Introduction: In recent years, evidence is accumulating in favour of the notion that endometriosis may (also) be an epigenetic disease. The most studied epigenetic mechanism is DNA methylation. The patterns of DNA methylation are established and maintained by enzymes called DNA methyltransferases (DNMTs). DNA methylation typically works in concert with histone modifications, controlling the chromatin structure by determining accessibility of the chromatin for transcription factors, and hence, gene expression. The crosstalk between DNA methylation and chromatin remodelling is established by methyl-CpG-binding domain proteins (MBDs). In the present study, we have assessed expression levels of DNMTs and MBDs in (i) normal endometrium throughout the menstrual cycle and (ii) in eutopic and ectopic endometrium of women with endometriosis. Furthermore, the effect of treatment with female sex steroid hormones on expression levels of DNMTs and MBDs was investigated in endometrial explant cultures.

Material and Methods: *Cyclic endometrium and explant cultures:* Endometrial tissue was collected from 24 women of 26-52 years of age with regular menstrual cycles who underwent surgery for benign indications other than endometriosis. Biopsies were obtained during the menstrual (n = 3), proliferative (n = 8) and secretory (n = 13) phase of the cycle. Proliferative phase samples (n = 8) were cultured in the presence of estrogen (E2) or a combination of estrogen and progesterone (E2 + P) for 24 hours. *Matched eutopic and ectopic endometrium of endometriosis patients:* Tissue samples of endometrium were obtained during the proliferative phase of the menstrual cycle at the time of laparoscopic surgery for infertility with or without pelvic pain in 34 women, including women with laparoscopically confirmed absence of endometriosis (n = 20) and women with laproscopically and histologically confirmed endometriosis (n = 14). In each patient with endometriosis, one lesion was excised for research purposes and used in this study. RNA was isolated from all tissue samples followed by cDNA synthesis and quantitative real-time PCR to determine expression levels of DNMT1, DNMT2, DNMT3B, MBD1, MBD2 and MeCP2.

Results: The relative expression levels of DNMT1 and MBD2 were significantly higher in secretory phase endometrium compared to proliferative endometrium ($p = 0.02$ and $p = 0.004$, respectively) and menstrual endometrium ($p = 0.009$ and $p = 0.018$, respectively). Mimicking the hormonal milieu in the secretory phase by treatment with E2 + P resulted in significant upregulation of expression levels of DNMT1 and MBD2 ($p = 0.021$ and $p = 0.038$, respectively). This effect was not observed after treatment with E2 alone. Furthermore, expression levels of DNMT1 and MBD2 were significantly higher in eutopic endometrium of controls compared to eutopic endometrium of endometriosis patients ($p = 0.007$ and $p = 0.001$, respectively) and ectopic endometrium ($p = 0.001$ and $p = 0.0002$, respectively). MeCP2 expression levels were significantly higher in eutopic endometrium of controls compared to eutopic endometrium of cases ($p = 0.02$), whereas expression levels of MBD1 were significantly lower in ectopic endometrium compared to eutopic endometrium of cases ($p = 0.017$) and controls ($p < 0.0001$)

Conclusion: Expression levels of DNMT1 and MBD2 are significantly higher in secretory phase endometrium compared to proliferative and menstrual phase endometrium. Concordantly, cultivation of proliferative phase endometrium with E2 + P results in increased expression levels of both proteins, suggesting hormonal regulation of these genes. Moreover, significant differences in DNMT and MBD expression are observed between patients with endometriosis and disease-free controls, providing support for the notion that endometriosis is indeed, in part, an epigenetic disaese.

SELECTED ORAL COMMUNICATION SESSION

SESSION 18: MISCARRIAGE: TREATMENT AND PROGNOSTIC FACTORS

Monday 28 June 2010 **15:15 - 16:30**

O-069 Aspirin alone or combined with low-molecular-weight heparin in women with unexplained recurrent miscarriage, a randomised placebo-controlled trial

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Introduction: Unexplained recurrent miscarriage (RM) is extremely stressful for women and effective treatment is eagerly awaited. Aspirin and (low-molecular-weight) heparin are being used increasingly, even though evidence to support its use is not available. In the ALIFE study we investigated whether aspirin combined with low-molecular-weight heparin or aspirin alone, compared to placebo, improves the live birth rate in women with unexplained recurrent miscarriage.

