxidase antibodies was associated with a lower live birth rate, but only in women that did not use levothyroxine.

What is known already: There is an established association between TPO-Ab and recurrent miscarriage, but very little is known about the actual chance of a live birth. Two studies reported on live birth rate and both concluded that TPO-Ab status does not affect live birth rate. A limitation from these studies was that the live birth rate was not calculated for the complete study or control group, but only for those women who actually became pregnant.

Study design, size, duration: This retrospective cohort study was conducted among 408 women that consulted the recurrent miscarriage clinic in a Centre for Reproductive Medicine, University Hospital, between 2005 and 2011.

Participants/materials, setting, methods: 408 women with RM were invited to participate and fill out a questionnaire about their subsequent pregnancies after the initial RM work-up. Serum thyroid stimulating hormone (TSH) concentration and presence of TPO-Ab were determined. Patients with other causes for RM were excluded. No official institutional review board approval was needed according to national legal requirements.

Main results and the role of chance: Data from 202 women with unexplained recurrent miscarriage were available for analysis. 28 women were TPO-Ab positive (13.8%). From these 28 women, 10 women were treated with levothyroxine and 18 women did not receive treatment. 174 women were TPO-Ab negative. Apart from a slightly higher TSH level in the group women with TPO-Ab without treatment ($p = 0.023$), baseline characteristics were not different between the groups. 29% of women with TPO-Ab without treatment had a pregnancy resulting in a live birth after 12 months compared to 51% of women without TPO-Ab (log rank test $p = 0.011$, hazard ratio 0.23, 95% CI 0.07–0.72, p 0.012). Women with TPO-Ab receiving levothyroxine had a live birth rate comparable to women without TPO-Ab, namely 60% (log rank test $p = 0.55$).

Limitations, reason for caution: The participation rate (59%), the chance for recall- and selection bias, and the small sample size might have influenced the results.

Wider implications of the findings: In clinical practice, it is important to inform women with RM and TPO-Ab about their prognosis for a live birth. Counseling patients remains difficult even including the results of this study. The results provide an extra argument for starting randomised trials to assess the effectiveness of levothyroxine treatment in women with RM and TPO-Ab.

Results of such trials might be included in future RM guidelines with a potential change in screening and treatment advice.
Study funding/competing interest(s): Funding by hospital/clinic(s), Academic Medical Centre, Centre for Reproductive Medicine, Amsterdam, The Netherlands.
Trial registration number: Not applicable.

P-498 Follicular development following transvaginal injection of autologous adipose-derived mesenchymal stromal cells to ovaries in premature ovarian failure patients

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Study question: Oocyte donation in POF patients cannot reach its popularity due to legal and ethical reasons in many countries. It is necessary to generate new options ameliorating diminished ovarian function and even may help patients have a genetically related child in the future.
Summary answer: The results have shown for the first time that transvaginal ovarian injection of autologous ADSCs is associated with encouraging hormone results and improved ovarian function, which may make ADSCs serve as a safe and feasible treatment modality to facilitate ovarian recovery.
What is known already: ADSCs are of autologous tissue origin with low immunogenicity, and are more easily available because of minimal ethical considerations. ADSCs have been tested in clinical practice to regenerate injured tissues with promising results, which makes it an extensive promising source of pluripotent stem cells. Before applying this new therapeutic technique to humans, experiments in POF model rats using ADSCs injection to confirm the therapeutic effect on the damaged ovarian function were established in our lab.
Study design, size, duration: Four female patients meet with our inclusion criteria show no responses to hormone replace treatment (HRT) were enrolled. All four patients underwent ovarian transplantation between December 2012 and April 2013. The follow-up lasted 8 to 12 months.
Participants/materials, setting, methods: The Baseline characteristics, hormonal profile, and ultrasound findings of four Chinese Han women patients presenting with POF seeking pregnancy were documented pre- and post-transplantation in University affiliated hospital. No severe side effects, such as were observed after the operation in all cases.
Main results and the role of chance: Six months After the ADSCs transplantation, FSH in the patients was 47.3 ± 10.65, significantly decreased compared to pre-transplantation (111.9 ± 12.75) ($P < 0.05$). A conspicuous increase of E_2 was found after the ADSCs injection surgery (13.74 ± 3.27 vs. 100.2 ± 29.69). All patients belonging to those with active signs of follicle-like structure after transplantation. The blood flow at the ovaries where ADSCs were injected was assessed by contrast enhanced transvaginal ultrasonography. After the injection of ADSCs, showing an increase of ovarian volume and blood flow in the ovaries. Ovary volume was also measured pre- and post-transplantation of ADSCs (1282 ± 124.7 vs. 2343 ± 200.1).
Limitations, reason for caution: Rigorous, double-blind clinical trials in large cohort of individuals will be performed in order to support and promote the long-term therapeutic uses of ADSCs. The pregnancy rate was not that satisfying, which could be related to the long duration of their POF history and/or to the severity of ovarian insufficiency.
Wider implications of the findings: Encouraging hormone results and improved ovarian function were found after ADSCs transplantation in women with POF, transvaginal ovarian injection of ADSCs may serve as a promising treatment approach to facilitate ovarian recovery.
Study funding/competing interest(s): Funding by national/international organization(s), State Key Development Program of Basic Research of China Grant (973 Project No. 2010CB945104).
Trial registration number:
The clinical trial registration number is NCT01853501.

P-499 Transforming growth factor-beta1 decreases progesterone production by down-regulating StAR expression through Smad2/3 and ERK1/2 signaling pathways in human granulosa cells

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Study question: The aim of this study was to investigate the effect of transforming growth factor-beta1 (TGF-β1) on progesterone production and the underlying molecular mechanisms in human granulosa cells.
Summary answer: TGF-β1 decreased progesterone production in human granulosa cells by down-regulating steroidogenic acute regulatory protein (StAR) expression through Smad2/3 and ERK1/2 signaling pathways. Interestingly, TGF-β1 did not affect the expression of cholesterol side chain cleavage enzyme (P450scc) or 3β-hydroxysteroid dehydrogenase (3β-HSD).
What is known already: Many animal studies have shown that maintaining a low progesterone level until the time of ovulation induction is critical for normal follicular development. In addition, elevation of serum progesterone levels in IVF patients before or on the day of human chorionic gonadotropin (hCG) administration is associated with significantly decreased implantation and pregnancy rates. However, to date, only a handful of studies have investigated the roles of TGF-β1 in the regulation of steroidogenesis in granulosa cells.
Study design, size, duration: SVOG cells are human granulosa cells that were obtained from women undergoing IVF and immortalized with SV40 large T antigen. Cultured SVOG cells were used to investigate the effects of TGF-β1 on the expression of steroidogenesis-related genes and proteins expression as well as the progesterone production.
Participants/materials, setting, methods: Cultured SVOG cells were treated with recombinant human TGF-β1 and the expressions of steroidogenesis-related genes and proteins were examined by RT-qPCR and western blotting, respectively. The accumulation levels of progesterone were measured by enzyme-linked immunosorbent assay (ELISA). The experiments were repeated on at least three independent occasions.
Main results and the role of chance: TGF-β1 treatment down-regulated StAR expression and decreased progesterone production without affecting the expression of P450scc or 3β-HSD in SVOG cells. The suppressive effects of TGF-β1 on StAR expression and progesterone production were abolished by pre-treating a potent and specific TGF-β1 type I receptor inhibitor, SB431542, and siRNA-mediated knockdown of TGF-β type I receptor. In addition, treatment with TGF-β1 activated the Smad2/3 and ERK1/2 signaling pathways. Inhibition of the Smad2/3 and ERK1/2 signaling pathways attenuated the TGF-β1-induced down-regulation of StAR expression and progesterone production.
Limitations, reason for caution: The limitation is that the effects of TGF-β1-induced down-regulation of StAR expression and progesterone production were only investigated in immortalized human granulosa cells. Testing of the findings in primary culture of human granulosa cells is needed to further confirm the role of TGF-β1 in the regulation of progesterone production.
Wider implications of the findings: The production of progesterone by granulosa cells plays a critical role in maintaining a successful pregnancy at early embryonic stage. Therefore, a better understanding of the regulation of progesterone production in human granulosa cells would provide significant insights into ovarian physiology and pathology, which may lead to new methods of infertility treatment. Our results suggest that TGF-β1 may prevent premature luteinization by inhibiting progesterone production before the time of ovulation.
Study funding/competing interest(s): Funding by national/international organization(s), This work was supported by an operating grant from the Canadian Institutes of Health Research to P.C.K.L. and the National Natural Science Foundation of China (31271605) to Y.P.S. F.L is the recipient of scholarship from China Scholarship Council.
Trial registration number: Not applicable.

P-500 Discrepancy between antimüllerian hormone and antral follicle count: "The winner is…"

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Study question: Antral follicle count (AFC) and antimüllerian hormone (AMH) have shown to be good predictors of poor ovarian response (POR) to stimulation treatment. However, what should clinicians expect to happen when a patient has normal AFC value but low AMH or the opposite?
Summary answer: The incidence of POR was 27% when only AMH was below its cut-off for POR and 28% when only AFC was below its cut-off. Pregnancy rates (PR) and embryo quality did not differ significantly between both settings (low AMH + normal AFC vs. normal AMH + low AFC).

What is known already: Prediction of ovarian stimulation outcomes is of interest in order to correctly identify patients with a very poor prognosis. AMH and AFC have an added value to age in predicting POR; however, concerning prediction of pregnancy after IVF, AFC is of limited value and AMH has shown conflicting results. Counselling patients is even harder when AMH and AFC show divergent values.
Study design, size, duration: Retrospective study carried out in a University-affiliated fertility clinic from 2009–2013. Cycles in which AMH and AFC values were available were analyzed (n = 863). POR was defined as cycle with <4 oocytes retrieved / cancelled. Cut-off values for POR prediction were: AMH < 0.4 ng/ml, AFC < 7 follicles.
Participants/materials, setting, methods: Four settings are distinguished: low AMH and AFC (I), low AMH with normal AFC (II), normal AMH with low AFC (III), normal AMH and AFC (IV). Patients' baseline characteristics and stimulation outcomes are compared among groups, focusing on II and III (discrepancies). A p value of <0.05 was considered significant.
Main results and the role of chance: AMH and AFC were discordant in 23% of cases; in 19% they were both low and in 58% both normal. Incidence of POR in each group: 64% (I), 27% (II), 28% (III), 8% (IV). Basal characteristics and stimulation outcomes were significantly different among 4 groups. When comparison was restricted to discrepant groups (II and III) no significant differences were observed regarding age, basal FSH, cancellation rates, oocytes retrieved, MII, fertilization rates and mean quality of transferred embryos. PR in each group was: 15% (I), 21% (II), 27% (III), 39% (IV). Statistically significant differences were observed between PR of group III vs. I and between IV vs. the rest of groups. PR did not differ significantly between group II vs. III nor II vs. I.
Limitations, reason for caution: Our results are limited by the retrospective nature of the study.
Wider implications of the findings: In cases of discrepancy between both tests, our results reveal that no one predicts significantly better than the other one in terms of oocyte quantity or quality. Nevertheless, a possible link between AMH and quality cannot be ruled out, as PR when AFC is low but AMH is normal was significantly higher than when both tests were low.
Study funding/competing interest(s): Funding by hospital/clinic(s), Department of Obstetrics, Gynecology and Reproductive Medicine; University Hospital Quirón - Dexeus.
Trial registration number: NA.

P-501 Corifollitropin alfa in a GnRH antagonist protocol for poor ovarian responder patients - a new alternative - a prospective, non randomized, controlled study

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Study question: Use Corifollitropin alfa in GnRH antagonist protocol will be beneficial in women with a history of poor responder?
Summary answer: Corifollitropin alfa in GnRH antagonist protocol in women with a history of poor responder, the results are very promising in terms of number of recruited follicles, oocytes retrieved, oocytes MII and pregnancy rates.
What is known already: Poor response is characterized by a low follicular recruitment, fewer oocyte retrieved, higher canceled cycle rate, lower pregnancy rate and is difficult to define the most appropriate stimulation protocol. Corifollitropin alfa has a short time to reach its peak serum concentration and it could benefit the poor responder patient.
Study design, size, duration: Prospective, non randomized, controlled study. We included patients with a previous IVF cycle canceled or with poor responder (53) treated with corifollitropin alfa in a GnRH antagonist protocol. They are matched with themselves and controls with the same background (47) treated with conventional protocol (October 2012 - November 2013)
Participants/materials, setting, methods: Patients received 100–150 µg corifollitropin on 2º - 3º day of the menstrual cycle followed by a daily dose of GnRH antagonist on day 7–8 of the cycle. Day 8 or 9 of the cycle, a variable daily dose of gonadotropin was administered until the day of ovulation triggering.
Main results and the role of chance: There was no difference in age between the two group (37.7 versus 38.8). 64.2% of corifollitropin group and 46.8% of control group had Bologna Criteria. Follicular recruitment, oocytes retrieved and MII oocytes were significantly higher in corifollitropin group (6.5 versus 4.3, 6.0 versus 4.2 and 5.0 versus 3.4 respectively) (p < 0.01). In the corifollitropin group, comparing the recruitment of follicles >15 mm in the previous cycle with the cycle of study, we observed a significant increase in the count of follicles recruited (3.2 versus 6.5) (p < 0.01). In both groups there were no significant differences in ovarian puncture rate, embryo transfer rate and average of embryos transferred. The pregnancy rate was greater in corifollitropin group (50.0% versus 37.5%) (p = 0.3).
Limitations, reason for caution: The results justify randomized controlled studies to have more consistent data to add this stimulation protocol to our routine work.
Wider implications of the findings: In the wide spectrum of alternative stimulation protocols in patients with low response, where none have the last word, corifollitropin alfa, its pharmacokinetics, ease management and our results open a new way of action in the treatment of the poor responder patients.
Study funding/competing interest(s): Funding by hospital/clinic(s), Reproduction Unit Centro Gutenberg, Málaga, Spain.
Trial registration number: Not applicable.

P-502 The exogenous progesterone free luteal phase in IVF – exploring a new concept

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Study question: Can the luteal phase be supported with small daily boluses of hCG without the administration of exogenous progesterone (P), while maintaining a good reproductive outcome? Do ongoing pregnancy rates (PR) correlate to mid-luteal P levels?
Summary answer: Excellent ongoing pregnancy rates can be obtained by luteal administration of small daily doses of hCG (125 IU/day) to stimulate the luteal phase and boost the endogenous P production – without exogenous progesterone administration. Mid-luteal P levels and their possible correlation with ongoing pregnancy rate will be discussed at the conference.
What is known already: Luteal phase support remains mandatory for IVF patients due to the luteal LH insufficiency induced by ovarian stimulation. Luteal P administration continues until confirmation of pregnancy, but in many cases until 10th week of gestation. Progesterone support is associated with inconvenient discharge or pain related to intramuscular injections. We report for the first time the use of a small daily dose of r-hCG for luteal phase support in patients who received no exogenous Progesterone.
Study design, size, duration: RCT (feasibility study) performed in a public IVF unit. A total of 90 IVF normo-responder patients co-treated with a GnRH antagonist between February 2012 and January 2014 were randomized to three different stimulation and luteal phase regimens including two study groups and one control group. N = 30 in each group.
Participants/materials, setting, methods: Three groups co-treated with GnRH antagonist, randomized to stimulation with either rFSH and rhCG (I and II) or r-FSH (III). Ovulation was induced with GnRHa (I and II) or r-hCG (III). In I and II the luteal phase consisted of 125 IU r-hCG daily, whereas III received standard vaginal progesterone support.
Main results and the role of chance: The biochemical pregnancy rate in groups I, II and III were 58%, 53% and 52%, respectively (NS); the ongoing pregnancy rate/embryo transfer in groups I, II and III was: 42%, 31% and 39%, respectively (NS). The early pregnancy loss rate: 29%, 43% and 25% in groups I, II and III, respectively (NS). Mid-luteal P levels will be presented at the conference and a possible correlation to ongoing PR will be discussed.
Limitations, reason for caution: This RCT shows the feasibility of a new low dose hCG driven luteal phase support protocol in normo-responder patients; however, a larger sufficiently powered RCT is mandatory to draw firm conclusions.
Wider implications of the findings: The implementation of this new protocol in the normo-responder patient would significantly reduce the treatment burden of the majority of IVF patients, although daily s.c. injections of r-hCG are necessary, Future dose finding studies should be performed to determine the minimal daily hCG dose necessary to secure the most optimal reproductive outcome without increasing the risk of OHSS.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Investigator driven study. External funding: ARTS Biologics.
Trial registration number: Project number: M-20110289.
CLINTRIAL.GOV NUMBER: NCT015041

P-503 Interest of serum leptin measurement during controlled ovarian stimulation in obese patients undergoing IVF cycles

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Study question: The main objective of this study was to describe serum leptin kinetics during IVF cycle, and to evaluate its correlation with cycle outcome in order to determine its potential interest as a biological predictive tool in the monitoring of ovarian stimulation for infertile obese patients.
Summary answer: Basal serum leptin level and its evolution throughout ovarian stimulation cycle were correlated with patients' characteristics, but were not correlated with ovarian response and cycle outcome in obese patients.
What is known already: At the interface of energy metabolism and fertility, leptin is the most intensively studied factor to explain the association between obesity and female infertility. High serum leptin levels have been reported to be deleterious on female fertility, with negative effects during IVF cycle on ovarian response, embryo quality and cycle outcome. However, this has not been confirmed up to now and no specific threshold has been reported.
Study design, size, duration: This monocentric prospective study was conducted between March 2012 and May 2013 on all obese patients undergoing controlled ovarian stimulation for IVF/ICSI cycle who had given their consent. Ovarian stimulation characteristics and cycle outcomes were recorded and analyzed.
Participants/materials, setting, methods: This study was conducted at the University Hospital of Nantes, France. Ovarian stimulation was performed according to antagonist protocol. Serum leptin was measured at the beginning (stimulation day 5, "basal") and at the end of the stimulation (hCG triggering ± 1 day, "hCG") with Human Leptin ELISA kit (Eurobio™, France).
Main results and the role of chance: A total of 58 obese patients were included. Mean basal serum leptin level was positively correlated with BMI ($p = 0.0016$). Mean basal leptin level was significantly lower than mean hCG leptin level (respectively 46.18 ± 20.41 vs. 51.77 ± 22.90 ng/mL, $p < 0.0001$). This difference significantly increased with BMI. Neither basal nor hCG leptin levels were correlated with ovarian response to stimulation and cycle outcome. Moreover, leptin dynamics throughout ovarian stimulation was not correlated with cycle outcome either. However, basal leptin level was significantly lower in cycles leading to a biochemical pregnancy than in cycles arrested because of poor ovarian response (35.16 ± 12.69 ng/mL vs. 55.63 ± 27.24 ng/mL). Moreover, very few pregnancies were observed for leptin levels above the threshold of 50ng/mL, with a negative predictive value of 90%.
Limitations, reason for caution: The main limitation of our study is the insufficient adjustment of FSH starting dose according to obesity stage, which could explain the lack of correlation between serum leptin level and cycle outcome. In addition, some women might not have been fasting at the time of blood sample.
Wider implications of the findings: Even if the predictive interest of serum leptin level in ovarian stimulation monitoring in obese patients was not confirmed in our study, further interventional studies should focus on basal leptin level on the 3rd day of the cycle preceding IVF in order to define if a relevant threshold predicting ovarian response can be established, allowing improved patients counselling and delayed IVF cycle after nutritional care if necessary.
Study funding/competing interest(s): Funding by hospital/clinic(s), CHU de Nantes.
Trial registration number: None.

P-504 Polycystic ovarian syndrome (PCOS) follicles produce more serum Anti Mullerian Hormone (AMH) per antral follicle than follicles in normal ovaries: an in-vivo comparative study

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Study question: Do women with polycystic ovary syndrome (PCOS) secrete more antimullerian hormone (AMH) per antral follicle than controls?
Summary answer: Women with PCOS secrete significantly more AMH per antral follicle than women with polycystic ovarian morphology only (PCOM) and matched controls.
What is known already: Women with PCOS have significantly higher levels of AMH than women with PCOM and controls. This is thought to be due to the greater number of antral follicles in these women. In vitro studies on granulosa cells from women with PCOS and controls show higher production of AMH per granulosa cell in women with PCOS.
Study design, size, duration: Prospective observational study analysing the data from 437 women attending the fertility clinic over a period of 13 months from September 2012 to September 2013.
Participants/materials, setting, methods: Women attending a tertiary referral university IVF centre were each examined for serum concentrations of AMH and the total antral follicle count (AFC). The ratio of AMH /AFC for each subject was calculated. Women were categorised into three groups: PCOS, PCOM and control. PCOS was defined based on Rotterdam ESHRE/ASRM consensus criteria.
Main results and the role of chance: Women in the three groups were statistically similar when compared for age, body mass index (BMI), and smoking status. The mean AMH/AFC ratios in the PCOS, PCOM and control groups were 2.14, 1.29 and 1.25. The AMH/AFC ratio was significantly higher in the PCOS group as compared to the PCOM and control groups [$F(2, 432) = 21.77$, $P < 0.001$].
Limitations, reason for caution: Although the estimation of serum AMH is standardised the estimation of the AFC is liable to operator dependant variation.
Wider implications of the findings: The study gives an insight into the pathophysiology of PCOS. The increased individual production of AMH by PCOS follicles could be intrinsic to PCOS or may be driven by other factors and this needs further investigation.
Study funding/competing interest(s): Funding by hospital/clinic(s), Homerton University Hospital NHS Foundation Trust.
Trial registration number: NA.

P-505 Timing of ovarian stimulation in patients prior to chemo- or radiotherapy – an analysis of 674 stimulations

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Study question: Is the number of fertilized oocytes and zygotes and the dosage of gonadotropins used different if ovarian stimulation is started in the early vs. mid-late proliferative or luteal phase of the menstrual cycle before chemo- or radiotherapy?
Summary answer: The number of fertilized oocytes and zygotes was not significantly different if stimulation was initiated during different phases of the menstrual cycle but the dosage of gonadotropins used was significantly higher if stimulation was started in the mid-late proliferative and the luteal phase of the menstrual cycle.
What is known already: Fertility preservation procedures should be performed as fast as possible in order not to postpone the oncological therapy. Initiation of ovarian stimulation has been shown to be possible at any time of the menstrual cycle as part of fertility preserving procedures. However, data about the efficacy of stimulations initiated in the mid-late and the luteal phase of the cycle are very limited.
Study design, size, duration: A retrospective analysis was performed with 4.282 patients counselled for fertility preservations procedures before chemo- or radiotherapy in 99 centers, being part of the network FertiPROTEKT (www.fertiprotekt.com) from 2007-2013. From these, 702 women performed ovarian stimulation before initiation of cancer therapy with complete information received from 674 women (96.0%).
Participants/materials, setting, methods: According to the time of stimulation initiation patients were grouped into A) initiation day 1–5, B) day 6–14, C) after day 14 (luteal phase). Days needed for stimulation, gonadotropin dosages, number of aspirated oocytes, cryopreserved oocytes and zygotes were collected and analysed with ANOVA followed by SNK post-hoc test.
Main results and the role of chance: Of these 481 (71.4%) were in group A, 96 (14.2%) in group B, 77 (11.4%) in group C. No difference was seen in the average age of women and the days of stimulation. The average dosage of gonadotropins necessary for stimulation were elevated in group B (2451 ± 110.7) and C (2680.6 ± 146.7) vs. group A (2029.1 ± 75.5). The number of oocytes collected by follicle aspiration was significantly higher in group B (12.72 ± 0.89) compared to group A (10.16 ± 0.32) and group C (12.47 ± 0.99) ($p < 0.05$),

whereas the average number of cryopreserved oocytes or zygotes were not significantly different (Group A: 3.63 ± 0.31 & 3.65 ± 0.21, B: 5.01 ± 0.79 & 4.0 ± 0.52, C: 3.72 ± 0.721 & 4.79 ± 0.61).
Limitations, reason for caution: As only few of the cryopreserved oocytes and zygotes have already been used for thawing cycles, data about pregnancy rates following initiation of stimulation in different phases of the menstrual cycle could not be generated.
Wider implications of the findings: Ovarian stimulation can be started as soon as possible irrespective of the menstrual phase of the cycle to avoid postponement of the chemo- or radiotherapy. However, the dosage of stimulation should be adapted according to the menstrual phase in which stimulation is initiated.

P-506 The effect of FSH administration on the day of hCG trigger in COH IVF cycles

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Study question: Does it matter whether deprivation of follicular stimulation hormone (FSH) over 24 hours? Does additional FSH administration on the day of hCG trigger improve cycle outcomes in COH IVF cycles?
Summary answer: FSH administration on the day of hCG trigger does not have beneficial effects on cycle outcomes, such as the number of oocyte, fertilization rate, embryo quality and pregnancy rate.
What is known already: Ovarian stimulation protocols are very diverse and there are some differences depending on clinicians. If women receive no FSH on the day of hCG trigger, the interval between last FSH administration and hCG trigger is more than 24 hours. On the contrary, the time gap is less than 24 hours with FSH administration on hCG day. Time gap might have adverse effect on cycle outcomes if it is increased.
Study design, size, duration: This retrospective study included 1289 women undergoing COH IVF cycles between January 2012 and December 2013.
Participants/materials, setting, methods: We retrospectively reviewed 1289 patients undergoing IVF using GnRH agonist and antagonist protocols. On the day of hCG trigger, 116 women received additional FSH (FSH Group) and 1172 women did not receive FSH (No FSH Group).
Main results and the role of chance: In each group, age (35.1 ± 4.5 vs. 36.0 ± 4.2), the number of oocytes (9.9 ± 6.2 vs. 10.0 ± 6.2), the number of transferred embryos (2.0 vs. 2.0) and fertilization rate (69.5% vs. 69.4%) did not show significant differences. There were no significant differences between two groups in the pregnancy rate [50.0% (58/116) vs. 49.5% (580/1172), $P = 0.332$] and ongoing pregnancy rate [44.0% (51/116) vs. 45.4% (532/1172), $P = 0.262$].
Limitations, reason for caution: This is a retrospective study. The sample size of FSH Group is small in this study. Prospective randomized studies with larger sample size will be needed to confirm our conclusions.
Wider implications of the findings: Many clinicians usually do not give additional FSH on the day of hCG trigger. In this situation, some women do not receive FSH over 36 hours like coasting. It could have adverse effect on cycle outcomes. However, additional FSH administration on the day of hCG trigger does not have beneficial effects on cycle outcomes, such as the number of oocyte, fertilization rate, embryo quality and pregnancy rate. The time gap between last FSH administration and hCG trigger does not impact on cycle outcomes of COH IVF cycles.
Study funding/competing interest(s): Funding by hospital/clinic(s), Mamapapa&baby Obstetrics & Gynecology clinic.
Trial registration number: None.

P-507 Correlation between the serum luteinizing hormone/follicle-stimulating hormone ratio and the anti-Müllerian hormone levels in normo-ovulatory women

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Study question: Does serum luteinizing hormone (LH)/follicle-stimulating hormone (FSH) ratio correlate with anti-Müllerian hormone (AMH) levels as a predictor of ovarian reserve in normo-ovulatory women?
Summary answer: There was a significant partial correlation between the serum LH/FSH ratio and AMH levels when adjusted by age.
What is known already: AMH is regarded as an age-specific marker for predicting ovarian reserve. Some study reported that elevated day 3 FSH/LH ratio ≥2 is associated with higher rates of cancellation in IVF cycles. Theoretically, the LH/FSH ratio will decrease as the ovarian reserve declines because FSH is known to increase more significantly than LH. Therefore we focused on the variation of LH/FSH ratio with aging and evaluated the correlation between LH/FSH ratio and AMH in normo-ovulatory women.
Study design, size, duration: Retrospective analysis of basal serum hormone levels was performed in 1,251 patients undergoing IVF who had regular menstrual cycles. Patients were classified into 6 groups according to age: Group 1, under 31 years; Group2, 32–34; Group 3, 35–37; Group 4, 38–40; Group 5, 41–43; Group 6, more than 44 years.
Participants/materials, setting, methods: The serum levels of AMH, LH, FSH, TSH and prolactin were obtained on the day 2–3 of menstrual period. Comparisons of hormone levels and LH/FSH ratio between age groups were performed using one-way analysis of variance. The partial correlation between AMH and LH/FSH ratio was analyzed after adjustment of age.
Main results and the role of chance: The serum AMH level was inversely correlated with age ($r = -0.400$, $p < 0.001$). A significant negative correlation was found between serum LH/FSH ratio and age ($r = -0.213$, $p < 0.001$). There was a significant partial correlation between serum LH/FSH ratio and AMH when adjusted by age ($r = 0.348$, $p < 0.001$). The LH/FSH ratio was related to the age and could be considered as a useful marker for ovarian reserve and could be applied to clinical evaluation. This is the first report on the age-related change of LH/FSH ratio and the correlation between the serum LH/FSH ratio and AMH levels in normo-ovulatory women.
Limitations, reason for caution: Investigation of the direct ovarian respond to controlled ovarian stimulation according to the LH/FSH ratio is needed combined with the present correlation results.
Wider implications of the findings: The day 3 LH/FSH ratio is a relatively easy test to obtain and adds more predictive power over the FSH level alone. The decreased basal LH/FSH ratio even with normal FSH might be a sign of diminished ovarian reserve. The LH/FSH ratio could be an age-related reference value for ovarian reserve instead of serum AMH in reproductive aged women. We can predict ovarian reserve using commonly checked tests with higher accuracy in reproductive aged women.
Study funding/competing interest(s): Funding by hospital/clinic(s), Dongguk University Ilsan Hospital/No conflict of interest exists.
Trial registration number: N/A.

P-508 Does multiple attempts at embryo transfer have an effect on clinical pregnancy rates

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Study question: Does multiple attempts at embryo transfer have an effect on clinical pregnancy rates?
Summary answer: Retained embryos with immediate re-transfer does not have any impact on clinical pregnancy rates during IVF treatment cycles. This provides some relief to both patients and clinicians when it occurs.
What is known already: The reported incidence of retained embryos following transfer varies in the literature between 1 and 8%. The incidence is trending downwards with the use of ultrasound guidance. It is difficult and very stressful to explain this unpleasant situation to patients when it occurs.
Study design, size, duration: A retrospective chart review of all IVF cycles with retained embryos that required retransfer between October 2009 and March 2012 was performed.
Participants/materials, setting, methods: We reviewed IVF cycles with or without ICSI, and included fresh and frozen embryo transfer cycles. All embryos were transferred on day 3 post oocyte retrieval. A Sydney cook catheter was used for the embryo transfer. Trans-abdominal ultrasound was used for guidance during the transfer.
Main results and the role of chance: A total of 49 IVF cycles were identified with retained embryos that required retransfer. This represents 7.5% (49/652) of all IVF cycles in that period of time. The total number of cycles with embryo transfers was 652 for the same time period. The clinical pregnancy rate in the

retransfer group was 30.6% (15/49). The clinical pregnancy rate in all cycles in the same time period was 34.8% (227/652). (p = 0.521 Chi-square test). There was no statistical difference found.
Limitations, reason for caution: 1- Small number of cycles limits the power of the study. further studies are needed to confirm these findings. 2- Higher incidence of retained embryos in this study (7.5%) could be due to senior trainees performing the procedure. Further studies are needed to clarify the incidence of retained embryos.
Wider implications of the findings: These findings helps with the councelling process with patients when retained embryos occurs at transfer.
Study funding/competing interest(s): Funding by hospital/clinic(s), The Fertility Clinic at LHSC, Department of Obstetrics & Gynaecology, Western University, London, ON.
Trial registration number: Not applicable.

P-509 Ovarian response and cumulative live birth rate of women undergoing in-vitro fertilisation who had discordant anti-Mullerian hormone level and antral follicle count

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Study question: What are the ovarian response and cumulative live birth rate of women undergoing in-vitro fertilization (IVF) treatment who had discordant baseline serum anti-Mullerian hormone (AMH) level and antral follicle count (AFC)?
Summary answer: When AMH and AFC are discordant, the ovarian responsiveness is intermediate between that when both are concordant on either end; women having higher AMH within the same AFC quartile had higher number of retrieved oocytes and cumulative live-birth rate.
What is known already: Many studies have consistently reported that AMH and AFC were among the best ovarian response markers for prediction of ovarian response in IVF treatment, and that their performance was probably comparable. Discordance in AMH and AFC categories has been reported but its implication remains unclear.
Study design, size, duration: Retrospective cohort study on 1,046 women undergoing the first IVF cycle from 2007 to 2009.
Participants/materials, setting, methods: Subjects treated on the long GnRH agonist protocol were analysed. They were classified according to their quartiles of AMH and AFC, which were correlated with the number of retrieved oocytes, ovarian sensitivity index (OSI = the number of retrieved oocytes per 1,000 IU of FSH administered) and cumulative live-birth rate.
Main results and the role of chance: 32.2% of our studied subjects were discordant in their AMH and AFC quartiles. Among them, those having higher AMH within the same AFC quartile had higher number of retrieved oocytes and cumulative live-birth rate ($p < 0.05$). Subjects discordant in AMH and AFC had intermediate OSI which differed significantly ($p < 0.05$) compared to those concordant in AMH and AFC on either end. OSI of those discordant in AMH and AFC did not differ significantly ($p > 0.05$) whether either AMH or AFC quartile was higher than the other.
Limitations, reason for caution: We limited our subjects to those treated on the long GnRH agonist protocol to avoid different protocols confounding on the outcome measures. The generalisability to the GnRH antagonist protocol and its clinical application to determination of FSH dosing need further clarification.
Wider implications of the findings: Both AMH and AFC would be recommended to be utilized for individualization of stimulation regimen; when the AMH and AFC fall into discordant categories, it would be sensible to adopt an intermediate dose of gonadotrophin as compared to those with concordant AMH and AFC categories on either end.
Study funding/competing interest(s): Funding by University(ies), The study was supported by a Small Project Funding from the University of Hong Kong.
Trial registration number: Registry of the Clinical Trials Centre, The University of Hong Kong: HKCTR-1477.

