Intensive Care Unit admission in one state (thus unable to include in multivari-
able analysis) and women's weight, a known risk factor among women with PCOS. Additionally we had no data to assess environmental exposure.

Wider implications of the findings: The hormonal profile of women/mothers with PCOS who underwent ART may explain the impact on their health, pregnancy and offspring, compared to women with TO. Prevention strategies targeted towards improving the metabolic and endocrine consequences of PCOS, such as optimizing the health of women with the syndrome prior to conception through changes in life-
style, diet and appropriate vitamin supplementation, should be considered. Doing so could reduce the health risk and economic burden of PCOS.

Study funding/competing interest(s): No funding and competing interest(s) to report

Trial registration number: Not applicable

O-020 The influence of sex steroids on the neuronal correlates of working memory in women with Polycystic Ovarian Syndrome

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Study question: The aim of the study was to investigate specific effects of an over-
production of androgens on brain activity during working memory processes in
women with a Polycystic Ovarian Syndrome (PCOS)

Summary answer: Our main conclusion is that women with PCOS need ad-
ditional resources during a working-memory task, suggesting less efficient execu-
tive functioning with regard to working-memory compared to women without
PCOS

What is known already: Working-memory is a specific cognitive component of
temporal storage and manipulation of information and is needed for multiple pro-
cesses of cognition, such as language, perceptual speed, but also verbal and visual
memory and planning. Possible deficits may affect daily functioning and behav-
ior. This is the first MRI study with a focus to working-memory in women with
PCOS and hyper androgenism.

Study design, size, duration: In this study we used a cross-sectional design
between women with PCOS with hyper androgenism compared to women
without PCOS (controls). In total, we investigated 34 participants (fourteen
women with PCOS and twenty controls with the use of functional MRI, a neuro-
psychological assessment and blood samples were withdrawn.

Participants/materials, setting, methods: Fourteen women with PCOS com-
bined with hyper androgenism and 20 controls underwent functional MRI scan-
ning while performing a working memory task (N-Back). During this task
participants monitored a series of letters and were asked to indicate whether this
letter was also presented n (n = 1, 2 or 3) steps previously.

Main results and the role of chance: Analysis of the blood samples showed
higher levels of androgens in the PCOS group (p = 0.009). The task performance
did not differ between the two groups for mean reaction time nor accuracy of
the answers. However, brain activation patterns did differ significantly between
the two groups. The group with PCOS showed more activation within the superior pari-
etal lobe right and the inferior parietal lobe left during all given N-Back stimuli.
This finding may suggest that women with PCOS need additional resources during
a working-memory task, suggesting less efficient executive functioning compared
to women without PCOS.

Limitations, reason for caution: Because we only wanted to include homoge-
neous groups of participants to reduce the risk of possible confounds, recruit-
ment of which proved to be burdensome.

Wider implications of the findings: This is the first study investigating the spe-
cific effects of PCOS on brain activity. These results suggest less efficient executive
functioning in women with PCOS. Although the changes are rather subtle it should
be investigated more in depth to investigate if the efficiency of brain activity is less
in more cognitive domains. Inefficiency in brain activity of working memory com-
bined with other cognitive deficits may affect daily life functioning in women with
PCOS more.

Study funding/competing interest(s): None

Trial registration number: None

SELECTED ORAL COMMUNICATION SESSION

Session 05: Sperm factors influencing ART outcome

Monday 8 July 2013 10:00 - 11:30

O-021 Does the extent of sperm DNA fragmentation affect IVF or ICSI outcome: a systematic review and meta analysis

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Study question: Does the literature suggest that high sperm DNA damage affect
IVF and ICSI outcome?

Summary answer: High sperm DNA damage detected with SCSA, TUNEL or
COMET assays has been shown to be associated with lower clinical pregnancy
rates in IVF cycles.

What is known already: Sperm DNA damage is found in infertile as well as fertile
men. The relationship between sperm DNA damage and fertilization rate, embryo
quality, pregnancy and miscarriage rates has been evaluated in the literature. It has
been demonstrated that DNA damaged sperm have the capacity to fertilize oocytes
but could be associated with an increase in the miscarriage rate following ART
(assisted reproductive technique).

Study design, size, duration: A systematic review and meta-analysis of studies on
the effect of high sperm DNA fragmentation rate on IVF/ICSI outcome was under-
taken. Medline, Embase and Cochrane library databases were searched from data-
base inception to January 2013. The search was limited to studies published in
English language.