Methods: We conducted a multicenter, randomised, placebo-controlled trial in 8 centres in The Netherlands. Women aged between 18 and 42 years were eligible if they were diagnosed with unexplained RM and attempted to conceive or were less than 6 weeks gestational age. RM was defined as at least 2 miscarriages with an upper gestational age of 20 weeks (calculated from the first day of the last menstrual period). Unexplained RM was diagnosed in case of normal parental karyotypes of both partners, the absence of uterine pathology on pelvic ultrasound, absence of antiphospholipid syndrome (lupus anticoagulant and anticardiolipin IgG and IgM), and a normal fasting level of homocysteine (< 16 umol/L). Exclusion criteria were previous venous or arterial thromboembolism, an indication for anticoagulant treatment during pregnancy or endocrine disorders.

After written informed consent was obtained, randomisation was performed centrally with stratification for age (< or ≥ 36 years), number of previous miscarriages (2 or ≥ 3), and centre.

Oral medication was started at the day of inclusion, either preconceptionally or less than 6 weeks gestational age. Aspirin was given as calcium carbasalate 100 mg (Ascal®, Vemedia BV, Diemen, The Netherlands), equivalent to 80 mg of acetylsalicylic acid. Aspirin and placebo study medication was packed in sachets of identical appearance. Patients, doctors, and trial nurses were blinded for these treatment allocations. Women allocated to receive open-label nadroparin 2850 IU s.c. received oral aspirin and started nadroparin injections when a viable intrauterine pregnancy was confirmed by ultrasound from 6 weeks of gestational age. Aspirin or placebo was given until 36 weeks gestational age or stopped at time of miscarriage, ectopic pregnancy or premature delivery. LMWH was given throughout gestation and stopped 12 hours before delivery.

The primary outcome measure was live birth rate. Secondary outcomes included miscarriage rate, obstetric complications, maternal and foetal safety. Differences in live birth rates will be expressed as absolute risk differences and relative risks, with their 95% confidence intervals, with the placebo group as reference. Data were analyzed according to the intention-to-treat principle.

Results: Between February 2004 and January 2008, 364 women were included in the trial.

The live birth rate did not differ between women assigned to aspirin combined with nadroparin (54.5%, absolute risk difference -2.6%, 95%CI -15.0 to 9.9; relative risk 0.96, 95%CI 0.76 to 1.19), aspirin alone (50.8%, absolute risk difference -6.2%, 95%CI -18.8 to 6.4; relative risk 0.89, 95%CI 0.71 to 1.13) and placebo (57.0%, reference). In 299 women who became pregnant, the live birth rates were 69.1% (absolute risk difference 2.1%, 95%CI -10.8 to 15.0;

relative risk 1.03, 95%CI 0.85 to 1.25), 61.6% (absolute risk difference -5.4%, 95%CI -18.6 to 7.8; relative risk 0.92, 95%CI 0.75 to 1.13), and 67.0% (reference) respectively. Side effects, most notably skin reactions, occurred more often in women assigned to aspirin and nadroparin.

Conclusions: Aspirin combined with nadroparin and aspirin alone did not improve live birth rate relative to placebo in women with unexplained recurrent miscarriage. (Current Controlled Trial number, ISRCTN58496168)

Funding: ZonMW, the Dutch Organization for Health Research and Development (945-27-003). Meda Pharma BV supplied study medication (calcium carbasalate and placebo). GlaxoSmithKline BV offered a grant. Funding sources were not involved in study protocol preparation, trial management or data analysis.

O-070 Low-molecular weight heparin: an effective therapy in the management of hyperhomocysteinemia-associated recurrent spontaneous miscarriage

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Introduction: The management of recurrent spontaneous miscarriage (RSM) still remains a great challenge. It is generally accepted that women with polycystic ovarian syndrome (PCOS) are at greater risk of spontaneous abortion. Few recent studies indicate an association between high level of homocysteine and RSM, especially in insulin resistant (IR) PCOS women. It is possible that high level of homocysteine causes placental thrombosis leading to RSM. Treatment with low-molecular-weight heparin (LMWH) has become an accepted treatment option for women with RSM; however, the subgroup of women with history of RSM likely to respond to LMWH has not been precisely identified. With this background, the objective of the present study was to evaluate the efficacy of LMWH (Fragmin) on pregnancy outcome in a group of women with RSM either with or without PCOS, insulin resistance and/or hyperhomocysteinemia.