P-510 A comparison of the second-generation Beckman Coulter IVD and first-generation AnshLabs ELISA assays for anti-Müllerian hormone in patients undergoing IVF treatment

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Study question: The objective of the study was to draw a comparison between the commonly used second-generation assay by Beckman Coulter and the Ultra-Sensitive first-generation assay by AnshLabs.
Summary answer: Although first and second generation assays show the comparable correlation with the clinical condition of the patient, the Ultra-Sensitive first generation kits seem better alternative for the unstable II generation kits. The results from the first generation assays are distributed on the wider range which facilitates the clinical interpretation.
What is known already: The ovarian reserve is the main factor influencing the efficacy of infertility treatment. Currently, the anti-Müllerian hormone is the main indicator of the ovarian reserve and has a wide spectrum of clinical importance. It achieved a high clinical value right after the introduction of the first commercial AMH assay in 2005.
Study design, size, duration: Serum samples (n = 520) were analysed, and 130 of them (showing lowest correlation with the stimulation) were examined both by the second-generation assay by Beckman Coulter and the first-generation assay by AnshLabs. Results of the patients were correlated with their number of antral follicles, follicles after stimulation and received cumuluses.
Participants/materials, setting, methods: Serum samples (n = 520) were chosen from female patients undergoing routine AMH tests before the IVF program. For a suitable comparison, we chose the serum samples of patients with the lowest correlation between the AMH serum level and the response to stimulation. The AMH serum levels of the patients were examined using two AMH tests, the second-generation assay by Beckman Coulter and the first-generation assay by AnshLabs. The precision and accuracy of both methods were determined and the results of AMH serum levels of 130 patients were correlated with their number of antral follicles (AFC), the amount of follicles after stimulation and the quantity of received cumuluses.
Main results and the role of chance: To estimate the relationship between results obtained with different analyses, the Pearson correlation analysis and regression equation Passing Bablok were used. In assessing the significance of differences, the Student's t-test for dependent pairs was used. In order to visualize the data scatter, Bland and Altman graphs were used. Both the precision and the accuracy of the compared methods were highly satisfactory. The coefficients of variation obtained in the study conducted on two different levels of control material were lower than 12% and the load did not exceed 9%. The study proved that both of the methods yielded comparable results and the coefficient of variation between the first-generation and the second-generation AMH assays was 0.871.
Limitations, reason for caution: The blood was sampled in the morning in all cases and the transport to the laboratory was very quick, the blood was not stored in room temperature for significantly long time but was tested as soon as possible.
Wider implications of the findings: It is important to use only one type of the AMH test (either the first generation assays by AnshLab or second generation assays by Beckman Coulter) as they give different AMH levels in the same blood sample. The first generation assays seem to have more clinical value as they show much wider range of the results, thus allowing the clinician to set more precise schedules of the treatment.
Study funding/competing interest(s): Funding by hospital/clinic(s), INVICTA Fertility Clinic.
Trial registration number: There is no trial registration number.

P-511 The effect of race and ethnicity on the outcome of conventional and mild IVF: multivariate analysis of 564 patients from a randomized clinical trial

Abstract withdrawn by the author

P-512 A randomized clinical trial comparing Minimal Stimulation IVF with single embryo transfer to conventional IVF with double embryo transfer

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Study question: What are the differences in clinical outcomes between minimal stimulation IVF with single embryo transfer and conventional IVF with double embryo transfer, including ongoing pregnancy rates, administration of drugs, ovarian hyper-stimulation syndrome (OHSS), and multiple pregnancy rates?
Summary answer: Minimal stimulation IVF resulted in lower ongoing pregnancy rates but multiple pregnancy rates were 9 fold lower. The gonadotropins use in minimal stimulation IVF was decreased by 75% and OHSS was completely prevented.
What is known already: In an ongoing effort to reduce multiple pregnancies, gonadotropins use and OHSS, minimal stimulation IVF and single embryo transfer was employed in our center. Conventional IVF with luteal phase down-regulation is a routine approach at most centers.
Study design, size, duration: An open-label, randomized controlled non-inferiority trial was completed among 564 infertile women between 2009 and 2013 at a single large fertility center. Couples were followed until either discontinuation of treatment or achieving 9 weeks of pregnancy with ultrasound findings of a positive fetal heart rate.
Participants/materials, setting, methods: Infertile women under the age of 38 who were undergoing their first IVF cycle were randomly allocated to either minimal or conventional stimulation IVF treatments. The primary outcome was ongoing pregnancy rate. Secondary outcomes were multiple pregnancy rates, OHSS and gonadotropin use.
Main results and the role of chance: A total of 564 women were included, of whom 286 were allocated to minimal stimulation IVF and 278 to conventional IVF. The cumulative ongoing pregnancy rate was 50% (143/286) for minimal stimulation IVF and 66% (184/278) for conventional IVF (RR 0.76, 95% CI 0.65–0.87). The ongoing multiple pregnancy rate was 4.2% in minimal stimulation IVF compared to 36% in conventional IVF (RR 0.12, 95% CI 0.052–0.26). There were no cases of OHSS in minimal stimulation IVF compared to 16 (5.8%) moderate/severe OHSS cases in the conventional arm. Gonadotropin consumption was significantly lower with minimal stimulation IVF than in conventional IVF (464 ± 161 versus 2074 ± 389 IU, $p < 0.0001$).
Limitations, reason for caution: This data is limited by the single embryo transfer in minimal stimulation IVF versus the double embryo transfer in conventional IVF.
Wider implications of the findings: Ongoing pregnancy rates achieved by minimal stimulation IVF are high but significantly lower than that of conventional IVF. However, minimal stimulation IVF completely eliminated OHSS, significantly decreased multiple pregnancy rate and significantly reduced gonadotropin use. In counseling patients for the choice between minimal stimulation IVF or conventional IVF, all these outcomes have to be presented to the patient to allow for optimal shared decision making.
Study funding/competing interest(s): Funding by hospital/clinic(s), New Hope Fertility Center, 4 Columbus Circle, New York, NY 10019, United States.
Trial registration number: NCT 00799929.

P-513 Evaluation of polymorphisms in BMP15 genein infertile women and its correlation with results of controlledovarian stimulation

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Study question: Identify the polymorphisms rs3810682 (–9C > G), rs38897937 (905A > G) and rs17003221 (901C > T) of the *BMP*15 gene in Brazilian infertile women undergoing assisted reproduction, correlating the polymorphisms to serum levels of estradiol, FSH and AMH and with ovarian stimulation outcomes (good response, inadequate response and ovarian hyperstimulation syndrome).
Summary answer: Polymorphisms of BMP15 gene were not correlated with Brazilian infertile women. We found the same result when compared to hormonal measurements (FSH, AMH and estradiol).
What is known already: Polymorphisms in the *BMP15* gene turn the protein less bioactive or inhibits secretion due to its action on FSHR of granulosa cells, theoretically increasing the sensitivity of the follicle to FSH. On the otherhand, an activator polymorphism may have the opposite effect. The literature suggests that improving our understanding of polymorphisms of the *BMP1*5 gene may be important for advancing the diagnosis and treatment of infertility.
Study design, size, duration: This is a prospective cohort which were screened so far, 161 Brazilian women within fertility caused by tuboperitoneal factor and male factor (without associated female factor) that under went assisted reproduction procedures. The samples were collected since September/2011 until December/2013.
Participants/materials, setting, methods: All patients were above38 years old, with normal of serum FSH, prolactin andTSH, presence of both the ovaries, regular ovulatory cycle, BMI ≤ 30, no history of poor response and no evidence of endocrine diseases Detection of polymorphisms were performed using *TaqMan* methodology by real time PCR.
Main results and the role of chance: For the polymorphism rs3897937, we did not find an association between the serum levels of estradiol, FSH and AMH ($p = 0.68$; $p = 0.61$; $p = 0.16$, respectively), we observed similar results for the polymorphisms rs17003221 ($p = 0.46$; $p = 0.27$; $p = 0.10$, respectively) and rs3810682 ($p = 0.21$; $p = 0.39$; $p = 0.37$, respectively). We could observe that ovarian response was not correlated with the polymorphisms rs3897937, rs17003221 and rs3810682 ($p = 0.15$; 0.62; 0.24, respectively). We showed in this study that polymorphisms of BMP15 were not correlated to ovarian stimulation outcomes, not either with hormonal measurements.
Limitations, reason for caution: Small sample size.
Wider implications of the findings: Studies in mice and sheep highlight the importance of*BMP15*genein regulating ovulation rate. In humans, studies have shown the relationship between *BMP15*with premature ovarian failure and controlled ovarian hyperstimulation. Moron et al. (2006) found a statistically positive association between the polymorphisms of *BMP15* and hyper response to ovarian stimulation. Another study is in agreement with the correlation between genotypes and ovarian hyper stimulation syndrome (Hanevik et al., 2011).
Study funding/competing interest(s): Funding by national/international organization(s), FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo.
Trial registration number: Not applied.

P-514 The effects of preconception TSH levels on ART outcome in 8657 cycles

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Study question: The aim of this study was to assess the effects of preconception TSH levels on the reproductive outcomes of women <40 years after assisted reproduction techniques.
Summary answer: There was an association between TSH levels and ART outcome. Confounding factors were evaluated.
What is known already: The relationship between TSH level and pregnancy rates has been investigated in many studies. Several studies have denied such an association whereas others have confirmed the presence of a negative relationship, the latter studies concluded that the probability of pregnancy decreases significantly when TSH increases. However there is no consensus on the effects of subclinical hypothyroidism on ART outcome.
Study design, size, duration: This is a retrospective cohort study includes 8657 fresh cycles performed between January 2000 to September 2013 at our hospital.
Participants/materials, setting, methods: Cycles in which women were <40 years with normal baseline ovarian reserve testing (FSH < 10 IU/L and basal antral follicle count >8) were analyzed. Cases using testicular sperm, or aspirated sperm were excluded from analysis. 8657 cycles were classified according to TSH level. Group A TSH ≤2.5 mIU/L ($n = 6672$) and group B 2.5 > TSH ($n = 1985$).
Main results and the role of chance: No differences were observed between two groups regarding mean female age, mean BMI, duration of infertility and mean previous trial number. Similarly, no differences were observed between group A and group B regarding duration of stimulation, estradiol (E_2) on the day of hCG administration, total gonadotropin dose, the mean endometrial thickness on hCG day, number of COC, number of MII oocytes, fertilisation rates, mean number of embryos transferred, mean day of embryo transfer and implantation rates. In

group A, clinical pregnancy rate (CPR) and ongoing pregnancy rate (OPR) were significantly higher than group B ($p < 0.0001$ and $p = 0.0049$ respectively). In group B miscarriage rate was significantly higher than group A ($p < 0.05$).
Limitations, reason for caution: Our results demonstrate a negative correlation between increasing TSH levels and reproductive outcome including CPR, miscarriage rates and OPR. However, our study does not consent to draw definitive conclusions and further evidence is required prior to show the increasing TSH level effects on reproductive outcome in ART.
Wider implications of the findings: TSH levels affects not only pregnancy rates but also abortion rates.
Study funding/competing interest(s): Funding by hospital/clinic(s), There is no conflict of interest. No funding source.
Trial registration number: Not RCT.

P-515 Investigation and resolution of the effect of an interfering factor in the Beckman Coulter Anti-Müllerian Hormone (AMH) Gen II ELISA assay

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Study question: Are the sample storage and dilution issues reported with the AMH Gen II ELISA assay due to complement interference and can these problems be prevented by pre-mixing samples with assay buffer prior to analysis?
Summary answer: We demonstrated that C1q in the sample binds to the immobilized anti-AMH antibody forming a complex, initiating the complement cascade. Subsequent C3 activation prevents AMH in the sample from binding to the capture antibody resulting in falsely lowered values. A pre-mix step resolves apparent perceived sample instability and dilution issues.
What is known already: Studies suggest sample storage conditions affect AMH Gen II assay results: undiluted fresh samples or samples stored at –20°C for <1 year or at –70°C result in falsely lowered AMH values. Calibrators, internal Beckman controls and the UK NEQAS AMH scheme using processed/stored samples do not demonstrate the issue. Complement is a recognized source of interference in some immunoassays causing a confusing pattern of interference easily mistaken for analyte instability in serum.
Study design, size, duration: Studies to demonstrate the mechanism of complement interference included using fresh/frozen samples with native AMH and also samples in complement-negative synthetic matrix spiked with C1q or AMH.
Participants/materials, setting, methods: The samples were tested in a modified AMH Gen II assay in which the AMH Gen II conjugate was substituted with anti-C1q or anti-C3 antibodies. A pre-mix step was introduced to eliminate complement interference. Its validity was investigated in dilution recovery experiments and by performing fresh sample comparisons versus both the original testing protocol and the alternative AMH assay with the pre-mix step.
Main results and the role of chance: C1q and C3b bind to immobilized anti-AMH antibody. C1q with both fresh and frozen samples, but C3 is cleaved and C3b deposited only with fresh samples. AMH results are falsely lowered in a matrix where the complement cascade can occur, but C1q alone doesn't cause significant interference which apparently needs the amplification effect of the C3 cleavage and C3b deposition. Samples stored for 8 months at –20°C demonstrated a significant decline in complement interference however interference was still present. The pre-mix step does not significantly affect binding of C1q but effectively prevents the activation of C3 and hence the binding of C3b. Using the pre-mix step, AMH Gen II compares well with the Immunotech (IOT) AMH assay (AMH Gen II = 1.01 IOT– 0.73 ng/mL).
Limitations, reason for caution: Complement interference explains observations that AMH values increased on storage and why fresh samples demonstrated under-recovery; however it does not answer other AMH measurement questions including repeatability of patient values over time. A better understanding of biological/pharmacological factors that may alter AMH levels is required, as is an international standard.
Wider implications of the findings: The pre-mix step restores dilution recovery and eliminates complement interference which will improve additional aspects of assay performance. The pre-mix step should be adopted by all users of the AMH Gen II method. Rigorous evaluation and audit of local clinical criteria against the new method should be performed. As with all diagnostic tests, it is necessary that the AMH test be interpreted in context with other laboratory, radiologic, and clinical findings.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Beckman Coulter Inc.
Trial registration number: Not applicable.

P-516 Pre-analytical assessment of AMH stability in human serum using a well characterized midpro-mature immunoassay

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Study question: How can the AMH stability concern be addressed in routine clinical practice?
Summary answer: The AMH in serum is mostly pro-mature associated form. The kinetics of association of pro and mature is rapid. Assay design that includes stable epitope antibodies and is not impacted by molecule association will measure reproducible results.
What is known already: AMH is a homodimeric glycoprotein composed of two 55 kDa N-terminal and two 12.5 kDa C-terminal homodimers, non-covalently linked by disulfide bridges. Recently, there have been multiple field safety notices for AMH Gen II ELISA (Beckman Coulter) related to sample stability and antibody complement binding interferences. This has generated multiple debates, publications related to reproducibility of AMH measurements. To date no publication has clearly stated if the AMH variability is related to process (pre-analytical) or the assay.
Study design, size, duration: Prospective study ($n = 16$) was designed in which serum samples were tested within 3 hrs of draw, aliquoted, stored at room temperature (RT), –20°C, 2–8°C and re-assayed at 7, 10, 24, 48 and 168 hours. Multiple samples were thawed up to 4 cycles and measured at two independent sites.
Participants/materials, setting, methods: A well characterized two-step, ELISA (Ansh Labs, US AMH, AL-105) was used to measure AMH levels in 25 µL of sample in <3 hours. The assay is specific for human and measures pro-mature AMH complex. The assay is calibrated (0.09–19.4 ng/mL) against standardized recombinant human AMH.
Main results and the role of chance: No significant changes were observed when samples were stored at RT, 2–8°C and –20°C. The median AMH concentration (16 serum samples, range 0.34–20 ng/mL) measured at 7, 10, 24, 48, 168 hrs were 5.2, 4.9, 5.1, 4.9, 5.0 ng/mL at RT, 5.0, 5.0, 4.7, 4.3, 4.4 ng/mL at –20°C and 4.8, 4.8, 5.0, 4.7, 4.9 ng/mL at 2–8°C. The average CV on multiple runs at RT, 2–8°C, -20°C was 8.7%, 6.9%, 9.3%, respectively. Total imprecision (all data points) on stored samples and two controls were 9.3%, 4.8% and 3.1%, respectively. Freeze thaw analysis showed that AMH levels were stable over 4 thaw cycles, with median levels of 4.5, 4.7, 4.8, 4.7 ng/mL obtained at site 1 ($n = 4$) and 1.2, 1.2, 1.3, 1.3 ng/mL, respectively at site 2 ($n = 5$).
Limitations, reason for caution: Strength: This is a prospective study and uses a well characterized AMH assay. The assay is human- specific, not impacted by complement and uses standardized recombinant human AMH calibrators. The samples were collected and tested under controlled conditions. Equilibration kinetics of AMH will be presented.
Wider implications of the findings: AMH as a biomolecule is very stable. Well characterized assays and good pre-analytical methods will produce reliable and reproducible results. This finding will help resolve the uncertainty related to AMH stability and will make measurement of AMH more convincing.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Ansh Labs.
Trial registration number: NA.

P-517 The relation between age, AMH and other ovarian reserve parameters: comparison of a healthy population of women with regular menstrual cycle with infertile patients

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Study question: This study asks whether 'Anti Mullerian Hormone' (AMH) were related best with age in assessing ovarian reserve in comparison with fertile and infertile populations. This study asks whether 'Anti Mullerian Hormone' (AMH) were related best with age in assessing ovarian reserve in comparison with fertile and infertile populations.
Summary answer: AMH was correlated with age in both infertile and fertile group. However, AFC (antral follicle count) had stronger correlation with age as compared to AMH. No significant difference was found between fertile and infertile groups with regards to AMH, basal FSH levels and AFC.
What is known already: The progressive decrease in ovarian reserve is associated with aging. AMH and AFC were thought to be best parameters to evaluate ovarian reserve. In infertile population ovarian reserve tests are used to predict outcome in terms of the number of oocytes retrieved and occurrence of pregnancy. Diminished ovarian reserve is a poor prognostic factor for achieving pregnancy in both fertile and subfertile populations. However, pregnancy may occur even at extreme abnormal test results.
Study design, size, duration: This is a prospective study included 177 primarily infertile patients who applied to our infertility clinic and initiated IVF treatment and 162 healthy fertile patients admitted to our clinic between October 2011-March 2013.
Participants/materials, setting, methods: Patients between 18-43 years old were included to the study. The participants was divided in to age categories of <30, 30–39, ≥40. FSH, AMH levels and AFC were compared between infertile and fertile population for each category. Correlations of AMH, basal FSH and AFC with age were evaluated.
Main results and the role of chance: There was no significant difference between fertile and infertile populations in terms of AMH and AFC (2.12 ± 1.7 vs. 2.18 ± 1.9, $p > 0.05$ and 8.4 ± 4.9 vs. 8.5 ± 5.3, $p > 0.05$; respectively). Basal FSH levels were higher in infertile patient compared to fertile patients (8.2 ± 7.1 vs. 6.5 ± 4.3, $p < 0.05$) in patients <40 years of age. According to age categories AFC, AMH was not different between fertile and infertile groups. AMH was negatively correlated with age in both fertile and infertile populations ($r = -0.60$, $p < 0.001$ and $r = -0.45$, $p < 0.001$; respectively). However, AFC revealed stronger correlation in both fertile and infertile population ($r = -0.68$, $p < 0.001$ and $r = -0.51$, $p < 0.001$; respectively) compared with FSH and AMH.
Limitations, reason for caution: Infertile patients with diminished ovarian reserve may tent to undergo IVF more quickly which may lead biasness in comparision with fertile population. However, no significant difference was observed between groups in terms of ovarian reserve markers. The existence of heterogeneity in infertile population with regards to etiology is another limitation.
Wider implications of the findings: The preliminary study results revealed that AMH and AFC as the best markers of ovarian reserve are identical in infertile and fertile population. The decrease in the ovarian reserve in infertile patients is directly related to age not the infertility. The correlation of AFC with age is better than AMH in both fertile and infertile population.
Study funding/competing interest(s): Funding by University(ies), There is no funding for this study and the authors report no conflicts of interest.
Trial registration number: This not a trial.

P-518 Prolactin may influence mechanical properties of large and small arteries in healthy regularly menstruating women of reproductive age

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Study question: The potential role of prolactin (PRL) in the regulation of central and peripheral arterial stiffness in reproductive age women.
Summary answer: Increase in the serum PRL concentrations (within normal range) in young, healthy women is related to the decrease in parameters describing arterial stiffness, both at the central (aorta) and peripheral level (small arteries).
What is known already: Most clinical and experimental studies postulate negative influence of PRL on cardiovascular system. PRL may play a role in the pathogenesis of primary and gestational hypertension and peripartum cardiomyopathy. Positive correlations between PRL concentrations and central & peripheral blood pressure and pulse wave velocity (marker of arterial stiffness) in early menopausal women were reported recently.
Study design, size, duration: Cross sectional study of 55 healthy and regularly menstruating women (30 ± 5 yrs of age; BMI 22.2 ± 3.7 kg/m^2), with excluded endocrinopathy - evaluated at the early follicular phase in the morning (EFP – 3rd-5th day of the menstrual cycle, 8–9 am, fasting).
Participants/materials, setting, methods: Prolactin serum concentrations were measured with chemiluminescent method. Simultaneously non-invasive continuous applanation tonometry (Colin BMP7000, Japan) to measure peripheral pulse pressure wave with subsequent on-line reconstruction of central pulse pressure wave (Sphygmocor Mx, Australia) was performed. Pearson and Spearman correlations were used in statistical analysis.
Main results and the role of chance: In the early follicular phase of normal menstrual cycle a significant inverse correlation between PRL levels and the parameters describing the peripheral (PRL: pAugmentation Index [AI], $r = -0.31$, $p = 0.02$) and central (PRL: cAI, $r = -0.32$, $p = 0.02$, PRL: cAugmentation Pressure [AP], $r = -0.32$, $p = 0.02$) vascular stiffness were observed.
Limitations, reason for caution: Applanation tonometry is an indirect method of arterial stiffness assessment however in experimental studies high concurrence of applanation tonometry and invasive methods was confirmed. Number of participants in the study is relatively low and strength of the correlations is not very high.
Wider implications of the findings: Presented results stay in disagreement with available literature which is rather sparse. Role of PRL in the regulation of cardiovascular system in reproductive age women and its influence on elastic properties of arteries remains unclear and needs further research.
Study funding/competing interest(s): Funding by national/international organization(s), National Science Center Poland.
Trial registration number: 407297640.

P-519 Low AMH levels as a marker of reduced ovarian reserve in young women affected by Down syndrome

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Study question: To evaluate the circulating Antimullerian Hormone (AMH) levels in women with Down Syndrome (DS) with regular menstrual cycles and to analyze their correlations with the clinical, hormonal and metabolic parameters, compared with those obtained from an age-matched population of healthy women.
Summary answer: AMH levels were significantly lower in DS subjects compared to age matched controls. This finding is in keeping with the early onset of menopause previously observed in these women. Sub-analysis of data in DS patients under 30 years of age suggests an early follicular depletion related to trisomy 21.
What is known already: AMH is a reliable marker of ovarian reserve in adult females. The gynecologic health of women with DS and other forms of mental disabilities has been largely understudied. DS patients experience menopause earlier than healthy subjects and are twice as likely to undergo premature ovarian insufficiency. Menopause accelerates the cognitive decline and is associated with a two-fold increased risk of mortality in DS women. Nonetheless, no previous studies investigated the ovarian reserve of these subjects.
Study design, size, duration: Fourteen regularly menstruating women with Down syndrome, confirmed by chromosomal analysis (age 25.43 ± 7.40 years; body mass index - BMI 26.12 ± 5.54 kg/m^2), were enrolled for this prospective case-control study. Data from DS patients were compared with those obtained from 20 normo-ovulatory volunteers matched for age and BMI.
Participants/materials, setting, methods: A general physical examination, including assessment of BMI, was done on each patient. AMH levels, gonadotrophins, 17beta-estradiol, prolactin, plasma androgens were analyzed during the early follicular phase of the menstrual cycles. Fasting insulin levels and HOMA-IR, for the assessment of peripheral insulin sensitivity, were also investigated in all participants.
Main results and the role of chance: AMH levels were significantly lower in DS subjects compared to controls (1.34 ± 1.11 vs. 3.01 ± 1.65 ng/ml; $P < 0.01$).

Prolactin concentrations, even if in the normal range, were higher in DS patients compared to controls ($P < 0.01$). No further differences were found in the hormonal and metabolic assessment. After dividing the participants on the basis of age, AMH resulted significantly lower in DS subjects compared to controls both under and above 30 years of age (1.77 vs. 3.73 ng/ml, $P < 0.01$; 0.28 vs. 2.20 ng/ml, $P < 0.01$, respectively). AMH was inversely correlated with age in all groups, and directly correlated with testosterone and DHEAS only in DS subjects ($P < 0.01$). In the same patients, AMH showed a tendency towards a direct correlation with insulin levels ($P = 0.055$).

Limitations, reason for caution: The relatively small number of DS subjects requires further studies to confirm our findings. The exclusion of DS women with oligomenorrhea and/or chronic anovulation may theoretically have led to an underestimation of the ovarian reserve defect in this syndrome.

Wider implications of the findings: There has been a welcome increase in longevity of DS women, who much more often reach the menopausal age. In accordance with our finding of reduced AMH levels, the cessation of ovarian activity frequently occurs earlier than in the general population and is associated with accelerated aging and reduced quality of life. The assessment of gonadal function, including AMH measurement, should become part of the challenge for an improved medical management of DS people.

Study funding/competing interest(s): Funding by national/international organization(s), This study was supported by a grant of Ministry of Public Health, 'The prevention of mental handicap' 'Oasi' Institute for Research – Troina (EN), Italy.

Trial registration number: Not applicable.

P-520 AMH and AMHR2 gene polymorphisms in infertile women and the correlation with assisted reproduction outcomes

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Study question: Evaluate the correlation between *AMH* (T146G/ Ile49Ser rs 10407022) and *AMHR2* (A-482G rs2002555; C1749G rs2071558; G4952A rs3741664; A intron 10G rs11170555) polymorphisms with measurement of serum AMH, estradiol and FSH, ovarian stimulation response and assisted reproduction outcomes.

Summary answer: We showed that polymorphisms of *AMHR2* gene are associated to the assisted reproductive outcomes and the serum level of AMH is correlated to controlled ovarian response.

What is known already: Recent studies have demonstrated that serum AMH levels reflect the size of the primordial follicle pool. Studies in *AMH* null mice showed that, in the absence of AMH, follicles are recruited at a faster rate, and are more sensitive to FSH. It has been suggested that polymorphisms in *AMH* and *AMHR*2 genes may influence hormone function in folliculogenesis and cause the arrest of follicular growth and so, leads to decreased of ovarian reserve.

Study design, size, duration: This is a prospective cohort which were screened, 157 Brazilian women with infertility caused by tuboperitoneal ($n = 62$) factor and male factor ($n = 95$) - without associated female factor - that underwent assisted reproduction procedures. The samples were collected from September of 2011 until December of 2013.

Participants/materials, setting, methods: All patients were above 38 years old, with normal of serum FSH, prolactin and TSH, presence of both ovaries, regular ovulatory cycle, BMI ≤ 30, no history of poor response and no evidence of endocrine diseases. Detection of polymorphisms were performed using *TaqMan* methodology by real time PCR.

Main results and the role of chance: We observed that patients that suffered ovarian hyper stimulation had a statistically increased AMH compared to normal and poor ovarian response ($p = 0.0002$). Serum FSH and estradiol were not associated to the polymorphisms. The genotype GG of polymorphism rs2002555 showed an association of the number of oocytes visualized on USG, number of embryos, and embryos transferred ($p = 0.018$; $p = 0.021$; 0.03, respectively). We did not found positive associations for rs10407022 of *AMH* gene and rs3741664 of *AMHR2*. The polymorphism rs11170555 and rs2071558 had similar results and both were associated with the number of oocytes visualized on USG, oocytes retrieved, and oocytes fertilized ($p = 0.006$; $p = 0.034$; $p = 0.022$) and ($p = 0.006$; $p = 0.031$; $p = 0.01$) respectively, when the patients presented ancestral allele.

Limitations, reason for caution: Small sample size due to the rigorous inclusion criteria.

Wider implications of the findings: Recent studies showed that T146G (rs10407022) and A-482G (rs2002555) polymorphisms of the *AMH* and *AMHR2* genes seems to affect the sensitivity of the ovaries to FSH in women who undergone assisted reproduction treatment, with conflict results. Besides, studies showed that AMH serum level is correlated to assisted reproduction outcomes such as number of oocytes and the number of mature follicles. However, they have not found a positive correlation between polymorphisms and ovarian stimulation.

Study funding/competing interest(s): Funding by national/international organization(s), Fapesp (Fundação de Amparo à Pesquisa do Estado de São Paulo).

Trial registration number: Not applied.

P-521 Does the duration of GnRHa use in long protocol IVF/ICSI affect the stimulation phase and outcome of therapy

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Study question: What is the impact of prolonged GnRHa use upon the stimulation phase of IVF/ICSI therapy and clinical pregnancy rates?

Summary answer: Prolonged down-regulation in ART does not affect pregnancy rates or treatment discontinuation due to poor response during stimulation. Where the downregulation lasted more than 21 days a higher rate of poor ovarian response during stimulation was observed.

What is known already: Long down-regulation protocol is the main therapy used worldwide in ART treatments. Some ART centers use the OCP to prevent cyst formation and prolonged down-regulation. Yet, patients dislike the idea of OCP use during fertility therapy. Programmes that start the GnRHa in the follicular phase encounter prolonged use of this medication. The effect of prolonged down-regulation upon the outcome of therapy has been poorly studied.

Study design, size, duration: A retrospective controlled data analysis of all patients who undertook an IVF or ICSI cycle utilizing the long protocol from January 2008 to January 2013. Data was obtained from our electronic database system. This data was processed in a Microsoft excel spreadsheet and analysed.

Participants/materials, setting, methods: Group 1 included treatments where GnRHa use was for ≤17 days. Group 2 included treatments where the GnRHa was used for >17 days. We compared the outcomes of treatment in patients belonging to the 2 groups. Parameters analysed included clinical pregnancies and cycles stopped due to poor ovarian response.

Main results and the role of chance: We analysed 2387 cycles that fulfilled the inclusion criteria. Of these, 1700 cycles were down regulated for 17 days or less and 687 cycles were down regulated for 18 days or more. Clinical pregnancy rates per embryo transfer were 39% (553/1413) for those down regulated for 17 days or less compared with 35% (198/565) for those down regulated for more than 17 days. Poor response to stimulation and discontinuation rates were similar between groups, 9% for Group 1 (148/1700) and 10% (68/687) for Group 2. Interestingly, we observed that in cases where downregulation was achieved after 21 days a significantly higher percentage had a poor response to stimulation resulting in treatment discontinuation (12% (48/386) compared to 8.5% (170/2001), $p < 0.0001$). Pregnancy rates were similar.

Limitations, reason for caution: The retrospective nature of our study is one of its limitations. We have not separated patients according to reason for prolonged downregulation (Group 2) due to the fact that vast majority had ovarian cysts (82%).

Wider implications of the findings: Our findings offer reassurance that outcomes of IVF/ICSI treatment are not affected by prolonged downregulation. Couples should be counselled that when this extends over 21 days there is an increased likelihood of treatment discontinuation due to poor ovarian response. Pregnancy rates are nevertheless not affected if treatment continues.

Study funding/competing interest(s): Funding by hospital/clinic(s). N/A.

Trial registration number: None.

P-522 Metformin versus myoinositol: which one is better in obese PCOS patients - a crossover study on clinical, endocrine and metabolic effects

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Study question: Which is the more effective insulin-sensitizing drug between metformin and myoinositol on hormonal, clinical and metabolic parameters in obese patients with polycystic ovary syndrome (PCOS)?
Summary answer: Both treatments were able to improve the glyco-insulinaemic balance, but only metformin resulted able to positively affect clinical and hormonal features in obese women affected by PCOS.
What is known already: Due to the central role of metabolic abnormalities in the pathophysiology of PCOS, insulin sensitizers have been proposed as a feasible treatment option. The biguanide metformin is still the most used, but alternative molecules were recently evaluated. Myoinositol, an insulin second messenger, seems able to restore spontaneous ovarian activity in PCOS patients. However, previous reports evaluated the effects of formulations containing myoinositol plus folic acid and no direct comparisons with metformin are available in literature.
Study design, size, duration: This is a crossover, active-treatment-controlled, randomized study. 21 PCOS obese women were randomized to receive metformin (850 mg twice a day) or myoinositol (500 mg three times a day) for 6 months. After a 3-month washout, the same subjects received the other compound for the following 6 months.
Participants/materials, setting, methods: We recruited 21 obese women with PCOS diagnosed according with the Rotterdam criteria (age: 25.62 ± 4.7; BMI: 32.55 ± 5.67). The investigations, performed during the early follicular phase, included menstrual pattern, anthropometric characteristics, hirsutism score evaluation, hormonal assays, oral glucose tolerance test, euglycemic hyperinsulinaemic[Q1] clamp and lipid profile.
Main results and the role of chance: 13 patients completed the study without protocol violations. Both metformin and myoinositol significantly reduced the insulinaemic response to OGTT ($p < 0.05$ and $p < 0.01$ respectively). Both treatments induced an improvement in insulin sensitivity documented by the increase of M value during the euglycaemic-hyperinsulinaemic clamp, though these differences did not reach the statistical significance. Metformin was able to significantly decrease body weight ($p < 0.01$), improve menstrual pattern ($p < 0.01$) and the Ferriman-Gallwey score ($p < 0.05$), to reduce and rostenedione ($p < 0.05$), FAI and AMH levels ($p < 0.01$), and to significantly decrease LH and estradiol levels ($p < 0.05$). None of these clinical and hormonal changes were observed during the myoinositol administration period.
Limitations, reason for caution: The sample size was limited for a randomized trial. Despite the considerable number of studies in literature, treatment schedules for both metformin and myoinositol are not well standardized. It could be conceivable that an higher myoinositol dosage could be more effective on PCOS features.
Wider implications of the findings: This is the first study directly comparing the efficacy of metformin vs. myo-inositol administration on hormonal, clinical and metabolic features in PCOS patients. At variance with previous reports, the novelty of the present trial relies on the use of a pure myoinositol formulation and on the cross over design, which allows to compare the efficacy of different drugs in the same patient.
Study funding/competing interest(s): Funding by University(ies), Università Cattolica del Sacro Cuore.
Trial registration number: NCT01791647.

P-523 Should women delay pregnancy following laparoscopic adjustable gastric banding

Abstract withdrawn by the author

P-524 Comparison of results of two flexible ovarian hiperstimulation protocols with two different initial doses (150 VS = 225 UI) in patients undergoing FIV/ICSI cycles

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Study question: Is a starting dose of ≥225 better than 150 IU in flexible stimulation protocols, in ≤35 years old patients regarding the number of retrieved oocytes, embryos available for freezing and pregnancy rate in cycles of IVF / ICSI?
Summary answer: There were no significant differences between the two starting doses (150 vs. ≥225) in relation to the number of retrieved oocytes, embryos available for freezing and pregnancy rate. We found significant difference in the total dose of FSH as well as the dose increased in the 150 stimulated group.
What is known already: Prospective randomized studies compared the effectiveness of different starting doses (100 vs. 200 IU, 150 IU versus ≥225 IU) in patients up to 41 years old, most of them reporting when compared doses ≤150 vs. ≥225 UI, this later associated to a greater number of retrieved oocytes, but both are equally effective doses regarding the pregnancy rate. We have not found comparative dose studies in patients under 35 years of age.
Study design, size, duration: 231 cycles of IVF/ICSI from January 1, 2011 to December 31, 2012 have been analyzed retrospectively. Two groups of patients stimulated with 150 IU of rFSH or HMG (group I = 72 cycles), and 225 IU of rFSH or HMG (group II = 159 cycles) were studied.
Participants/materials, setting, methods: Infertile couples candidates for assisted reproductive techniques attended in the Instituto Nacional de Perinatologia. The couples had ovarian ovulation disorders, altered tubo-peritoneal factor, endometriosis, altered male factor. The cycles realized for the couples were assigned in one of the two groups of study depending of the initial gonadotrophin dose.
Main results and the role of chance: The total dose used of FSH in group II was significantly higher versus group I (2096.0 vs. 1447.9 IU). The percentage of patients in whom the dose was increased was higher in Group I vs. Group II (26.4% vs. 9.1%, $p = 0.001$). The number of retrieved oocytes was similar between the groups (10.5 vs. 10.2, $p = 0.76$) as well as the number of embryos available the day of the transfer and the number of embryos that were vitrified was 6.73 vs. 6.08 $p = 0.97$ and 1.5 and 1.3 $p = 0.820$ for Group I and II respectively. The population total clinical pregnancy rate was 39.3% ($n = 91$), and for group I and II 41.6% and 37.7% respectively ($p \geq 0.05$).
Limitations, reason for caution: The research implies situations of bias, for being a retrospective study. Prospective studies should be performed in our population.
Wider implications of the findings: Since the study population comes mostly from a low-income-level, we wanted to establish the dose- benefit of ≥225 vs. 150 IU, which, although involved higher cost, could ultimately reflect greater benefits for the patient capturing more oocytes and therefore a larger number of available embryos for vitrification. This would allow the patient the benefit of one or more vitrified embryos cycles. However, the initial ≥225 UI dose did not achieve the aforementioned benefits.
Study funding/competing interest(s): Funding by hospital/clinic(s), Instituto Nacional de Perinatología, México, D.F., México.
Trial registration number: N/A.

