

ORAL PRESENTATIONS

INVITED SESSION

SESSION 01: ROBERT G. EDWARDS' MEMORIAL KEYNOTE SESSION

Monday 15 June 2015

08:30–09:30

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

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Study question: Are women's stress levels prospectively associated with fecundity and infertility?

Summary answer: Higher levels of stress as measured by salivary alpha-amylase are associated with a longer time-to-pregnancy (TTP) and an increased risk of infertility.

What is known already: Data suggest that stress and reproduction are inter-related; however, the directionality of that association is unclear.

Study design, size, duration: In 2005–2009, we enrolled 501 couples preconceptionally in a prospective cohort study in Michigan and Texas, USA. Couples were followed for up to 12 months and through pregnancy if it occurred. A total of 401 (80%) couples completed the study and 373 (93%) had complete data available for this analysis.

Participants/materials, setting, methods: Enrolled women collected saliva the morning following enrollment and then the morning following their first observed study menses for the measurement of cortisol and alpha-amylase, which are biomarkers of stress. TTP was measured in cycles. Covariate data were captured on both a baseline questionnaire and daily journals.

Main results and the role of chance: Among the 401 (80%) women who completed the protocol, 347 (87%) became pregnant and 54 (13%) did not. After adjustment for female age, race, income, and use of alcohol, caffeine, and cigarettes while trying to conceive, women in the highest tertile of alpha-amylase exhibited a 29% reduction in fecundity (longer TTP) compared with women in the lowest tertile [fecundability odds ratio = 0.71; 95% confidence interval (CI) = (0.51, 1.00); $p < 0.05$]. This reduction in fecundity translated into a more than twofold increased risk of infertility among women these women [Relative Risk = 2.07; 95% CI = (1.04, 4.11)]. In contrast, we found no association between salivary cortisol and fecundability.

Limitations, reasons for caution: Due to fiscal and logistical concerns, we were unable to collect repeated saliva samples and perceived stress questionnaire data throughout the duration of follow-up. Therefore, we were unable to examine whether stress levels increased as women continued to fail to get pregnant.

Wider implications of the findings: This is the first US study to demonstrate a prospective association between salivary stress biomarkers and TTP, and the first in the world to observe an association with infertility.

Study funding/competing interest(s): This study was supported by the Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (contracts #N01-HD-3-3355, N01-HD-3-3356, N01-HD-3358). There are no conflicts of interest to declare.

Trial registration number: NA.

Keywords: fecundity, infertility, stress

O-002 How a dead duck can be fertile

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For witnessing and publishing 'The first case of homosexual necrophilia in the mallard duck (*Anas platyrhynchos*)' the speaker was awarded the 2003 Ig Nobel Prize in the field of Biology. Thanks to this much coveted award he became known as 'The Duck Guy' and people from all over the globe send him their own observations of and/or publications on non-reproductive sexual behavior in animals. Here he presents highlights from his ever growing 'Necrophilia Files', including some severe cases that were overlooked for decades.

To address the topic of human reproduction too, the speaker will also share his insights in possibly the most dramatic example of current habitat destruction and loss of biodiversity – the disappearance of the once-ubiquitous crab louse (*Phthirus pubis*) due to excessive removal of pubic hair among sexually active people.

Keywords: ducks, animal behaviour, non-reproductive sexual behaviour, necrophilia, pubic louse

SELECTED ORAL COMMUNICATIONS

SESSION 02: NEW PERSPECTIVES ON EMBRYO SCORING

Monday 15 June 2015

10:00–11:30

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

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Study question: Blastocysts cultured for IVF are graded based on stage of hatching and organization of the inner cell mass and trophectoderm. Common practice is to implant embryos based on quality score in cases where preimplantation genetic screening (PGS) is not used. Is blastocyst embryo quality score correlated with chromosomal ploidy?

Summary answer: PGS is critical to improving success rates in implantation and live birth during IVF. In cases where PGS is not used prior to implantation, embryo quality score can be used to determine the likelihood of embryo aneuploidy based on strong correlations between embryo aneuploidy and *in vitro* trophectoderm development.

What is known already: It is common to select embryos for transfer based on morphology during IVF. A retrospective study assessing ploidy and general blastocyst morphology suggests that blastocysts graded "excellent" have a higher euploid rate than embryos graded "good," "average," and "poor," but the value of this information is limited to the general definitions of morphology used. A better understanding of how ploidy effects blastocyst morphology and organization may help improve efficacy in non-PGS related IVF transfers.