Participants/materials, setting, methods: Study selection and data extraction
were conducted independently by two reviewers. We used ‘sperm DNA damage’,
‘DNA fragmentation’, ‘IVF’, ‘ICSI’, ‘outcome’, ‘pregnancy’ to generate the relevant
citation. Studies with clinical pregnancy outcome (gestational sac or fetal heart on
USG) were included. Revman 5 statistical software was used for analysis.

Main results and the role of chance: The search identified 25 studies involving
3360 couples.

For SCSA assay: Meta-analyses of 14 studies (n = 1621) showed a significant-
ly higher clinical pregnancy rate (CPR) in the group who had a DNA fragmentation
index (DFI) score of <30% (Relative risk (RR) 1.17, 95% CI 1.05-1.30; P =
0.004) compared to the group who had a DFI >30%.

For TUNEL assay: Meta-analyses of 8 studies (n = 1233) showed a signifi-
cantly higher CPR in the group who had a low DNA fragmentation compared to
the group who had a high DNA fragmentation (RR 0.69, 95%CI 0.52-0.90, P =
0.006).

For Comet assay: Meta-analyses of 3 studies (n = 506) showed a significantly
higher CPR in the group who had a low DNA fragmentation compared to the group
with high DNA fragmentation (RR 1.16, 95%CI 1.03-1.30, P = 0.01).

Limitations, reason for caution: There has been several assays described in the
literature to define low and high DNA fragmentation. The diagnostic accuracy of
these assays has been widely debated. There is inconsistency in defining the
threshold for high DNA fragmentation test. The studies included were heteroge-
neous and not adequately powered.

Wider implications of the findings: Increased sperm DNA fragmentation may
detrimental effect on IVF outcome. Adequately powered studies with strict
inclusion criteria to further evaluate the effect of DNA fragmentation testing in
IVF and the role of antioxidants as well as IMSI (intracytoplasmic mor-
phologically selected sperm injected) as treatment options are needed.

Study funding/competing interest(s): None

Trial registration number: None
**O-022** Novel detection of intratubular germ cell neoplasia in non-obstructive azoospermia using OCT3/4 and PLAP in AgarCytos of testicular sperm extraction specimens

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**Study question:** Can we diagnose intratubular germ cell neoplasia (IGCN) using the immunohistochemical markers placental-like alkaline phosphatase (PLAP) and OCT3/4 on AgarCytos, made of the remnants of the testicular sperm extraction (TESE) specimen, and what is the incidence of such (pre)malignancies in men with non-obstructive azoospermia (NOA) undergoing TESE for fertility treatment?

**Summary answer:** For the first time, IGCN can be successfully detected by immunohistochemical evaluation of AgarCytos, made of the remnants of TESE biopsies. The observed incidence of a germ cell (pre)malignancy in this specific population was 4.4%.

**What is known already:** Infertile men are at higher risk for testicular cancer compared to the general population. IGCN can be detected by immunohistochemistry in standard testicular biopsies and, albeit less accurate, in semen using PLAP and OCT3/4.

**Study design, size, duration:** Between January 2011 and April 2012 a prospective cohort study was conducted at a Dutch tertiary care academic training hospital. All males with NOA (n = 182) undergoing a urological work-up followed by a diagnostic TESE (n = 251) for fertility treatment were included.

**Participants/materials, setting, methods:** After cryopreservation of sperm, if present, an AgarCyt was made of the remnants of these TESE biopsies. Sections were stained with haematoxylin-eosin for pathological examination as well as PLAP and OCT3/4 for immunohistochemistry to detect IGCN.

**Main results and the role of chance:** Eight men (4.4%) were diagnosed with a germ cell (pre)malignancy: six with seminoma, of which two without and four with concomitant IGCN, and two with IGCN only. Microscopic evaluation including immunohistochemical analysis of the AgarCytos diagnosed three (1.6%) more cases of a germ cell (pre)malignancy compared to scrotal ultrasound alone (one case of bilateral seminoma with concomitant IGCN and two cases of IGCN alone). No false-positive cytology results were found upon conventional histological evaluation.

**Limitations, reason for caution:** The main limitation of this study is lack of a simultaneously taken standard testicular biopsy, for comparison with our novel diagnostic method. Nevertheless, in all but one of our cases orchidectomy followed and the diagnosis was confirmed by histology, in the remaining case repeat TESE showed similar results.