P-525 Comparison of metformin and simvastatin administration in women with polycystic ovary syndrome before intracytoplasmic sperm injection cycle; a prospective, randomized, clinical trial

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Study question: The aim of this study was to compare the effectiveness of metformin with simvastatin administration prior to intracytoplasmic sperm injection (ICSI) in patients with polycystic ovary syndrome (PCOS) who are candidates for ICSI.
Summary answer: The results showed that simvastatin effectively improved hyperandrogenism signs and symptoms in PCOS patients, but the impressive effects of simvastatin as a pretreatment regimen were not significantly different from metformin with regards to ICSI cycle outcome.
What is known already: According to the latest Cochrane published study about metformin treatment before and during ICSI cycles, metformin did not improve live birth or pregnancy rates; also the Cochrane database announced that although simvastatin improved lipid profiles and hyperandrogenism

signs, it did not have a significant effect on the chance of pregnancy in natural cycles.
Study design, size, duration: Prospective, double blind, randomized clinical trial/ 40 women based on a closed enveloped randomization method between December 2010 and November 2012.
Participants/materials, setting, methods: Women with PCOS and a history of infertility referred for their first ICSI cycle.
Main results and the role of chance: The results showed that both drugs significantly improved patients' hirsutism scores, and that simvastatin had a greater effect than metformin (Group A; *p*: 0.0001 vs. Group B; *p*: 0.003). The reduction in body mass index (BMI) was not significant in any of the groups. Simvastatin reduced some biochemical parameters, such as FSH, LH, testosterone, total cholesterol, and LDL, and significantly increased the HDL levels, whereas metformin significantly decreased FSH, TG, testosterone and total cholesterol. Overall, 35% and 30% of patients treated with metformin and simvastatin, respectively, became pregnant. There was no significant difference between the effects of these two drugs on the ICSI cycle results, such as the number of oocytes in meiosis 2 (M2) phase (*p* value: 0.4) and the number of grade A embryos (*p* value: 0.7).
Limitations, reason for caution: If our finding are evaluated in a larger sample of patients or if treatment is administered longer or at higher doses, may be the results in ICSI outcome will be different.
Wider implications of the findings: We found that the outcome of the ICSI cycle was not significantly improved with simvastatin or metformin treatment; however, biochemical parameters as well as the signs and symptoms in PCOS women were improved.
Study funding/competing interest(s): Funding by University(ies), Ahvaz Jundishapur University of Medical Science (AJUMS), Emam Khomini Hospital.
Trial registration number: The study was submitted to the Iranian health ministry web site for clinical trials (www.IRRCT.IR). (Registration number: IRCT 201202138994).

P-526 Is there a role of progesterone elevation on the day of hCG trigger on IVF cycle outcomes?

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Study question: Do plasma progesterone levels on the day of hCG administration effect IVF cycle outcomes?
Summary answer: Although higher plasma progesterone levels before the hCG triggering have been associated with lower pregnancy and live birth rates, in our study population we did not observe significant effect of high progesterone levels on the IVF cycle outcomes in patients whose age ≤40 years old and basal FSH level <15 mIU/ml.
What is known already: One of the known drawbacks for controlled ovarian stimulation (COH)-ART treatment is lower implantation and live birth rates after embryo transfer. The implantation process is a limiting factor for COH-ART cycles. The inverse relationship between progesterone elevation before hCG triggering and pregnancy rates were shown already in previous studies but in contrast no influence on ART outcomes was also reported.
Study design, size, duration: Retrospective cohort study. Total of 330 ICSI/ embryo transfer cycles which have been performed at Ege University IVF Center between January–September 2013 are analyzed.
Participants/materials, setting, methods: The only embryo transfer cycles which have been performed in patients whose age ≤40, basal FSH level <15 and evaluated plasma progesterone(P) levels on the day of hCG triggering were included to this study.
Main results and the role of chance: A total of 330 ICSI/ET cycles were analyzed. Of those 311 cycles (94.2%) were antagonist, 16 were agonist (4.8%) and 3 were (0.9%) hypogonadotropic/hypogonadism cycles. SET was performed in 65.8% and DET was performed in 34.2% cycles. According to the cutoff value of 1.5 ng/ml of P levels on the day of hCG, cycle outcomes were compared. The group I (P level ≤ 1.5 ng/ml) consisted of 239 cycle (72.4%) and group II (P level > 1.5 ng/ml) 91 cycle (27.6%). The clinical pregnancy rates were 33.9 vs. 30.8% ($p = 0.59$), ongoing pregnancy and live birth rates were 26.4 vs. 25.3% ($p = 0.84$), for groups I and II; respectively. The first trimester abortion rate was also comparable at 5.4% in the group I and 4.4% in the group II.
Limitations, reason for caution: Retrospective nature and small sample size are the main limitations for this study.
Wider implications of the findings: Understanding the follicular phase endocrine characteristics is a key component for the COH-ART treatment success. Decreased implantation rates with higher progesterone levels were reported before. In contrast with these reports, our results demonstrated that, higher plasma progesterone levels on the day of hCG might not have a negative impact on the ART outcomes. In conclusion; further studies are needed to understand the relation and the effect of the progesterone rise on the ART outcomes.
Study funding/competing interest(s): Funding by University(ies). None.
Trial registration number: None.

P-527 Optimal ovarian stimulation for good prognosis patients in the *in vitro* fertilization program

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Study question: The aim of this study was to elucidate the optimal ovarian stimulation in good prognosis patients (younger than 38 years, 1st or 2nd attempt) at the beginning of their IVF treatment in order to choose the most effective and rational (cost effective) stimulation.
Summary answer: Our data show that the mild and short protocols of ovarian stimulation are an optimal selection for good prognosis patients in our program in terms of pregnancy and embryo cryopreservation. The mild and short protocols are optimal for classic IVF, whereas only the short protocol is advised for ICSI treatment.
What is known already: There are different protocols of ovarian stimulation in good prognosis patients worldwide; however, it is still unclear which type of stimulation is considered to be the best in these patients. It is known that fewer, but higher quality oocytes are retrieved using mild and short protocols. Nevertheless, it is not known how these protocols affect the embryo cryopreservation program and the cumulative pregnancy rate.
Study design, size, duration: Our retrospective study included 2,928 *in vitro* fertilization cycles (1,451 IVF and 1,477 ICSI) in good prognosis patients (<38 years, first or second attempt). The patients were treated with three different protocols of ovarian stimulation: mild (225 cycles), short (GnRH antagonists; 1,411 cycles) or long (GnRH agonists; 1,292 cycles) protocol
Participants/materials, setting, methods: The number of oocytes and embryos, embryo cryopreservation and achievement of pregnancy were followed in all patients to elucidate the optimal ovarian stimulation to yield the best clinical outcome. The differences between the groups were evaluated by chi-square test and multiple regression.
Main results and the role of chance: There was a tendency towards a lower number of oocytes per patient after mild and short protocols in comparison with the long protocol of ovarian stimulation, although there was no statistically significant difference in the mean number of oocytes and embryos per cycle and pregnancy rates between the groups. Interestingly, there was a significantly higher proportion of cycles with embryo cryopreservation and a higher proportion of cryopreserved embryos after mild and short protocols of ovarian stimulation, thus indicating a better quality of oocytes and embryos. After IVF both mild and short protocols were found to be favorable, while after ICSI only short protocol could be advised. The long protocol seems to be less optimal and more expensive.
Limitations, reason for caution: The embryo thawing cycles are still in progress and the cumulative pregnancy rate cannot be evaluated at present.
Wider implications of the findings: In good prognosis patients who undergo the first or second attempt of *in vitro* fertilization the mild and short protocols of stimulation are an optimal choice without impairing the overall clinical outcome and at much lower expense. The mild and short protocols yield high quality oocytes and embryos, therefore even improving the embryo cryopreservation program and cumulative pregnancy rate.
Study funding/competing interest(s): Funding by hospital/clinic(s).
Trial registration number: None.

P-528 Comparing the endocrine secretion of cultured human granulosa luteinized cells of women undergoing FSH- and FSH/LH-stimulation

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Study question: Many investigations show, that there is a benefit of using combined FSH/LH-stimulation by over 35-year old women undergoing IVF-treatment, but doesn't show the reason. Is there any difference between the granulosa cells of women, which are stimulated with FSH/LH or FSH only?
Summary answer: Granulosa cells of the combined FSH/LH-stimulation show higher secretion rates of the most important factors for the early pregnancy – progesterone, Vascular Endothelial Growth Factor (VEGF) and cGMP. These effects are strongest by the oocyte near granulosa cells.
What is known already: Currently there are different treatment approaches for the stimulation of multifollicular maturation of oocytes in IVF treatment. One treatment approach, the combined stimulation of FSH and LH, which is closer to the natural cycle, should improve the oocyte quality, the fertilization and pregnancy rate. These effects are stronger by elderly women. The authors convene only to the closeness to the natural cycle, but don't discuss the molecular processes, which cause these positive effects.
Study design, size, duration: Primary granulosa luteinized cells were isolated from ovarian follicles of women aged over 35 years and undergoing short-antagonist protocol with recombinant FSH (Gonal-f) or combined recombinant FSH/LH (Pergoveris) and oocyte retrieval for ICSI treatment because of male subfertility. A matched pair analysis of 39 women of each stimulation was performed.
Participants/materials, setting, methods: Granulosa cells were collected from follicle puncture fluid and from denudation of the oocyte-cumulus complex and cultured for 1–5 days. Secretion rates of progesterone, VEGF and Inhibin A had been determined for three different time points at a minimum of three independent runs. Also cAMP and cGMP concentrations were measured.
Main results and the role of chance: Higher secretion rates of progesterone and VEGF were observed by the granulosa cells of the FSH/LH stimulation, especially by the granulosa cells of the oocyte-cumulus complex, where a two-fold secretion rate of progesterone either VEGF were noticed. Contrary the secretion rate of Inhibin A is decreased by 25 % by the granulosa cells of the FSH/LH stimulation. cAMP measurement show no difference between the two stimulations, but cGMP concentrations were higher by the granulosa cells of the FSH/LH stimulation. A combined FSH/LH stimulation results a better endocrine function of the granulosa cells. The results show that the type of ovarian stimulation determines the quality of the luteal phase to a large extent.
Limitations, reason for caution: High fluctuations of the individual cases cause a high variance, so that no difference between the two stimulations was statistically significant. A higher number of cases has to be investigated.
Wider implications of the findings: The findings conclude a supported luteal phase, which is awarded to a higher pregnancy rate. Therefore the combined FSH/LH-stimulation represents an interesting therapeutic option for all women aged over 35 years. Whether these improvements are also associated with increased pregnancy rates – which appears highly desirable for this group of patients – must be verified by further studies.
Study funding/competing interest(s): Funding by hospital/clinic(s), Gynaecological Endocrinology and Reproductive Medicine, University Hospital Mainz, Germany, Fertility Center Wiesbaden, Germany.
Trial registration number: None.

P-529 High-fat diet in the development of polycystic ovary syndrome in a rat model

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Study question: What is the role of high-fat diet (HFD) in the development of polycystic ovary syndrome in a rat model? The present study investigated the effect of high-fat diet (HFD) exposure in a rat model of PCOS established by 20-consecutive-day injection of dehydroepiandrosterone (DHEA, 6 mg/kg body weight).
Summary answer: These data indicated that high fat diet (HFD) exposure significantly affected the development of various endocrinal and behavioral phenotypes in PCOS, with several metabolic measurements worsened by HFD treatment.
What is known already: Obesity exerts a major impact on the PCOS phenotype, particularly on the metabolic associations and complications, and significantly worsens metabolic and reproductive outcomes in these women. However, the exact role of fat in polycystic ovary syndrome (PCOS) remains unclear.
Study design, size, duration: Female SD rats were administered with DHEA (6 mg/100 g body weight in 0.1 ml oil) and fed on a HFD for 20 consecutive days (from days 27 to 46). Administration with DHEA or the vehicle sesame oil (0.1 ml) on the normal chow was performed in parallel as control groups.
Participants/materials, setting, methods: Markers of ovarian function including ovarian morphology, estrous cycle, serum testosterone (T), estradiol (E), progesterone (P) and homocysteine (HCY) were evaluated. Oral Glucose Tolerance Test (OGTT), fat distribution test, oil red O staining of liver, magnetic resonance imaging and (MRI) and behavioral tests of locomotion and anxiety were also performed.
Main results and the role of chance: Both DHEA and DHEA + high-fat diet (HFD) treatments showed anovulation and increased serum T and E levels. DHEA + HFD rats showed higher level of P and homocysteine (HCY), and displayed metabolic alterations that were absent in both control and DHEA rats, such as impaired glucose tolerance, pronounced lipid accumulation in the liver, enlarged adipocytes. DHEA + HFD treatment but not DHEA treatment alone increased the body mass index (BMI) and fat concentration. Interestingly, open field and elevated plus maze tests indicated increased anxiety of DHEA rats, which was reversed by HFD exposure. Both DHEA and DHEA + HFD rats exhibited decreased locomotive activities.
Limitations, reason for caution: The complicated interactions between endocrinal changes induced by DHEA and fat metabolism require further investigation.
Wider implications of the findings: Diet and fat may be significant influencing factors in the development of PCOS and require more attention in both laboratory and clinical situations.
Study funding/competing interest(s): Funding by national/international organization(s), National Natural Science Foundation.
Trial registration number: N/A.

P-530 Prevention of ovarian hyperstimulation syndrome by administration of gonadotropin-releasing hormone antagonist in high-risk patients after oocyte retrieval

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Study question: To discuss the efficacy of gonadotropin-releasing hormone (GnRH) antagonist in high-risk patients after oocyte retrieval for prevention of the ovarian hyperstimulation syndrome (OHSS).
Summary answer: The administration of GnRH antagonist after oocyte retrieval can reduce the incidence of severe early OHSS.
What is known already: OHSS is a serious iatrogenic complication of ovarian stimulation in patients undergoing *in-vitro* fertilization (IVF) treatment, triggered by human chorionic gonadotropin. Although cryopreservation of all embryos and their transfer in subsequent cycles can prevent pregnancy-associated late OHSS, early OHSS, may still occur in high-responder patients. It had been reported that administration of GnRH antagonist in severe OHSS patients, resulted in a rapid reduction of oestradiol concentrations and rapid regression of the syndrome.
Study design, size, duration: A prospective, randomized and controlled study was conducted from Sept 2011 to June 2013. 64 high-responder patients were divided into two groups randomly: 32 patients were administered with GnRH antagonist (Cetrotide, 0.25 mg) for 3 consecutive days after oocyte retrieval, and 32 cases were the control group.
Participants/materials, setting, methods: On Day 0, 3 & 7 after oocyte retrieval, collected blood samples to respectively observe the variances in E2 and P levels, performed ultrasound to assess the ovarian size and ascetic fluid, and followed up the numbers of patients with moderate and severe OHSS in both groups.
Main results and the role of chance: The proportions of patients with moderate OHSS in both groups, had no significant difference (31.3% and 30% respectively), but the incidence of severe OHSS in the Group treated with Cetrotide was

lower than the control group (9.4% and 46.7% respectively), with significant difference ($P < 0.05$). The ovarian size in both groups started to enlarge respectively from the day of hCG administration, Day 0 & 3 after oocyte retrieval, however, on Day 7, it decreased, without significant difference ($P > 0.05\%$). The E2 level in both group increased gradually on Day 0–3 after oocyte retrieval, but having the increase rate in the group treated with Cetrotide lower than the control group ($P = 0.065$), and showed a declined tendency on Day 7, without significant difference between both groups.

Limitations, reason for caution: Two cases failed to be followed up in the control group.

Wider implications of the findings: The current study is the first time, that administration with GnRH antagonist in high-risk patients after oocyte retrieval for preventing early OHSS. The role of GnRH antagonist for preventing OHSS or management OHSS, might act on the ovary by accelerating corpus luteum regression, and then, reducing the expression of locally produced ovarian angiogenic factors, like vascular endothelial growth factor (VEGF).

Study funding/competing interest(s): Funding by hospital/clinic(s). Seeds funding by Peking University Third Hospital.

Trial registration number: We did not register my study in ICMJE online, but had registered in our hospital Ethics Committee in 2011.

P-531 Disturbed serum adipokines levels in normal-weight, normoinsulinemic four phenotypes of polycystic ovary syndrome

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Study question: Altered adipose tissue secretion plays a central role in the metabolic abnormalities observed in PCOS. This study was conducted to determine the concentrations of adipokines in different phenotypes of PCOS women, hypothesizing that normal-weight, normoinsulinemic PCOS women, with mildest phenotype also present with changes in adipokine secretion compared to controls.

Summary answer: There was no difference in adipokines serum levels between different PCOS phenotypes. Lower levels of adiponectin and ghrelin, and higher levels of leptin and resistin were observed in all lean PCOS phenotypes without hyperinsulinemia compared to controls suggesting that other intrinsic PCOS factor is in the culprit of these abnormalities.

What is known already: Women with PCOS have significantly elevated leptin and resistin, and lower adiponectin and ghrelin serum levels compared to healthy women. Whether these abnormalities are secondary to obesity/abdominal fat distribution/hyperandrogenism/hyperinsulinemia or represents the intrinsic PCOS abnormality is yet to be determined.

There is a limited data on the adipokine serum levels in four main PCOS phenotypes defined by the Rotterdam Criteria. The majority of them observed positive association of serum adipokine concentrations and PCOS phenotype severity.

Study design, size, duration: Observational, prospective study of 186 women with PCOS fulfilling Rotterdam criteria and 162 weight and age-matched women was conducted from 2009 to 2013. Five groups were created: A) O (Oligoovulation) + H (Hyperandrogenism) + P (Polycystic ovaries) ($n = 102$); B) O + H ($n = 15$), C) H + P ($n = 27$); D) O + P ($n = 42$) and E) (control group).

Participants/materials, setting, methods: Body mass index (BMI), waist/hip ratio (WHR) and hirsutism were evaluated. Serum concentrations of a leptin, adiponectin, resistin, ghrelin, androgens, sex hormone binding globulin (SHBG), glucose and insulin were measured. Free testosterone and homeostatic model assessment of insulin resistance (HOMA-IR) were calculated. Results were analyzed using SPSS 17 for Windows.

Main results and the role of chance: No association was found between the leptin, resistin, adiponectin and ghrelin serum levels with severity of PCOS phenotype (all $p > 0.05$). We observed higher levels of leptin and resistin and lower levels of adiponectin and ghrelin in all PCOS phenotypes compared to control group (all $p < 0.001$). The most prominent finding was the lower adiponectin ($p < 0.001$) and ghrelin ($p < 0.001$) serum levels and higher levels of leptin ($p < 0.001$) and resistin ($p < 0.001$) in lean PCOS patients without hyperandrogenemia and insulin resistency (phenotype D) compared to healthy controls (group E).

Limitations, reason for caution: Small sample size in group B. HOMA-IR was used instead of more reliable but technically difficult hyperinsulinemic-euglycemic clamp technique. With significant variations in PCOS presentation seen in different ethnic populations, generalizing data obtained from any single ethnic group to other population groups should be approached with caution.

Wider implications of the findings: Although the O + P phenotype itself is under dispute, our data show that the normal-weight, normoinsulinemic PCOS women, who share this phenotype present with alterations in adipokines metabolism, the finding that was previously observed in PCOS women in general. This may suggest that some other intrinsic PCOS factor other than obesity, hyperandrogenism or hyperinsulinemia modulates the adipose tissue dysfunction which merit further research.

Study funding/competing interest(s): Funding by national/international organization(s). Ministry of Science, Education and Sport of the Republic of Croatia (No. 108-0000000-0388).

Trial registration number: None.

P-532 Involvement of bone marrow-derived vascular progenitor cells in neovascularization during follicular development in mice

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Study question: Do the bone marrow-derived progenitor cells contribute to neovascularization during follicular growth?

Summary answer: The bone marrow-derived endothelial cells and pericytes partially contribute to the neovascularization during follicular growth.

What is known already: Neovascularization is necessary for follicular growth. Vascularization is first observed in preantral follicles, and thereafter the vasculature markedly increases in follicles. Neovascularization includes angiogenesis and vasculogenesis. Vasculogenesis is the formation of new blood vessels by bone marrow-derived endothelial progenitor cells. It is unclear whether vasculogenesis occurs during follicular growth. Mature blood vessels are characterized by recruitment of pericytes. However, it is unclear where pericytes come from and whether they contribute to neovascularization in the follicle.

Study design, size, duration: A parabiosis model was used in this study. Six-week-old wild-type and transgenic female mice expressing green fluorescent protein (GFP) were conjoined between the lateral abdominal regions to create a shared circulatory system. For immunohistochemistry, six to twelve ovaries obtained from three to six parabiosis models were used, and immunostaining was evaluated on three to four tissue sections in each ovary.

Participants/materials, setting, methods: After 6 weeks, the ovaries were obtained and immunostained for CD31/CD34 (a vascular endothelial cell marker), platelet-derived growth factor receptor (PDGFR) a pericyte marker, and GFP (a bone marrow-derived cell marker). Immunostaining was evaluated on three to four tissue sections in each ovary in each developmental stage of the follicles.

Main results and the role of chance: Cells that were positive for both CD34 and PDGFR were observed in the stroma adjacent to the primary or early preantral follicles and in the theca cell layer of the follicles from the late preantral stage to the preovulatory stage. CD31/CD34 and GFP double-positive cells were observed in the theca cell layer of the follicle from the antral stage to the preovulatory stage while the number of double-positive cells in the preovulatory follicles did not increase. PDGFR and GFP double-positive cells were observed in the theca cell layer of the preovulatory follicle but not in the smaller follicle.

Limitations, reason for caution: The study used the mouse as a model and the applicability of the observed phenomena in humans warrants further investigation.

Wider implications of the findings: Locally existing endothelial cells and pericytes in the stroma play a central role in the neovascularization during follicular growth, while bone marrow-derived endothelial cells and pericytes partially contribute to this process.

Study funding/competing interest(s): Funding by University(ies). Scientific Research from the Ministry of Education, Science, and Culture, Japan. This work was supported in part by JSPS KAKENHI Grant Number 20591918, 21592099, and 21791559 for Scientific Research from the Ministry of Education, Science, and Culture, Japan.

Trial registration number: Clinical Trial Registration Number UMIN000007959.

P-533 Simplified consecutive natural cycles IVF compare favorably to stimulated ones in good prognosis patients

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Study question: The aim of this study is to compare clinical pregnancy rates and implantation rates using consecutive natural cycles (NC) to those of an antagonist stimulated (aCOH) cycle in good prognosis patients.
Summary answer: Implantation and cumulative clinical pregnancy rates are similar when two good quality embryos are transferred either together in a stimulated cycle or one by one in the context of consecutive natural cycles. Number of natural cycles needed to achieve the goal of transferring two embryos ranged from two-five.
What is known already: NC's have a high cancellation rate and usually it takes 4–5 consecutive menstrual cycles in order to have 1–2 embryos available to transfer. On the other hand, it is estimated that the NC costs 75–80% less than a stimulated cycle. In a recent Cochrane review, NC IVF in clinical trials compares favorably to stimulated cycles.
Study design, size, duration: A total of 576 patients below 38 years old treated with IVF at a private Assisted Conception Unit from January 2010 until December 2013, were included in this retrospective study using a 1:1 study group/control group analysis.
Participants/materials, setting, methods: Group A (N = 288) were patients that had in total two embryo transfers using embryos derived from consecutive NC (had a second if first ET has failed). Group B (N = 288) had elective double embryo transfer (DET) after (aCOH). All patients had intracytoplasmic sperm injection used for fertilization and Day 3 ET.
Main results and the role of chance: Out of 288 patients using a fresh embryo derived from NC, 84 had a clinical pregnancy after their first embryo transfer (29%). Patients (N = 204) that failed on their first attempt, had a subsequent embryo transfer with a NC derived embryo and 51 had a clinical pregnancy (25%). The cumulative pregnancy rate in this group after 2 embryo transfers was 47%, with a cumulative implantation rate 27%. In the control group, out of 288 cases that had DET with aCOH, 145 achieved a clinical pregnancy (50%) with an implantation rate of 28.3%. There was no statistical significance when comparing IVF outcome among these two groups.
Limitations, reason for caution: The only drawback is that it might need several NC (up to five) to achieve a pregnancy
Wider implications of the findings: NC can be a reasonable alternative to stimulated IVF if at least in total two embryos are transferred (one by one). The benefits are, more friendly approach, significantly less cost, avoidance of anesthesia, twins and OHSS.
Study funding/competing interest(s): Funding by hospital/clinic(s). Embryogenesis Assisted Conception Unit Athens – Greece
Trial registration number: None.

P-534 Age at natural menopause in women with clinically manifest vascular disease

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Study question: Is clinically manifest vascular disease (CVD) associated with an earlier menopause compared to women with a specific risk factor for vascular disease and compared to women from a healthy reference population?
Summary answer: Women with a history of CVD at any age had a 30% higher risk to experience menopause compared to women with risk factors for vascular disease.
What is known already: In healthy women, early menopause is an event that is associated with the exacerbation of several risk factors of cardiovascular disease. Cardiovascular disease is a major cause of morbidity and mortality in the Western world. The possible role for vascular disease as a causative factor in early reproductive ageing has recently received attention. The underlying mechanism may be an irreversible vascular change leading to advanced vascular ageing which potentially modifies the ovarian ageing process.
Study design, size, duration: The current study was designed as a retrospective cohort study. Clinical data of the women included in the Second Manifestations of ARTerial Disease (SMART) study with CVD or risk factors (hypertension of hyperlipidemia) were used. As healthy reference population we used the Prospect-EPIC cohort, including 3808 women with a natural menopause.
Participants/materials, setting, methods: In SMART were 1266 women with CVD and 623 women with risk factors included. Study participants filled out standardized questionnaires on age at last menstrual period. Differences in menopausal age were analysed using Kaplan Meier and Cox proportional hazard analysis with age, starting from birth as the time variable, with adjustment for possible confounders.
Main results and the role of chance: The Kaplan Meier estimate of median age at natural menopause was 50 years for women with CVD and 51 years for women with hypertension or hyperlipidemia. Cox proportional hazard analysis showed that with women with CVD were more likely to experience menopause compared to women with risk factors for vascular disease, adjusted for confounders (HR. 1.30, 95%CI 1.14–1.47). The median age at menopause in a healthy reference population is 51 years.
Limitations, reason for caution: Age at menopause was self-reported and assessed retrospectively.
Wider implications of the findings: These results support the hypothesis that compromised vascular health and ovarian ageing are related to each other.
Study funding/competing interest(s): Funding by hospital/clinic(s), University Medical Center Utrecht.
Trial registration number: Not applicable.

P-535 The utility of Anti-Müllerian Hormone in diagnosing Polycystic Ovary Syndrome amongst women presenting to an infertility clinic

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Study question: Is AMH a useful diagnostic tool for diagnosing Polycystic Ovary Syndrome (PCOS) using the revised Rotterdam criteria?
Summary answer: AMH is a useful diagnostic test for predicting PCOS. AMH declines more slowly with age in those women with PCOS compared with non-PCOS. The diagnostic utility of AMH for PCOS is increased with increasing age.
What is known already: AMH has a strong association with the underlying pathophysiology of PCOS including initiation of primordial follicle growth, reduction in aromatase expression and contribution to anovulation. AMH is increased in PCOS women due to increased production from the elevated number of small antral follicles and intrinsic factors within the granulosa cells. Serum AMH corresponds with severity of hyperandrogenism (HA) and oligoanovulation (OA) in PCOS. A universal diagnostic threshold for PCOS remains unconfirmed.
Study design, size, duration: A diagnostic utility study of AMH as a marker of PCOS in a consecutive series of women presenting to a tertiary infertility clinic, recruited between June 2012 and May 2013.
Participants/materials, setting, methods: 164 women presenting to a tertiary infertility clinic had complete clinical, hormonal and ultrasound features recorded. Serum AMH was measured using the Generation II assay (Beckmann Coulter). 67 (40.8%) had PCOS and 97 did not. Statistical tests included receiver operator characteristics (ROC) and Student's t test.
Main results and the role of chance: PCOS women were younger (29.1 vs. 32.6 years, $p < 0.0001$) but had similar BMI. AMH differed between women with PCOS (69.3 ± 47.7 pmol/L), PCOM alone (45.7 ± 24.7 pmol/L) and those without either PCOS/PCOM (17.3 ± 12.1 pmol/L) ($p < 0.0001$). The AUC for AMH in PCOS diagnosis was 0.84 (95% CI 0.77–0.90). Testosterone using mass spectrometry had an AUC of 0.73 (95% CI 0.65–0.81).

Grouped by age (≤25, 26–30, 31–35, >35 years), the serum AMH significantly dropped in the non-PCOS group (≤25 vs. >35 years: 41.5 vs. 21.7; $p = 0.028$) but not in PCOS (≤25 vs. >35 years: 78.6 vs. 64.4; $p = 0.61$). The AUC for AMH diagnostic utility increased with increasing age (≤25 years: 0.74 95% CI 0.52–0.95 vs. >35 years 0.91 95% CI 0.77–1.0).

No significant difference was shown between phenotypic presentations and AMH.

Limitations, reason for caution: Results should be viewed with caution as they are based on an infertility population with a high incidence of PCOS. With respect to age, larger cohorts are needed to confirm age findings.

Wider implications of the findings: This study confirms that AMH and age are dependent factors in diagnosis of PCOS. Age specific reference ranges for AMH are crucial if AMH is to be adopted within the diagnostic criteria for PCOS.

Study funding/competing interest(s): Funding by commercial/corporate company(ies). Beckmann Coulter supplied the AMH reagent kits free of charge.

Trial registration number: na.

P-536 Ovarian response prediction in controlled ovarian stimulation with 150 µg corifollitropin alfa in a GnRH-antagonist protocol

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Study question: Can high and low ovarian response to 150 µg corifollitropin alfa be predicted and what are the best single predictor variables out of an array of demographic, sonographic and endocrine parameters assessed?

Summary answer: Anti-Müllerian Hormone (AMH) and Antral follicle count (AFC) have good predictive capacity for high and low response and should be taken into account when planning a patient for corifollitropin alfa 150 µg stimulation.

What is known already: Corifollitropin alfa 150 µg is available as a single dose with 7-day action for patients >60 kg body weight. The number of oocytes obtained following ovarian stimulation varies strongly between individuals. Extreme responses can theoretically be reduced by defining patients with a predicted optimal response. AMH has been reported as a good predictor of ovarian response, however, AMH assessment has so far mostly been missed in the phase III trial program on corifollitropin alfa.

Study design, size, duration: Multi-centric ($n = 5$), multi-national, investigator-initiated, prospective, observational cohort study, 9/2010–7/2013, NCT01206803. 294 women recruited and centrally registered. 212 patients included in the primary analysis, 45 are not in label for weight (<60 kg) (only considered for secondary analyses). 37 patients excluded due to protocol violations/ missing serum samples.

Participants/materials, setting, methods: Infertile women ($n = 212$); natural luteal phase prior to treatment; cycle day 2/3: assessment of endocrine (AMH, FSH, LH, E2, P), sonographic (total AFC) and demographic (bodyweight, cycle-length, age, duration of infertility) variables; fixed stimulation regimen; agonist triggering in case of hyper response; laboratory analyses performed centrally.

Main results and the role of chance: AMH and AFC are the best predictors for low (<6 oocytes) and high (>18 oocytes) response. In the prediction of high response, the area under the curve of the receiver operating characteristic [AUC (95%)] for AMH was 0.844 (0.758–0.931) and 0.752 (0.644–0.860) for AFC. The optimal thresholds for identifying excessive respond were 2.02 ng/mL for AMH (sensitivity = 77.8%, specificity = 72.2%, Positive Predictive Value, PPV = 31.3%, Negative Predictive Value, NPV = 95.2%) and 14 for AFC (sensitivity = 69.0%, specificity = 70.4%, PPV = 27.5%, NPV = 93.3%). AMH and AFC also predicted low ovarian response. The AUC for AMH was 0.857 (0.778–0.937) and 0.748 (0.658–0.838) for AFC. The optimal thresholds for predicting low response were 0.88 ng/mL for AMH (sensitivity 81.3%, specificity 81.5%, PPV = 50.7%, NPV = 94.9%) and 9 for AFC (sensitivity 70.7%, specificity 71.9%, PPV = 37.1%, NPV = 91.3%).

Limitations, reason for caution: AMH values depend on assay method and might need conversion with future assays. Other definitions of low/high ovarian response exist. The optimality of the selected thresholds needs validation in further independent data. Multivariate analyses taking into account possible interdependencies between the predictors may lead to an even more precise prediction.

Wider implications of the findings: Identification of patients with suboptimal response to corifollitropin alfa 150 µg allows comparative assessment of other stimulation protocols in such patients in order to explore potential improvements by treatment individualization.

Study funding/competing interest(s): Funding by University(ies), University of Lübeck.

Trial registration number: ClinicalTrials.gov identifier NCT01206803.

P-537 Cochrane review: Androgens (dehydroepiandrosterone or testosterone) for women undergoing assisted reproduction

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Study question: Is pre-treatment with dehydroepiandrosterone (DHEA) and testosterone effective and safe in subfertile women undergoing assisted reproduction?

Summary answer: Testosterone pre-treatment increases the live birth rate in women who are poor responders but the effect is small. We did not find an effect for DHEA.

What is known already: Dehydroepiandrosterone (DHEA) was first reported as a treatment for assisted reproduction in 2000 and has been used as an adjunct to *in vitro* fertilisation (IVF) in women with premature ovarian failure (POF), premature ovarian aging (POA) and diminished ovarian reserve (DOR). Testosterone has also been used as an adjunct in assisted reproduction. Both are postulated to increase conception rates, by stimulating follicular development and leading to greater oocyte yields.

Study design, size, duration: Our Cochrane systematic review included all randomised controlled trials (RCTs) comparing DHEA or testosterone with any other active intervention, placebo or no treatment. Primary outcomes were live birth and miscarriage rates. Secondary outcomes were clinical pregnancy rates and adverse events to woman and fetus.

Participants/materials, setting, methods: We included 13 studies of women undergoing IVF or intra-cytoplasmic sperm injection (ICSI). Exclusions: peri or post menopausal, already taking DHEA or testosterone at time of enrolment, or undergoing ovulation induction or intrauterine insemination. Two authors independently screened search results, selected studies for inclusion, and extracted data.