Material and Methods: This randomised study was conducted at Institute of Reproductive Medicine, Kolkata, India. A total of 756 women with history of 2 or more consecutive first trimester abortions were screened and 156 were selected for the study. The exclusion criteria included age >40 years, uterine anomalies, specific chromosomal defects, hypothyroidism, hyperprolactinemia and antiphospholipid syndrome. The presence or absence of PCOS, as judged by clinical, biochemical and ultrasound observations, was the initial dividing criteria between 2 groups in the selected population, while subsequent stratification was based on hyperhomocysteinemia. IR was evaluated by oral glucose tolerance test and PP levels of insulin. Total homocysteine levels were measured by automated chemiluminescence immunoassay. LMWH at a prophylactic dose of 2500 IU sc everyday in concomitant with low dose acetylsalicylacid (ASA 75mgm/day) was administered to all subjects after foetal cardiac activity was observed by USG and continued up to 12 weeks of gestation. Like in their previous unsuccessful pregnancies, all patients also received intravaginal micronised progesterone (100mgm) twice daily. Women diagnosed with IR had continued treatment with metformin at a dose of 500mg/day. Live birth rate was the only outcome measure.

Results: Of the 156 selected subjects, 75 had bilateral PCOS, while 81 women were unexplained cases of RSM. The plasma homocysteine concentration (mean + SEM) was significantly ($P < 0.04$) higher in the PCOS group (12.12 ± 0.87μmol/l) as compared to that of the non-PCOS group (10.09 + 0.57μmol/l), and hyperhomocysteinemia was documented in a greater segment of the PCOS population (PCOS: 57.33% vs. non-PCOS: 23.5%). The overall rate of successful term pregnancy in the PCOS group (72%) was higher than that of non-PCOS group (59.2%). However, the rates of pregnancy salvage were comparable between the hyperhomocysteinemic populations of both groups (PCOS: 83.7% *vs.* non-PCOS: 78.9%), which was significantly higher than that of the respective normohomocysteinemic segments (PCOS: 56.3% *vs.* non-PCOS: 53.2%). Regardless of body mass index, insulin resistant PCOS patients had significantly higher ($p < 0.0288$) plasma homocysteine level (13.27 ± 0.87μmol/l) than that of the non-IR PCOS patients (9.6 ± 1.43μmol/l). PCOS group when subdivided keeping homocysteine level as the decisive factor, the incidence of IR was found to be preponderant (72.1%) in the hyperhomocysteinemic segment as compared with 25% in the normo-homocysteinemic subgroup. Nevertheless, uneventful term pregnancy rates were comparable between the IR (83.8%) and non-IR (83.3%) population of the hyperhomocysteinemic PCOS subgroup; and no correlation was evident between IR and the therapeutic response to LMWH with respect to pregnancy salvage.

Conclusion: With regard to prevention of pregnancy loss in our RSM cohort, LMWH conferred added benefit to those with hyperhomocysteinemia irrespective of presence or absence of PCOS and/or IR. Since significant placental thrombosis leading to miscarriage is reported to be rare beyond first trimester, duration of treatment with Fragmin was restricted only up to 12 weeks to avoid untoward side effects.

O-071 "How long should we wait?" effect of inter-pregnancy interval on outcomes of pregnancy following miscarriage

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Introduction: Based on a single large scale study set in Latin America, current WHO guidelines recommend a minimum interpregnancy interval of 6 months for optimal reproductive outcomes following miscarriage. There is however, no evidence to support this in developed countries, where access to health care and abortion legislation are very different.

Objective: To determine the optimum interpregnancy interval (IPI) following miscarriage in a first pregnancy in a population of women living in Scotland, UK.

Material and Methods: After receiving approval from the Privacy Advisory Committee of the Information and Services Division, National Health Service, Scotland, data were extracted on the first two pregnancies occurring in Scotland between 1981 and 2000.

Study Design Population based retrospective cohort study

Setting Scottish hospitals between 1981 and 2000

Participants Women (n = 30.937) who had a miscarriage in their first pregnancy and then had a subsequent pregnancy recorded in the Scottish Morbidity Registers (SMR).