**Wider implications of the findings:** Men undergoing TESE because of NOA should be offered simultaneous screening for IGCN because of the increased incidence of germ cell (pre)malignancies in this specific population. The principal advantage of our new method is that all available testicular tissue can be used for both sperm recovery and pathological evaluation, increasing the yield of spermatozoa as well as the chance to find (pre)malignant cells.

**Study funding/competing interest(s):** This study was (partially) funded by Merck Sero (Schiphol-Rijk, the Netherlands), but there are no conflicting interests to disclose.

**Trial registration number:** N/A

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**O-023** Evaluating the etiopathogenic factors of DNA damage in sperm with large nuclear vacuoles

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**Study question:** Is there a relationship between the etiopathogenic factors of DNA damage and the presence of large nuclear vacuoles (vacuoles occupy >50% of the nuclear area) in sperm?

**Summary answer:** Our results support the idea that the presence of large nuclear vacuoles in sperm could reflect molecular anomalies, such as abnormal chromatin packaging (underprotamination), mitochondrial damage and apoptosis.

**What is known already:** There is strong clinical evidence supporting an association between increased DNA damage in sperm and abnormalities in several characteristics of semen. Several studies have linked the presence of large nuclear vacuoles (LVN) and increased DNA damage in sperm based on high magnification analyses. Different mechanisms may explain the presence of damaged DNA in human spermatozoa, including abnormal chromatin packaging (underprotamination), mitochondrial damage and apoptosis.

**Study design, size, duration:** A cross-sectional study of semen samples obtained from 608 men who underwent infertility evaluations was conducted from August 2010 to August 2012. Percentages were treated as continuous variables. Correlation analyses were performed using Spearman’s rank test.

**Participants/materials, setting, methods:** At least 200 spermatozoa per evaluation were examined from each semen sample. The percentages for the following groups were determined:
- Normal and LVN spermatozoa by motile sperm organelle morphology examination (MSOME/15,000x magnification);
- Spermatozoa with abnormal chromatin packaging (chromomycin A3);
- Spermatozoa with mitochondrial damage (MitoTracker Green);
- Spermatozoa in apoptosis (positive for annexin-V).

**Main results and the role of chance:** Regression analysis revealed that abnormal chromatin packaging, mitochondrial damage and apoptosis were positively correlated with the percentage of LVN spermatozoa. Conversely, regression analysis revealed that abnormal chromatin packaging and mitochondrial damage were negatively correlated with the percentage of normal sperm. A significant relationship with apoptosis was not observed (Table 1).

**Limitations, reason for caution:** This descriptive study was based on in vitro evaluations. Cross-sectional analyses do not allow inferences regarding directionality between observed associations. Therefore, determining the cause and effect between variables is not possible.

**Wider implications of the findings:** Based on the clinical and laboratory findings regarding the repercussions of possible DNA damage in offspring, these data indicate that the use of spermatozoa with LVN should be avoided to obtain embryos with higher implantation and development potential.

**Study funding/competing interest(s):** The authors declare that they have no competing interests.

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

**O-024** Punalpin® increases the numbers of motile sperm in men with reduced semen quality: a prospective, randomized, controlled, double-blinded trial

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**Study question:** Does oral consumption of tablets containing standardized amounts of greater galangal (Alpinia galanga) and extract of pomegranate (Punica granatum) increase the total number of motile spermatozoa?

**Summary answer:** After three months of daily intake of Punalpin® consisting of tablets containing standardized amounts of rhizome of greater galangal and extract of pomegranate fruit skin the total number of motile spermatozoa was significantly increased compared to placebo.
What is known already: Juice from pomegranate fruit has been shown to stimulate spermatogenesis and to increase sperm motility in rats after seven weeks of gavage (Türk et al. 2008), and increased sperm counts and motility have been shown in mice, which were given galangal extract for 90 days (Qureshi et al. 1992). Within traditional medicine, pomegranate fruit has long been used to increase fertility, however studies on the effect on spermatogenesis in humans have never been published.

Study design, size, duration: The study was designed as a prospective, randomized, controlled, double-blinded trial. Seventy men with base-line total motile sperm less than 40 million were randomized to take tablets corresponding to approximately 3.5 g fresh galangal rhizome and 1500 ml pomegranate fruit juice or placebo daily for three months.

Participants/materials, setting, methods: Participants were recruited from an advertisement in Nordic Cryobank and local newspapers. Enrollment was based on mean total number of motile spermatozoa of two ejaculates (with 4-10 days of abstinence). The participants delivered an ejaculate after 4-8 days of tablet-intake and two ejaculates just before they stopped taking the tablets.