Main results and the role of chance: 6 studies reported our primary outcome of live birth, and 5 reported miscarriage rates. 10 studies reported clinical pregnancy rates. There was evidence of a significant difference in LBR between androgen treatment and control (pooled odds ratio (OR) = 2.54 [CI 1.33–4.82]). This suggests that in women with a 9% chance of having a live birth without pre-treatment, the live birth rate in women having pre-treatment of DHEA or testosterone will be between 11% and 31%.

There was no evidence of a significant difference in live birth for DHEA (OR = 1.38 [CI 0.26 to 7.47]), but evidence of a significant difference in live birth for testosterone (OR = 2.80 [CI 1.39–5.64]).

There was no evidence of a significant difference in miscarriage with androgen pre-treatment (OR = 2.31 [CI 0.70–7.64]).

Limitations, reason for caution: The evidence to date is limited by small sample sizes and inadequate study methodology. These results can only be applied to women who are poor responders. Larger well-conducted trials are needed. In particular, investigators need to recruit an adequate number of participants for meaningful analysis to be possible.

Wider implications of the findings: In spite of evidence showing androgen pre-treatment enhances ovarian response, we have only demonstrated a small

improvement in live birth or pregnancy rates. Women who are poor responders may increase their chance of delivering a live infant with pre-treatment with androgens. This benefit cannot be generalised to all women until it has been demonstrated by further research.
Study funding/competing interest(s): Funding by University(ies), University of Auckland.
Trial registration number: N/A.

P-538 Effect of anti-Müllerian hormone (AMH) and bone morphogenetic protein 15 (BMP-15) on steroidogenesis in primary-cultured human luteinized granulosa cells

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Study question: Is there any effect of AMH and BMP-15 on estradiol and progesterone production from primary-cultured human luteinized granulosa cells? What is the effect of FSH on their actions? Which are the possible mechanisms involved?
Summary answer: The AMH and BMP-15 by interacting with FSH affect the production of estradiol and progesterone from cultured luteinized granulosa cells possibly via Smad5-protein phosphorylation.
What is known already: AMH and BMP-15, both members of TGF-β superfamily, regulate follicular development. AMH inhibits the follicular activation and growth as well as its sensitivity to FSH. BMP-15 contributes to follicle survival by reducing the FSH-induced progesterone production. Nevertheless, it is not known whether AMH and BMP-15 affect basal and FSH-induced progesterone and estradiol release.
Study design, size, duration: Luteinized granulosa cells (GCs) were obtained from follicular fluid of 30 women, undergoing *in vitro* fertilization. The effects of AMH and BMP-15 alone or in combination in the presence or absence of FSH on estradiol and progesterone production, Smad5 phosphorylation and StAR expression were studied in parallel.
Participants/materials, setting, methods: GCs were preincubated for 24 h in medium + serum and subsequently cultured in serum-free medium for 48 h in the presence/absence of various concentrations of AMH, BMP-15 and FSH alone or in combinations. Steroids were measured in culture-supernatant using enzyme-immunoassays, while Smad5-pathway activation and StAR expression were assessed immunocytochemically.
Main results and the role of chance: We found that AMH attenuated the FSH-induced estradiol production ($p < 0.001$) with no effect on basal levels, and decreased the basal and FSH-induced progesterone production ($p < 0.001$). The FSH-induced StAR expression was attenuated by AMH ($p < 0.001$). Besides, BMP-15 attenuated the FSH-induced estradiol production ($p < 0.001$) but have no effect on basal levels. Progesterone basal secretion was reduced by BMP-15 ($p < 0.001$) but this effect was reversed with the addition of FSH ($p < 0.01$), probably via increasing StAR expression ($p < 0.001$). The FSH-induced StAR expression was also attenuated by BMP-15 ($p < 0.001$). In all combinations of FSH, AMH and BMP-15 the Smad-signaling pathway was confirmed, since the Smad5 protein level was higher compared to control ($p < 0.001$).
Limitations, reason for caution: These are *in vitro* findings and do not necessarily apply to the physiology of folliculogenesis.
Wider implications of the findings: They provide the background for further investigation of the ovarian steroidogenesis.
Study funding/competing interest(s): Funding by University(ies), University of Thessaly, Medical school, programme number:4503.
Trial registration number: We have no trial registration number in our study.

P-539 Inadequacy of initiating rosuvastatin then Metformin on biochemical profile of polycystic ovarian syndrome patients

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Study question: To assess whether metformin would have a role in potentiating the effect of rosuvastatin in PCOS patients when administered concomitantly.
Summary answer: Using statins for 3 months then administering metformin may not be an optimal protocol for the management of PCOS.
What is known already: Conflicting results about the statin's role in management of PCOS. Some studies suggest superiority of metformin to statin, while other groups showed different biochemical modifications for each drug.
Study design, size, duration: This is a prospective, randomized, double-blinded, placebo controlled study conducted over 6 months. The study involved 40 women with PCOS, 3 of which dropped out of the study.
Participants/materials, setting, methods: Biochemical and inflammatory markers were measured for all participants before initiation of study and at each time point. All patients received rosuvastatin for 3 months, then were randomized to either receive rosuvastatin plus metformin, or rosuvastatin plus placebo for 3 months.
Main results and the role of chance: Age, lipid and biochemical inflammatory profiles were similar in both groups. No significant differences in blood studies were found between the intervention and placebo groups at 3 and 6 months after treatment. Significant differences in the outcome variables of LDL ($p = 0.007$) and total cholesterol ($p = 0.005$) emerged within the intervention group, with significantly higher levels at 6 months compared to LDL and total cholesterol levels at 3 months. FBS levels were significantly higher but to a lesser extent ($p = 0.02$) within the intervention group at 6 months compared to 3 months. From baseline to 6 months, however, LDL levels had decreased for both groups and there was a significant difference in the unit decrease.
Limitations, reason for caution: Lack of metformin-only group and few clinical parameters. The former is attributed to the small sample size.
Wider implications of the findings: Using statins for 3 months then administering metformin may not be an optimal protocol for the management of PCOS. For further confirmation of our results, larger study population should be recruited and longer follow-up for CVD and PCOS clinical features should be monitored.
Study funding/competing interest(s): Funding by University(ies), American University of Beirut Medical Center Department of Obstetrics and Gynecology.
Trial registration number: N/A.

P-540 Is COMT polymorphism a risk factor to premature ovarian insufficiency

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Study question: Is COMT polymorphism a risk factor associated to Premature Ovarian Insufficiency?
Summary answer: Polymorphism analysis in patients with established premature insufficiency showed a statistically significant difference in the incidence of the mutated allele in women with POI compared with the control.
What is known already: The cause of POI is still unknown in most of the cases, when karyotype is normal. Also there is no study associating *COMT* gene to POI. However, some studies pointed to the importance of estradiol metabolism in the pathway of follicle development.
Study design, size, duration: Case control study. Sample collection started in 2012 and continued to November 2013. Case group was composed by 100 women diagnosed with POI under 40 years old and increased levels of FSH. Control group was composed by 123 women older than 45 years old, fertile, menopaused in the regular age.
Participants/materials, setting, methods: Cytogenetic analysis and dosage of basal FSH levels were performed for all the patients in the case group to confirm the diagnosis. Genotyping of *COMT* polymorphism (Val/Met – rs4680) was performed by Taqman methodology; Statistical analysis was performed by chi-square test and p value <0.05 was considered significant.
Main results and the role of chance: The results of cytogenetic analysis showed one patient with mosaicism and one with a translocation, both involving chromosome X. These patients were excluded from genotyping analysis. Genotyping

results showed that patients with POI presented statistical difference concerning genotype and allele distribution ($p = 0.0033$ and $p = 0.0146$ respectively).
Limitations, reason for caution: Premature Ovarian Insufficiency ovarian dysfunction is a multifactorial disease that may be secondary to autoimmune diseases, infections, iatrogenic exposure and different genetic alterations. Despite of the large evidence observed in the association data there is no study correlating POI with *COMT*.
Wider implications of the findings: The finding enhances the knowledge about genetic pathways associated to the development of POI.
Study funding/competing interest(s): Funding by national/international organization(s). This study is supported by CNPq (process 470333/2013-8) and Instituto Ideia Fertil.
Trial registration number: None.

P-541 Investigation of PPAR signalling in human granulosa cells (hGCs) obtained from IVF patients under different regimens of ovarian stimulation

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Study question: Can different hormonal treatments affect PPARs (Peroxisome Proliferator Activated Receptors) and PPAR-regulated steroidogenic enzymes in ovarian hGCs from IVF patients stimulated with urinary (HMG) or recombinant gonadotropin, with FSH and LH activities, compared with recombinant FSH alone?
Summary answer: Our results showed different expression levels of PPARs in hGCs from IVF patients stimulated with HMG, rFSH + rLH, compared to rFSH alone. Enzymes regulated by PPARα, such us HMG-CoA, 17-βHSD4, 3-βHSD, Aromatase, exhibited changes in their expression levels which might indicate effects on hGCs steroidogenic activity in response to LH activity.
What is known already: All three PPARα PPARβ and PPARγ isotypes are expressed in the ovary and can be activated by endogenous and exogenous ligand, including toxic molecules, with subsequent effects on steroidogenesis, follicle development, ovulation and implantation. PPARs are a family of nuclear hormone receptors that require the interaction with RXRs (Retinoic X Receptors) for their activation.
Study design, size, duration: Five IVF patients stimulated with different protocols: rFSH (group A), HMG (group B) or rFSH-rLH (group C), were enrolled in this study. After oocyte pick-up, hGCs isolated from follicular fluid were analysed for gene and protein expression of PPAR isoforms and their targets involved in steroidogenic activity.
Participants/materials, setting, methods: Protein extracts from hGCs were subjected to Western blotting and analyzed by using antibodies against PPARs, RXRs, 3βHSD, 17-βHSD4 and Aromatase. RNA extracts were reverse transcribed to cDNA, and PPARs, HMG-CoA, 17-βHSD4, 3-βHSD, and Aromatase expression levels were analysed with real-time quantitative PCR. Cultured hGCs were subjected to immunocytochemistry.
Main results and the role of chance: Western blotting revealed that in control group (group A), PPARβ and its RXR were the most expressed isotypes. Increased expression of PPARα, 17-βHSD4, 3-βHSD and decreased expression of Aromatase, was observed in group C, (Student Newman Keuls Method, $p < 0.05$). RT-qPCRs revealed comparative levels of HMG-CoA in all experimental groups. Higher levels of PPARα, 17-βHSD4, 3-βHSD, and decreased levels of Aromatase were found in group C (One Way Anova: $p < 0.001$). Immunofluorescence analysis showed cytoplasmic and nuclear pattern of PPARs and their receptors RXRs in all cellular groups, with a prevalence of nuclear localization of all activated complex.
Limitations, reason for caution: The relationship between the expression of PPAR and steroidogenic enzymes in hGCs from IVF patient stimulated with the three hormonal protocols needs to be confirmed by assessing enzymatic activities and performing a large scale study.
Wider implications of the findings: Our results revealing that different sources or composition of gonadotropins in ovarian stimulation affect the expression of PPARs and their targets in hGCs, agree well with previously results showing upregulation of PPARα gene in cumulus cells from patients stimulated with HMG. Since PPARs modulators are known to have beneficial effects on PCOS (Polycystic Ovary Syndrome) our results can help to improve criteria for adapting FSH and LH administration to individual patients.
Study funding/competing interest(s): Funding by University(ies). The study was funded by MIUR.
Trial registration number: Not requested. Basic science study.

P-542 Factors affecting IVF outcome after conservative treatment with oral progesterone with or without LNG-IUS in patients with stage Ia endometrial adenocarcinoma

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Study question: What factors affect the IVF outcome in patients with well-differentiated early stage endometrial adenocarcinoma (EC) treated with conservative methods, i.e. oral progesterone only or combined oral medroxyprogesterone acetate/levonorgesterl-intrauterine system (MPA/LNG-IUS)?
Summary answer: The factors such as endometrial condition on hCG day and number of Dilatation and curettage (D&C) during EC treatment seem to affect the IVF outcome in patients who had undergone conservative management for early stage EC.
What is known already: Hormone therapy is the most common method for well-differentiated early-stage EC in patients who wish to preserve fertility. IVF treatment in this condition is highly successful for certain women but not for others. Some investigations suggest EC patients might have an impaired endometrial (EM) response. It is critical to select patients carefully for conservative treatment of early stage EC and it's also important to assess the fertility potential before initiation of ART.
Study design, size, duration: This is a retrospective study reviewing total seven patients enrolled our fertility center between May, 2005 and December, 2013 after complete remission(CR) of early stage EC by conservative management. The patients are divided in two groups (oral progesterone only (Group A) = 3 patients; combined oral MPA/LNG-IUS (Group B) = 4 patients).
Participants/materials, setting, methods: All patients underwent IVF/ET (fresh or cryopreserved) cycles using short GnRH antagonist protocol. The possible affecting factors of IVF outcome were analyzed; age, ovarian reserve, EMT on hCG day, time interval between the onset of conservative EC treatment and CR, time interval between CR and IVF onset, and number of D&C.
Main results and the role of chance: The patients(mean age 32.4 ± 4.9) underwent IVF/ET trials(total seventeen) immediately after CR except one woman(12 months after CR, Group B, live birth). All clinical pregnancies (total ten) occurred within the third ET cycle in six women (Group A = 2, Group B = 4 women). There were eight healthy live births (2 singleton, 3 sets of twins), one ongoing pregnancy (Group B) and one first trimester pregnancy loss (Group B). Only one patient failed to achieve pregnancy during nine ET trials (Group A). The mean number of D&C was 3.6 in all women, 3.0 in successfully pregnant women ($n = 5$) whereas 5.0 in women with repeated implantation failure (RIF) or pregnancy loss ($n = 2$). The EMT was thin (median 5 mm) in a woman with RIF or irregular (7 mm with fluid) in a woman with pregnancy loss.
Limitations, reason for caution: The study size is small and retrospective. And follow-up periods were short-term, especially in group B for applying new treatment modality. The long-term randomized controlled study is needed to confirm this study for wider application.
Wider implications of the findings: Our results suggests the possibility of successful pregnancy by IVF/ET will be higher in certain patients who desire fertility after conservative treatment for early-stage EC; i.e., women with appropriate EM thickness and shape on hCG day, the less number of D&C during treatment which refers to relatively short time interval between the onset of EC treatment and CR.
Study funding/competing interest(s): Funding by hospital/clinic(s). The authors have received no funding for this study. There are no competing interests.
Trial registration number: This is not an RCT study, therefore we received no trial registration number.

P-543 Effect of ovarian stimulation on embryo implantation: analysis of 402,185 stimulated and 1981 unstimulated IVF cycles

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Study question: Does ovarian stimulation cause significant impairment to embryo implantation in *in vitro* fertilisation (IVF) treatment.
Summary answer: The overall implantation rate (IR) was significantly higher in stimulated compared to unstimulated (natural) IVF cycles. Among women with <3 oocytes retrieved the IR was significantly higher in unstimulated compared to stimulated cycles.
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 in IVF causes significant detriment to embryo implantation over unstimulated cycles is debated.
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 in the UK since 1991. Data involving 402,185 stimulated fresh IVF cycles and 1981 unstimulated cycles were analysed.
Participants/materials, setting, methods: Data on women undergoing either an unstimulated or a stimulated fresh IVF treatment cycle with at least one oocyte retrieved during the period from 1991 to June 2008 were analysed to compare IRs and live birth rates. IR was defined as the proportion of transferred embryos resulting in clinical pregnancies.
Main results and the role of chance: Majority of women were aged ≤34 years, 50.3% and 46% among women undergoing stimulated and unstimulated IVF. The median number of oocytes retrieved was 9 (IQR 6–13) in stimulated cycles. Among women undergoing unstimulated IVF 83.7% had one oocyte and 16.3% had two oocytes. The IR was significantly higher in stimulated compared to unstimulated cycles: 16.6%; (95% CI 16.5%, 16.7%) versus 12.2%; (95% CI 10.7%, 13.6%); $p < 0.001$. There was a decline in IR with increasing female age in both groups. Live birth and multiple pregnancy rates were 22.8%, 26.1% and 6.6%, 6.1% for stimulated and unstimulated cycles. Among women with <3 oocytes retrieved, the IR was significantly lower in stimulated than unstimulated cycles: 9.3%; (95% CI 8.0%, 10.6%) versus 12.2%; (95% CI 10.7%, 13.6%), $p = 0.0013$.
Limitations, reason for caution: Although the analysis was performed on unstimulated and stimulated IVF cycles, the data had the limitation that there was no information on the total dose of gonadotropin consumption.
Wider implications of the findings: Analysis of this dataset demonstrates that ovarian stimulation in IVF does not cause detriment to embryo implantation over natural cycles as ovarian stimulation allowed for embryo selection. However, stimulated cycles with poor ovarian response (<3 oocytes) had significantly lower IR compared to unstimulated cycles. This could result from better embryo quality from a natural selection of oocytes and a better endometrial receptivity in natural cycles suggesting a role for natural cycle IVF in poor responders.
Study funding/competing interest(s): Funding by hospital/clinic(s). Not applicable.
Trial registration number: Not applicable.

P-544 hMG induced LH-activity in short flare GnRH-agonist protocol: Is there any effect on follicular fluid adiponectin levels and adiponectin receptors expression in cumulus cells?

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Study question: What is questioned in this study is whether follicular fluid adiponectin levels and adiponectin receptors' expression in cumulus cells are affected by hMG induced LH-activity in short flare GnRH-agonist protocol.
Summary answer: Although adiponectin receptor AdipoR1 expression was higher in hMG group, hMG induced LH-activity was not found to affect follicular fluid adiponectin levels as they did not differ among hMG and rec-FSH group.
What is known already: The addition of rec-LH during the late follicular phase has been associated with higher adiponectin follicular fluid levels, suggesting that LH-activity may enhance follicular insulin sensitivity, resulting in decreased androgen levels through a cascade mediated by increased production of adiponectin.
Study design, size, duration: This prospective randomized experimental study was performed on a study population consisted of 24 women, who underwent IVF/ICSI. The participants were assigned into two groups of 12 women each, to undergo controlled ovarian stimulation through a short flare GnRH-agonist protocol with either hMG or rec-FSH. The study lasted 1 year.
Participants/materials, setting, methods: During oocyte retrieval, follicular fluid was collected for adiponectin levels determination. For the determination of adiponectin receptors' expression, cumulus cells were collected on the day of oocyte retrieval, RNA was extracted, cDNA was synthesized and Real-time PCR was applied using specific primers for adiponectin receptor (AdipoR1 and AdipoR2) genes.
Main results and the role of chance: Adiponectin receptors' expression was normalized through comparison with house-keeping gene expression, which is considered to be unchanged. AdipoR1 expression was higher in hMG group (0.303 ± 0.193 versus 0.084 ± 0.102, t-test p-value 0.002, Mann-Whitney U p-value 0.001), whereas AdipoR2 expression did not differ among the study groups. Significant differences were found in follicles >12 mm, number of oocytes retrieved, number of mature oocytes and number of embryos produced in favor of rec-FSH. However, given that follicular adiponectin levels did not differ among hMG and rec-FSH group (1.48 ± 0.22 versus 1.62 ± 0.33 mcg/ml, t-test p-value 0.252, Mann-Whitney U p-value 0.126), the superiority of rec-FSH cannot be attributed to a favourable follicular milieu at least in terms of adiponectin levels.
Limitations, reason for caution: The limited number of participants may have resulted in failing to detect the real differences that existed in the population. Furthermore, adiponectin was measured in the follicular fluid but not in the serum, rendering possible correlations with systematic effects of LH-activity on this adipokine levels obscure.
Wider implications of the findings: Given that LH-activity during the late follicular phase has been associated with higher adiponectin follicular fluid levels, whereas our findings imply that the addition of LH-activity throughout the follicular phase have no impact on adiponectin levels, further research on the effect of LH-activity on this adipokine levels during the early follicular phase are encouraged.
Study funding/competing interest(s): Funding by University(ies), National and Kapodistrian University of Athens.
Trial registration number: Master of Science program entitled "Regenerative & Reproductive Medicine."

P-545 Ovarian endometrioma is associated with increased AMH levels

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Study question: Is AMH levels reduced in patients with endometrioma?
Summary answer: In contrast to current understanding, serum AMH levels appear to be significantly higher in the presence of endometrioma.
What is known already: It is generally believed that endometriosis has significant damaging effect to ovarian reserve. Regards to the effect of endometrioma to AMH levels currently available evidence provide conflicting views; ranging significantly lower AMH measurements, in smaller studies, to no association between AMH and endometrioma in more robust studies.
Study design, size, duration: All women 20 to 45 years of age referred to tertiary centre for management of infertility from 01.09.2008 to 16.11.2010 and had measurement of AMH-DSL assay were included. Following patients were excluded: a) referral for fertility preservation, b) history of tubal or ovarian surgery, c) patients with PCO.
Participants/materials, setting, methods: The AMH measurements of patients with endometrioma were compared to that of without disease using robust multivariable regression analysis following adjusting for relevant covariates such as age, ethnicity, endometriosis (without endometrioma) and causes of infertility.

Main results and the role of chance: In total of 2816 patients met inclusion criteria. 2627 women did not have endometriosis, whilst 189 were diagnosed with endometriosis and 46 of which had unilateral or bilateral endometrioma. The mean and median ages of patients were 32.8(±4.5) and 33.2 (IQR: 29.5–36.5), respectively. Mean AMH measurement was 17.5(±5.01) pmol/L, median 14.2 (IQR: 7.6–23.2) pmol/L.

Our study found that patients with endometrioma have in average 31% higher AMH compared to that of without which was statistically significant ($p = 0.034$).

Contrary to widely accepted view, endometrioma does not appear to be associated with reduced AMH measurements, which is supported by a large study, which has been published recently. Furthermore our study, which is based on a robust multivariable analysis, suggests that presence of endometrioma may be associated with higher AMH levels.

Limitations, reason for caution: The study population is derived from the patients referred for management of infertility and hence these findings may not apply to general population. We recommend further studies should be conducted to understand this association further.

Wider implications of the findings: Increased level of AMH may be due to following factors. Presence of endometriotic cyst in ovaries may result in increased expression AMH in granulose cells and/or increased rate of recruitment growing follicles. Furthermore, AMH also be expressed in endometriotic cells, which is supported by a published research study.

Study funding/competing interest(s): Funding by University(ies), University of Manchester.

Trial registration number: (UK-NHS 10/H1015/22).

P-546 Effect of addition of growth hormone to gonadotropins in ovarian stimulation of poor responders in IVF

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Study question: Is there a statistical significant difference favouring the use of growth hormone adjuvant in poor responders undergoing IVF, compared to a placebo group?

Summary answer: This study suggests that growth hormone addition increases the probability of live birth in poor responders undergoing ovarian stimulation with gonadotropin releasing hormone (GnRH) analogues and gonadotropins for IVF.

What is known already: Poor ovarian response results in decreased oocyte production, cycle cancellation and is ultimately associated with a significantly diminished probability of live pregnancy. Both animal and human data indicate that addition growth hormone is fundamentally involved in ovarian steroidogenesis and follicular development, as well as increasing intra-ovarian insulin-like growth factor I production, which in turn is important to overall ovarian function.

Study design, size, duration: Case control study where 100 poor responder patients were taken and divided into two equal groups, one being the control group. The study was conducted over the period of 1 year.

Participants/materials, setting, methods: Two groups of participants formed equally out of the 100 patients involved in the study. In the first group of 50 patients, GH was added to stimulation protocol with human menopausal gonadotropin (HMG) and in the second group of 50 patients GH was not used alongside the HMG.

Main results and the role of chance: It was shown that the probability of clinical pregnancy was significantly increased. GH addition led to an absolute increase of clinical pregnancy rate. 19 out of 50 (38%) poor responders achieved clinical pregnancy. In the control group 7 out of 50 (14%) poor responders achieved clinical pregnancy. A significantly lower requirement for gonadotropins was observed in the GH group as compared with the control group. It was observed that COCs retrieved was higher in GH treated group. A significantly higher proportion of patients treated by GH reached embryo transfer as compared with those treated by gonadotropins only. Furthermore, decrease in the cost of treatment with GH addition was observed.

Limitations, reason for caution: The total number of patients involved in the study is small (100) and therefore further test and trials are required to compare or contrast with the findings presented here.

Wider implications of the findings: The prevalence of poor responders varies in the literature between 9–24% and remains a largely unsolved issue in IVF, often leading couples to exit their IVF treatment or seek oocyte donation. Addition of GH in stimulation protocol for poor responders is likely to be a solution to increase IVF success rates, reduce oocyte donation thus improving emotional satisfaction, in turn increasing the acceptance of IVF as a priority treatment to infertility in poor responders.

Study funding/competing interest(s): Funding by hospital/clinic(s), A. H IVF & Infertility Research Centre.

Trial registration number: N/A.

P-547 Is the addition of DHEA in the poor responders top or flop?

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Study question: Does dehydroepiandroste (DHEA) improve ovarian function and increase the chances of pregnancy? Is there a correlation between the use of DHEA in assisted reproductive medicine (ARM) and the dose of gonadotropins in ovarian stimulation, number of oocytes obtained, fertilized eggs, quality of the embryos, day of embryo transfer (ET) and pregnancy?

Summary answer: There is a non-significant trend for a positive correlation between the use of DHEA in the ARM and the number of oocytes obtained, the number of fertilized eggs, the quality of the embryos, the day of ET, androgens concentration in serum and the pregnancy rates (35.84% clinical pregnancies in DHEA group compared to 17.33% controls)

What is known already: It has been reported by several research groups that DHEA improves the response of the ovary to stimulation, quantitatively as well as qualitatively with more ET. This leads to improved pregnancy rates. DHEA patients show significantly higher antral follicles, MII oocytes, reduced stimulus duration, improved quality embryos and significantly improved pregnancy rates in women with poor ovarian reserve (POR).

Study design, size, duration: Retrospectively, from January 2009 to April 2012, 428 POR patients were treated with IVF/ICSI in our IVF center. Only patients where enrolled who had at least one negative IVF/ICSI cycle. The group with concentration of androgens below normal or in the lower third of normal (226 patients) received DHEA 75 mg/day, while the other group with a normal concentration of androgens (202 patients) did not get DHEA.

Participants/materials, setting, methods: All of our patients were consented before receiving the DHEA. The short protocol of GnRH-agonists and GnRH-antagonists was used. The dose of FSH used was between 75 and 300 IU/day and for hMG, 2–4 ampoules. The duration of stimulation was looked; also the number of oocytes obtained, PN stage, the number and the quality of the embryos, the day of ET and only clinical pregnancies (positive embryonic heart action) were recorded.

Main results and the role of chance: The average age was 37.69, (24.03 BMI). 536 cycles were performed, 334 in the DHEA group, 202 the control group. The duration of treatment was 3.799 months. The concentration of GnRH and the duration of ovarian stimulation before and after administration of DHEA showed no significant differences. Our statistics showed that after administration of DHEA more oocytes were obtained and more fertilized eggs achieved but with no significant difference. The number of transferred ideal embryos in d 2,3 and 5 before and after administration of DHEA was not significantly different but the number of ET on day 3 was higher in the DHEA group with positive pregnancy. In the DHEA group 284 ETs were performed in 334 IVF/ICSI cycles. Percentage relationship between clinical pregnancy and number ET resulted 28.52%. In the control group there were 202 ET performed with 17.33% clinical pregnancies per ET. According to DIR 2011, clinical pregnancy per ET in the ICSI group between 35 and 39 years old women was 26.15%. Concentration of androgens in the serum of patients with clinical pregnancies were over the normal range.

Limitations, reason for caution: Known variability of androgen levels/day, therefore the data presented should be considered as provisional.

Wider implications of the findings: DHEA could be useful in POF women during IVF treatment. The origin of low androgens in the serum should be further investigated in these women (adrenal versus ovarian); in which setting DHEA would me more useful? However, it is not disputed that DHEA really affects IVF/ICSI outcome in women with POR.

Study funding/competing interest(s): Funding by hospital/clinic(s). The research was funded itself. Praxisklinik Frauenstrasse. The authors declare no conflict of interest in this study.

Trial registration number: For this retrospective study, we needed no trial registration number.

P-548 The change in serum AMH levels during ovulation induction in anovulatory infertility is related to mono- versus multifollicular development

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Study question: Is the change in serum anti-Müllerian hormone (AMH) levels during ovulation induction with gonadotropins in WHO group II anovulatory infertility related to mono- versus multifollicular response?
Summary answer: AMH levels declined significantly during ovulation induction and the change in AMH was correlated with the change in the antral follicle count (AFC) and the number of mature follicles on the day of hCG. The change in AMH was significantly higher in multifollicular compared with mono-follicular cycles.
What is known already: It is well-established that serum AMH is correlated with the AFC and that pre-treatment AMH levels show a marked decline during controlled ovarian stimulation for IVF (Fanchin et al., 2003, Eldar-Geva et al., 2005). However, few studies have investigated AMH level and follicular dynamics during ovulation induction in anovulatory infertility and with contrasting results (Catteau-Jonard et al., 2007, Fong et al., 2011).
Study design, size, duration: This prospective, clinical study performed between 2010 and 2012 included 71 normogonadotrophic, anovulatory women who were consecutively treated with an individualized, nomogram-based, starting dose of highly purified human menopausal gonadotropin (HP-hMG) (Nyboe Andersen et al., 2008). All women underwent one ovulation induction cycle according to a flexible, low-dose step-up regimen.
Participants/materials, setting, methods: Serum levels of AMH and oestradiol and sonographic parameters were assessed at baseline, on the day of a dominant follicle, and on the day of hCG (dhCG). Longitudinal changes in AMH were assessed by repeated measures ANOVA. Further analyses were performed using Wilcoxon-Mann-Whitney test and Pearson's rank correlation coefficient (r).
Main results and the role of chance: In all, 66 women were included. Mean (SD) age was 29.9 ± 3.1 years, baseline median (IQR) AMH 55.5 (34.5–99.8) pmol/l, median (IQR) oestradiol 0.19 (0.13–0.25) nmol/l, and mean (SD) total AFC (2–9 mm) 47.4 ± 23.9. All but 6 women had polycystic ovaries. In total, 34 women achieved mono-ovulation and 32 had multifollicular growth. Mean serum AMH declined significantly between time points for both mono- and multifollicular growth ($P = 0.01$ and $P < 0.001$, respectively), and the mean relative AMH value (dhCG/baseline) differed significantly between mono- and multifollicular cycles ($P = 0.001$). Furthermore, the relative change in AMH was significantly correlated with the relative change in AFC during stimulation ($r = 0.481$, $P < 0.001$), with the number of ≥15 mm follicles on the day of hCG ($r = –0.357$, $P = 0.003$), and with oestradiol levels on the day of hCG (–0.394, $P = 0.001$).
Limitations, reason for caution: The relatively small sample size of the study may reduce the power of the results. Thus, further studies of AMH dynamics during ovulation induction in anovulatory infertility are required.
Wider implications of the findings: AMH levels showed a slight, but significant change during ovulation induction in the group of women who achieved mono-ovulation. A more marked decline in AMH and AFC was observed in cycles with multifollicular response, reflecting the stimulation of antral follicle growth beyond the AMH-secreting window. In mono-follicular growth this effect is minimal, but may still be present due to the excess of small antral follicles in anovulatory infertility compared with normal ovulatory cycles.
Study funding/competing interest(s): Funding by commercial/corporate company(ies). The study was partially supported by a Ph.D. grant from Ferring Pharmaceuticals. The study medication was supplied by Ferring Pharmaceuticals.
Trial registration number: EudraCT-number 2010-021459-16. ClinicalTrials.gov Identifier: NCT01250821.

P-549 Strategy to prevent the failure of GnRH agonist (GnRH-a) to trigger final oocyte maturation in patients with high risk of OHSS stimulated with antagonist protocol

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Study question: Why does GnRH agonist rarely fail to trigger oocyte maturation in subjects with high risk of OHSS in antagonist protocol? Can we identify patients with high risk of GnRH-a trigger failure? What kind of strategies can we use to prevent the trigger failure without increasing the risk of late OHSS?
Summary answer: Strategy based on identification of subjects with high-risk of GnRH-a trigger failure, investigation of progesterone and LH levels following GnRH-a administration, in tandem with an application of GnRH-a combined with low dose of hCG, represent a safe approach to save the stimulation cycle.
What is known already: In patients with high risk of developing OHSS antagonist protocol combined with agonist trigger and embryo freezing represent the most recommended treatment to avoid OHSS onset. Some authors reported a failure of such strategy in limited groups of patients and the option of subsequent hCG administration and repeated egg retrieval for cycle salvation have been reported in literature.
Study design, size, duration: Prospective non randomised study. Based on the observation of 3 subjects with GnRH-a trigger failure and documented lack of progesterone and LH surge. In the period from 6/2011 to 12/2013 a total group of 115 egg donors and 17 IVF patients with a high risk of OHSS development (multifollicular development by ultrasound, more than 25 follicles) were evaluated.
Participants/materials, setting, methods: All subjects underwent stimulation with antagonist protocol. GnRH-a (triptorelin 0.2) was used to trigger the final oocyte maturation combined with simultaneous application of 1500 IU of hCG. The number of oocytes, intensity of abdominal discomfort, ultrasound measurement of ovarian volume, presence of ascites and severity of OHSS symptoms were evaluated.
Main results and the role of chance: We have not observed any case of retrieval failure. Average amount of eggs retrieved was 28 for egg donors and 32 for IVF patients. Proportion of MII oocytes was not compromised. OHSS symptoms were evaluated according to Golan classification. Percentage of subjects with OHSS was low, main symptoms included abdominal discomfort and ovarian enlargements, confirmed by ultrasound. Ascites evacuation was not necessary. 13 IVF patients underwent fresh single blastocyst transfer, 6 pregnancies were documented. 3 from these with retrieval failure following GnRH-a trigger only in previous attempt, 2 subjects underwent new stimulation in antagonistic protocol and combination of GnRH-a and hCG 1500 IU was used for the trigger**.** In both cases, oocytes were retrieved and no signs of OHSS was observed.
Limitations, reason for caution: As frequency of GnRH-a trigger failure is estimated to affect 3% of antagonist/agonist cycles, larger numbers are needed to confirm our results. We must identify failures caused by mistakes in administration or timing of GnRH-a.
Wider implications of the findings: GnRH-a trigger failure might represent a major disappointment for patients facing multifollicular development and a risk of OHSS which causes stress for involved gynaecologists, as identification of reasons for retrieval failure is difficult. As safety is a major concern of ovarian stimulation, proposed strategy needs to be confirmed by a large randomised (multicenter) trial to achieve higher statistical significance.
Study funding/competing interest(s): Funding by commercial/corporate company(ies). Self funding, no competing interests.
Trial registration number: N/A.

P-550 Corticotropine-releasing hormone receptor 1 (CRHR1) is differentially expressed during human follicle maturation and is up-regulated in ovarian endometriosis

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Study question: As Corticotropine-Releasing Hormone (CRH) signalling is transduced via CRH receptors (CRHRs) this work aimed to analyse whether CRHR1 is present in the human ovary. With endometriosis being a chronic inflammatory process and hence a condition of stress it was examined whether CRHR1 expression is altered in ovarian endometriosis.
Summary answer: We herein highlight CRHR1 to be differentially expressed during human follicle maturation and to be overexpressed in ovaries affected by endometriosis.