Main outcome measures: Miscarriage, live birth, termination, stillbirth, or ectopic second pregnancy. Rates of caesarean and preterm delivery, low birthweight, pre-eclampsia, placenta praevia, placental abruption or induced labour in a subsequent continuing pregnancy.

Results: Compared with IPI of 6 to 12 months, women with an IPI of < 6months were less likely to have another miscarriage {adj. OR 0.79, 95%CI 0.73.0.87)}, termination of pregnancy {adj. OR 0.49, (95%CI 0.42.0.57)} and ectopic pregnancy {adj. OR 0.51(95%CI 0.35, 0.73)}. Women with IPI >24 months were more likely to have an ectopic second pregnancy {adj. OR 1.72(95% CI 1.24.2.38)} or termination {adj. OR 2.18 (95% CI 1.89.2.52)}. Those with a live birth in the second pregnancy and IPI < 6months, were less likely to experience caesarean {adj OR 0.91 (95% CI 0.84.0.98)}or preterm delivery {adj. OR 0.89 (95% CI 0.81.0.98)} of low birth weight babies{adj. OR 0.84 (95% CI 0.72.0.99)}., but more likely to have labour induced {adj. OR 1.14(95%CI 1.05, 1.23)} compared to IPI of 6 to 12 months.

Conclusions: Women who conceive within 6 months of an initial miscarriage have the best outcomes and lowest complication rates.

O-072 Is pre-clinical pregnancy loss a ëcauseí of unexplained infertility?

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Introduction: Early pre-clinical pregnancy loss (EPL) occurring a few days post-implantation accounts for up to 25 % of pregnancy losses in the normal population. We hypothesised that a proportion of women with 'unexplained infertility'may present with subfertility because their pregnancies fail before they are clinically recognized. If this were true, then the incidence of EPL would be higher in women with unexplained infertility. By studying the pattern of elevation and decline of human chorionic gonadotropin (hCG) levels around the time of implantation we examined the incidence of EPL in patients with unexplained infertility compared with that observed in healthy volunteers.
Material and Methods: 60 patients with unexplained infertility and 60 normal, healthy controls with regular menstrual cycles who were trying to conceive, were recruited to participate in this prospective cohort study. 3 cycles were analysed intentionally for both groups, providing sufficient statistical power to detect a 25% increase in the incidence of EPL in the study group Starting on cycle day 10, a daily home LH ovulation predictor test (Ovulady, Clindia Benelux, The Netherlands) was performed to detect the LH-surge in urine. All participants were asked to collect and freeze first morning urine samples daily from the day on which the LH surge was detected, until the following menstruation or a positive pregnancy test.

Following collection and thawing, urine samples were analysed in the Immunolite 1000® immunoanalyser (Siemens, the Netherlands) for hCG levels. In order to correct for variations in urinary concentration, hCG levels were corrected for creatinine concentrations. In order to produce a reference curve of the luteal level of urinary hCG in the absence of implantation, daily urine samples were similarly collected from 12 regularly cycling women who could not conceive.

Implantation was determined by two methods; first the rise of hCG above the highest level in the references for three consecutive days ('Wilcox' method) and secondly a linear mixed model on logarithmically transformed hCG/creatinine data. Early pregnancy loss was defined as implantation without subsequent pregnancy. An ongoing pregnancy was defined by a positive urine pregnancy test followed by positive heartbeat on ultrasound at nine weeks amenorrhoea.
Results: In the 133 cycles of 60 women with unexplained infertility for which complete urine sets were available, just one implantation was detected with both methods, and became an ongoing pregnancy. In contrast in the 107 completely collected cycles of 48 control patients, 30 implantations were detected. Analysis of urinary hCG profiles using the linear mixed model indicated that 24 clinical pregnancies and 6 cases of EPL occured. The difference in EPL between patients and controls was –0.20 (95% CI: -0.58 to 0.66).5 of these 6 implantations were also identified with the Wilcox method including all the ongoing pregnancies. In the subsequent cycle, half the patients who experienced EPL became clinically pregnant.
Conclusions: Our data do not support the hypothesis that recurrent EPL may be an underlying 'cause' of unexplained infertility. EPL is often followed by a vital pregnancy in the subsequent cycle, and is as such a positive predictor of subsequent fecundity. Post-implantation failure is unlikely to contribute significantly to subfertility. Further research should focus on the pre-implantation period if the causes of 'unexplained' infertility are to be further elucidated.