Study design, size, duration: Embryo quality scores have been obtained for 528 blastocysts from 112 patients all using the same IVF center. Trophectoderm biopsies from 528 blastocysts underwent PGS for chromosomal abnormalities using array CGH or Next Generation Sequencing and the results were compared to embryo quality score to determine any existent correlations.

Participants/materials, setting, methods: Blastocyst stage embryos were scored according to the standard Gardner blastocyst grading system by a single embryologist. Amplified trophectoderm biopsies were analyzed using either Illumina's 24-sure array CGH or VeriSeq NGS protocols and chromosomal profiles were compared to embryo score to determine correlates.

Study question: This study aims at investigating the relationship between seminal plasma growth factors (TGF β 1, IGF I and EGF) and semen parameters, sperm DNA fragmentation, and ART outcome among idiopathic infertile men and normal men.

Summary answer: The increase of Seminal TGF β 1 levels was significantly associated with low semen characteristics such as total sperm count, progressive motility and normal morphology, and with high rate of sperm DNA fragmentation in idiopathic infertile men. Nevertheless, we didn't found any correlation between growth factors and IVF or ICSI outcomes.

What is known already: It is becoming apparent that seminal growth factors have a definite role in fertility and their modulation can cause infertility in both men and women. These factors have also been shown to have both positive and negative effects on the function of spermatozoa in vitro. Therefore, their exploration may provide useful information in cases of male idiopathic infertility.

Study design, size, duration: A prospective study conducted during one year that included 104 couples undergoing ART cycles (IVF or ICSI). Two groups were identified according to semen analysis and infertility related factor. A first group ($n = 67$) with only male infertility factor (idiopathic OAT) and a control group ($n = 37$) with normal semen parameters.

Participants/materials, setting, methods: A part of each sample was prepared using a discontinuous sperm gradient after parameters analyze, for oocyte insemination or injection and the remaining pellet was fixed for subsequent measure of DNA fragmentation index (TUNEL assay). Subsequently, seminal plasma growth factors concentrations were measured by ELISA using commercially available kits.

Main results and the role of chance: The mean concentration of seminal plasma TGF β 1 was significantly higher in OAT group 234.68 ± 41.32 vs. 97.25 ± 36.85 ng/ml in controls ($p = 0.001$). TGF β 1 levels were correlated negatively with sperm total count, motility ($r = -0.437$, $p = 0.000$; $r = -0.556$, $p = 0.000$) but positively with teratozoospermia ($r = 0.705$; $p = 0.000$). No evident correlation was found between these growth factors and ART outcomes such as fertilization, cleavage, top embryos and pregnancy rates. Idiopathic infertile men showed a high degree of DNA damage 20.21% as compared to controls 12.96% ($p = 0.02$). A strong significant negative correlation was found between DNA fragmentation and all semen parameters ($p < 0.01$). Moreover, a significant positive correlation was noted between seminal TGF β 1 and sperm DNA fragmentation levels ($r = 0.350$, $p = 0.01$) but not with IVF or ICSI outcomes.

Limitations, reason for caution: EGF tended to be higher in OAT group but the difference was not significant. EGF levels were lower among patients with clinical pregnancy compared to those with ART failure but IGFI levels were higher in controls and in cases of pregnancy. This may be due to our small sample size.

Wider implications of the findings: Seminal TGF β 1 may have a direct or indirect role in spermatogenesis-steroidogenesis and its derangement may be involved in male idiopathic infertility especially when mediated through low semen characteristics and high DFI which can be explained by reactive oxygen species (ROS) generated with excessive seminal TGF β 1 that leads to DNA disintegration and therefore to increase sperm DNA damage. However, the exact molecular mechanism of seminal TGF β 1 on spermatid cells inducing ROS production requires further investigation.

Study funding/competing interest(s): Funding by hospital/clinic(s) – University Farhat Hachad Hospital-unit of reproductive medicine.

Trial registration number: NA.