Main results and the role of chance: From baseline to after three months of Punalpin® intake corresponding to a daily dose of 11 mg ellagic acid, 100 mg punicalagin A, 265 mg punicalagin B (from pomegranate) and 16 mg 1’-acetoxychavicol acetate (from galangal) the average total number of motile sperm increased with 62% (from 23.4 millions to 37.8 millions), while for the placebo group the number of motile sperm increased with 20% (from 19.9 millions to 23.9 millions). This difference in increase between the study and placebo groups was statistically significant (p = 0.026). There was no increase in number of motile sperm after one week of tablet consumption for either of the groups, indicating that the pomegranate and galangal preparations exert their positive effect during spermatogenesis rather than on epididymal sperm.

Limitations, reason for caution: It cannot be expected that every participant take 100% of the tablets for the three-months period. The counting of the remaining tablets of all bottles showed that more than 85% of the participants had taken more than 80% of the tablets.

Wider implications of the findings: Our findings may help subfertile men to gain more that 80% of the tablets. Almost one half the MII oocytes were selected sperm injection (IMSI) with ejaculated spermatozoa was significantly higher than that by ICSI. However, most of these thresholds are arbitrarily determined and not evaluated.

Study design, size, duration: This is a prospective observational bicentric study setting in two public assisted reproductive technologies (ART) units in France between January and July 2012. A Spearman correlation test was performed to validate MSOME reproducibility between ART units. Normal morphology rate and MSOME done on selected spermatozoa were respectively determined according to David’s and Vanderzwalmen’s classifications the day of ICSI. ROC curves analysis was performed to determine thresholds associated with the occurrence of a pregnancy.

Main results and the role of chance: We observed an excellent correlation between the two operators (r = 0.98) validating MSOME data of the study. Percentages of normal morphology grade spermatozoa using standard classification and first-best morphology grade spermatozoa determined by MSOME were not significantly associated with (i) clinical pregnancy (p = 0.58; 0.05/ Area under curve (AUC) = 0.532; 0.507), (ii) fertilization (p = 0.88; 0.90), (iii) top quality embryo (p = 0.27; 0.98) and (iv) good quality embryo rates (p = 0.73; 0.98), respectively.

Limitations, reason for caution: This study is limited by its statistical power which is not very high due to the sample size (111 ICSI cycles, 41 clinical pregnancies).
Wider implications of the findings: There is a long time controversy about the meaning of sperm morphology in ART practices and now with the ultrastructure morphology assessed by MSOME. This study tries to give an answer in this decade-old controversy. Because there is no threshold of sperm head normalcy associated with the occurrence of a pregnancy, standard morphology evaluation and/or MSOME should not be used to predict ICSE outcome.

Study funding/competing interest(s): authors declare no funding or competing interests.

Trial registration number: No Trial registration number.

SELECTED ORAL COMMUNICATION SESSION

Session 06: Endometriosis

Monday 8 July 2013 10:00 - 11:30

O-027 Endometriosis symptom variation by mutually adjusted demographic characteristics in a cohort of 1000 women

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Study question: Are demographic characteristics associated with the presentation of pain symptoms (including severe dysmenorrhea, severe acyclic pelvic pain, intestinal symptoms, cyclic urinary symptoms, deep dyspareunia) inpatients with endometriosis?

Summary answer: Age, race, education, and age at menarche were significant predictors, independent of each other, of severe dysmenorrhea. Severe acyclic pelvic pain was significantly associated with age, education, marital status, and parity. Intestinal symptoms were significantly associated with race and age at menarche.

What is known already: Severe dysmenorrhea is the symptom most commonly related to the reduced quality of life in patients with endometriosis. Although the literature has focused on quantifying pain symptoms associated with endometriosis, little is known about the distribution and variation of these symptoms by the patient's demographic characteristics.

Study design, size, duration: This prospective cohort study from 1998-2010 of 1002 patients (median age 33; range 29-38) with laparoscopic and histological confirmation of endometriosis who were unsuccessful treat by medication.

Participants/materials, setting, methods: Relative risks(RR) and 95%-confidence intervals(CI) from multivariable Poisson regression models adjusted for number of disease locations(1,2,3,4,5+), age at surgical diagnosis(14-24;25-29;30-34;35-39;40+), race (white;black;Asian), educatio(n (elementary;high school;university), marital status (single;married;partnered;divorced/widowed), age at menarche(<11;12-13;14+), and parity(0;1-2;3+). This mutual adjustment yields associations independent of the other characteristics investigated.