O-073 Influence of BMI on miscarriage rate after elective single blastocyst transfer

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Introduction: Although evidence suggests that increased Body Mass Index (BMI), defined by the World Health Organization as a BMI of ≥ 25 kg/m^2, increases the risk of miscarriage after spontaneous conception, there is insufficient evidence to describe the effect of increased BMI on the outcome of pregnancies occurring after assisted reproductive technology (ART). Most of the available studies assess the outcome of ART in relation to BMI following transfer of multiple embryos at various stages of development and without adjusting for important confounders. The purpose of this study was to assess the effect of BMI on miscarriage rate in women conceiving following elective single blastocyst transfer (eSBT) in IVF/ICSI cycles after controlling for confounding factors.
Material and Methods: All pregnancies occurring after eSBT between April 2006 and October 2009 were included in our analysis. Patients were divided into two groups according to their BMI at the start of the treatment cycle, group 1 had a BMI of 18.5-24.9 and group 2 had a BMI of 25 or above. Confounding variables studied included female age, duration of infertility, cause of infertility, smoking status, ovarian reserve, history of previous miscarriage, method of oocyte fertilisation and grade of blastocyst replaced according to Gardner's grading system. Miscarriage was defined as pregnancy loss before 20 weeks of gestation. Oocyte donation and PGD cycles were excluded.
Results: A total of 318 women conceived following eSBT in fresh (n = 255) and cryo-thawed (n = 63) IVF/ICSI cycles. Among these, 185 (58%) had a BMI of 18.5-24.9 (mean = 21.7, group 1) and 133 (42%) had a BMI of 25-35 (mean = 28.2, group 2). Only 14% of group 2 women (19/133) were obese (defined as BMI ≥ 30). Overall, 26% (85/318) of women miscarried before 20 weeks gestation. The miscarriage rate was significantly lower in group 1 compared with group 2 (22% (41/185) vs 33% (44/133), OR = 1.74, 95% CI = 1.1-2.9, P = 0.03). After adjusting for confounding variables, having a BMI of 25 or above more than doubled the risk of miscarriage before 20 weeks gestation (adjusted OR = 2.3, 95% CI = 1.2-4.4, P = 0.01).
Conclusions: This study clearly demonstrates that increased BMI is independently associated with higher miscarriage rate after IVF/ICSI treatment. This information should be included in counselling of patients prior to ART treatments.

SELECTED ORAL COMMUNICATION SESSION

SESSION 19: REPRODUCTION AND GENETICS

Monday 28 June 2010 **15:15 - 16:30**

O-074 Increased incidence of mosaicism detected by FISH in murine blastocyst cultured in vitro

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Introduction: It is now well accepted that the majority of *in-vitro* derived human preimplantation embryos are chromosomally abnormal. Whether the same pattern exists in human embryos *in-vivo* is unknown. This would be impossible to demonstrate in humans. Hence we chose murine embryos to study this difference owing to their ease of manipulation. The aims of the study were set as follows (1) to establish if murine embryos (MF1 strain) could be used to study aneuploidy (2) comparing the incidence of mosaicism between the *in-vivo* and *in-vitro* cultured embryos.
Material and Methods: Dual-colour FISH was developed on murine embryonic nuclei. Two groups of embryos were analysed: Group A (*in-vitro*) were obtained 48 hours following superovulation and mating of the female mice and cultured *in-vitro* until the blastocyst stage. FISH was performed at different stages that included the cleavage, morula and blastocyst stage. Group B (*in-vivo*) were obtained on day 5 and FISH was performed immediately without culture.
Results: There was an increase in chromosomal mosaicism seen from the 2-cell stage up to the blastocyst stage in the *in-vitro* cultured group. Overall chromosomal abnormality from day 1 - day 5 was found to be 20% (29/144) in group A. The incidence of chromosomal abnormalities in group B was significantly lower than group A (blastocysts) [8% (3/40) and 31% (20/64) respectively; $p < 0.05$].
Conclusion: Murine embryos were shown to be an efficient model system to investigate chromosomal abnormalities. Our data shows that extended culture of embryos until day 5 leads to an increase in mosaicism and the incidence recorded was augmented in comparision to *in-vivo* obtained blastocysts.
Abbreviations: FISH: fluorescent in situ hybridization

O-075 IVF outcome in couples with meiotic anomalies in testicular biopsy

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