

# 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

C. D. Lynch<sup>1</sup>, R. Sundaram<sup>2</sup>, J. M. Maisog<sup>2</sup>, A. M. Sweeney<sup>3</sup>, G. M. Buck Louis<sup>2</sup>

<sup>1</sup>The Ohio State University College of Medicine, Department of Obstetrics and Gynecology, Columbus, U.S.A.

<sup>2</sup>Eunice Kennedy Shriver National Institute of Child Health and Human Development, Division of Intramural Population Health Research, Rockville, U.S.A.

<sup>3</sup>Texas A&M Health Science Center, Department of Epidemiology and Biostatistics, College Station, U.S.A.

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

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

**What is known already:** Data suggest that stress and reproduction are 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

Moeliker Kees<sup>1</sup>

<sup>1</sup>Natural History Museum Rotterdam, Rotterdam, The Netherlands

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

J. Blazek<sup>1</sup>, M. Large<sup>1</sup>, V. Pham<sup>2</sup>, M. Hughes<sup>3</sup>, T. Gordon<sup>4</sup>

<sup>1</sup>Genesis Genetics, R&D/PGS, Houston, TX, U.S.A.

<sup>2</sup>Fertility Specialists of Houston, Embryology, Houston, TX, U.S.A.

<sup>3</sup>Genesis Genetics, PGS/PGD, Plymouth, MI, U.S.A.

<sup>4</sup>Genesis Genetics, PGS/PGD, London, United Kingdom

**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.

binding globulin (SHBG), oestradiol (E) and inhibin B) in men attending reproductive medicine clinic?

**Summary answer:** Selected endocrine disrupting chemicals (EDCs), as phthalates and their metabolites, can adversely affect the levels of reproductive hormones measured in serum of men attending reproductive medicine clinic, however, there was no evidence confirming the adverse effects of BPA or alkylphenols.

**What is known already:** Reproductive toxicities of BPA, phthalates and alkylphenols have been extensively studied in laboratory animals. A number of recent epidemiologic studies have also suggested toxic effects of BPA and certain phthalates on human male reproductive system, but the results of these are not conclusive, since there is considerable amount of contradicting data. There are no epidemiologic studies to investigate the influence of environmental exposure to alkylphenols on human health.

**Study design, size, duration:** Prospective cohort study included 140 men of subfertile couples seeking fertility treatment at university-based tertiary centre. Data was collected from February 2011 until June 2012. Single-spot urine samples were obtained to measure EDCs and creatinine concentration to account for urinary dilution. Non-fasting blood samples were taken to measure hormone levels.

**Participants/materials, setting, methods:** Gas chromatography/mass spectrometry was used to measure: BPA, nonylphenol (NP), octylphenol (OP), di(2-ethylhexyl)-phthalate (DEHP), dibutyl-phthalate (DBP), diethyl-phthalate (DEP) and their metabolites. Additionally to the measured hormones, free androgen index (FAI), inhibin B/FSH and T/LH ratios were calculated to evaluate Sertoli and Leydig cell function, respectively.

**Main results and the role of chance:** BPA was detected in 98% of all measured samples. Phthalates with their metabolites were detected in >95% of the urine samples, OP was detected in 90% and NP was detected in 79% of samples with 0.3 ng/mL limit of detection. After adjusting for confounding factors using linear regression models, DEHP and its metabolites showed negative association with T (primary metabolite mono-(2-ethyl-5-oxohexyl)phthalate  $b = -0.61$ , 95% CI -1.13 to -0.10), inhibin B (DEHP  $\beta = -12.24$ , 95% CI -23.06 to -1.44) and with inhibin B/FSH ratio (DEHP  $\beta = -0.19$ , 95% CI -0.32 to -0.05). DEP has shown inverse correlation with LH in univariate analysis, however this was insignificant in multivariable model ( $\beta = -0.05$ , 95% CI -0.11 to 0.01). There was no influence of BPA or alkylphenols on any of the observed outcomes.

**Limitations, reason for caution:** Urinary EDC exposure measurements are subject to temporal variability. EDCs can also cause negative reproductive effects by other pathways that are not reflected in altered serum hormone levels. Many studied outcomes provide a potential for incidental correlations.

**Wider implications of the findings:** We have shown that phthalates can adversely influence male reproductive hormone levels. This is in agreement with the previous laboratory and some epidemiologic studies showing negative effects on male reproductive function. BPA and alkylphenols do not seem to have a comparable effect, which suggests their minor relevance at environmentally present concentrations compared to phthalates. Still, our study was comprised of men attending fertility clinic and the results may not be representative of the general population.

**Study funding/competing interest(s):** Funding by national/international organization(s) – Slovenian Research Foundation (P3-334-0327).