Main results and the role of chance: Patients aged 14-24 (RR = 1.27,CI = 1.06-1.51) and 25-29 (RR = 1.18,CI = 1.04-1.35) were more likely to report severe dysmenorrhea compared to patients 30-34 at diagnosis. Black race compared to white (RR = 1.20,CI = 1.06-1.36), elementary education compared to college (RR = 1.18,CI = 1.02-1.36), and ≤11 years old at menarche compared to 12-13 (RR = 1.15,CI = 1.04-1.28) were also significantly associated with greater risk of severe dysmenorrhea. Increased risk of severe acyclic pelvic pain was found among younger patients (RR = 1.57,CI = 1.05-2.35) for those aged 14-24 versus 30-34) and parous patients (RR = 1.47,CI = 1.15-1.87 versus nulliparous). Black race as compared to white (RR = 1.40,CI = 1.07-1.84) and younger age at menarche (RR = 1.26,CI = 1.03-1.55 for ≤11 versus 12-13) were associated with increased risk of intestinal symptoms. No significant associations were found between demographic characteristics and cyclic urinary symptoms or deep dyspareunia.

Limitations, reason for caution: Due to missing data, we were only able to consider body mass index, smoking, physical activity, and family history of endometriosis as predictors of pain on a subset of patients, reducing power and potentially generalizability for these additional demographic characteristics.

Wider implications of the findings: Not only do pain symptoms vary among women with endometriosis, these symptoms may be more common with specific demographic characteristics such as age at diagnosis, education, race, parity, and age at menarche. This variation may lend insight into disease etiology or patterns of diagnostic delay/bias.

Study funding/competing interest(s): There was no fundingsupport for this project.

Trial registration number: This was no a registered trial

O-028 Interleukin-8 in follicular fluid: a promising prognostic marker for IVF success in women with endometriosis

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Study question: Altered expression of intra-follicular cytokines and angiogenic molecules is known to influence oocyte and embryo quality in women undergoing in vitro fertilization (IVF). The objective of the present study is to explore which molecule plays the most significant role in affecting the IVF outcome in women with endometriosis.

Summary answer: Interleukin-8 (IL-8) in follicular fluid is identified as the key molecule which plays the main contributory role in determining oocyte quality, fertilization rate and embryo competence in women with endometriosis undergoing IVF.

What is known already: A broad range of cytokines and angiogenic factors present in the follicular fluid are involved in follicular development. What still remains unknown is which particular marker, out of these molecules, most significantly affects IVF outcome.

Study design, size, duration: 200 women with advanced endometriosis (study group) and 140 normal ovulating women with tubal infertility (controls), aged 26-40 years, undergoing IVF were recruited during February 2011-December 2012 for this prospective case-controlled study. Follicular fluid samples were collected at the time of oocyte retrieval from all the patients.

Participants/materials, setting, methods: Cytokines and angiogenic factors were measured using ELISA. Multivariate statistical analyses were used to find the principal factor as predictive marker for IVF outcome. ROC curve was applied to determine the optimum cut-off point for identifying the best oocytes in women with endometriosis. The study was conducted at our institute.

Main results and the role of chance: Significant increase in levels of cytokines (pro-inflammatory: IL-1β, TNF-α, IL-2,8,12, IFN-γ and anti-inflammatory: IL-4,6,10) and angiogenic molecules (VEGF, adrenomedullin and angiogenin) were observed in women with endometriosis, compared to controls. Endometriosis cases could be well-differentiated from controls based on PCA and PLS-DA analysis. Further, IL-8 has been identified as the strongest contributing factor and a potential marker for IVF outcome in endometriosis. IL-8 negatively correlated with oocyte quality (r = -0.67, P < 0.001), fertilization rate (r = -0.54, P < 0.001) and embryo grading (r = -0.58, P < 0.001). ROC curve of IL-8 indicated a threshold level of 18.7 pg/ml(90.0% sensitivity, 85.7% specificity and AUC 0.872). This marker could identify the best quality oocytes in women with endometriosis. There is little role of chance, as most of the studies had similar outcomes.

Limitations, reason for caution: Intra-follicular IL-8 as a marker, apart from endometriosis, also warrants validation in other infertility cases. Multivariate techniques are complex and should be used with adequate interpretation skills.

Wider implications of the findings: IL-8 holds promise to be a potential marker for non-invasive assessment of oocyte quality that needs to be evaluated to select the oocytes with high fertilization potential, and hence the embryo with high developmental and implantation potential for transfer.