What is known already: CRH is well known to be one of the bodies major stress hormones. Since CRH has been highlighted to regulate ovarian steroidogenesis, follicle maturation, luteolysis and ovulation, it is discussed to profoundly interfere with controlled ovarian stimulation protocols.
Study design, size, duration: Normal ovaries (n = 8) as well as ovaries affected by endometriosis (n = 14) were retrieved from the archives of the Department of Gynaecology and Obstetrics, LMU, Munich and stained for CRHR1 by immunohistochemistry.
Participants/materials, setting, methods: Formalin fixed paraffin embedded tissue (normal ovaries: n = 8, ovarian endometriosis: n = 14) was stained for CRHR1 using a standardized immunohistochemistry protocol. Appropriate positive and negative were included into each experiment. CRHR1 immunoreactivity was quantified by two independent observers by consensus. The study duration is 1 year so far.
Main results and the role of chance: CRHR1 was exclusively expressed in ovarian follicles (n = 8) with the ovarian stroma staining negative. Regarding both normal ovaries and cases of ovarian endometriosis (n = 14) CRHR1 was identified in primordial, primary and tertiary follicles. The only secondary follicle detected did not show CRHR1 positivity. Regarding normal ovaries the mean fraction of CRHR1 positive follicles was significantly ($p < 0.01$) higher in tertiary follicles (fraction CRHR1 positive = 83 ± 26%) as compared to primordial (fraction CRHR1 positive = 21 ± 22%) or primary (fraction CRHR1 positive = 17 ± 24%) ones. No such observation was made in case of ovarian endometriosis. Interestingly CRHR1 was detected to be up-regulated at least threefold ($p < 0.001$) in ovarian endometriosis cases as compared to normal ovaries. Normal ovaries and ovarian endometriosis samples did not significantly differ regarding patient age.
Limitations, reason for caution: Since material form normal premenopausal ovaries is quite rare, only eight samples of normal ovaries were examined within this work. A larger study panel will be needed in order to confirm our results.
Wider implications of the findings: Since hyper-activation of the hypothalamic-pituitary-adrenal gland axis has been observed in endometriosis patients, we hypothesize that CRHR1 up-regulation in ovarian endometriosis may cause increased stress receptivity and thus hormonal misbalance of ovaries affected by ovarian endometriosis. Whether there might be a clinical benefit of blocking CRHR1 signalling in endometriosis patients undergoing controlled ovarian stimulation remains to be examined.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), FöFoLe, Deutschlandstipendium.
Trial registration number: The study has been approved by the ethics committee of the Ludwig-Maximilians-University of Munich.

P-551 Frozen thawed embryo transfer: natural or artificial cycle?

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Study question: Is there a difference in clinical and ongoing pregnancy rates as well as live birth rates between natural cycle frozen thawed embryo transfer (FET) versus artificial cycle FET?
Summary answer: Based on current literature the natural cycle it is not possible to prefer one method of endometrium preparation over the other. Further studies, also addressing e.g. cost-efficiency should be performed.
What is known already: FET is now considered an effective and cost-efficient component of IVF or IVF-ICSI treatment. The introduction of elective single embryo transfer have emphasized the importance of FET. However, it remains unclear which endometrial preparation method can best be employed. While several methods have been compared with regards to pregnancy and live birth rates, little data exists regarding the performance of natural versus artificial cycle FET or the role of luteal support in this context.
Study design, size, duration: A systematic review of MEDLINE and EMBASE databases identified 43 studies comparing frozen thawed embryo transfer protocols. After careful selection, 8 retrospective studies and 1 prospective study comparing natural cycle FET with artificial cycle FET were subject to analysis.
Participants/materials, setting, methods: The included studies comparing natural cycle FET with artificial cycle FET allowed the analysis of 8152 FET cycles. Subgroup analyses with regard to modified or true natural cycle as well as the use of luteal support was performed.
Main results and the role of chance: No difference with regard to clinical pregnancy (OR 1.1, 95% CI 0.9–1.6), ongoing pregnancy (OR 1.1, 95% CI 0.9–1.5) or live birth (OR 1.2, 95% CI 0.9–1.6) was found. Subgroup analyses also showed no differences in true natural cycle FET or modified natural cycle FET versus artificial cycle FET. Moreover, the use of luteal support had no significant impact on any of the selected endpoints.
Limitations, reason for caution: Most of the studies included in this review are retrospective studies which are subject to high degree of bias. The results of this review should therefore be interpreted with caution.
Wider implications of the findings: Appropriately powered randomized studies remain necessary to guide practice in FET. More over cost-efficiency analyses as well as patients preference studies are required. The Dutch 'ANTARCTICA' randomised trial which is nearing completion will address these questions.
Study funding/competing interest(s): Funding by hospital/clinic(s). None.
Trial registration number: No registration number.

P-552 Does FSH surge at the time of HCG trigger improves IVF/ICSI outcomes - a randomized, double-blind, placebo-controlled study

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Study question: Dose an artificially induced FSH surge at the time of HCG trigger can improve the IVF/ICSI outcomes?
Summary answer: An additional FSH bolus administered at the time of the HCG trigger has no effect on clinical pregnancy rate, good quality embryo, fertilization rate, and implantation rate in women undergoing long gonadotropin-releasing hormone agonist protocol IVF/ICSI.
What is known already: Normal ovulation is preceded by a surge in both LH and FSH. In a standard IVF stimulation cycle, HCG is administered 36 h before oocyte retrieval to simulate the LH surge and promote final oocyte maturation. Yet the last dose of FSH may be given up to 2 days before retrieval. Whether an artificially induced FSH surge, concurrent with the LH surge, would improves cycle outcomes was controversial in the current studies.
Study design, size, duration: It was a randomized, double blind trial with intervention and placebo group. A sample size calculation indicated 347 women per group would be adequate. Recruitment took place during June 2012 and November 2013. Computerized random table were obtained from statistician, and blinded to participants and clinical doctors.
Participants/materials, setting, methods: All groups received a standard long GnRH agonist protocol IVF/ICSI, and HCG 10000 IU to trigger oocyte maturation. In addition, participants were randomized to uFSH bolus (6 A) versus placebo at the time of HCG trigger. Of 738 subjects randomized, 369 received placebo and 369 received uFSH.
Main results and the role of chance: There was no differences in baseline demographic characteristics between the two study groups. There were also no differences in cycle characteristics such as mean number of stimulation days, total gonadotropin dose, peak estradiol. The pregnancy rate in placebo group was 53.2%, while 56.1% in uFSH group. The number of oocytes collected were 10.45 ± 4.46 vs. 10.48 ± 4.55, good quality embryo rate were 36.99% vs. 33.86%, fertilization rate were 63.8% vs. 63% in the placebo group and uFSH group respectively. However, none of these reached a statistical significance.
Limitations, reason for caution: It was a single-center study which may effects the effectiveness.
Wider implications of the findings: Given previous data and our own results, the evidence indicates that an additional FSH bolus administered at the time of the HCG trigger has no benefit on clinical pregnancy rates in women undergoing long GnRH agonist protocol IVF/ICSI.
Study funding/competing interest(s): Funding by national/international organization(s). This study was supported by Science Technology Research Project of Guangdong Province (S2011010004662).
Trial registration number: The trial was registered in the Chinese Clinical Trial Registry (ChiCTR-TRC-12002246).

P-553 In women with reduced ovarian reserve does stimulation with 600 IU of gonadotropin offer any advantage over a lower 300–450 IU dose

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Study question: To check the outcomes of treatment in women with poor ovarian reserve who were commenced on a maximum dose of 600 IU gonadotropins and to compare them with those who were commenced on lower dose gonadotropin, 300–450 IU.
Summary answer: Women with poor ovarian reserve have marginally better clinical pregnancy rates, even when stratified by age, if a lower dose of gonadotropin is used rather than the maximum 600 IU. High dose gonadotropins might be considered for those who are above 40 years, especially if they did not respond to lower dose and prior to the egg donation option.
What is known already: Three decades after the birth of the first IVF baby, poor response to controlled ovarian hyper stimulation still remains a frustrating limiting factor for IVF programs throughout the world. Uncertainty regarding the ideal dose and protocol for women with predicted poor ovarian reserve does remain a challenge
Study design, size, duration: This is a retrospective cohort study (n = 161) of patients with low ovarian reserve and expected poor response, comparing the outcome of IVF treatments between patients that started ovarian stimulation on 600 IU to ones that received 300–450 IU from January 2011 to January 2013.
Participants/materials, setting, methods: Within an academic setting we identified patients that had IVF/ ICSI with the background of low ovarian reserve: AMH < 5.0 pmol/L, (Antral Follicle Count) AFC ≤ 6 and FSH ≥ 10 IU/L and expected poor response to stimulation. We analysed female age, gonadotropin dose, stopped cycles and clinical pregnancies.
Main results and the role of chance: Of the 1758 IVF/ ICSI treatments performed, 161 (9.2%) met the inclusion criteria. A 600 IU dose was used by 143 patients, 53 (37%) had treatment discontinuation due to poor ovarian response. The clinical pregnancy rate (CPR) was 13% (18/143) per cycle started and 20% (18/90) per embryo transfer. Stratified by age, the CPR per cycle started was 14% (5/37) in ≤35 years, 10% (7/67) in 36–40 years and 15.3% (6/39) in >40s.

In the 300–450 IU group (n = 18), 28% were stopped due to poor response. The CPR per cycle started was 33.3% (6/18) and 54.5% (6/11) per transfer. Age stratified, the CPR per cycle started was 60% (≤35 years, 3/5) and 30% in 36–40 years. No pregnancies established in over 40s.
Limitations, reason for caution: The power of the study is limited due to the small sample size.
Wider implications of the findings: In the absence of large randomized trials, we are considering recommending a lower dose of gonadotropins to patients with low ovarian reserve and expected poor ovarian response during ART. The maximum 600 IU dose could be used as last resort prior to donor eggs.
Study funding/competing interest(s): Funding by hospital/clinic(s). HARI Clinic – Dublin.
Trial registration number: 64151.

P-554 Multicenter, prospective study evaluating the first fully automated immunoassay for anti-Müllerian hormone (AMH) for the assessment of ovarian reserve on the cobas® e analyzers

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Study question: To investigate the value of AMH measurement in the assessment of ovarian reserve as expressed by antral follicle count (AFC) using the first fully automated immunoassay for anti-Müllerian hormone (AMH) on the cobas® e analyzers.
Summary answer: 500 patients have been enrolled from December 2012 to December 2013 at 7 study sites in Europe (Belgium, France, Germany, Spain, United Kingdom) and Australia. Correlation analysis will be performed for AFC and AMH as well as for follicle stimulation hormone (FSH) and AMH.
What is known already: AMH and AFC are the best biomarkers for assessing ovarian reserve and show good correlation in single center studies but lower correlation in multicenter studies. Both determination of AMH using the available AMH enzyme linked immunosorbent assays (ELISA) and determination of AFC seems to be dependent on the investigating observer/study site.
Study design, size, duration: Multicenter, prospective cohort study enrolling 500 women planned for transvaginal sonography (TVS) and hormone status on day 2–4 of menstrual cycle from December 2012 to December 2013 at 7 study sites in Europe (Belgium, France, Germany, Spain, United Kingdom) and Australia.
Participants/materials, setting, methods: Women planned for TVS and hormone status on day 2–4 of menstrual cycle for AFC determination had AFC determined including antral follicles of 2–10 mm in size using 2-dimensional TVS. AMH and follicle stimulation hormone (FSH) was quantified in serum samples collected on the same day of AFC determination using the fully automated Elecsys® AMH assay, Elecsys® FSH assay, and Elecsys® estradiol (E2) assay respectively.
Main results and the role of chance: AFC was classified in the following groups: AFC low (0–7), AFC medium (8–15) and AFC high (>15). To show the value of fully automated Elecsys® AMH assay for the assessment of ovarian reserve expressed by AFC the following analysis will be done for all patients and separately for each study site: a scatterplot of AFC versus AMH together with correlation coefficients (Spearman and Kendall), an agreement table will be generated using two cutoffs for AFC: 7 and 15, and hence three AFC groups are defined 0–7, 8–15, >15. According to the prevalences within these groups, quantiles on AMH will be computed to define three groups. Receiver Operator Analysis (ROC) curves will be calculated for the prediction of low and high AFC (≤7, >15) of AMH, FSH and E2.
Limitations, reason for caution: Although in- and exclusion criteria for the target patients have been the same for all study centers target population may differ somewhat between centers. At all study sites 2-dimensional TVS was used, however the ultrasound equipment was not exactly the same potentially leading to different AFC values between sites.
Wider implications of the findings: Currently, AMH is measured using manual ELISA assays. However, conflicting results were observed in some cases, raising the question concerning the assays' reliability. The first fully automated immunoassays for anti-Müllerian hormone (AMH) on the cobas® e analyzers is a reliable assay for the determination of AMH in serum or plasma. Due to advancement of ultrasound technology AFC values determined nowadays using state of the art 2-dimensional TVS are higher than those published 5 and more years ago.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Roche Diagnostics GmbH, Germany.
Trial registration number: N/A.

P-555 First fully automated immunoassay for anti-Müllerian hormone (AMH) on the cobas® e analyzers

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Study question: Development of a high sensitive, fast and fully automated AMH assay on the cobas® e electrochemiluminescence immunoassay platform.
Summary answer: The Elesys® AMH immunoassay on the fully automated cobas® e platform yields reliable quantitative results with high sensitivity in approximately 18 min. Repeatability and intermediate precision ranged from 1.0 to 2.2% and 1.5 to 2.8%, respectively. Limit of Detection (LoD) was 0.01 ng/mL, Limit of Quantitation (LoQ) was 0.03 ng/mL.
What is known already: AMH gained importance in the assessment of ovarian reserve in women. So far, only manual AMH enzyme linked immunosorbent assays (ELISA) assays are available to determine the AMH level in serum or plasma. However, conflicting results were observed in some cases, raising the question with regard to assay reliability. The accuracy and reproducibility issues of currently available manual AMH assays limit the widespread application of AMH in clinical practice.
Study design, size, duration: Repeatability and intermediate precision were calculated based on 14 serum samples. Experiments comparing Elecsys® AMH immunoassay to Beckman AMH Gen II assay or Ansh Labs ultrasensitive AMH ELISA assay used 57 serum samples.
Participants/materials, setting, methods: Elecsys® AMH is an immunoassay using two AMH specific antibodies in a sandwich format using serum or lithium heparin plasma. Primarily the 140 kDa total AMH is detected. 50 µL of sample volume is needed and the quantitative result is available after approximately 18 min.

Main results and the role of chance: Elecsys® AMH assay was standardized against Beckman AMH Gen II covering a measuring range from 0.01 to 23 ng/mL. The overall recovery against the Immunotech calibration was about 90%. Repeatability and intermediate precision ranged from 1.0 to 2.2% and 1.5 to 2.8%, respectively. LoD (LoQ) was 0.01 ng/mL (0.03 ng/mL). Percentage deviation of recovery was always below 10% of initial value. Elecsys® AMH, when compared to Beckman AMH Gen II (or Ansh Labs ultrasensitive AMH ELISA) <8 ng/mL yielded a correlation coefficient of 0.98 (0.97) and a slope of 0.81 (0.73). Sample storage at 20–25°C or 2–8°C up to 7 days or freeze thaw cycles or sample storage at –20°C for up to 9 months was without effect on measured AMH.
Limitations, reason for caution: Due to high variability between laboratories using manual AMH assays the results from the experiments comparing Elecsys® AMH immunoassay to Beckman AMH Gen II assay or Ansh Labs ultrasensitive AMH ELISA assay need to be confirmed by other researchers.
Wider implications of the findings: The development of a high sensitive, accurate fully automated AMH immunoassay for the reliable determination of AMH in serum and Lithium-heparin plasma is a prerequisite for routine use in clinical practice and research.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), Roche Diagnostics GmbH, Germany.
Trial registration number: N/A.

P-556 Which results in IVF/ICSI when the rate of AMH and FSH are discordant?

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Study question: How to interpret results of AMH and FSH when they are discordant to estimate the ovarian reserve (OR) of a patient under 38 years old?
Summary answer: On women under 38 years old, in case of discordance between the level of AMH and FSH, only the level of AMH must be taken to judge the OR and adapt the treatment of stimulation in ART.
What is known already: Decrease of women's fertility is induced by reduction of the OR. Age is considered to be the most important factor in determining OR. Since the age of first birth increases every year, we need to determine markers that would enable to foresee OR and chances of success of an ART. FSH and more recently AMH are known as markers of this OR: normal level of AMH and FSH is considered as normal OR (NOR).
Study design, size, duration: A retrospective study was performed in the center of Dijon and Strasbourg in France and included all of the IVF or ICSI cycles on patients under 38 years old, with attempt of fresh embryo transfer between the 01.01.2008 and the 12.31.2011 whose AMH, FSH and Estradiol rates were available.
Participants/materials, setting, methods: According to ESHRE Bologna standard definition, we compared 2 control groups: NOR/DOR (Decreased OR) to 2 research groups: NAMH (AMH normal, FSH increased)/DAMH (AMH decreased, FSH normal) in the number of punctured oocytes, rate of cycle annulation, rate of pregnancy per cycle and per transfer.
Main results and the role of chance: We analyzed 1707 cycles. Whatsoever were FSH and Estradiol levels, the number of punctured oocytes was lower in case of decreased AMH level (DOR 5.6 ± 3.2; DAMH 6.4 ± 4.3) than in case of normal AMH level (NOR 12.3 ± 6.3; NAMH 10.5 ± 5.6). Whatsoever were the levels of FSH and Estradiol, the rate of cycle annulation was statistically higher in case of decreased AMH level (DOR 38.2%, DAMH 33.7%), compared to normal AMH level (NOR 13.3%, NAMH 16%). However, the rates of pregnancy per cycle and per transfer were not different, whatsoever was the level of the different hormones. The total units of used gonadotropins and the rate of estradiol the day of HCG weren't significantly different when we compared the groups with similar level of AMH (NOR/NAMH, DOR/DAMH).
Limitations, reason for caution: We do not find a difference of rate of pregnancies and live births between the groups. It might be due to the size of some groups. However, we find the same results on the two different centers.
Wider implications of the findings: AMH is only a good quantitative marker of OR to stimulation in ART in patient under 38 years old. Whatsoever is the level of FSH, we should consider women with decreased level of AMH as poor responder and women with normal level of AMH as normal responder to an ovarian stimulation. Only the rates of AMH must be taken into account to judge the ovarian reserve and adapt the treatment of stimulation.
Study funding/competing interest(s): Funding by hospital/clinic(s), CMCO, Schiltigheim.
Trial registration number: No trial registration number.

P-557 Variability between centers in antral follicle counts and numbers of oocytes retrieved following ovarian stimulation

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Study question: Does the correlation between stimulation day 1 antral follicle counts (AFC) and oocyte yields vary between centers in a gonadotropin-releasing hormone (GnRH) antagonist protocol?
Summary answer: Correlation between AFC and oocytes ranges between –0.28 and 0.85. As a consequence, prediction errors range between centers from almost zero to over 7 oocytes.
What is known already: AFC is one of the most commonly used tests of ovarian reserve prior to ovarian stimulation for *in vitro* fertilization and is a predictor of the resultant ovarian response.
Study design, size, duration: Retrospective analysis of 3 randomized controlled trials, Pursue (33 centers in the US, 1388 women, aged 35–42 years, ≥50 kg), Engage (34 centers in North America and Europe, 1476 women, aged 18–36 years, >60 kg), and Ensure (19 centers in Europe and Asia, 395 women, aged 18–36 years, ≤60 kg).
Participants/materials, setting, methods: For the first 7 days of ovarian stimulation in a GnRH antagonist protocol, participants were randomized to a single injection of corifollitropin alfa or daily recombinant follicle-stimulating hormone. Per center, Pearson correlation coefficients (σ) were calculated and predicted numbers of oocytes were obtained from linear regression.
Main results and the role of chance: Mean (SD) AFCs were 10.7 (3.9), 12.4 (4.5), and 11.2 (4.4), and numbers of oocytes retrieved were 10.5 (7.0), 13.2 (7.6), and 12.4 (7.0) in Pursue, Engage, and Ensure, respectively. There was considerable variability between centers in AFCs and the number of oocytes: mean center AFCs (and oocytes) ranged from 6.1–13.9 (6.0–14.6), 8.1–17.8 (8.5–18.4), and 6.7–16.2 (7.1–15.8) in Pursue, Engage, and Ensure, respectively. AFC-oocyte correlation coefficients ranged between centers from 0.22 to 0.85, –0.28 to 0.76, and 0.15 to 0.78, respectively. When considering AFC as a predictor of the number of oocytes, prediction errors (absolute difference between observed and predicted oocytes) averaged by center ranged from 1.4–6.1, 2.0–7.8, and 0.3–6.3 in Pursue, Engage, and Ensure, respectively.
Limitations, reason for caution: This was a retrospective analysis. Equipment used to measure AFC was not standardized across centers.
Wider implications of the findings: There is considerable variability between centers in the accuracy of AFC. The between-center variability observed in this analysis casts serious doubts on the capacity of AFC to reliably predict ovarian response in ovarian stimulation as a single parameter.
Study funding/competing interest(s): Funding by commercial/corporate company(ies). Financial support for this study was provided by Merck & Co., Inc., Whitehouse Station, NJ, USA.
Trial registration number: NCT01144416, NCT00696800, NCT00702845.

P-558 Impact of dose of recombinant FSH on the number of oocytes retrieved in women with high antral follicle counts

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Study question: What is the impact of the dose of recombinant follicle-stimulating hormone (rFSH) administered for ovarian stimulation in a gonadotropin-releasing hormone (GnRH) antagonist protocol on the number of oocytes retrieved (NOR) in women with a high antral follicle count (AFC)?
Summary answer: There was an upward trend in the mean NOR with increasing rFSH dose (150 IU/d to 300 IU/d) in association with higher AFC.
What is known already: AFC is one of the most commonly used tests of ovarian reserve prior to ovarian stimulation for *in vitro* fertilization (IVF). A high AFC predicts a good ovarian response with gonadotropin stimulation with subsequent high oocyte retrieval rates. It is not known whether or not there is an interaction between the dose of rFSH administered for ovarian stimulation and the oocyte retrieval rate in subjects with a high AFC.
Study design, size, duration: This is a retrospective analysis of the rFSH arms of 3 RCTs of similar design with different dosing for rFSH according to the study population: Ensure (n = 128, women aged 18–36 years, ≤60 kg), Engage (n = 750, women aged 18–36 years, >60 kg), and Pursue (n = 696, women aged 35–42 years >50 kg).
Participants/materials, setting, methods: rFSH doses were 150 IU/d (Ensure), 200 IU/d (Engage), and 300 IU/d (Pursue). The number of oocytes retrieved was compared between rFSH doses by ANOVA, adjusted for age, weight, AFC, and baseline estradiol. Subgroup analyses were performed based on AFC (<10, 10–14, and ≥15) and the interaction with dose investigated.
Main results and the role of chance: For 150 IU, 200 IU, and 300 IU rFSH, respectively, mean AFC was 11.4, 12.4, and 10.6 (P = 0.0038; corrected for age, weight), geometric mean baseline estradiol levels were 126.7, 119.4, and 142.3 pmol/L (P = 0.0001; corrected for age, weight), mean NOR was 10.6, 12.6, and 10.3, with adjusted estimates, 9.9, 10.8, and 12.1 (P = 0.0061), geometric mean estradiol levels (day of hCG) were 4420.6, 4245.0, and 4357.3 pmol/L (increase from baseline with a factor of 35.4, 35.5, and 30.6), with adjusted increases differing among doses without a clear trend: 31.8, 30.2, and 36.4, respectively (P = 0.0036). Subgroup analyses by AFC category confirmed that NOR and estradiol levels were higher with higher AFC, but differences among doses were more pronounced (AFC by dose interaction P = 0.024, NOR; P = 0.017 estradiol).
Limitations, reason for caution: This was a retrospective analysis and the 3 rFSH doses were tested in 3 different studies. Adjusting for confounding factors may not have fully accounted for this difference. Anti-Müllerian hormone levels were not included in this analysis.
Wider implications of the findings: A higher oocyte yield with increased rFSH dose from 150 IU/d to 300 IU/d may allow for increased chance for selection of good-quality embryos following IVF. Care to protect against ovarian hyperstimulation syndrome must also be part of the decision on the rFSH dose.
Study funding/competing interest(s): Funding by commercial/corporate company(ies). Financial support for this study was provided by Merck & Co., Inc., Whitehouse Station, NJ, USA.
Trial registration number: NCT00702845, NCT00696800, NCT01144416.

P-559 Impact of oral contraceptives on luteinizing hormone levels during ovarian stimulation with recombinant follicle-stimulating hormone in gonadotropin-releasing hormone antagonist cycles: data from the Xpect trial

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Study question: What is the impact of oral contraceptive (OC) pretreatment on luteinizing hormone (LH) levels during ovarian stimulation with recombinant follicle-stimulating hormone (rFSH) in a gonadotropin-releasing hormone (GnRH) antagonist protocol, and does this impact ongoing pregnancy rates (OPR)?
Summary answer: During ovarian stimulation, serum LH levels recovered after cessation of OC and there was no relation between LH levels and OPR, therefore the apparent lower OPR in subjects with OC pretreatment could not be explained by LH levels.
What is known already: In the Xpect trial, the implantation rate was significantly lower in women who received OC treatment prior to ovarian stimulation for *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) compared with those who did not (23.4% vs. 30.4%).This resulted in an OPR of 26.3% compared with 35.7% in the non-OC group (Nyboe Andersen et al. *Hum Rep*. 2011; 26:3413–3423).
Study design, size, duration: Women were randomized to OC treatment (30 µg ethinyl E_2/150 µg desogestrel) for 14–21 days prior to ovarian stimulation, or no OC pretreatment. rFSH (200 IU/d) was started 5 days after stopping OC treatment (on stimulation day 1, next cycle) with ganirelix (0.25 mg/d) from stimulation day (StimD) 5.
Participants/materials, setting, methods: In women aged 18–39 years with body mass index ≤32 kg/m^2, and a menstrual cycle of 24–35 days undergoing ovarian stimulation for IVF/ICSI, serum LH levels at StimD 1, 5, and 8 (n = 199 and n = 188, OC and non-OC groups, respectively) and OPR were analyzed.
Main results and the role of chance: LH levels were similar in the OC and non-OC groups at StimD 1 (median, 4.8 vs. 5.0 IU/L; P = 0.51, Wilcoxon test), although LH levels <2 IU/L were more common in the OC group (6.5% vs. 1.6%). LH levels on StimD 5 were also similar in the 2 groups (1.8 vs. 1.8 IU/L; P = 0.21); however, on StimD 8, LH levels in the OC group were lower (0.9 vs. 1.8; P < 0.001). There was no significant trend in OPR associated with LH level for any of the time points or pretreatment groups. OPR with LH < 2, 2-<4, and ≥6 IU/L on StimD 8 were 25.2%, 30.0%, and 30.0%, respectively, in the OC group (trend test, P = 0.57), and 43.0%, 28.6%, and 32.1%, respectively, in the non-OC group (P = 0.16).
Limitations, reason for caution: This study only included ovulatory women, and the results may be different in anovulatory women.
Wider implications of the findings: As far as ongoing pregnancy rates are concerned, treatment with OC in the cycle prior to ovarian stimulation does not appear to be advantageous. The lower pregnancy rates with OC pretreatment are not associated with suppression of LH levels after cessation of OC, so other possible mechanisms should be explored.
Study funding/competing interest(s): Funding by commercial/corporate company(ies). Financial support for this study was provided by Merck & Co., Inc., Whitehouse Station, NJ, USA.
Trial registration number: NCT 00778999.

P-560 Psychopathological profile in polycystic ovary syndrome (PCOS): an integrated interdisciplinary approach

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Study question: The aim was to explore if women affected by PCOS are characterized by a specific psychological profile in regard to clinical features and particularly to traits like anger and aggressiveness. Also to investigate the relationship between the psychological traits and the phenotypical and biochemical features of the syndrome.
Summary answer: We report a high prevalence of several psychopathological traits such as anxiety, depression, hostility and obsessive-compulsive disorder. Although the phenotypical features of PCOS were not related to the psychological manifestations, we found a significant correlation between obsessive and anger traits, anger expressiveness and plasma androgen levels.
What is known already: PCOS is the most common endocrine disorder in reproductive-aged women. It is associated with infertility, obesity, hirsutism, hyperandrogenemia, insulin-resistance; an increased prevalence of psychological morbidities has already been reported. Little is known about the involvement of the clinical and hormonal features of the syndrome in the psychological trait especially at the light of the suggested role of hyperandrogenemia in the aggressive behavior and in the impaired impulse control.
Study design, size, duration: Prospective uncontrolled correlational trial involving 30 PCOS patients of the outpatient clinics of the Reproductive Medicine Unit, San Paolo Hospital - University of Milan, Italy in the period March - December 2013.
Participants/materials, setting, methods: The study included 30 patients meeting NIH (1990) and Rotterdam (2003) criteria. As a consequence of a complete clinical and biochemical screening the self-reported psychological data [Symptom Check List 90-R (SCL-90-R); Short-Form Health Survey 36

(SF-36); State-Trait Anger Expression Inventory-2 (STAXI-2)] were collected. Statistical analyses were performed with SPSS-19.

Main results and the role of chance: Our results showed a remarkable prevalence of psychopathological outcomes: Obsessive-compulsive disorder (30%), Depression and Anxiety (23,3%), Hostility (30%), Paranoid Ideation (26,7%), Global Severity Index (23,3%) and Positive Symptom Total (40%). The data from STAXI-2 revealed impaired scores in: Trait Anger Sub-scale (μ = 52.9), Anger Expression-Out (μ = 50.7), Anger Control-In (μ = 52.5). Most of the women reported low quality of life. These psychological traits were not related to the body mass index, body fat distribution, insulin-resistance rate and hirsutism score. The plasmatic levels of total testosterone and the free androgen index were inversely related to the obsessive disorder and the anger trait. Moreover, about anger expressiveness, the plasmatic level of testosterone showed a positive correlation to the outward and a negative correlation to the inward manifestations.

Limitations, reason for caution: Although the use of validated psychological tests with a normative sample and the definition of inclusion criteria aimed at a rigorous selection of PCOS patients, the small sample size and the lack of a control group represent limitations of this study but also a strong motivation to persuid.

Wider implications of the findings: Clinicians should be aware of a possible interaction between biochemical and psychosocial aspects of PCOS and should consider the interdisciplinary approach in the patients' care, involving not only gynecologists and endocrinologists, but also psychologists and psychiatrists.

Study funding/competing interest(s): Funding by University(ies), San Paolo University-Hospital, Milan, Italy.

Trial registration number: The study protocol was approved by the S. Paolo University Hospital Institute Board with the number 2/2013.

P-561 Prolonged estradiol exposure may compromise pregnancy rates in embryo transfers in artificially prepared frozen-thawed cycles

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Study question: What is the value of endocrine profile assessment, endometrial thickness and estradiol valerate exposure in women scheduled for an artificial endometrial preparation cycle for vitrified cleavage stage embryo transfers?

Summary answer: The only factor inversely related with ongoing pregnancy rates in women undergoing embryo transfers in artificially prepared frozen-thawed cycles is the duration of estradiol valerate exposure.

What is known already: A Cochrane review by Ghobara et al. (2010) found significant higher live birth rates in artificially prepared endometrium for frozen-thawed embryo transfers when associated with a GnRH-agonist. Although estradiol in the early follicular phase inhibits ovulation in non-GnRH down regulated cycles, complete pituitary suppression may not be achieved and some follicular activity can still occur. It remains unknown to what extend variations duration of estradiol exposure nor variations in luteinizing hormone (LH), follicular stimulation hormone (FSH), progesterone (P) or estradiol (E2) influence embryo implantation.

Study design, size, duration: In this retrospective cohort study 293 consecutive vitrified-warmed cleavage stage embryo transfers between February 2010 and December 2012 were included.

Participants/materials, setting, methods: Cycles of 176 patients with mean age of 32.1 (SD 5.26) at a tertiary referral centre with exclusion of from oocyte donation or in-vitro maturation cycles. Endometrium preparation was performed by administering 7 days of estradiol valerate at 4 mg, followed by a minimum of 6 days at 6 mg. Progesterone was initiated as soon as endometrial thickness was >7 mm.

Continuous variables were compared with Mann-Whitney U test. Logistic regression analysis was performed with ongoing pregnancy rate as the dependent variable and E2, P, LH, FSH, endometrial thickness and duration of estradiol valerate exposure as independent variables.

Main results and the role of chance: According to our results, serum levels of LH, FSH, P, or E2 on the day before initiation of progesterone administration did not significantly differ between patients with or without an ongoing pregnancy.

Women with an ongoing pregnancy had a shorter duration of administration of estradiol valerate (13.4 vs. 14.7 days; $p = 0.04$), higher endometrial thickness (8.92 vs. 8.17 mm; $p = 0.044$) and lower basal FSH levels at initiation of estradiol valerate (CD 2) (5.57 vs. 6.4; $p = 0.048$) compared to non-pregnant women. Multivariable logistic regression analysis demonstrated that the only variable independently associated with a significant reduction in ongoing pregnancy rates was the duration of estradiol valerate administration (OR 0.85; 95% CI (0.745–0.974).

Limitations, reason for caution: A limitation of this study is its retrospective design. Prospective randomized trials need to establish whether embryo transfer in an endometrium thinner as 7 mm should be preferred over prolongation of estradiol exposure.

Wider implications of the findings: Our findings demonstrate that assessment of endocrine profile is not essential prior to scheduling vitrified warmed embryo transfers, given that LH, FSH, E2, or P values were not associated with ongoing pregnancy rates in artificially prepared frozen-thawed cycles. Unlike endometrial thickness, longer duration of exposure to estradiol was associated with lower ongoing pregnancy rates.

Study funding/competing interest(s): Funding by hospital/clinic(s), Centre for Reproductive Medicine, UZ Brussels.

Trial registration number: None.

P-562 ThyrART: embryo quality and reproductive outcome after IVF according to maternal thyroid function and autoimmunity

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Study question: Do embryo quality and reproductive outcomes [number of oocytes retrieved, number of oocytes that underwent intra-cytoplasmic sperm injection (ICSI), biochemical pregnancy rate, live birth rate] after in-vitro fertilization (IVF) are affected by maternal thyroid function and / or thyroid autoimmunity?

Summary answer: Changes in maternal thyroid-releasing hormone (TSH) concentrations are associated with pregnancy outcome (negative correlation with biochemical pregnancy and live birth rates). Changes in thyroid hormones [thyroxine (fT_4) and triiodothyronine (fT_3)] are associated with embryo parameters (negative correlation with number of oocytes retrieved / underwent ICSI, embryo score at day 3).

What is known already: The role of thyroxine (T_4) during embryo development has been recognized as crucial. However, the evidence of the impact of ovarian stimulation (OS) for IVF on thyroid function or autoimmunity is inconclusive, based mainly on assessments just before and after OS.