Keywords: growth factors, semen parameters, DNA fragmentation, ART outcome

P-029 Efficacy of follicle-stimulating hormone treatment in male idiopathic infertility: a meta-analysis

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Study question: To comprehensively evaluate whether follicle stimulating hormone (FSH) administration to the male partner of idiopathic infertile couples improves pregnancy rate, both spontaneous and after assisted reproductive technique (ART), using a meta-analytic approach and including controlled clinical trial.

Summary answer: The administration of FSH to infertile men is reported in the literature since 1991, improving fertilisation and pregnancy rate. A significant

increase in pregnancy rate after ART and male treatment with FSH was already shown in several studies, since FSH improves the sperm quality. FSH treatment could improve sperm quality and pregnancy rate in idiopathic infertile men.

What is known already: The Cochrane Collaboration recently estimated the overall effect of FSH treatment of the man in couples attending ART, enrolled in randomised, controlled, clinical-trials. That meta-analysis demonstrated that FSH treatment significantly improves spontaneous pregnancy rate, whereas no improvement of pregnancy rate was observed after ART, using fixed and strict inclusion criteria. They excluded all trials in which the randomization was not provided leading to potential loss of useful information that could help clinicians in their routinely practice.

Study design, size, duration: We conducted a comprehensive literature search for controlled clinical trials in which FSH was administered for male idiopathic infertility, compared with placebo or no treatment. The randomization was not considered as inclusion criterion. We considered studies in which men with idiopathic infertility or subfertility were enrolled, chronically treated with any type of FSH, compared with placebo or no treatment.

Participants/materials, setting, methods: We found 15 controlled clinical studies. Concerning the type of FSH, eight studies included in the meta-analysis used recombinant FSH, whereas seven studies used purified FSH. Pregnancy rate, when evaluated, was considered spontaneous or after ART. Selected trials gave details about 1275 infertile-men, 614 treated with FSH and 661 not-treated.

Main results and the role of chance: Among the 15 studies included, nine studies evaluated the spontaneous pregnancy rate, resulting in an overall improvement of about 4.5 (CI 2.17–9.33 and $I^2 = 0\%$) ($p < 0.001$). Eight studies evaluated pregnancy rate after ART, showing a significant improvement of about 1.60 (CI 1.08–2.37 and $I^2 = 43\%$) ($p = 0.002$). Sub-dividing studies according to the FSH preparations (purified or recombinant), the pregnancy rate improvement remained significant ($p = 0.007$ and $p = 0.002$, respectively). Eleven studies considered sperm quality after FSH treatment, finding a significant improvement of sperm concentration (mean improvement of 2.66×10^6 millions/mL, with CI 0.47–4.84, $p = 0.02$), but not of sperm motility (mean improvement of 1.22×10^6 millions/mL, with CI -0.07–2.52, $p = 0.06$). Finally, three trials evaluated testicular volume, showing a non-significant increase in men treated (mean increase of 1.35 mL, with CI -0.44–3.14, $p = 0.14$).

Limitations, reason for caution: The heterogeneity of studies, together with the high risk of biases in this field of research could limit the strength of these results.

Wider implications of the findings: The results of controlled clinical trials available in literature indicate an improvement of pregnancy rate after FSH administration to the male partner of infertile couples, both spontaneous and after ART.

Study funding/competing interest(s): Funding by University(ies) – University of Modena and Reggio Emilia.

Trial registration number: NA.

Keywords: FSH, idiopathic male infertility, reproduction

P-030 Are obese men subfertile?

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Study question: Does obesity affects male fertility potentials?

Summary answer: BMI was proved, in our study, to have a significant effect on sperm concentration.

What is known already: Overweight and obesity can affect female fertility, in men the negative effects on reproductive system attributed to obesity are less evident and have been less often studied.

Study design, size, duration: Prospective, 439 male partners of couples presenting for evaluation of infertility, in the period from January 2013 to November 2014.

Participants/materials, setting, methods: Semen samples from 439 male partners of couples presenting for evaluation of their infertility, were collected. Men were divided into three BMI groups; normal, overweight and obese. Samples has been analyzed based on the WHO 5th edition guidelines. Meticulous scrotal examination and scrotal ultrasound were performed. Hormonal profile, including testosterone, FSH, LH and prolactin was done to exclude any possible causative hormonal factor.

Main results and the role of chance: Mean BMI was 29.67 ± 5.89 . ANOVA testing revealed no significant differences in semen parameters between the 3 different BMI groups. Also, pair-wise multiple comparisons were found to be