Introduction: Sperm DNA fragmentation and MSOME (motile sperm organelle morphology examination) are good predictors of In Vitro Fertilization (IVF) outcomes. It has been proved by other authors the negative effect of DNA fragmentation and sperm vacuolization on embryo quality and developmental rate of embryos.

In this study we try to correlate these parameters with pregnancy rate in order to determine their predictive value to properly inform our patients.

Material and Methods: We included 150 infertile couples undergoing an initial IVF treatment, without severe male factor. All patients underwent a full seminologic study, following the recommendations of WHO 2010, a morphological determination using a high magnification inverted microscope Leica AM6000, and an analysis of DNA fragmentation by SCD technique (Halosperm, Halotech DNA, SL), according to manufacturer's protocol. Fragmentation rate is considered altered over 30% (Fernandez JL, et al., 2003).

Taking into account the results of pregnancy with gestational sac, we studied the relationship between pregnancy rate and sperm abnormalities considering level of DNA fragmentation and presence of large nuclear vacuoles evaluated by high magnification. The statistical comparison was conducted by ROS curves to determine the sensitivity and specificity of these two diagnostic tools.

Results: The accumulative clinical pregnancy rate was 54.7%. The average male patient age was 37.7, and the female one was 36.7 years.

The most predictive cut-off of pregnancy was 25.5% of DNA fragmentation with a negative predictive value 67.6% ($p < 0.002$). Regarding the degree of vacuolization, the best predictor of pregnancy was about 73.5% of vacuolated sperm grade III + IV, with a negative predictive value of 39.4%. ($P = 0.09$), being not statistically significant.

Conclusions: In our center, a sperm DNA fragmentation above 25.5%, indicates high probability of failure in IVF treatment. Taking exclusively into account the result of the high magnification analysis of spermatozoa does not allow us to predict whether patients will become pregnant or not.

Nowadays our center is developing additional studies with donors to establish accurately the predictive degree of such analysis.

SELECTED ORAL COMMUNICATION SESSION

SESSION 05: RECURRENT MISCARRIAGE

Monday 4 July 2011 **10:00 - 11:30**

O-021 Long-term prognosis for live birth in women with recurrent miscarriage: a descriptive follow up study of a cohort of 987 women

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Introduction: Previous research has focused on miscarriage risk in the next pregnancy as a prognostic marker in women with recurrent miscarriage (RM) but the range of reported risk estimates is wide. We found no long-term follow-up studies on live birth outcome in patients with RM. We propose that live birth rate per time unit is a more reproducible outcome and more relevant from the patients' perspective than future miscarriage rate per pregnancy. Therefore we conducted a long-term follow up study on live birth outcome after first consultation to a tertiary recurrent miscarriage clinic during a 24 year period.

Material and Methods: From 1986 to 2008, 1312 patients were referred of which 987 women fulfilled inclusion criteria of a minimum of three consecutive miscarriages before week 21 + 6 and primary or secondary RM. Information on live birth outcome after first consultation was obtained by linkage to the National Danish Birth Registry. We studied: a) the proportion of women with a live birth after first consultation b) the average number of live births including those before first consultation in the cohort and in the general Danish population and c) the fecundity (defined as the probability of pregnancy resulting in birth) rates among women in the cohort compared with those of the general Danish population.

Results: Of the women fulfilling inclusion criteria, 33.3% (95% CI: 30.3 to 36.3) had not achieved a subsequent live birth five years after first consultation decreasing to 28.9% (25.9 to 32.1) 20 years after first consultation. At attained age 30, 40 and 49 the average number of children born by women in the cohort was 1.23, 1.54 and 1.47 children, respectively. In the general population a comparable measure was 1.13, 1.70 and 1.71 children per woman, respectively. Women in the RM cohort were, compared with women in the general population, 3.84 (3.44 to 4.28) times more likely to give birth during the first year after first consultation. This figure declined towards the level of the general population over the following five years.