Study design, size, duration: Prospective study (ThyrART), assessing embryo quality and reproductive outcome of women undergoing OS (flexible GnRH-antagonist protocol) for IVF (classic or ICSI) whose thyroid function (TSH, fT_3, fT_4) and autoimmunity (anti-TPO, anti-Tg) are closely observed throughout OS.

Participants/materials, setting, methods: Thyroid function and autoimmunity of 42 women were assessed at baseline and throughout OS. Overall change was calculated for all thyroid parameters [Δx = (Concentration of x at the pregnancy test day) – (Concentration of x at menstrual cycle day 3)]. Embryo quality scores were calculated and reproductive outcome were recorded.

Main results and the role of chance: A significant increase was recorded in TSH concentrations between OPU day and the day of the pregnancy test ($p = 0.017$), whereas anti-TPO concentrations were decreased ($p = 0.043$). The number of oocytes retrieved as well as the number of oocytes that underwent ICSI were significantly negatively correlated with Δ_{fT3} ($r = –0.701$ $p = 0.008$ and $r = –0.673$, $p = 0.012$ respectively). Best embryo score at day 2 was positively correlated with Δ_{fT4} ($r = 0.64$, $p = 0.048$) and best embryo score at day 3 was negatively correlated with fT_3 at the day of OPU ($r = –0.43$, $p = 0.05$). Biochemical pregnancy and live birth rates were negatively correlated with Δ_{TSH} ($r = –0.741$, $p = 0.004$, and $r = –0.534$, $p = 0.06$, respectively).

Limitations, reason for caution: Sample size comprises the major limitation of this study.

Wider implications of the findings: The fact that changes in maternal TSH concentrations are associated with pregnancy outcome and changes in thyroid hormones (fT_4 and fT_3) are associated with embryo parameters needs further confirmation. As the threshold for TSH concentrations for the first trimester of pregnancy is particularly low (<2.5 μ?U/mL), special care, such as universal

baseline screening and levo-thyroxine supplementation in selected cases, have to be considered in women undergoing OS for IVF.
Study funding/competing interest(s): Funding by national/international organization(s), Hellenic Endocrine Society.
Trial registration number: Not applicable.

P-563 Successful pregnancies with postprandial glycemia reduction using sitagliptin in women with repeated assisted reproductive technology failure

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Study question: We sought to improve outcomes in women with repeated failure of assisted reproductive technology (ART) by decreasing postprandial glycemia with sitagliptin, a new antihyperglycemic agent.
Summary answer: Sitagliptin significantly improved follicular and embryonic development and pregnancy rates in metformin-unresponsive, older ART repeaters.
What is known already: This is the first report of the above sitagliptin-related improvement.
Study design, size, duration: Case-control study. Treatment and control groups included 44 women each. Duration was the most recent ART cycle with or without sitagliptin, followed to its outcome.
Participants/materials, setting, methods: Matching criteria included age, number of past ART failures, and day-3 follicle-stimulating hormone values. All subjects in both groups had failed using metformin. Mean age was 41.0 ± 0.5 (SEM) and previous failed ARTs were 5.8 ± 0.6. The number of controls per case was 1.
Main results and the role of chance: Sitagliptin significantly enhanced follicular and embryonic developments. Rates of clinical and ongoing pregnancy were significantly and dramatically improved with sitagliptin (20% and 14%, respectively), compared with matched controls (2.3% and 0%). Among 44 various parameters before sitagliptin, triglyceride and free testosterone alone showed significant positive correlations with achievement of ongoing pregnancy (logistic regression). Sitagliptin significantly decreased plasma glucose levels at 0, 30, 60, and 120 min after oral administration of 75 g glucose in oGTT. Change ratio of serum TAGE by sitagliptin administration were significantly correlated with increment of day-2 superior embryos ($\beta = -0.32$, multiple regression).
Limitations, reason for caution: This was a case-control study rather than a randomized controlled trial.
Wider implications of the findings: We believe that similar sitagliptin-dependent outcomes would prevail in populations fulfilling the matching criteria above.
Study funding/competing interest(s): Funding by hospital/clinic(s), Women's Clinic Jinno.
Trial registration number: Not applicable.

P-564 Sleep disturbances in a community-based sample of women with polycystic ovary syndrome

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Study question: The aim of this study was to see whether there is an excess of sleep disturbances in women with PCOS in a community-based sample, and to investigate the degree to which this is accounted for by obesity, depression or other potentially modifiable risk factors.
Summary answer: PCOS was associated with the occurrence of difficulty falling asleep (OR 1.94 95% CI 1.28–2.95), even after adjustment for body mass index (BMI) and depressive symptoms. An association with frequency of waking and not being able to get back to sleep quickly (OR 1.92 95% CI 1.12–3.31) was not significant after adjustment.
What is known already: Clinical samples of women with PCOS have a high prevalence of sleep disorders. Whether this applies in a community sample of women with PCOS is unknown.
Study design, size, duration: Data were obtained from the Lucina cohort of women born during 1973–1975 at a large hospital in Adelaide, South Australia. At age 30 years, 2,046 eligible cohort members (93%) could be traced and 974 (49% after excluding $n = 62$ identified as deceased or disabled) participated, with 724 seen in person.
Participants/materials, setting, methods: The 724 women interviewed in person provided core information, including medical history, and completed additional questionnaires and a clinical examination. PCOS was diagnosed by Rotterdam criteria. Confounders assessed included obesity, depression, smoking, alcohol intake and physical activity. Odd ratios were calculated.
Main results and the role of chance: PCOS was associated with increasing occurrence of difficulty falling asleep (OR 1.94 95% CI 1.28–2.95), attenuated but still statistically significant after adjustment for body mass index (BMI) and depressive symptoms. PCOS was associated with increasing frequency of waking up at night for no reason and not being able to get back to sleep quickly (OR 1.92 95% CI 1.12–3.31) but not after adjustment for BMI and depressive symptoms.
Limitations, reason for caution: This is a representative community cohort. Sleep disturbances were assessed by questionnaire. The findings need to be replicated elsewhere due to geographic and clinical variation in the prevalence and severity of specific PCOS symptoms.
Wider implications of the findings: The excess of sleep disturbance in women with PCOS was at least partly explained by the high prevalence of obesity and, and especially, depressive symptoms in this group. However, the data also support the presence of a perturbed process related to arousal.
Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), University of Adelaide, Robinson Institute, NHMRC, Australian Research Council.
Trial registration number: Not applicable.

P-565 Dual oocyte retrievals in multiple waves of folliculogenesis in patients with poor response for controlled ovarian stimulation

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Study question: Is dual oocyte retrieval in multiple waves of folliculogenesis in poor responders feasible?
Summary answer: Dual oocyte retrieval in multiple waves of folliculogenesis was feasible in patients with poor response for controlled ovarian stimulation (COS).
What is known already: There has been emerging evidence for the existence of multiple antral follicular waves which are recruited throughout the menstrual cycle. Based on the theory of multiple folliculogenesis, new treatment regimens for assisted reproduction technology (ART) are now promoted.
Study design, size, duration: Nine poor responders for COS were included in this preliminary retrospective comparative study. Poor responder was defined according to Bologna criteria.
Participants/materials, setting, methods: Minimal stimulation was performed for the recruitment of the 1st wave follicles. Gonadotropins at a dose of 150 IU for the recruitment of the 2nd wave follicles was started on the day after oocyte retrieval of the 1st wave follicles. GnRH antagonist 0.25 mg was added daily when the leading follicle of the 2nd wave reached 13–14 mm. Oocyte retrievals for the 2nd wave follicles were performed 36 h after hCG administration when the leading follicle reached 16 mm or larger. All embryos were cryopreserved 3 days after oocyte retrieval. Cryopreserved-thawed embryo transfer was performed.
Main results and the role of chance: There were no significant differences in number of oocytes retrieved, fertilization rate, number of embryos transferrable and cumulative embryo score per embryo between 1st and 2nd retrieval. Dual oocyte retrieval showed no significant differences in number of oocytes retrieved, number of embryos transferrable, and cumulative embryo score per embryo when compared with previous single conventional oocyte retrieval. In the dual oocyte retrieval, however, only 2 cycles were cancelled due to no embryos transferrable, which were significantly different from the single oocyte retrieval (2/9 vs. 3/7, $P = 0.023$). Two patients achieved a pregnancy after cryopreserved-thawed embryo transfer in patients with dual oocyte retrieval.

Limitations, reason for caution: Since this was a preliminary retrospective study with small sample size, randomized study in a larger scale will be necessary.
Wider implications of the findings: These findings may indicate that dual oocyte retrieval in multiple follicular waves is feasible in poor responders. Additionally, it may reduce cancellation due to no embryos transferrable.
Study funding/competing interest(s): Funding by hospital/clinic(s), Seoul Rachel Fertility Center.
Trial registration number: None.

P-566 The effect of preceding ovulation and FSH on bone metabolism in women undergoing controlled ovarian stimulation

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Study question: Do bone markers (CTX, TRAP) differ depending on preceding ovulation versus anovulation and how does endogenous and therapeutic FSH influence female bone metabolism?
Summary answer: All bone markers were slightly lower in ovulatory cycles at baseline. No significant correlation could be found between CTX and FSH in this short-term study. The course of osteocalcin correlated positively with the course of FSH in the ovulatory group ($p = 0.021$), while the courses of TRAP ($p = 0.015$) and CTX ($p = 0.037$) correlated positively with estrogen in the anovulatory group.
What is known already: Direct effects of FSH on bone are under discussion since 2006, as ovarectomised rats experienced more bone loss than ovarectomised and hypophysectomised rats (Sun et al., 2006). FSH with or without LH is used in therapeutic dosages for IVF. Whether FSH stimulates bone resorption in women undergoing gonadotropin-therapy for assisted reproduction was unknown.
Study design, size, duration: 100 women undergoing ART were recruited after informed consent to have serum samples taken, measuring their bone markers on 4 visits during controlled ovarian stimulation. GnRH analogues were used for down-regulation before stimulation start and during stimulation. A combination of FSH and HMG or FSH alone was given for stimulation, starting around day 3.
Participants/materials, setting, methods: In 30–45-year-old IVF-patients, bone alkaline phosphatase (BAP), osteocalcin (OC) c-terminal peptide (CTX) and tartrate-resistant acid phosphatase (TRAP) were measured on: V1 = second half of the preceding cycle, V2 = beginning of stimulation cycle, V3 = oocyte retrieval day, V4 = luteal phase of stimulation cycle.
Main results and the role of chance: Of 59 long protocol patients (average age: 38 years, vitamin D3: 20.5 ng/ml), 27 participants were ovulatory and 32 participants anovulatory (progesterone ≤6 ng/ml) at V1. All bone markers were slightly lower in ovulatory cycles than in anovulatory cycles, CTX ($p = 0{,}055$). Estrogen was comparable in both groups ($p = 0{,}867$), initial FSH levels were lower in ovulatory cycles ($p = 0{,}002$). While ovulatory patients showed lower average FSH levels in V1 (median FSH 4.4 mIU/ml), anovulatory started with median values of 6.3 mIU/ml which decreased to 4.85 mIU/ml ($p = 0.027$) at V2. BAP ($p = 0.962$) and osteocalcin ($p = 0.140$) did not change significantly V1 to V2 in the ovulatory group, TRAP decreased ($p = 0.004$), CTX increased ($p = 0.084$). After anovulation, TRAP ($p = 0.054$) and BAP ($p = 0.049$) decreased from V1 to V2.
Limitations, reason for caution: Since the life cycle of a single BMU (bone mineral unit) lasts 3 months, this small and short term study may not have captured possible effects of repeated treatments in long-term fertility patients.
Wider implications of the findings: The courses of FSH and CTX seemed to be associated, but no significant positive correlation between both was found. As osteocalcin is interpreted as a marker for bone formation in particular but also as a marker for bone turnover in general [3], the positive correlation with FSH could be interpreted as a reaction of bone turnover to FSH. A significant correlation however was only seen in the ovulatory group.
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by commercial/corporate company(ies), ART Bogenhausen, aided by a grant from Ferring Arzneimittel GmbH.
Trial registration number: None.

P-567 Pregnancy and neonatal outcome following ovulation induction with the aromatase inhibitor letrozole and clomiphene citrate

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Study question: To evaluate the pregnancy and neonatal outcome after ovulation induction with the aromatase inhibitor letrozole or clomiphene citrate (CC).
Summary answer: There were no significant differences between the letrozole and CC groups.
What is known already: Aromatase inhibition has recently been focused on as an effective means of ovulation induction in the management of fertility. Letrozole, a highly selective, non-steroidal aromatase inhibitor, can successfully induce ovulation in women, including even those who are resistant to clomiphene citrate.
Study design, size, duration: The study included couples directed to the IVF unit at Kyono ART clinic, Japan, for IVF/ICSI treatment from January, 2002 to November, 2012. The subjects were 1389 couples with 2461 oocyte pick up cycles, divided into two groups: letrozole in 1113 cycles and CC in 1348 cycles.
Participants/materials, setting, methods: We compared the results of assisted reproductive technology (ART) and neonatal conditions in the letrozole and CC groups. We assessed the fertilization rate (FR), embryo development, cumulative pregnancy rate (PR), neonatal conditions at birth and development of movement and language between the two groups.
Main results and the role of chance: There were no significant differences between letrozole and CC in mean age (38.8 ± 4.3 vs. 39.7 ± 4.2), FR (67.8% vs. 70.2%), good quality embryo on day 3 (46.2% vs. 44.2%) or blastocyst formation rate (39.7% vs. 39.6%). PR was significantly higher in the letrozole group compared with the CC group (22.7% vs. 16.8%, $P = 0.00047$). There were no differences in the results of neonatal outcomes between the two groups (letrozole group, 137 singletons and 8 sets of twins; CC group, 72 singletons and 6 sets of twins). In the letrozole group, there were two cases of congenital abnormality: one of 21 trisomy and one of polymelia. In CC group, there was one case of congenital abnormality: a neonatal death stemming from congenital cardiac disease and congenital clubfoot.
Limitations, reason for caution: None.
Wider implications of the findings: Aromatase inhibitors and CC resulted in favorable pregnancy and neonatal outcomes and indicated that children were growing normally. It is shown that the two drugs are safe for mothers and fetuses. However, we need to consider further long-term follow up in the future.
Study funding/competing interest(s): Funding by hospital/clinic(s), Kyono ART Clinic.
Trial registration number: This study is not RCT.

P-568 Clinical pregnancy and live birth rates in *in vitro* fertilization are dependent on the physician performing the embryo transfer; a study of 10780 cycles

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Study question: The aim of our study is to investigate the influence of the physician performing the embryo transfer with a standardized procedure on the clinical pregnancy and live birth rates in an IVF-ET program. Confounding factors were minimized by selecting day 5 blastocyst transfers of patients under 35.
Summary answer: Clinical pregnancy and live birth rates were significantly different amongst embryo transfer providers. There was not a correlation between clinical pregnancy and live birth rates and number of physicians' embryo transfer cycles. The skills of the physicians performing the embryo transfer and the patients' demographic characteristics appear to influence the outcome.
What is known already: Embryo transfer is a crucial step in IVF and there are a lot of factors which appear to influence pregnancy rates. The physician's impact on the success of treatment is less certain, because conflicting results have been reported. Several studies have denied such an association whereas others suggested that the physician factor may be an important variable in the outcome. However there is no consensus yet.

Study design, size, duration: This retrospective cohort study includes 10780 IVF/ICSI cycles performed between the years of 2000 and 2011 at Istanbul Memorial Hospital (IMH). The differences between the clinical pregnancy and live birth rates of eight individual physicians were analyzed.
Participants/materials, setting, methods: The study was performed using the electronic IVF database of IMH. From the computerized patient charts, data were retrospectively collected on 10780 embryo transfer cycles. All transfers were performed under ultrasound guidance by use of a soft Wallace catheter with patients who had a full bladder.
Main results and the role of chance: Clinical pregnancy rates and live birth rates varied among the individual physicians performing the embryo transfer. The patient and cycle characteristics (Age, BMI, number of previous trials, number of total oocytes and day of embryo transfer) were significantly different among providers. In an ideal patient group, in which the patient demographics were more uniform and confounding factors were minimized by selecting day 5 blastocyst transfers of patients under 35. There were statistical differences in individual physician performances within the same IVF program. Among 8 physicians, the clinical pregnancy rate in the patients under 35 group ranged from 46.5 % to 62.3% and the live birth rates from 23.2% to 55.0% which were statistically different. There are statistically significant differences in individual outcomes among providers.
Limitations, reason for caution: The limitation of our study was in a retrospective nature of study and many confounding factors have affected the ART outcome.
Wider implications of the findings: The skill of the physician performing the embryo transfer may influence the outcome of the IVF. Experience did not seem to correlate with outcome. Although other factors such as embryo quality, cycle and patient's characteristics played a significant role, the present results particularly emphasised the importance of the role of the embryo transfer provider on the outcome of IVF/ICSI. These findings is supported by the results of previous studies, however contradictory data does also exist.
Study funding/competing interest(s): Funding by hospital/clinic(s), Istanbul Memorial Hospital.
Trial registration number: None.

P-569 Efficacy of GnRH agonist supplementation for luteal phase support

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Study question: The purpose of this study was to assess in IVF/ICSI cycles the efficacy, as pregnancy and implantation rates, of a single administration of GnRh agonist after embryo transfer, in addition to progesterone therapy.
Summary answer: In both IVF and ICSI cycles, Implantation, clinical and ongoing pregnancy rates were significantly higher in patient group who received GnRh agonist in the luteal phase.
What is known already: In addition to progesterone therapy, GnRH agonist administration has been proposed as intervention to support the luteal phase in IVF and ICSI cycles. It is hypothesized that GnRH analogs can promote corpus luteum maintenance and/or have a direct effect on the embryo, improving the success rates.
Study design, size, duration: Five hundred twenty IVF/ICSI patients undergoing embryo transfer, from April to December 2013, were analyzed and divided in two groups (Group A: GnRh agonist and progesterone, Group B: only progesterone) in a retrospective study.
Participants/materials, setting, methods: Two hundred forty IVF/ICSI patients undergoing embryo transfer, were included in Group A (progesterone plus GnRH agonist) and two hundred eighty in Group B (progesterone alone). Chi square analysis was used for statistical comparison.
Main results and the role of chance: Patients characteristics, age, number of oocytes, transferred embryo quality and number, were similar, with no statistical differences between the two groups. Also, there was no significant difference in the fertilization rate. However clinical and ongoing pregnancy rates were higher in Group A: 46.7% vs. 29.2, $P < 0.02$ and 41.8% vs. 24.2%, $P < 0.02$ respectively. Furthermore, implantation rate was significantly increased in group A (31.8% vs. 16.1%, $P < 0.05$)
Limitations, reason for caution: Although our findings confirm previous experiences in this subject, large RCT are necessary to precisely assess the impact of luteal GnRH agonist administration on IVF success. Probably, GnRH agonists could improve success rates only on cleavage stage embryo transfer.
Wider implications of the findings: Pregnancy and implantation rates, in IVF and ICSI cycles, could be significantly improved by a single administration of GnRH agonist during the luteal phase. These treatment could be adopted as a routine luteal phase support for assisted reproduction treatments.
Study funding/competing interest(s): Funding by hospital/clinic(s), Biogenesi Reproductive Medicine Centre.
Trial registration number: Not applicable.

POSTER VIEWING

REPRODUCTIVE EPIDEMIOLOGY AND HEALTH ECONOMY

P-570 Awareness of factors affecting future fertility in young men and women

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Study question: What is the level of awareness about reproductive risk factors among a group of young men and women (aged 18–25 years) representing a variety of social and educational backgrounds?
Summary answer: Young adults seem to have a good level of awareness about important factors affecting their future fertility. However there are gaps in their knowledge and many believe in fertility myths. Education is a key determinant of awareness levels.
What is known already: Previous studies have exclusively assessed the views of individuals attending higher education institutions and have mainly addressed perceptions of the effect of advanced female age and postponement of pregnancy on future fertility. Most have demonstrated lack of awareness about the negative impact of female age on future fertility. Few studies have assessed the views of young adults on other key factors which could affect fertility
Study design, size, duration: A questionnaire based crossectional study was undertaken in 2009. Eight hundred questionnaires were distributed; 487 completed and returned (61% response rate), 414 (52% of the total distributed) were valid for analysis (204 males; 210 females).
Participants/materials, setting, methods: Young adults (18–25 years old) were recruited from three settings (two U.K. National Health Service clinics and a University campus). The questionnaire included: demographic details, questions about factors suggested in the published literature to have an impact on future fertility and some "fertility myths" (a total of 24 factors with questions about their effects on males and females). Logistic regression was used to compare the proportions of males and females identifying >50% and >75% of the important fertility risk factors correctly, adjusted for potential confounders.
Main results and the role of chance: The mean (standard deviation) age in years of male and female participants were 21.7 (2.2) and 21.2 (2.0). The females were significantly more qualified than males-[25% holding a university degree compared to 17% of males]. 89% and 86% of females and 92% and 93% of males respectively recognised advanced female age and male smoking as risks to fertility. Some misconceptions existed such as putting higher emphasis on poor diet and emotional stress compared to chlamydial infection for females. The level of correct knowledge was associated with level of education ($p < 0.001$). When adjusted for potential confounders (age, recruitment site, and level of education), a comparable proportion of males and females identified >50%, and >75% of the recognised risk factors correctly. Study strengths included a good sample size, participants of varying backgrounds and adjustment for confounders.
Limitations, reason for caution: Suggesting risk factors in questionnaires can imply the answers to unaware participants. To minimize this chance we

included factors which clearly have no relationship with fertility. Answer options included (enhances, reduces, has no effect on future fertility, or don't know.) Thus, the answers are more likely to reflect the real knowledge of participants.
Wider implications of the findings: Although the awareness level to fertility risks was high, there are still some knowledge gaps with an overemphasis on the fertility enhancing effect of adopting a healthy lifestyle among young adults. Additionally many seem to believe in fertility myths. As prevention is the best cure, these areas should be tackled by future awareness raising programmes.
Study funding/competing interest(s): Funding by University(ies), University of Aberdeen, Aberdeen, Scotland, UK.
Trial registration number: Not applicable.

P-571 Association between the number of oocytes collected in a single oocyte pick-up (OPU) and the success of assisted reproductive technology (ART) treatment

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Study question: What number of oocytes collected in a single OPU is associated with the highest crude productivity rate (defined as live deliveries resulted from either fresh or subsequent thaw cycles arising from a single OPU)?
Summary answer: OPUs with 17 oocytes had the highest crude productivity rate. Extra oocytes (>17) were not significantly associated with an increase in the crude productivity rates. The majority of live deliveries were achieved within the first three embryo transfer attempts.
What is known already: The number of oocytes collected in a single OPU is one of the most important determinants in optimizing ART treatment outcomes. A randomised controlled trial is not an ethical or feasible study design to investigate the association between the number of oocytes collected in a single OPU and subsequent successful ART treatment. Instead, a large population cohort study would provide the best evidence regarding this association.
Study design, size, duration: A population cohort study used the Australian and New Zealand Assisted Reproduction Database. This study included 65904 OPUs in which at least one oocyte was collected in Australia and New Zealand during 2009 to 2010.
Participants/materials, setting, methods: Initiated OPUs and subsequent thaw cycles were followed until 31 Dec 2011 or a live delivery before 31 Oct 2012. The crude productivity rates were compared by women's age, number of oocytes, and number of transfer attempts using Chi-square test, linear regression and life table method.
Main results and the role of chance: Of the 65904 OPUs, 15.7% had 1–3 oocytes, 79.1% had 4–20 oocytes, and 5.2% had ≥20 oocytes. There were 17602 live deliveries, with 98.0% of them achieved within the first three transfer attempts (77.8% following initiated fresh cycles, 16.0% following the first thaw cycles and 4.3% following the second thaw cycles). The crude productivity rate was 39.7% for women aged 25–29 years, significantly higher than the 8.5% for women aged ≥40 years ($p < 0.01$). A significant linear trend ($p < 0.01$) was observed between the number of oocytes and the crude productivity rate following the first three transfer attempts, from 16.1% for OPUs with 4 oocytes to 39.0% for OPUs with 17 oocyte. Further increase of oocytes (>17) did not significantly increase the crude productivity rates.
Limitations, reason for caution: Stimulation protocol was not available in the database. The association between number of oocytes and productivity rate across age and parity needs further multivariate analysis. In this cohort study, subsequent thaw cycles were followed between 12 and 36 months. The productivity rate would increase with a longer period of follow-up.
Wider implications of the findings: The highest crude productivity rate was 39.0% following the first three transfer attempts for OPUs with 17 oocytes. An OPU with more than 17 oocytes was associated with an increase in the transfer potential, but not the crude productivity rate. Further study is needed to investigate the best protocol of controlled stimulation to achieve the optimum number of oocytes collected in a single OPU according to woman's/couple's demographics and specific conditions.
Study funding/competing interest(s): Funding by University(ies), UNSW.
Trial registration number: N/A.

P-572 Analysis of factors associated with spontaneous live birth following cessation of IVF and ICSI treatments

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Study question: What are the factors associated with spontaneous conception following cessation of IVF and ICSI treatments?
Summary answer: Our study showed that the cumulative live birth rates over a 6-year period were 22% for couples who have had successful IVF & ICSI treatments compared to 31% who had unsuccessful IVF & ICSI. Unexplained infertility and ovulation dysfunction were the most important factors associated with the live births.
What is known already: Few studies have reported spontaneous conception following cessation of IVF and ICSI treatments. However the rates of conception varied between these studies due to differences in patient cohort, duration of follow-up and the treatment received. Furthermore, most studies had a small sample size and were from a single IVF centre.
Study design, size, duration: An anonymous internet-based survey. All registered users of www.ivf-infertility.com received an electronic questionnaire and covering letter over a 6-week period ending in July 2013. The letter invited users to participate if they had received IVF or ICSI in the past. The covering letter explained the background of the study and acted as a form of informal consent.
Participants/materials, setting, methods: The questionnaire-addressed issues relating to information before, during and after IVF treatment included: female age, duration of infertility, parity, if patency of fallopian tubes were checked and the cause of infertility. The number of IVF & ICSI cycles, whether the patient conceived by IVF and the outcome. Whether the patient conceived spontaneously, the time taken to conceive, and the outcome of the pregnancy. Statistical analysis was performed using Chi Square, Fisher exact test and logistic regression where applicable.
Main results and the role of chance: 484 patients responded. Of these, 11 responded that they made active effort not to get pregnant again as their family was complete or there had been changes in circumstances. A further 70 couples had received IVF using donated egg, donor sperm, donor embryos or had surgical sperm retrieval – these were excluded from the study. The remaining 403 patents met the criteria for inclusion of the study. The cumulative live birth rates over a 6-year period following cessation of IVF & ICSI were 22% following successful IVF and 31% following unsuccessful IVF. The two groups of patients were analyzed separately in relation to the women's age, parity before IVF, number of treatment cycles, duration of infertility prior to IVF, cause of infertility, women's BMI, smoking habit and spontaneous conception. Unexplained infertility ($P = 0.02$) and ovulation dysfunction ($P = 0.01$) were the most significant factors associated positively with spontaneous conception. There was a trend that women age ≤35 and those had successful IVF had a higher chance of spontaneous conception compared to women aged ≥35 ($P = 0.07$). This is possibly a reflection of the study being underpowered.
Limitations, reason for caution: Our study is retrospective and relied on patient self-reporting with a potential for bias, as pregnant women are more likely to participate than disappointed patients. Also, as an internet based survey it may not be a representative of all infertile couples seeking IVF & ICSI treatment.
Wider implications of the findings: Most infertile couples believe that IVF/ICSI is the only option available to them to conceive whereas our study shows that approximately one in four couples may conceive spontaneously. It is important to emphasise that this is cumulative rate over a 6-year period. Probability of conception was significantly higher if the cause of infertility was unexplained or ovulation dysfunction. There was a trend that those women age ≤35 and those who had successful IVF were more likely to achieve spontaneous conception.
Study funding/competing interest(s): Funding by hospital/clinic(s). No funding was obtained.
Trial registration number: Following enquiry with NHS Health Research Authority, no ethics committee approval was required.

P-573 Relationship between causes of infertility and ethnic differences in IVF outcome – a retrospective study

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Study question: Is the cause of infertility responsible for ethnic differences in assisted reproduction outcome?
Summary answer: There are significant differences in both clinical pregnancy rates and the causes of infertility among ethnic groups.
What is known already: The number of IVF cycles performed each year has increased steadily since 1991 and in 2011 was over 60,000 in the UK. The live birth rate after IVF has increased from 14%, to 25% by 2010. Maternal ethnicity is a significant determinant in successful outcome of ART. Live birth rates, clinical pregnancy rates and implantation rates following fertility treatment particularly IVF, are significantly lower in all ethnic groups when compared to white Europeans. As such, the relationship between patient ethnicity and the likelihood of pregnancy from IVF remains an important area of interest.
Study design, size, duration: In a retrospective cohort study. 867 IVF cycles were reviewed over a period of 1 year between January 1, 2012 and December 31, 2012. Main outcome measure was clinical pregnancy rate, ethnicity and causes of infertility in a retrospective cohort study. 867 IVF cycles were reviewed over a period of 1 year between January 1, 2012 and December 31, 2012. Main outcome measure was clinical pregnancy rate, ethnicity and causes of infertility in a retrospective cohort study. 867 IVF cycles were reviewed over a period of 1 year between January 1, 2012 and December 31, 2012. Main outcome measure was clinical pregnancy rate, ethnicity and causes of infertility.
Participants/materials, setting, methods: Of 867 patients, 54.2% were Caucasian, 25.7% were Asian and 20.1% were Afro-Caribbean. Homerton Fertility Unit is a tertiary fertility centre located in East London serving a multiethnic population. Data was collected in an SPSS spreadsheet and results generated using SPSS commercial software (SPSS version-22, Chicago, IL). Statistical tests of Anova and Chi-square were used for analysis.
Main results and the role of chance: Caucasian couples had a better clinical pregnancy rate (35.1%) in comparison to Asian couples (25.9%) (chi-squared test X (2) = 12.989, $p = 0.002$). When controlled for age the results maintained significance. Afro-Caribbean women had the lowest pregnancy rate (21.2%). There was no significant difference in age, AMH, D3 FSH, and total FSH dose required, total duration of stimulation and total number of eggs collected. However differences in BMI between the ethnic groups were statistically significant ($P = 0.019$): Afro-Caribbeans had a mean BMI of 24.22 ± 7.32, Caucasians 22.47 ± 6.99 and Asians 22.39 ± 7.59. The finding of significance differences in clinical pregnancy rate may be attributed to the fact that different ethnic groups have varying fertility problems. Caucasians had a higher percentage of low ovarian reserve (24.9%) compared to Asian (21.2%) and afro-Caribbean (20.0%). Asians had a higher prevalence of unexplained infertility (33.2%) compared to Caucasians (24.2%) and Afro-Caribbeans (14.8%). Afro-Caribbeans had a higher incidence of male factor (31.1%) than Caucasians (20.9%) and Asians (16.3%). There is a significant difference in the causes of infertility between the ethnic groups (Chi-squared: X (10) = 29.985, $P = 0.001$) also maintained when controlled for age (X (10) = 29.655, $p = 0.001$).
Limitations, reason for caution: Retrospective cohort study.
Wider implications of the findings: Differences in the success rates of IVF between the ethnic groups are influenced by the different respective prevalences of the causes of infertility between these groups.
Study funding/competing interest(s): Funding by hospital/clinic(s). Homerton Fertility Centre, Homerton University Hospital, Homerton Row, E9 6SR, United Kingdom.
Trial registration number: Not applicable.

P-574 Investments in contraceptive services and climate change mitigation: a win-win approach

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Study question: Will expanding access to contraceptive services in developing countries contribute to global climate change mitigation?
Summary answer: The income-related effects of expanding contraceptive use on greenhouse gas emissions could potentially be larger than its demographic effects, challenging earlier assumptions on the link between climate change mitigation and family planning.
What is known already: Available estimates (including Population-Environment literature) stress the indirect climate effects of family planning services through its effects on fertility, but disregard the impact of associated economic benefits.
Study design, size, duration: An extensive systematic search was conducted in October 2013, to review the literature on the topic of this study. The duration of the study was 5 months (August 2013–January 2014).
Participants/materials, setting, methods: An extensive systematic search was conducted using Pubmed, ScienceDirect, Google Scholar, Web of Knowledge and the Population-Environment Research Network. The review focused on the cross-section between population dynamics, environmental science and reproductive health services, including articles that combined at least two targeted topics.
Main results and the role of chance:

- Increased contraceptive use has an impact on population size, age structures, household size, and other demographic parameters.
- Complementing population numbers with other demographic characteristics provide a better understanding of changing preferences for consumer goods, though studies are inconclusive about the carbon outlook of dominant demographic trends.
- When estimating the climate impact of contraceptive services, current models include the indirect climate effects of reduced fertility rates. These models exclude the 'demographic dividend' effect on per capita incomes which in turn affect the environment.
- The economic effects of increased contraceptive use could potentially have a larger impact on the environment than its demographic effects. Therefore, greenhouse gas emissions reductions caused by expanded access to contraceptive services remain highly speculative.
- Access to preferred contraceptive methods is fully justified on human rights and individual health grounds, and should be strongly promoted as a development priority. Preventing millions of maternal and infant deaths provides a clear evidence-based motivation to increase investments in contraceptive services and supplies.
- Further research could advance understanding of the environmental impact of demographic changes brought about by increased contraceptive coverage.

Limitations, reason for caution: Since available articles on the environmental benefits of family planning mainly present projections of CO_2-levels, this review focused on the effects on climate change mitigation, which is only one aspect of environmental change.
Wider implications of the findings: In the context of the international ambition to systematically include the environmental pillar into the Post-2015 Development Agenda, a clear assessment of environmental gains associated with increased contraceptive use is recommended. Although there is a high degree of uncertainty regarding these climate co-benefits, one should keep in mind the strong evidence regarding its impact on reducing maternal and children mortality, economic development and human rights, which clearly benefits the reduction of global social and economic inequalities.
Study funding/competing interest(s): Funding by national/international organization(s). World Health Organisation.
Trial registration number: Not applicable.