**Trial registration number:** NA.

**Keywords:** endocrine disrupting chemicals, bisphenol A, phthalates, alkylphenols, male reproductive hormones

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

S. Manuela<sup>1</sup>, D. Santi<sup>1</sup>, F. Linari<sup>1</sup>, E. Baldi<sup>2</sup>, M. Cambi<sup>2</sup>, A. Ferlin<sup>3</sup>, L. Gandini<sup>4</sup>, P. E. Levi Setti<sup>5</sup>, F. Lombardo<sup>6</sup>, M. Marino<sup>1</sup>, M. Muratori<sup>2</sup>, L. Negri<sup>7</sup>, E. Pignatti<sup>1</sup>, G. Rastrelli<sup>2</sup>, H. M. Behre<sup>8</sup>

<sup>1</sup>Unit of Endocrinology, Department of Biomedical Metabolic and Neural Sciences University of Modena and Reggio Emilia Italy, Modena, Italy

<sup>2</sup>Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy

<sup>3</sup>University of Padova, Department of Medicine Unit of Andrology and Reproductive Medicine, Padova, Italy

<sup>4</sup>Laboratory of Seminology – Sperm Bank, Department of Experimental Medicine University of Rome “La Sapienza”, Rome, Italy

<sup>5</sup>Humanitas Research Hospital, Department of Gynaecology Division of Gynaecology and Reproductive Medicine Humanitas Fertility Center Rozzano, Milan, Italy

<sup>6</sup>Laboratory of Seminology – Sperm Bank, Department of Experimental Medicine University of Rome “La Sapienza”, Rome, Italy

<sup>7</sup>Humanitas Research Hospital, Division of Gynaecology and Reproductive Medicine Humanitas Fertility Center Rozzano, Milan, Italy

<sup>8</sup>Center for Reproductive Medicine and Andrology, University Ernst-Grube-Str. 40, Halle D-06120, Germany

**Study question:** To assess whether in men with idiopathic infertility, the sperm DNA fragmentation (sDF) improves depending on the FSH receptor (FSHR) genotype as assessed by the non-synonymous polymorphisms (SNP) rs6166 (wild type or p.N680S).

**Summary answer:** FSH treatment improves sDF in a subgroup of idiopathic infertile men, although 40% of these men do not show any significant improvement. The response of sDF, a surrogate marker of sperm quality, together with the evaluation of FSHR SNP p.N680S might be useful to predict the response to FSH treatment.

**What is known already:** FSH is fundamental for spermatogenesis and is empirically used to treat male idiopathic infertility. Several studies suggest that sDF could be a candidate predictor of response to FSH treatment, in terms of probability to conceive. Furthermore, it is widely accepted that the FSHR SNP p.N680S influences ovarian response in women and testicular volume in men.

**Study design, size, duration:** Multicenter, longitudinal, prospective, open-label, two-arms clinical trial. Subjects enrolled were idiopathic infertile men and received 150 IU of recombinant FSH (Gonal f®) every other day for 12 weeks and were then followed-up for further 12 weeks after FSH-withdrawal. Patients were evaluated at baseline and at the end of the two phases.

**Participants/materials, setting, methods:** Eighty-eight men with idiopathic male infertility carrier of the homozygous FSHR p.N680S N or S genotype, FSH < 8 IU/L and sDF > 15%, were enrolled. 66 patients completed the sDF analysis. sDF was centrally evaluated by TUNEL/PI assay coupled to flow cytometry, resolving two different sperm populations, namely: Pibrighter and Pldimmer.

**Main results and the role of chance:** Thirty-seven men (56%) were carriers of the p.N680S homozygous-N and 29 (44%) of the homozygous-S genotype, respectively. Total sDF (Pibrighter + Pldimmer) was significantly lower at the end of the study in patients carriers of the p.N680S-N allele than patients carriers of p.N680S-S allele ( $p = 0.008$ ). Only in patients carriers of the p.N680S-N allele, total sDF decreased significantly from baseline to the end of the study ( $p = 0.021$ ) and this decrease was entirely sustained by the sperm population containing vital sperms (i.e., Pibrighter fraction) ( $p = 0.008$ ). Pldimmer fraction, including only non-vital sperms, was significantly higher in patients carriers of the p.N680S-S allele than in carriers of N allele ( $p = 0.018$ ). Total sDF was inversely related to total sperm number ( $p = 0.020$ ) and progressive sperm motility ( $p = 0.014$ ).

**Limitations, reason for caution:** The statistical power of the results obtained so far is 86.9%, with alpha-error 0.05. This is an interim-analysis.

**Wider implications of the findings:** The study suggests that FSH treatment induces a significant improvement of total sDF in men carriers of the p.N680S homozygous N allele. This sDF decrease awaits confirmation, since the study will be completed by June 2015.

**Study funding/competing interest(s):** Funding by commercial/corporate company(ies) – The study was supported by unrestricted grant by Merck Serono.

**Trial registration number:** EudraCT number 2010-020240-35.

**Keywords:** FSH treatment, male infertility, Sperm-DNA fragmentation

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

S. Ma<sup>1</sup>, G. Kirkpatrick<sup>1</sup>, A. H. Ren<sup>1</sup>, T. Liehr<sup>1</sup>, V. Chow<sup>1</sup>

<sup>1</sup>University of British Columbia, Department of Obstetrics and Gynecology, Vancouver, Canada

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