Conclusions: Approximately two thirds of women with RM referred to a tertiary miscarriage clinic succeed in having at least one live birth after referral. However, the average number of children born by women with RM seems low taking into account the strong wish for a child of these women. Fecundity rates are markedly increased during the first year after referral, which presumably is primarily a result of differences in pregnancy attempts in women with RM and women of the general population (the women are encouraged to become pregnant when monitored at a dedicated RM clinic) but also due to an increased live birth rate per pregnancy of women with RM (through relevant treatment of established risk factors). Further research into the specific risk factor profile of the subgroup of women with RM without a subsequent live birth is needed as well as comparison of fecundity rates in women with RM with a population of women with an equally strong wish for a child.

O-022 Time to pregnancy resulting in a live birth in women with unexplained recurrent miscarriage

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Introduction: No evidence based treatment is available for couples with unexplained recurrent miscarriage (RM). Proper counseling regarding chances on live birth is therefore crucial. So far, literature provides no data on how long it takes to a live birth after two or more miscarriages. The aim of this study is to assess the time to pregnancy resulting in a live birth in a cohort of women with unexplained recurrent miscarriage.

Material and Methods: A nested prospective cohort study was performed from February 2004 through July 2009 in 8 hospitals within a multicenter randomized controlled trial on anticoagulant treatment in women with unexplained recurrent miscarriage (ALIFE trial)[1]. Women in the trial were randomly assigned to receive aspirin combined with low-molecular-weight heparin, aspirin alone, or placebo. Women not pregnant at the moment that unexplained RM was diagnosed were included in the present cohort study. Unexplained RM, defined as at least two miscarriages, 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 (lower than 16 μmol/L).

The primary outcome was time to spontaneous pregnancy resulting in a live birth (in weeks), calculated from the moment of diagnosis until conception measured by a urinary HCG. Stepwise backward Cox proportional hazard analysis was used to evaluate the relative prognostic significance of maternal age (< 36 years or ≥ 36 years), BMI (< 25, 25-30, > 30), the number of preceding miscarriages (2 or ≥ 3), the presence or absence of a previous live birth and the presence or absence of factor V Leiden and/or prothrombin G20210A mutation, on time to pregnancy resulting in a live birth.

Results: 251 women were included. The mean age at time of diagnosing unexplained RM was 34 years. The median number of preceding miscarriages was 3 with a median gestational age of 8 weeks. 213 women became pregnant during the study period. 139 women had a live birth, 69 a miscarriage, 2 an ectopic pregnancy, 2 a termination of pregnancy and 1 had an intra-uterine fetal death. The median time to a subsequent pregnancy irrespective of outcome was 21 weeks (95% CI 16-26). The cumulative pregnancy rate was 56% after 6 months, 74% after 12 months and 86% after 24 months. The median time to a

subsequent pregnancy resulting in a live birth was 41 weeks (95% CI 29-53). None of the potential prognostic factors including maternal age, BMI and the presence or absence of factor V Leiden and/or prothrombin G20210A mutation showed significant differences.

Conclusions: In women with unexplained RM we found that 50% had a subsequent pregnancy that resulted in a live birth after trying to conceive for 41 weeks. 14% of the women did not become pregnant in 24 months. This information should be discussed in the counselling of women with unexplained RM regarding future pregnancy attempts.

Reference:

1 Kaandorp SP, Goddijn M, van der Post JA, Hutten BA, Verhoeve HR, Hamulyak K, Mol BW, Folkeringa N, Nahuis M, Papatsonis DN et al (2010) Aspirin plus heparin or aspirin alone in women with recurrent miscarriage. N Engl J Med, 362, 1586-1596.

O-023 Biochemical pregnancies in women with recurrent miscarriage - do they matter?

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Introduction: Biochemical pregnancies (BP) also called pregnancies of unknown location are pregnancies identified by a positive urine/serum hCG that terminate before a gestational sac can be detected by ultrasound. BPs can in theory be very early miscarriages or spontaneously resorbed ectopic pregnancies. BPs constitute a significant proportion of the pregnancy losses of women referred with a diagnosis of recurrent miscarriage (RM) but it is unclear to what extent they should be included in the diagnostic criteria of RM and whether they have prognostic impact.