P-575 Cost-effectiveness of a structured lifestyle program in overweight and obese subfertile women. Preliminary data from a randomised controlled trial – the LIFEstyle study

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Study question: Is a structured lifestyle program for overweight and obese subfertile women aiming at weight reduction prior to fertility treatment more cost-effective per ongoing pregnancy than fertility treatment only?
Summary answer: Despite the delay of fertility treatment for six months caused by lifestyle intervention in overweight and obese subfertile women, the ongoing pregnancy rate did not differ significantly and ongoing pregnancies were achieved at much lower costs.
What is known already: Overweight and obese women are at increased risk of subfertility and therefore may require fertility treatment more often. Small intervention studies have shown that lifestyle intervention prior to fertility treatment in overweight and obese subfertile women could improve spontaneous pregnancy chances and therefore prevent unnecessary fertility treatment. Cost-effectiveness studies of lifestyle programs for subfertile women have not been performed yet.
Study design, size, duration: We performed a cost-effectiveness analysis based on a randomised controlled trial (RCT – the LIFEstyle study). Subfertile women with a BMI of ≥29 kg/m^2 were allocated to a six-month lifestyle intervention program followed by fertility treatment (intervention group) or to fertility treatment only (control group). Follow-up was 24 months.
Participants/materials, setting, methods: Direct medical costs of the intervention program and fertility treatments were calculated based on methods of the Dutch Health Authority and Insurance Board and medication costs. Ongoing pregnancy rates are reported by intention to treat; data on the primary outcome (vaginal birth of a healthy singleton) is not yet complete.
Main results and the role of chance: We randomly allocated 582 women and the data of 447 women (77%) were available for the current analysis since follow-up is still ongoing (intervention group: *N* = 223, control group: *N* = 224). Ongoing pregnancy rates were 51.1% in the intervention group versus 58.9% in the control group (relative risk 0.87 95% confidence interval 0.73–1.03). Mean direct medical costs per patient were 2175 for the intervention group and 3155 for the control group. Direct medical costs per ongoing pregnancy were 4254 for the intervention group and 5354 for the control group. The incremental cost-effectiveness ratio (ICER), i.e. the ratio of the cost difference (– 980) and effect difference (−7.8%), was 28.428 per percent additional ongoing pregnancy.
Limitations, reason for caution: These results are based on direct medical costs from randomisation until an ongoing pregnancy had been achieved. Data on costs of pregnancy, labour, perinatal morbidity and complications follow during 2014 and therefore are not yet included. Sensitivity analyses are required to evaluate the impact of differences in resource use and costs.
Wider implications of the findings: These preliminary data suggest that overweight and obese subfertile women who participated in a lifestyle intervention program may require less fertility treatments to achieve an ongoing pregnancy compared to fertility treatment only. Full economic evaluation from a societal perspective, not only including direct medical costs, and with a healthy singleton as primary endpoint will determine if lifestyle intervention prior to fertility treatment will be cost-effective.
Study funding/competing interest(s): Funding by national/international organization(s). The study was supported by a grant from ZonMW, the Dutch Organization for Health Research and Development.
Trial registration number: The trial was registered at the Dutch trial registry (NTR 1530).

P-576 Hair mercury concentrations and ovarian reserve parameters in women undergoing fertility treatments

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Study question: In women undergoing fertility treatments, is there an association between hair mercury (Hg) concentrations and ovarian reserve parameters (i.e., antral follicle count)?
Summary answer: Increased hair mercury concentrations were associated with decreased antral follicle counts (AFC).
What is known already: Toxic metals such as Hg are widespread in the environment, and accumulate and biomagnify in the food chain (aquatic and terrestrial). Humans are ubiquitously exposed to Hg primarily through dietary sources (i.e., seafood). Increasing animal and human evidence links toxic metals with reproductive toxicity. However, little is known about the effects of toxic metals such as Hg on ovarian reserve and hence on women's reproductive health.
Study design, size, duration: Prospective cohort; 202 women [median, interquartile range (IQR): 35 years (33.0–39.0)] undergoing fertility treatments (12/2004-04/2012) at the Massachusetts General Hospital Fertility Center in Boston, MA, USA. Analysis was restricted to hair samples collected either during the 12 months preceding or the 5 months following the AFC determination.
Participants/materials, setting, methods: Total Hg was measured in the proximal 3 cm (representing exposure during the previous 3–6 months) using a Direct Mercury Analyzer 80 with a matrix matched calibration curve.
Statistics: Linear regression models adjusted for age, BMI, day-3 FSH, diagnosis, treatment protocol, fish consumption.
Outcome Measures: AFC determined by transvaginal ultrasonography.
Main results and the role of chance: Median Hg concentration was 0.6 ppm (IQR: 0.3–1.1) with 30% of the hair samples having concentrations >1.0 ppm safety limit. Mercury concentrations showed a strong negative correlation with AFC. The multivariate-adjusted AFC (95% CI) for women in increasing quartiles of mercury concentrations were: 11.3 (10.3–12.5), 11.3 (10.3–12.5), 10.1 (9.1–11.1) and 10.1 (9.1–11.2), (*P*-trend: <0.01). This association was stronger among women <35 years. The corresponding multivariate-adjusted AFC (95% CI) were: 13.9 (10.1–19), 11.9 (8.7–16.2), 11.4 (8.4–15.5), 9.6 (7.1–13.0), (*P*-trend: ≤0.01). Among women with similar fish consumption (either >1.40 or ≤1.40 servings/week), those with high mercury levels had significantly lower AFC than those with low levels (>0.55 vs. ≤0.55 ppm, $P < 0.05$).
Limitations, reason for caution: Analysis was limited to women undergoing fertility treatments and results may therefore not be generalizable to other women. Anti-Mullerian hormone levels were not available and therefore not included in the analysis. However, AFC is one of the most sensitive indicators of ovarian reserve.
Wider implications of the findings: We measured mercury levels in the hair, a reliable reflection of long term environmental exposure primarily through seafood consumption, to correlate with AFC (a sensitive indicator of ovarian reserve). We found a significant negative correlation that was stronger among younger women. Our results suggest that mercury has a deleterious effect on ovarian reserve and exposure to trace levels of Hg may lead to adverse female reproductive outcomes.
Study funding/competing interest(s): Funding by national/international organization(s), This work was supported by grants ES009718, ES000002, and T32DK007703 from the National Institute of Environmental Health Sciences (NIEHS).
Trial registration number: None.

P-577 A predictive model of live birth in IVF/ICSI limited to three predictors proves non inferiority compared with referent templeton and Nelson models

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Study question: The existing predicting models in IVF are based on national data bases, providing large sample size but offering a limited choice of predictors. Can we improve the precision of these models by using new markers, and define a simple index allowing an additive predictive value of the IVF past experience?
Summary answer: We demonstrate that the History Score HS of the patients defined by the sum-score of negative and positive LBs occurrences constitutes a key predictor in three groups of Positive (HS ≥ 1), negative (HS ≤ 2), and uncertain/unknown history (–2 < HS < 1), age being as second predictor, whereas AMH shown only useful for patients without IVF history.
What is known already: The most referenced Templeton and Nelson necessitate 6 and 15 predictors, respectively. AMH constitutes a new marker not yet documented in national databases. In contrast with the referent models, Khader et al. (2013) built a model only constituted by AMH and age that reached a good discrimination (AuR = 0.68). Rongières et al. (2012) added AMH, age, and previous IVF life birth, with higher discrimination (auR = .71). However, both studies were monocenter, and without controlling for referent models.
Study design, size, duration: We conducted a retrospective analysis based on the IVF/ICSI cycles performed into our centre. 1225 patients are needed to test non inferiority 5% difference on discrimination compared with referent model with a power of 0.8 at 95% two-sided level.
Participants/materials, setting, methods: The last recently 1225 cycles were selected on an intention-to-treat basis (age = 33.9 ± 4.3years, BMI = 23.5 ± 4.32 kg/m^2, 2.1 ± 1.2 unsuccessful attempts, AMH = 2.2 ± 2.1 ng/L). 210 (16.5%) out of them had a live birth. A stepwise hierarchical regression was first conducted to detect interaction between predictors, instead of the usual logistic regression method.
Main results and the role of chance: Fitting Templeton and Nelson models to our data provided non significantly different discrimination of 0.68 [0.63–0.73] and 0.67[0.62–73.0]. The stepwise regression identified three groups of medical history HS ≥ 1, HS ≤ –2, and –2 < HS < 1, corresponding to positive, negative and unknown/uncertain history summary. In each group, age significantly interacted on LB with unequal weights in each group, and AMH was the only determinant for the unknown group. Compared with the referent Templeton model, our prediction was characterized by a slightly better discrimination (R = 0.71, [0.65–0.77]), with a non significant difference of 0.04 [–0.02, 0.11], p = .28.
Limitations, reason for caution: Our mono-center study requires external validation to confirm these findings. Our results constitute an illustration of our proposal of definition of History Index. Although better than referent models, the precision remains insufficient for regular medical practice, in spite of a satisfactory calibration.
Wider implications of the findings: Our model involves three predictors (HI, Age, AMH) compared with models needing more predictors. The reason of this performance lies in the quantification of history into one continuous score HS and accounting for the interaction between HS and age, confirming the need to predict LB differently according to history. Finally AMH claimed in previous works as a potent predictor, has only a significant effect for *de novo* patients.
Study funding/competing interest(s): Funding by commercial/corporate company(ies), The authors are grateful to Merck-Serono, for providing help in data management and statistical analysis.
Trial registration number: None.

P-578 Nutrient patterns and risk of asthenozoospermia: a case-control study

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Study question: Is there any association between nutrient patterns and idiopathic asthenozoospermia risk?
Summary answer: Adherence to the pattern including sodium, cholesterol, saturated fatty acids, trans fatty acids and etc. is potentially an unfavorable indicator of asthenozoospermia risk while a diet comprising mainly of antioxidant nutrients may be associated with a reduced risk.
What is known already: The association of nutrient patterns and semen quality is not yet well studied, and the rare studies have been conducted in developed countries. Previous studies have reported some antioxidants may maintain or improve semen quality whereas high intake of saturated fatty acids and trans fatty acids may negatively associated with semen quality in humans.
Study design, size, duration: In this case control study, 107 incident asthenozoospermic men and 235 age-matched controls were interviewed through the infertility clinics in Tehran, Iran, from January 2011 to November 2012.
Participants/materials, setting, methods: Usual dietary intakes were collected using a semi-quantitative food frequency questionnaire and semen quality data were analyzed according to the fifth edition of WHO guideline. Dietary patterns were derived using factor analysis. The first tertile served as the reference category for regression analyses.
Main results and the role of chance: In principal component analysis two nutrient patterns emerged: Factor 1 explained 32.7% of the total variance and had high loadings for riboflavin, niacin, pyridoxine, thiamin, magnesium, pantothenic acid, cobalamin, vitamin C, folate, vitamin D, total fiber, selenium, phosphorus, vitamin E, manganese, vitamin K, monounsaturated fatty acids, polyunsaturated fatty acids and potassium. Factor 2 displayed high loadings for sodium, cholesterol, saturated fatty acid, fat, biotin, carbohydrate, iron, fluoride, zinc, copper, calcium and protein. After adjustment for potential confounders, participants in the highest tertile of the "Factor 1" scores, had 59% lower risk of asthenozoospermia compared to those in the lowest (p-trend: 0.002). Being in the highest tertile of the "Factor 2" was positively associated with the asthenozoospermia risk (OR: 2.77, 95% CI: 1.75–3.11).
Limitations, reason for caution: Measurement bias, selection bias and recall bias were inevitable.
Wider implications of the findings: Patients with asthenozoospermia should be advised to adhere to prudent pattern rich in fruits, vegetables, poultry, skimmed milk and sea foods as well as low in processed meat, high-fat dairy products and sweets.
Study funding/competing interest(s): Funding by University(ies), This study was financially supported by the National Nutrition and Food Technology Research Institute, Faculty of Nutrition and Food Technology, Shahid Beheshti University of Medical Science, Tehran, Iran. No conflict of interests to declare.
Trial registration number: None.

P-579 From the couple to the family: how many infertile couples become actually parents due to assisted reproductive treatments

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Study question: This study aims to analyze the 2 years' medical course of 232 couples with primary infertility, having undergone their first IVF or ICSI treatment in 2011, in order to evaluate how many of them will actually achieve their desire objective of having a child.
Summary answer: One hundred forty two couples, given 61.2% of the studied population has achieved parenthood due to fresh or frozen embryo transfers at the latest 2 years after their first attempt: only deliveries or clinical pregnancies of more than 6 months were taken into account in this result.
What is known already: Several studies have investigated the take home baby rate after ART treatments. They usually concerned large populations of couples presenting different infertility profiles, followed during a long period of time. As in France only 4 IVF attempts were allowed by law, couples' medical course could be short and few studies considered the cumulative parenthood rate in this particular situation.

Study design, size, duration: This study concerned 232 couples with primary infertility having undergone their first IVF or ICSI treatment in 2011 and whose medical course was carefully followed until December 2013. For each couple each ART attempt was documented: fresh and frozen embryo transfers; follow-up of all initiated pregnancies until delivery.
Participants/materials, setting, methods: All the 232 couples had undergone their different ART treatments in our center. An Excel file was constituted to collect all clinical and biological data, in case of missing data couples were contacted by phone or mail resulting in a dropout rate of only 7.7%.
Main results and the role of chance: Of the 232 couples, 92 achieved parenthood after their first IVF attempt, 40 after their second one, 8 after their third one and 2 after their last attempt. For 19 among these 142 parents, a second pregnancy was even obtained. Only twelve twin pregnancies were observed (8.4% of the deliveries) due to the high rate of elective single embryo transfer (eSET: 55% of all fresh ET). For nine couples a spontaneous pregnancy occurred despite unsuccessful IVF attempts (3.9%). Seventeen couples decided to abandon without using their 4 authorized attempts (7.3%). A divorce occurred for five couples. Given the high number of still frozen embryos (543) the cumulative take home baby rate of 61.2% obtained will certainly increase in the future.
Limitations, reason for caution: As this study focused on a specific population (primary infertility, first IVF attempt performed during a 1 year period), the number of cases is lower than other published studies. The young age of the women at their first IVF cycle (31.5 ± 4.6 years) must also be considered in the analysis.
Wider implications of the findings: This study allows us to give precise information concerning their real chances of being parents for these young couples with primary infertility who arrive for the first time in our center. More precisely we encourage them to continue even after a first unsuccessful attempt for 35% of the pregnancies were obtained secondarily. This work justifies the French medical reimbursement support system for very few couples undergone the fourth IVF attempt with very low success.
Study funding/competing interest(s): Funding by hospital/clinic(s), Laboratoire ESPACEBIO/CHR-Metz.
Trial registration number: This is an observational study.

POSTER VIEWING

REPRODUCTIVE SURGERY

P-580 Endovascular embolization of uterine arteries before surgical delivery in women with placenta previa and placenta accreta

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Study question: To compare the effectiveness of the traditional surgical treatment combined with the use of endovascular techniques in surgical abdominal delivery in women with placenta previa and accreta. To estimate the effects of endovascular embolization of uterine arteries accomplished before surgical delivery in women with placenta previa and placenta accreta.
Summary answer: Comparing the results of conventional surgical treatment and combined operations using UAE in group of patients without placenta accreta (subgroup CONna and UAEna) there were no significant differences in the amount of blood loss and necessity for blood transfusion (Table 2). The main and control group did not differ in frequency of the hysterectomy. There were no differences between the group in ICU stay duration and hospital stay duration.
What is known already: Disturbance of placental attachment is associated with an increased risk of premature detachment, post-partum bleeding, intrapartum fetal death [1]. Placenta accreta occurs in 9% of patients with placenta previa and 0.004% of women without previa.
Study design, size, duration: 51 patient with a complete placenta previa which were delivered in 2 hospitals during 2013 year.
Participants/materials, setting, methods: The main group (Gr UAE) consisted of 16 pregnant women with abnormal placentation who was operated. 9 patients had placenta accreta (the subgroup UAEa.), 7 women had placenta previa without evidence of accreta (the subgroup UAEna.)
Main results and the role of chance: The combined use of UAE allowed to avoid complete cases of hysterectomy in patients of the main group. In the control group all interventions in cases of placenta accreta lead to hysterectomy. The use of temporary UAE in operative abdominal delivery in women with placenta accreta significantly reduces blood loss, need for blood transfusion and women disability. This study did not reveal any benefits of combined technique over the conventional abdominal operative delivery in patients with placenta praevia with no signs placenta accreta.
Limitations, reason for caution: Average time of radioscope was 7.6 ± 7.8 min.
Wider implications of the findings: There are increasing number of reports about a successful use of uterine artery embolization techniques, which allows to save the uterus.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Almazov Federal Heart, Blood and Endocrinology Centre.
Trial registration number: Has no registration number.

P-581 Hysteroscopic outpatient metroplasty to expand dysmorphic uteri (HOME-DU) technique

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Study question: Could *Hysteroscopic Outpatient Metroplasty to Expand Dysmorphic Uteri (*HOME-DU technique) be safe and effective to increasing the volume and improving the morphology of the uterine cavity of dysmorphic uteri (i.e., T-shaped uteri and tubular-shaped/infantilis uteri).
Summary answer: Our findings would support the HOME-DU technique to be safe and effective in expanding the volume and normalizing the morphology of the uterine cavity of dysmorphic uteri.
What is known already: The novel ESHRE/ESGE classification system of female genital tract congenital anomalies incorporates in Class I all uteri with normal outline but with an abnormal lateral wall's shape of the uterine cavity. These uteri (i.e., T-shaped uterus and tubular-shaped/infantilis uteri) are defined as "dysmorphic". Several studies have showed very poor reproductive performance when these uterine malformations are not treated, since altered volume and shape of the uterine cavity are likely to contribute to defective endometrial receptivity.
Study design, size, duration: This is a prospective trial (Canadian Task Force classification II-A) conducted on 30 women enrolled from June 2011 to March 2012.
Participants/materials, setting, methods: The study was conducted in the Department of Obstetrics and Gynecology, University *of* Naples *Federico II*, Italy. Thirty women with dysmorphic uterus, as diagnosed by office hysteroscopy and three-dimensional trans-vaginal ultrasound (3D-TVS) underwent surgery. The HOME-DU technique consists in making two deep incisions on the fibromuscular constriction rings in the isthmic area with a 5 Fr bipolar electrode and, subsequently, on the anterior and posterior uterine walls, from the fundus up to the isthmus. At the end of the procedure a polyethylene oxide-sodium carboxymethyl cellulose gel is applied to prevent intrauterine adhesions. Surgical procedures were performed in the outpatient setting under conscious sedation, using a 5 mm continuous flow office operative hysteroscope. Follow-up consisted in office diagnostic hysteroscopy and 3D-TVS in the immediately following menstrual cycle.
Main results and the role of chance: The HOME-DU technique was successfully performed in all cases without any significant complication. A subjective increase of the uterine volume was noted in all patients, as measured at hysteroscopy by using the opening of the jaws of the grasping forceps as reference measure. At 3D-TVS follow-up, the uterine cavity volume significantly increased (from 2.1 ± 0.4 cm^3 to 1.4 ± 0.2 cm^3 $P = 0.00$) and the uterine morphology improved in all patients, with the exception of one woman.
Limitations, reason for caution: The post-surgical reproductive outcome was not assessed.
Wider implications of the findings: Our data support the safety and efficacy of this novel outpatient technique in order to improve the volume and shape of dysmorphic uteri without the need of general anesthesia and traditional resectoscopic surgery. This represents the pre-requisite for advocate a wider use of such outpatient technique in women affected by such peculiar uterine anomalies with a history of primary or secondary infertility, recurrent abortion and pre-term

delivery. Preliminary (not reported) data seem also to show that such improved uterine cavity may result in better reproductive outcomes. However the completion of 18 months follow-up is required in order to draw definitive conclusions.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Università degli studi di Napoli Federico II.
Trial registration number: No.

P-582 A comparative analysis between two aspiration pressures for oocyte retrieval in egg donation cycles: a pilot trial

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Study question: Is there any difference in terms of oocyte recovery rate between two different aspiration pressures for egg retrieval?
Summary answer: Aspiration pressure (150 vs 200 mmHg) does not seem to affect oocyte yield in egg donors.
What is known already: Transvaginal ultrasound-guided follicular aspiration rapidly replaced laparoscopic or transabdominal route for oocyte recovery in IVF cycles. Initially, aspiration pressures up to 120 mmHg were used in order to avoid 'egg damage'. Nowadays, aspiration pressures ranging from 150–200 mmHg are usually employed in many IVF units. Surprisingly, to the best of our knowledge, there is no report of prospective, comparative trials addressing the optimal aspiration pressure in IVF cycles.
Study design, size, duration: A prospective comparative operator-blind trial including 20 egg donors, in November 2013.
Participants/materials, setting, methods: Twenty egg-donors were divided into two groups: Group 1 (150 mmHg; $n = 10$) and Group 2 (200 mmHg; $n = 10$). A 17-G aspiration needle was used. The study was powered to detect differences in the number of eggs retrieved, but also the aspiration time and oocyte structural damage rate were analyzed.
Main results and the role of chance: Age, total dose of FSH received, days of antagonist used and number of follicles >11 mm on the last US scan, all these pre randomization parameters were statistically similar. The number of eggs retrieved was 20.7 ± 7.4 vs 19.1 ± 5.2 ($p = 0.5$); aspiration time (min) was 7.1 ± 1.7 vs 6.9 ± 1.3 ($p = 0.7$); egg structural damage rate was 9/207 (4.3%) vs 10/191 (5.2%) in group 1 vs 2 respectively, being these results not significantly different.
Limitations, reason for caution: The participants in our study consisted of patients with favorable prognosis; hence, our findings cannot be necessarily, extrapolated to a more adversely selected patient population (e.g., obese, endometriosis or diminished ovarian reserve patients). The sample size available restricted statistical power to detect differences in aspiration time.
Wider implications of the findings: In this pilot study the aspiration pressure does not seem to affect oocyte yield in egg donors. Future trials aimed to detect differences in operating time may help to fine-tune the optimal aspiration pressure. Statistical descriptive data such as the operating time (mean 7 min in the current trial) and follicular aspiration rate (20 s per follicle in this trail) may be useful for a better organization and logistics in the operating room.
Study funding/competing interest(s): Funding by hospital/clinic(s), Instituto Bernabeu Alicante, Spain.
Trial registration number: None.

P-583 Fertility after uterine artery embolization for symptomatic fibroids in women without associated infertility

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Study question: What is the fertility of young women eligible for surgical myomectomy but who decided to have a uterine artery embolization (UAE)?
Summary answer: In the absence of other infertility factor, UAE improves symptoms and quality of life and is associated with a 38% term-pregnancy rate comparing favorably with surgical series of myomectomy.
What is known already: Treating symptomatic fibroids with UAE in women seeking future fertility remains a matter of debate. Complications such as hysterectomy for necrosis or ovarian failure could compromise future fertility. At present, UAE is recommended in young patients only if surgical myomectomy is contraindicated. In a preliminary study, 66 young women not eligible for surgical myomectomy were treated with UAE. No birth had been obtained in these women with associated infertility factors.
Study design, size, duration: Prospective cohort study, from March 2009 to January 2011 on 16 patients.
Participants/materials, setting, methods: Patients <41 years old, with multiple symptomatic fibroids and no associated infertility factors were included. Bilateral limited UAE using Tris-acryl microspheres >500 µm were performed. Laparoscopies with dye test and hysteroscopies were performed 3 months after UAE. Pelvic MRI, quality of life (UFS-QoL), ovarian function and fertility were prospectively followed.
Main results and the role of chance: Women were 35 (26–41) years old. After UAE, symptoms score was reduced by 64 % (95%CI 48%–74 %), the quality of life score was improved by 131 % (95%CI 44%–218 %) and the uterine volume was reduced by 32 % (95%CI 24%–40 %). Ovarian reserve remained stable. Two women had complication following UAE: an early endometritis was treated conservatively with antibiotics. A localized uterine rupture was diagnosed during the dye test and was easily sutured during laparoscopy. During the 14 months of follow-up, 6 pregnancies occurred (fertility rate 38%, 95%CI 13%–62%). Two pregnancies were complicated: one case of intrauterine growth restriction with favorable full-term delivery and one case of HELLP syndrome with a delivery at 34 weeks. The birth weight ranged from 1980 g to 4030 g.
Limitations, reason for caution: The size of our cohort is limited.
Wider implications of the findings: A phase II randomized controlled trial comparing UAE and surgical myomectomy in this population is required.
Study funding/competing interest(s): Funding by national/international organization(s), CIRC AP-HP.
Trial registration number: Clinical trial P071006.

P-584 Should surgery be performed in women with endometriosis prior to ART to improve reproductive outcome

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Study question: What is the impact of endometriosis on reproductive outcome on women undergoing ART?

Does surgical treatment of endometriosis prior to IVF impact on the outcomes of Live Birth (LBR), Clinical Pregnancy (CPR), oocyte retrieval (MOR) and miscarriage rate (MR)?
Summary answer: Women with endometriosis were found to have comparable LBR per woman compared to those without endometriosis. Severe endometriotic disease may have a detrimental impact on the success of ART and surgical treatment especially of more severe disease may potentially contribute adversely to the success of ART.
What is known already: A poorer reproductive outcome has been associated with subfertile women with endometriosis undergoing assisted conception treatment. Surgical treatment of women with minimal and mild endometriosis is recommended as it improves spontaneous pregnancy. However the impact of surgery on the reproductive success rate of women undergoing IVF (*in vitro* fertilisation)/ICSI (intra-cytoplasmic sperm injection) is less clear.
Study design, size, duration: Meta-Analysis and systematic review.
Participants/materials, setting, methods: A total of 1346 papers were identified through electronic search and after screening, 36 studies were eligible to be included for data synthesis.
Main results and the role of chance: Compared to controls, women with endometriosis have comparable LBR (OR 0.94, 95% CI [0.84 to 1.06], $n = 13$, 12.682 patients, $I^2 = 35\%$), lower CPR (OR 0.78, 95% CI [0.65, 0.94], $n = 20.757$, $I^2 = 66\%$), lower MOR (MD −1.98, 95%CI [−2.87 to −1.09], $n = 17.593$, $I^2 = 97\%$) but a similar MR (OR 1.26, 95% CI [0.92.1.70], $n = 1259$, I^2 0%). Women with ASRM III-IV endometriosis had a lower LBR (OR 0.77, 95% CI [0.64 to 0.92], $n = 3849$, $I^2 = 0\%$), CPR (OR 0.60, 95% CI [0.44 to 0.81], $n = 9471$, $I^2 = 62\%$) and MOR (MD −1.76, 95% CI [−2.73 to 0.79], $n = 9172$ patients, $I^2 = 92\%$) when compared to controls. Sensitivity analysis

suggests that women with more severe disease (ASRM III–IV) had lower LBR (OR 0.78 95% CI [0.65 to 0.95], n = 2550, I^2 = 43%), CPR (OR 0.53 95% CI [0.33 to 0.84], n = 3470, I^2 = 85%), and lower MOR (MD −2.46 95% CI [−3.42 to −1.51], n = 3592, I^2 = 91%).

Limitations, reason for caution: Due to the observational nature of the included studies, the result of this study is subjected to confounders relating to clinical heterogeneity.

Wider implications of the findings: Severe endometriotic disease may have detrimental impact on the outcome of ART. Surgical treatment especially of more severe disease may potentially be detrimental to the success rate of ART. The role of nonsurgical/medical management needs to be further explored.

Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Ministry of Higher Education, University of Malaya, Malaysia; Complete Fertility Centre Southampton.

Trial registration number: Not applicable.

P-585 Results of microsurgical refertilisation after vasectomy and validation of factors predicting overall success

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Study question: Which factors can be used as a predictive tool for forecasting a successful clinical outcome after microsurgical refertilisation following vasectomy?

Summary answer: Intraoperative semen analysis, postoperative glucosidase levels and preoperative FSH level have a high prognostic value regarding vas patency after vasectomy reversal irrespective of post-vasectomy interval. Moreover, the chances for pregnancy seem to be higher if patency can be achieved after a vasectomy interval <10 years.

What is known already: Prior studies have suggested that the obstructive interval negatively affects the patency and pregnancy rates after microsurgical refertilisation. Some researchers have found out that intraoperative semen analysis classified according to the Silber score plays an important role in the clinical outcome but its predictive value is still heavily debated. No data are available on the relevance of biochemical seminal markers for predicting success of vasectomy reversal. Limited data suggest an influence of female partners age.

Study design, size, duration: This is a retrospective analysis of 191 patients that underwent a microsurgical refertilisation after vasectomy in our department between 2000 and 2011 with a follow up of at least 18 months.

Participants/materials, setting, methods: Preoperative hormones levels, testis volume and fatherhood were analysed. Intraoperative semen smears were classified according to Silber I (motile spermatozoa) to V (only cell debris). Postoperative findings included semen analysis (WHO), patency status, pregnancy and/or delivery status and the use of ART or not in order to achieve pregnancy.

Main results and the role of chance: Mean age of patients was 43.1 ± 6.3 years, while female partners were younger (33.9 ± 5.8 years). A significantly positive correlation was observed between patency rate and Silber classification ($p < 0.01$), postoperative a-glucosidase ($p < 0.01$) and preoperative normal FSH serum levels ($p = 0.03$). Overall patency was confirmed in 85.1% of patients through semen analysis. Our data did not show any significant association between obstructive interval and postoperative vas patency (>10 years: 83.7%; ≤10 years: 86.2%). The natural pregnancy rate of all patients was 34.4%. By additional ART pregnancy rate increased to 48.9%. Obstruction interval influenced significantly spontaneous pregnancy rates (≤10 years: 45.3%; >10 years: 18.9%). No correlation between female age and pregnancy could be shown.

Limitations, reason for caution: The retrospective study design and the inhomogeneous consecutive follow up are the main limitations of the present study.

Wider implications of the findings: We demonstrated that intraoperative Silber score, preoperative FSH serum concentration and postoperative a-glucosidase levels have a high predictive value concerning the clinical outcome after vasectomy reversal. Time interval between vasectomy and vasovasostomy >10 years influences pregnancy outcome and should be taken into consideration for counselling patients in respect to early and additional ART procedures after VV.

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: None.

P-586 Uterine fibroid pseudocapsule thickness differs among fibroid location: a pilot study

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Study question: To examine whether fibroid pseudocapsule measurements differ between fibroid uterine location and whether measurements correlate between different techniques.

Summary answer: Pseudocapsules of fibroids near the endometrial cavity are considerably thicker with a clear cut-off (2mm) than pseudocapsules from both intramural and subserous fibroids. High correlation between histology and US measurements was found in all pseudocapsules independently of fibroid location, indicating that ultrasonography measurements is an accurate technique.

What is known already: Pseudocapsule is a neurovascular bundle surrounding fibroid containing neuropeptides and involved possibly in myomectomy wound healing.

Study design, size, duration: A prospective single center, single operator surgical intervention trial of two hundred (200) consecutive patients with symptomatic uterine myomas.

Participants/materials, setting, methods: Two hundred consecutive patients underwent hysterectomy for uterine myomas. Before surgery, fibroids have been measured and recorded their pseudocapsule thickness by a single ultrasonographer. After surgery, all myoma-pseudocapsule-myometrium specimens analyzed by a single pathologist blinded for patients data. All interventions took place in a University-affiliated hospital.

Main results and the role of chance: The total enucleated myomas were 200. Pseudocapsules of fibroids near the endometrial cavity (n = 35) was considerably thicker (2.62 ± 0.31 mm) than those of both intramural fibroids (n = 68, 1.68 ± 0.13 mm) and subserous fibroids (n = 97, 0.98 ± 0.37mm) measured by US (P = 0.0001) and histology (P = 0.0001). On exploratory analysis, a clear cut-of measurement at 2 mm (P = 0.0001) was found between endometrial fibroid pseudocapsules and all other pseudocapsules.

Area under the curve was 1 for US and 0.999 for histology for endometrial cavity fibroids. Correlation between ultrasound and histology measurements was high (0.859 P = 0.00) (paired samples test). The large number of patients (200) and the single operator, single ultrasonographer and single pathologist, involved, minimizes the bias in this study.

Limitations, reason for caution: No limitation exists from the design and implementation of this study.

Wider implications of the findings: It is not known yet, another use of these findings, except improving surgical technique during myomectomy. Improved technique might assist to faster wound healing and better embryo implantation after myomectomy. This is in accordance with the pseudocapsule preservation concept, especially from those fibroids, located near uterine cavity.

Study funding/competing interest(s): Funding by hospital/clinic(s), Department of Obstetrics and Gynaecology, Vito Fazzi Hospital, Lecce, Italy.

Trial registration number: None.

P-587 Hysteroscopic Adhesiolysis: efficacy and safety

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Study question: To evaluate the efficacy and safety of hysteroscopic adhesiolysis in patients with intrauterine adhesions (IUAs), reproductive parameters (pregnancy rate, duration of pregnancies, life births rate, time lag between the intervention and diagnosis of pregnancy).

Summary answer: The reproductive outcome of hysteroscopic adhesiolysis is an effective and safe method for IUAs and significantly affected by degree of intrauterine adhesions rather than the main complaint before the procedure.

What is known already: Intrauterine adhesions result after trauma to the basal layer of the endometrium. This may occur as a complication of pregnancy-related

curettage. Also injury to the endometrium of a non-gravid uterus, including dilatation and curettage for diagnostic purposes, myomectomy, and hysteroscopic surgery, can also lead to intrauterine adhesions. Symptoms of intrauterine adhesions vary from no symptoms to menstrual complaints, reproductive failure, as infertility and recurrent pregnancy loss. Hysteroscopy is ideal for diagnosis, treatment and follow up.

Study design, size, duration: It is prospective cohort study, included 61 patients. The study conducted between January 2008 and December 2013.

Participants/materials, setting, methods: This study included 72 Patients presented with infertility (primary or secondary) or recurrent pregnancy losses caused by IUAs were included in the study.

Intervention(s): The adhesions were divided by semi-rigid scissors introduced under direct vision through hysteroscopy. One month later, second look hysteroscopy was performed.

Main results and the role of chance: Pregnancy rate changed from 18% to 65.5%, while live birth rate improved from 14.7% to 36%. The mean time until the first conception was 10.2 months (range: 2–40 months) after the operation. Nine women got pregnant twice during the study period. There was significant negative correlation between the degree of IUAs and the improvement in reproductive performance. Hysteroscopic adhesiolysis significantly improved menstrual pattern in 60.7% of patients complaining of hypomenorrhea or amenorrhea (P = 0.0017). The average operative time was 29 ± 10.2 (10–52) minutes and the hospital stay was 12.5 ± 2.1 (9–24) (hours). Uterine perforation occurred on 3 (4.9%), and cervical laceration occurred in one case (1.6%).

Limitations, reason for caution: There were 11 cases lost during follow up.

Wider implications of the findings: Hysteroscopy is safe and effective method for treatment of intrauterine adhesions (IUAs).

Study funding/competing interest(s): Funding by hospital/clinic(s), The study funded by research unit of Minia Maternity University Hospital.

Trial registration number: It is cohort study not RCT.

P-588 The characteristic features of pelvic peritoneal adhesions' morphology in women of reproductive age

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Study question: To examine the morphological structure of the pelvic peritoneal adhesions at infertile women and forecast scientifically the future behavior of formed adhesions after adhesiolysis.

Summary answer: Morphological examination of the pelvic peritoneal adhesions revealed differences in cellular and fiber composition depending on the genesis.

What is known already: One of the reason identifies the high frequency of adhesion formation is the presence of inflammation in the abdominal cavity with different severity and origin. Activation of intercellular relationships in the peritoneum becomes promoter of further adhesions when inflammation. A special role is given to macrophages. Data on the cells' distribution in the pelvic peritoneal adhesions in connection with their prescription, localization and origin is absent at accessible literature.

Study design, size, duration: The materials of this study were fragments of surgical specimens (adhesions and their parts) n = 50, obtained from fertile women who suffered with infertility during operative laparoscopy. The morphological and immunohistochemical study of adhesions were carried out by standard techniques using monoclonal CD 68 antibodies.

Participants/materials, setting, methods: Morphological examination of the pelvic peritoneal adhesions revealed differences in cellular and fiber composition depending on the genesis. The different activity of CD68 positive macrophages was found, indicating that moderate inflammatory reaction and self-sustaining progress of adhesions.

Main results and the role of chance: Postoperative adhesions are found in previous surgery. Morphologically, their structure is characterized by low cells and the extensive growth of the coarse-fibered connective tissue, poor vascularization and poor expression of CD68 (n = 6.7 ± 0.99). Pelvic adhesions in women with chronic pelvic inflammatory diseases were mainly located between the uterine tubes and/or ovaries and mesodesma. These adhesions contain a moderate or small number of CD68-positive macrophages (n = 10.4 ± 2.12), poor lymphoplasmocytic cell infiltration and vascularization; connective tissue had a coarse-fibered structure and had a well-developed layer of mesothelial cells. A result of endometriosis, a friable fibrous connective tissue with relatively small number of mesothelial cells, focal hemorrhages and massive vascularization was morphologically found in the adhesions. Immunohistochemical method found the increased activity and predominance of mature CD68-positive macrophages (n = 23.8 ± 1.38).

Limitations, reason for caution: Age limitation, only women aged 19–49 years took part in this study. Exclusion criteria were the following for the groups: acute gynecological diseases, malignant diseases of female genitalia and ovarian tumors.

Wider implications of the findings: Morphological examination of the pelvic peritoneal adhesions revealed differences in cellular and fiber composition depending on the genesis. CD68-positive cells were found in all observations, but their number varied indicating a moderate inflammatory reaction and self-sustaining progress of adhesions. These differences may partly explain the unequal dynamics of adhesions formation process, as well as their different sensitivity to the preventive and curative effects.

Study funding/competing interest(s): Funding by University(ies), Crimean State Medical University named after S. I. Georgievky.

Trial registration number: Case control study.

P-589 Influence of endometrial polyp-like lesions detected during ultrasound at ovarian stimulation on IUI results

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Study question: To ascertain the influence of endometrial polyp like lesion (EPLL) visualized by vaginal ultrasound during ovarian stimulation on IUI results.

Summary answer: A non significant trend to somewhat lower PR was observed in patients with EPLL. In our opinion, the cycle where EPLL was observed should not be canceled. However, probably before starting the following cycle performing a diagnostic/operative hysteroscopy could increase PR.

What is known already: Although a number of theorized mechanisms by which polyps could adversely affect reproductive performance have been proposed, their influence on assisted reproduction treatment is not well known. Regarding IUI, there is paucity of reports on this topic.

Study design, size, duration: The population under study consisted of 224 consecutive women subjected to 587 IUI cycles at our unit during a 1 year period, where the hysterosalpingography (HSG) showed normal tubal patency as well as a normal uterine cavity. Hysteroscopy was not performed in such cases. Cases with abnormal cavity at HSG were not included.

Participants/materials, setting, methods: Ovarian stimulation was performed with gonadotropins, and during standard follicular monitoring, endometrial evaluation was also performed. In cases were an EPLL was observed, the cycle was not cancelled and IUI was performed as usual. If pregnancy was not achieved, the following cycle was started without any additional studies. Hysteroscopy was performed when EPLL persisted after 3 cycles, if pregnancy was not reached. Pregnancy rates were compared in cases with EPPL and in cases with normal endometrial ultrasound.

Main results and the role of chance: The EPLL prevalence in women with normal HSG was of 20.53% (46/224). Per cycle PR was somewhat lower in EPLL cases (18.6%) compared with cases with normal endometrial cavity (22.4%), without statistical significance. Endometrial polyps were confirmed in 14/21 cases subjected to hysteroscopy. In 2/21 submucous myoma were observed, and in 5/21 no abnormal findings were observed (in 1 of these pseudopolypoid endometrium was observed).

Limitations, reason for caution: Non randomized study. Cases were an EP was found at HSG were subjected to hysteroscopic polipectomy and were excluded from the study. Cases where an EP was suspected by HSG or vaginal ultrasound but discarded by hysteroscopy were also excluded from the study.

Wider implications of the findings: A trend towards lower PR was observed in women were an EPLL was detected during ovarian stimulation, compared with those with an endometrium without EPLL. Since the PR even in presence of EPLL were considerable (18.6%), we do not recommend to cancel the current cycle, but to perform hysteroscopy, if pregnancy is not achieved, before starting the next cycle.
Study funding/competing interest(s): Funding by hospital/clinic(s), I confirm that I have no conflict of interest in relation to this work.
Trial registration number: Unnecessary.

P-590 Perfusion with cryoprotectants *in vivo* and *in vitro* of whole ovine ovaries as a model for human

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Study question: Cryopreservation of whole ovine ovaries with vascular pedicles is an important focal point of research in medicine when ovine ovary is an animal model for human ovary. The aim of research was to study the effectiveness of perfusion of whole ovine ovaries with vascular pedicle *in vivo* and *in vitro*.
Summary answer: It was concluded that *in vitro* perfusion of ovine intact ovaries with vascular pedicle by freezing medium is more effective than this manipulation performed *in vivo.*
What is known already: Cryopreservation and transplantation of whole ovaries with vascular pedicle would be helpful to prevent post-transplantation ischemia. Cryoprotectant diffusion into perfused ovine ovaries is a potentially limiting factor that has not been adequately investigated. After cryopreservation of whole ovine ovaries, their viability is low.
Study design, size, duration: For *in vivo* perfusion, 13 ovaries with pedicle were expelled to operation field. Fallopian tubes were not separated, mesoovarium was not removed and utero-ovarian anastomosis was not ligated. For *in vitro* perfusion (n = 23) at the room temperature (22°C), catheter through aorta was introduced to arteria ovarica and fixed inside.
Participants/materials, setting, methods: *Arteria ovarica* was cannuled and ovaries perfused by Leibovitz L-15 medium + 100 IU/ml heparin + 5% bovine calf serum + dimethyl sulfoxide + ethylene glycol + sucrose + Indian ink *in vivo* and in *vitro*. In the first and second cycle of experiments, ovaries (n = 13 and n = 23) were perfused *in vivo* and *in vitro*, respectively, during 60 min.
Main results and the role of chance: We guessed that if we connect *arteria ovarica* through perfusor with cryopreservation medium, it will create the stably increasing pressure of this medium, and then blood slowly will be replaced by freezing medium. We have presupposed that we will perform our perfusion manipulations *in vivo* when normal physiological negative pressure in the ovary from site of *vena ovarica* is observing. However, after *in vivo* perfusion only about 10% of ovarian tissues were perfused due to an appearance of multiple anastomoses when the perfusion medium goes from *arteria ovarica* to *arteria uterina* without fulfilling into the ovaries. It was technically difficult to close these multiple anastomoses during the perfusion. After successful *in vitro* perfusion, approximately 100% of ovarian tissue and its vascular pedicle obtained blue colour.
Limitations, reason for caution: No limitation.
Wider implications of the findings: We did presuppose that blood which goes through the capillaries of ovarian tissue will be removed from these capillaries more effectively *in vivo* than *in vitro.* Theoretically, by *in vivo* perfusion of ovary the herd can play the role of additional pump for perfusion solution: will help perfusion medium to penetrate through the capillaries of the ovary.
Study funding/competing interest(s): Funding by University(ies), Cologne University.
Trial registration number: No trial registration number.

P-591 Does surgery prior to ART affect IVF/ICSI outcome in women with endometrioma or deep infiltrating endometriosis (DIE) – a result from meta-analysis and systematic review

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Study question: Do women with endometrioma or DIE have poorer IVF/ICSI outcome when compared to those without disease? Does surgery prior to ART affect IVF/ICSI outcome in women with endometrioma or DIE?
Summary answer: Women with endometrioma or DIE have similar reproductive outcome compared to those without disease. There is no evidence of benefit for surgical treatment of endometrioma before ART. There is insufficient evidence to support surgical treatment in women with DIE prior to ART.
What is known already: The presence of endometriosis is known to be detrimental to fertility. Many patients with endometrioma or DIE may eventually require ART to achieve a pregnancy. Two previous systematic reviews published more than 4 years ago found no evidence of benefit between surgical treatment and expectant management of endometrioma prior to ART; the benefit for surgical treatment of DIE prior to ART is unclear.
Study design, size, duration: Systematic review and meta-analysis. We searched all published and unpublished studies from 1980–2014. We included participants who had surgical management of endometrioma or DIE prior to ART. The quality of each paper was assessed and scored according to Newcastle-Ottawa Assessment scale. All suitable data were extracted and analysed using RevMan5.
Participants/materials, setting, methods: Literature search yielded 621 studies; 35 studies were included with total number of 4657 women were involved.
Main results and the role of chance: Compared to controls, women with endometrioma undergoing ART had a similar live birth rate (LBR) (OR 0.98, 95% CI [0.71, 1.36], n = 928, I^2 = 0%), clinical pregnancy rate (CPR) (OR 1.17 95% CI [0.87, 1.58], n = 928, I^2 = 0%) and a lower mean number of oocytes retrieved (MOR) (MD –0.98 95% CI [–1.85, –0.10], 941 cycles, I^2 = 65%). Compared to controls, those with surgically treated endometrioma have similar LBR (OR 1.09 CI 95% [0.64, 1.86], n = 302, I^2 = 49%), CPR (OR 0.97 95% CI [0.77, 1.21], n = 1411, I^2 = 0%) and a lower MOR (MD –0.24 CI 95% [–0.39, –0.09], 837 cycles, I^2 = 0%). Women with DIE had a similar LBR (OR 1.12 95% CI [0.48, 2.60], 1 study, n = 526), CPR (OR 1.53 95% CI [0.71, 3.28] and MOR when compared to controls. One study showed that surgically treated women with DIE had a higher pregnancy rate (OR 2.19 [1.12, 4.28], n = 169) compared to no treatment.
Limitations, reason for caution: Due to observational nature of the included studies, heterogeneity between these studies is high and therefore the results are subjected to confounders and bias.
Wider implications of the findings: In infertile women with endometrioma, there is no evidence that surgical treatment prior to ART improves pregnancy rates. This is in agreement with previous reviews and the recent ESHRE guidance. A single study reported evidence of benefit for surgery prior to ART in DIE.
Study funding/competing interest(s): Funding by University(ies), Ministry of Higher Education, University of Malaya, Malaysia, Complete Fertility Centre, Southampton.
Trial registration number: No Applicable.

POSTER VIEWING

STEM CELLS

P-592 Expression patterns of the meiotic commitment protein, STRA8, differ in adult human and mouse ovaries

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Study question: The principal objective of this study was to evaluate expression of the germ cell-specific meiotic commitment protein, Stimulated by Retinoic Acid Gene 8 (STRA8), in ovaries of reproductive age women, and compare this pattern of expression to that observed in adult mouse ovaries.
Summary answer: Our data provide the fist evidence that STRA8 is present in ovaries of women, being localized to the cytoplasm of non-follicle enclosed germ cells (similar to mice) but remaining detectable in Balbiani bodies of oocytes contained within primordial follicles (unlike mice, which show no oocyte expression of the protein).
What is known already: In mice, Stra8, a retinoic acid-responsive and germ cell-specific gene, is required for meiotic commitment during oogenesis and

spermatogenesis. Recent genetic studies have demonstrated that Stra8 plays an important role in both prenatal and postnatal oogenesis in mice; however, conserved functionality of human STRA8 is unknown. While human STRA8 shares 55% sequence homology and several conserved phosphorylation domains with mouse Stra8, it lacks a central 51-amino acid glutamine-rich region, which may alter protein function.

Study design, size, duration: Ovarian cortical tissue was collected from reproductive aged women (ages 22–33 years) and vitrified. Following warming, the cortex was divided into 2-mm sample pieces and used for endpoint analysis.

Participants/materials, setting, methods: Ovarian cortex was processed for transmission electron microscopy (TEM), immunohistochemistry, and gene expression analysis. A custom rabbit polyclonal antibody specific for the human STRA8 protein was generated and validated for specificity using human testicular biopsy material.

Main results and the role of chance: Immunohistochemistry revealed distinct non-follicle enclosed cells positive for STRA8 protein in adult human ovaries. In these cells, STRA8 was localized to the cytoplasm. However, unlike in adult mouse ovaries in which Stra8 expression is restricted to non-follicle-enclosed premeiotic germ cells, oocytes within primordial follicles of human ovaries were also positive for STRA8 protein. By TEM, the distinct punctate staining for STRA8 in primordial oocytes was found to be localized to the Balbiani body. Gene expression analysis (RT-PCR) of total RNA extracted from human ovarian cortex confirmed that the endogenous *STRA8* gene is expressed at the mRNA level.

Limitations, reason for caution: The present studies are correlative. Future studies, including *in vitro* modeling using oogonial stem cells (OSCs) and cortical strip culture systems, will be needed to directly evaluate the role of STRA8 in human oogenesis through genetic manipulation.

Wider implications of the findings: STRA8 is expressed in non-follicle enclosed cells – presumably premeiotic germ cells, in adult human ovaries, although our data have uncovered distinct differences in the pattern of STRA8 expression between mice and humans. Whether or not these differences are functionally important to oocyte development remain to be determined. However, combined with the marked sequence homology differences in mouse versus human STRA8, these data suggest that STRA8 may play multiple roles in human oogenesis.

Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), J.T. discloses interest in intellectual property described in U.S. Patent 7,195,775, U.S. Patent 7,850,984 and U.S. Patent 7,955,846, and is a co-founder of OvaScience, Inc. D.W., C.S. and Y.K. have nothing to disclose. Funded by Northeastern University, the Glenn Foundation for Medical Research, NIH R37-AG012279.

Trial registration number: Not required.

P-593 Generation of mouse androgenetic haploid embryonic stem cells from a single sperm

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Study question: The aim of this study was to establish and characterize the embryonic stem (ES) cell line from mouse androgenetic haploid (AH) embryos derived from a single sperm.

Summary answer: ES cell-like cells with a haploid chromosome set derived from a single sperm were able to be established and successfully maintained for a long-term period.

What is known already: Androgenetic embryonic stem cells with paternal haploid genome are a valuable cell source for the reproductive and the regenerative medicine as well as the genetic analysis. However, available information about haploid embryonic stem cells is very limited.

Study design, size, duration: Mouse AH embryos were produced by the injection of a single sperm into enucleated oocyte, and the embryos cultured until the blastocyst stage. ES-like cells with a haploid genome were established from the inner cell mass (ICM) of the AH blastocyst and determined their biological characteristics.

Participants/materials, setting, methods: The expression of ES cell markers (Oct4, Nanog, Sox2, and Rex1) in ES-like colonies obtained from AH embryos was examined by immunohistochemical staining. Further, the DNA content of ES-like cells derived from AH embryo was measured by flow cytometry, and karyotype analysis was also performed.

Main results and the role of chance: After the culture for 4 days, 7.4% of AH embryos reached to the blastocyst stage, and ICM outgrowth was observed in approximately 40% of these. The ICM cells were dispersed, seeded over feeder cells, and spherical colonies were observed. Subsequently, these colonies were passaged every 5–6 days, and two ES-like cell lines were generated. In these cells, colony formation and proliferation were maintained for 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).

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 AH 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: Not applicable.

P-594 Inhibition of TGFβ signaling supports embryonic stem cell derivation and the ground state of pluripotency in mouse

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Study question: Does culturing pre-implantation embryos in the presence of a TGF (transforming growth factor) β pathway inhibitor support the mouse embryonic stem cells (mESCs) derivation and does the extra inhibition of TGFβ signaling during culture of mESCs derived in '2i' conditions impose an altered state of pluripotency in stem cells?

Summary answer: Culturing mouse embryos in the presence of the TGFβ pathway inhibitor, SB431542 (SB) permits the derivation of mESCs with efficiency up to 100%. mESCs derived in 2i conditions show significantly higher expression of naive and other pluripotency markers when additionally exposed to SB during culture.

What is known already: ESCs exist in a naive or primed state of pluripotency regulated by distinct signaling pathways, mostly antagonizing each other. Although naive ESCs are more homogeneous in terms of pluripotency, culture conditions can influence this phenomenon. For example, mESCs derived in 2i conditions (dual inhibition of fibroblast-growth-factor and glycogen-synthase-kinase pathways with PD0325901 and CHIR99021 respectively) are designated to be in the ground-state of pluripotency with better homogeneity than those derived in leukemia-inhibitory-factor and serum conditions.

Study design, size, duration: Embryos were cultured with/without SB supplementation from the morula stage onwards. Blastocysts were plated in the following mESCs derivation-conditions: 1) 2i, 2) 3i(2i + SB), 3) SB and 4) control without small molecule inhibitors. mESCs derived in 2i were further cultured in 2i vs 3i for six passages. Naive-pluripotency characteristics were evaluated by RT-qPCR.

Participants/materials, setting, methods: 5 control embryos and 77 embryos cultured in SB from the morula stage were plated in the aforementioned conditions. Levels of expression of Oct4, Sox2, Nanog, Rex1, Pecam1, Stella, Fgf5 and Gbx2 were examined by RT-qPCR in three 2i-derived mESCs lines and in subsequent mESCs cultures further maintained in 3i.

Main results and the role of chance: The derivation efficiency of mESCs from control embryos was 63.3%, 83.3%, 0%, 0% and that from embryos cultured in SB was 91.3%, 100%, 0%, 0% in the four aforementioned conditions respectively. When 2i lines were subsequently cultured in 3i, naïve and pluripotency genes were expressed more than 2-fold higher in 3i compared to those maintained in 2i. Naïve markers such as Pecam-1 and Stella and the pluripotency marker Nanog increased significantly ($p < 0.05$) in all cases in 3i. Oct4, Sox2 and Rex1 were also significantly up-regulated while the mesodermal marker FGF5 was significantly down-regulated in 2 out of 3 cell lines cultured in 3i. No significant difference between the two conditions was seen in the level of Gbx2, a post-implantation primitive ectodermal marker.

Limitations, reason for caution: Our results show that inhibition of TGFβ signaling in existing mESCs can modulate the pluripotency state of cells. Additional experiments such as functional differentiation tests, heterogeneity and larger-scale transcriptome-analysis of the cell lines maintained in 2i and 3i must be performed to better understand the differences between these pluripotent states.
Wider implications of the findings: Naive mESCs exhibit heterogeneity to a lesser extent than primed ones. So, devising a condition in which the cell population shows least heterogeneity merits further investigation and would be beneficial for future clinical use of ESCs. Our work will also serve to maintain a more robust pluripotent state in mESCs.
Study funding/competing interest(s): Funding by University(ies), This study was funded by a Special Research Fund (BOF) of Ghent University awarded to SG. The authors declare no competing interest.
Trial registration number: Not applicable.

P-595 Isolation, propagation and characterisation of oogonial stem cells from human and bovine ovarian cortex

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Study question: Is there a population of mitotic cells with germ line potential i.e., oogonial stem cells (OSCs) within the human and bovine ovarian cortex and if so, can they be isolated and cultured *in vitro* long term?
Summary answer: This is the first report of female germline stem cells (OSCs) being isolated from bovine ovarian cortex and corroborates a previous report demonstrating the isolation of OSCs from the human ovary.
What is known already: There is now an increasing body of experimental evidence indicating that a rare population of mitotically active germ cells referred to as OSCs exist in adult ovaries. These cells, described in mice and humans, seemingly have the ability to generate oocytes when cultured under specific conditions, however, only one lab has isolated these cells from human ovaries. Given the controversy surrounding OSCs it is important to determine whether these findings can be independently corroborated.
Study design, size, duration: Experimental laboratory study to develop isolation of OSCs from human and bovine ovarian cortex and to investigate propagation and expression of germ and pluripotency-associated genes. Biopsies of human ovarian cortex (n = 3, ages 13–33 years) were obtained with informed consent and Ethical Committee approval.
Participants/materials, setting, methods: Fluorescence-activated cell sorting (FACS) was used to isolate cells that externally expressed VASA after disaggregation of human and bovine ovarian cortex. After *in vitro* propagation RT-PCR and immunocytochemistry were used to assess the presence of germline-specific markers. Cells were also transduced with green fluorescent protein (GFP)- and mCherry lentiviruses.
Main results and the role of chance: A rare population of mitotically-active VASA-positive cells was isolated from both bovine and human tissue and grown *in vitro* for several months. To date, cells have been retrieved from three human samples and on five separate occasions from bovine ovary. RT-PCR and immunocytochemistry have demonstrated consistent expression of several pluripotency-associated and germline markers at both the mRNA and protein level, including LIN28, OCT-4, fragilis (IFITM3), STELLA, BLIMP1, and CKIT. It has been possible to cryopreserve the cells with successful reinitiation of growth on thawing. Stable expression of GFP and mCherry was achieved after lentivirus transduction.
Limitations, reason for caution: This study demonstrates that OSCs can be consistently isolated from human and bovine ovaries. Whilst the expression of stem cell and germline markers indicates characteristics of germline, or oogonial, stem cells, their capacity to enter meiosis and form functional oocytes has yet to be determined.
Wider implications of the findings: This is the first report of OSCs being isolated from bovine ovarian cortex and corroborates a previous report showing the isolation of human OSCs. These cells provide a novel model for investigating germ cell development. Future experiments will assess the capability of these cells to undergo oogenesis in an *in vitro* environment. If their potential can be harnessed, then OSCs may have a role in clinical applications, for example in fertility preservation, in the future.
Study funding/competing interest(s): Funding by national/international organization(s), UK Medical Research Council grants G0901839 and G1100357, No competing interests.
Trial registration number: N/A.

P-596 Human amniotic membrane-derived mesenchymal stem cells are effective in improving ovarian function

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Study question: The potential of human amniotic membrane-derived mesenchymal stem cells (hAMSCs) to improve ovarian function.
Summary answer: hAMSCs are effective in improving rather than reconstructing ovarian function.
What is known already: Hormonal replacement therapy (HRT) has been used to treat common menopausal problems, but it increases risks of cancer or recurrence in cancer survivors, forcing physicians to use alternative treatments. In recent years, interest has rapidly grown in the therapeutic potential of stem cells. Fetal mesenchymal stem cells are potential therapy material for those intractable diseases.
Study design, size, duration: Animal study, 8 for each group.
Participants/materials, setting, methods: Female C57B6 mice aged 32-weeks were used as the model of natural ovarian dysfunction. Female C57B6 mice aged 8-weeks treated with 4Gy X-irradiation were used as the sterilization model. hAMSCs were successfully developed in our center. hAMSCs were injected into mice via tale vein. After 1 month of transplantation, ovaries were excised. Ovarian function was evaluated by monitoring ovulation, estrous cycle, serum gonadal hormone and categorized follicles. Cell tracking, microarray analysis on cytokines, and transmission electron microscope were used to assess the repairmen roles of hAMSCs.
Main results and the role of chance: Compared to young mice, the aged mice had higher level of FSH and lower levels of estrogen and AMH, and less follicles. Mice irradiated by X-ray exhibited not only ovarian failure but also whole-body premature aging. The hAMSCs injection significant increase the oocytes number, fertilization and blastocyst formation rate in natural aging mice. Some immature primary follicles like structure and reduced degree of ovarian stromal fibrosis presented in sterilized mice (5 out of 8). Serum levels of estrogen in all hAMSCs-treated groups were higher than those in controls. However, cell tracking analysis revealed that hAMSCs did not directly differentiate into follicle component. Microarray analysis indicated that hAMSCs secreted kinds of growth factors, immunomodulatory factors, and chemokines.
Limitations, reason for caution: Descriptive, shown only in one species.
Wider implications of the findings: The increased hormones and hAMSCs-secreted factors may be benefit for follicle development and resisting fibrosis in ovarian dysfunction, which highlight the possibility of hAMSCs therapy in ovarian dysfunction.
Study funding/competing interest(s): Funding by University(ies), Jiangsu people hospital.
Trial registration number: No.

POSTER VIEWING

TRANSLATIONAL RESEARCH

P-597 INSC94Y transgenic pigs – a novel large animal model for studying developmental consequences of pre-conceptional diabetes mellitus

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Study question: To investigate the impact of pre-conceptional diabetes mellitus, we generated genetically diabetic *INS*^{C94Y} transgenic pigs, considerably

reflecting human conditions of permanent neonatal diabetes mellitus (Renner, S., et al. (2013), *Diabetes* 62, 1505–11). To clarify effects of maternal hyperglycaemia on glucose tolerance of the offspring, oral glucose tolerance tests were performed post-partum.

Summary answer: Although continuative studies need to investigate insulin levels and effects on pancreatic β-cells of the offspring, we could emphasize that maternal hyperglycaemia in this pig model potentially effects glycemic control of the offspring.

What is known already: Diabetic pregnancies have been shown to implicate developmental consequences and have prenatal programming effects on the offspring. The underlying pathomechanisms have mainly been investigated in diabetic rodent models and remained largely unknown. Important similarities between human and porcine reproductive biology underline the importance of the pig as excellent biomedical model for reproductive medicine. *INS*^{C94Y} transgenic pigs show fasting hyperglycaemia due to a clinically relevant failure of pancreatic β-cells but can develop normally under insulin treatment. Female *INS*^{C94Y} transgenic pigs have proven to get pregnant and deliver offspring.

Study design, size, duration: To clarify potential effects of maternal hyperglycaemia on glucose tolerance and insulin sensitivity of the offspring, oral glucose tolerance tests were performed post-partum before the first food intake.

Participants/materials, setting, methods: In this preliminary study oral glucose tolerance was investigated in transgenic and wild-type offspring of diabetic *INS*^{C94Y} transgenic sows mated to a non-transgenic boar and compared to the offspring of a non-diabetic wild-type sow mated to an *INS*^{C94Y} transgenic boar as well as of wild-type sow × wild-type boar matings.

Main results and the role of chance: We observed that both transgenic and wild-type offspring of diabetic *INS*^{C94Y} transgenic sows mated to a non-transgenic boar exhibited higher fasted blood glucose levels compared to the offspring born to a non-diabetic wild-type sows mated to an *INS*^{C94Y} transgenic boar as well as of wild-type sows mated to a wild-type boar. Oral glucose tolerance of the wild-type offspring born to diabetic *INS*^{C94Y} transgenic sows seem to be improved indicated by a decelerated rise of glucose levels after glucose administration and an apparently reduced area under the curve (AUC) for glucose when compared to transgenic littermates as well as the offspring born to non-diabetic sows.

Limitations, reason for caution: So far, six transgenic and twelve wild-type littermates born to *INS*^{C94Y} transgenic sows and five offspring of non-diabetic wild-type sows × wild-type boar matings were investigated using described method. Investigation larger animal cohorts is ongoing.

Wider implications of the findings: *INS*^{C94Y} transgenic pigs develop an exceedingly stable diabetic phenotype and therefore constitute a suitable environment to study developmental implications of pre-conceptional diabetes mellitus on oogenesis, uterine receptivity, embryonic and fetal development as well as fetal programming effects on the offspring in an organism recapitulating the human conditions.

Study funding/competing interest(s): Funding by national/international organization(s), BioSysNet.

Trial registration number: Trial registration number is only needed for clinical trials.

P-598 Expression and cytoplasmic localization of Calreticulin in human maturing oocytes

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Study question: We hypothesized that *Calreticulin* (CALR) is expressed during oocyte maturation and its localization changes during the meiotic transition from Germinal Vesicle (GV) to Metaphase II (MII) stage interacting with the endoplasmic reticulum whose relocalization is central to the process of oocyte maturation.

Summary answer: CALR is constantly expressed throughout maturation process and displays a specific localization in association with the MII spindle.

What is known already: CALR is a multifunctional protein that acts as a major Ca^{2+}-binding (storage) protein in the lumen of the endoplasmic reticulum (ER). In mammalian oocytes, CALR seems to play an important role in calcium homeostasis during oogenesis and development. The Ca^{2+} binding activity of CALR is crucial to the role played by this ion in important cellular processes, such as adjusting the cytoskeleton, modulate gene expression and protein synthesis. Nothing is known in the human oocyte.

Study design, size, duration: Supernumerary oocytes, donated from consenting patients undergoing in-vitro fertilization (IVF) treatment and stimulated by long standard protocol, were assigned to molecular analysis or immunofluorescence. Gene expression was conducted on pools of 5 oocytes and Immuno-cytochemical analysis was carried out on 15 oocytes per each stage of maturation (GV and MII).

Participants/materials, setting, methods: For molecular studies oocytes were subjected to semi-quantitative real time polymerase chain reaction (RT-PCR) to screen for CALR expression pattern. Immunostaining was performed using a specific anti-human CALR rabbit antibody. Equatorial sections were obtained with a Leica TCS SP2 Laser Scanning Confocal Microscope. Images were processed with ImageJ dedicated software.

Main results and the role of chance: As a control CALR expression was linked to HSPA5 (member of the heat shock protein 70 family and involved in the folding and assembly of proteins in the ER) and 28S the latter used with the function of "house keeping" gene and therefore as a positive control in opposition to Nestin used as negative control. CALR is expressed at comparable levels both in GV and MII oocytes. CALR immunolocalization at the GV stage reveals that the cytoplasm is marked by a finely granular green staining distributed throughout the cytoplasm and surrounds the nucleus, confirming its connection with the ER. In the MII stage appears coincident with the presumptive position and morphology of the meiotic spindle, as confirmed by the relative position of the chromosomes.

Limitations, reason for caution: The relatively small sample size suggests to carry out larger studies. In addition GV stage data should be confirmed using immature oocytes recovered from unstimulated ovaries.

Wider implications of the findings: Our findings are in agreement with recent data emerging in the mouse model where at the MII stage the ER acquires a highly specific conformation enveloping the meiotic spindle. CALR specific localization in association with the meiotic spindle suggests the hypothesis of different ER subdomains and an important involvement of Ca^{2+} in the regulation of the meiotic spindle. This opens entirely new scenarios for studies on the ER-cytoskeleton regulative interaction in the human oocyte.

Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Ospedale San Raffaele and Univerità degli Studi, Milan Italy.

Trial registration number: N.A.

P-599 Comet assay on mouse oocytes: technical adaptation to study genotoxicity of environmental factors on cumulus oocyte complexes

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Study question: How to improve oocyte Comet Assay to study genotoxicity of environmental agents on oocytes?

Summary answer: Comet Assay after *in vitro* exposure of mouse cumulus oocyte complexes (COC) to genotoxic agents offers an interesting tool to study the impact of environmental factors on oocyte DNA.

What is known already: Many tests are validated to study genotoxicity on somatic cells as micronucleus test, comet assay or Ames test. However, results obtained with somatic cells cannot be extrapolated to gametes. Comet Assay (CA) on oocytes was usually described after removal of zona pellucida. We adapted previously CA to mouse oocytes with zona pellucida in order to study genotoxicity of cryoprotectants used for vitrification. Nevertheless, oocyte exposed *in vivo* to environmental agents is surrounded by cumulus cells.

Study design, size, duration: CA was performed after *in vitro* exposure to genotoxic agents on two groups of mouse metaphase II oocytes: *(i)* oocytes with ZP (OZP-CA) *(ii)* COC (COC-CA). Experiments were performed in triplicate using at least 40 oocytes per condition. DNA damage was assessed using Olive Tail Moment (OTM).

Participants/materials, setting, methods: For OZP-CA, oocytes were exposed *in vitro* after hyaluronidase treatment of COC. For COC-CA, whole COC were exposed *in vitro* directly after removal from oviducts. Three conditions were studied: a negative control group; a positive control groups (250 µMol H_2O_2); an environmental agent, cerium dioxide nanoparticles (100 mg/L CeO_2 NPs).
Main results and the role of chance: For each test, an average of 100 oocytes was analyzed by condition. With OZP-CA, significant DNA damage was observed following H_2O_2 (mean OTM ± SD = 38 ± 1.3) and CeO_2 NP (11.6 ± 0.8) exposures compared to negative control (2.9 ± 0.2). With COC-CA, significant DNA damage was observed following H_2O_2 treatment (36 ± 1.3) and CeO_2 NP (20 ± 1.6) treatments compared to negative control (5.4 ± 0.6). When we compared OZP-CA to COC-CA, OTM were similar between negative control groups and between positive control groups. However, after exposure to CeO_2 NP, DNA damage was significantly higher with COC-CA ($p < 0.0001$).
Limitations, reason for caution: Although the COC-CA protocol is closer to *in vivo* condition than OZP-CA, any extrapolation to human *in vivo* exposure should be done with caution.
Wider implications of the findings: Even surrounded by cumulus cells, oocyte seems not to be totally protected from exogenous toxic agents. This adaptation of Comet Assay is a new interesting approach to assess the genotoxicity of environmental agents towards oocytes. First, this method gets closer to *in vivo* conditions of exposure in the female genital tract. The COC-CA also simplifies the protocol of the oocyte Comet Assay and likely provides a better inter-operator reproducibility, limiting cell manipulation.
Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s), F.G awarded a scholarship from 'Foundation, health, sport and development', Aix-marseille University for master's degree, This work is a contribution to the LABEX SERENADE (n° ANR-11-LABX-0064) funded by the «Investissements d'Avenir», French Government program of the French National Research Agency (ANR) through the A*Midex project (n° ANR-11-IDEX-0001-02)'.
Trial registration number: None.

P-600 Transcutaneous electrical acupoint stimulation improves clinical pregnancy rate in embryo transfer

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Study question: Does transcutaneous electrical acupoint stimulation (TEAS, an acupuncture-related technique) facilitate *in vitro* fertilization-embryo transfer (IVF-ET) and what are the optimum parameters to be used? What are the potential mechanisms of action?
Summary answer: TEAS at low frequency significantly increased the success rate of clinical pregnancy due to improved endometrial receptivity.
What is known already: The effect of acupuncture on pregnancy rate of IVF-ET is controversial. In a previous study, we found that TEAS 24 h prior and 30 min after ET increased clinical pregnancy rate significantly (Fertility and Sterility 2011; 96:912-6).
Study design, size, duration: To identify the optimal stimulation frequency for TEAS on pregnancy rates and to study the underlying mechanisms, large scale, multi-centered, randomized and controlled clinical trials were started in December 2011 and ended in March 2013.
Participants/materials, setting, methods: A total of 900 patients from three reproductive centers prepared to undergo fresh embryos transplant were recruited for frequency exploring trial (2 Hz, 100 Hz, mock TEAS and blank control), and 120 patients from one reproductive center prepared to receive frozen-cryopreservation embryos transplant were recruited for endometrial receptivity trial (2 Hz TEAS and blank group).
Main results and the role of chance: The results showed that TEAS was effective in increasing success rate of IVF-ET. 2 Hz TEAS was superior to 100 Hz in improving the clinical pregnancy rate (56.5% vs 44.4%, $P < 0.05$) and embryos implantation rate (34.4% vs 24.6%, $P < 0.01$). 2 Hz TEAS (but not 100 Hz) increased live birth rate compared with blank group (42.0% vs 28.2%, $P < 0.01$). 2 Hz TEAS decreased serum cortisol level in the ET day and increased endometrial expression of pinopode, integrin $\alpha_1\beta_1$, $\alpha_3\beta_3$, leukemia inhibitory factor (LIF) and serum progesterone levels on the theoretical embryo implantation day. Positive correlation was found between the serum progesterone level and the endometrial integrin $\alpha_1\beta_1$, $\alpha_3\beta_3$ as well as the pinopode expression levels.
Limitations, reason for caution: Center random system and independent data management were not applied in this study.
Wider implications of the findings: TEAS technique is a non-invasive safe method which has been used clinically for many years. We now report that it is also effective to improve clinical pregnancy rate in ET.
Study funding/competing interest(s): Funding by national/international organization(s), National Health and Family Planning Commission of the People's Republic of China.
Trial registration number: Registration was completed at Chinese Clinical Trial Registry which was a World Health Organization International Clinical Trial Registration Platform (Registration number: ChiCTR-TRC-11001780).

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