We wanted to assess the proportion of BPs of all pregnancy losses in the history of a large cohort of patients with unexplained RM and evaluate their impact on subsequent pregnancy outcome.

Material and Methods: The study group comprised all patients referred to the Danish RM clinic from January 2000 to July 2010 with 3 or more confirmed consecutive pregnancy losses before week 22 who had no births or stillbirths (primary RM) and who did not meet the exclusion criteria. Exclusion criteria were: incomplete pregnancy records, age ≥ 42 years at referral, presence of parental chromosome abnormality, uterine anomalies, lupus anticoagulant, irregular menstrual periods < 21 days or > 35 days or pregnancy after IVF/ICSI.

When reading the pregnancy records obtained from hospitals and practitioners we defined miscarriage (MI) as 1) a loss of pregnancy after 6th completed gestational week irrespective of findings by ultrasound/histology or 2) a loss prior to 6th completed gestational week with documentation of intrauterine pregnancy by ultrasound or histology of tissue from the uterus. We defined a BP as a documented pregnancy loss not meeting these criteria and not being confirmed as ectopic. All patients were monitored in their next pregnancy with repeated hCG measurements from day two after the first day of missed period and vaginal ultrasound from gestational week 5 + 5.

Results: Among 209 RM patients, 264 (32.6%) of their pregnancies before referral were classified as BPs and 545 (67.4%) were MIs according to the aforementioned definition.

In 22 patients with a history of only BPs, 27.1% of all pregnancies were ectopic pregnancies confirmed by laparoscopy/laparotomy whereas among RM patients with at least one confirmed MI the rate of ectopics were only 3.6% ($p < 0.0001$).

In three subsets of patients with exactly 3 pregnancy losses but less than 3 MIs (0 MIs + 3 BPs; 1 MI + 2 BPs or 2 MIs + 1 BP) no significant differences were found between the subsets regarding the risks of pregnancy loss (MI, BP or ectopic) in the next pregnancy, which were low. However, in patients with at least 3 MIs, the pregnancy loss rate in the next pregnancy for subsets with 3 MIs, 3 MIs + ≥ 1 BP(s), 4 MIs, 4 MIs + ≥ 1 BP(s), 5 MIs and 5 MIs + ≥ 1 BP(s) were 27%, 50%, 40%, 57%, 67% and 100%, respectively ($p < 0.01$ for the increase).

Conclusions: BPs comprise a significant proportion of pregnancy losses among patients referred to our clinic with a diagnosis of RM. RM patients with exclusively BPs are at a considerable risk of clinical ectopic pregnancy and many of their BPs may be spontaneously resorbed ectopics. In patients with ≥ 3 MIs, increased number of MIs but also a history of BPs significantly increase the risk of a new pregnancy loss in the next pregnancy. In these patients it is important to record information about BPs since they contribute negatively to the prognosis.

O-024 Comparison between two preventive treatments in women with recurrent miscarriages carrying a C677T methylenetetrahydrofolate reductase mutation: a prospective study

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Introduction: Recurrent miscarriage (RM) is defined as three or more consecutive spontaneous fetal losses. It affects 0.3% to 1% of pregnancies. The increase in the risk of miscarriage with the number of previous incidents argues in favor of the existence of an RM syndrome. Many studies have described correlations between RM and thrombophilia. The aim of this study was to compare the effect of the association of aspirin, low-molecular-weight heparin and folic acid versus aspirin and folic acid in women with recurrent miscarriages carrying a C677T methylenetetrahydrofolate reductase mutation.

Material and Methods: Hundred sixty-four women with a C677T MTHFR mutation were included in a prospective study between 2006 and 2009. All the women were screened for uterine malformations (by hysteroscopy), chromosomal abnormalities (karyotype) and endocrine disorders (fasting and post-prandial blood glucose, thyroid hormones). A prospective study with two preventive treatments was performed: first group with low-dose aspirin (100 mg/day) in 82 patients, and the second group with the same aspirin treatment and low-molecular-weight heparin (40 mg/d enoxaparin) (n: 82). All these women received 10 mg/d folic acid. An age-matched control group of 78 triparous women without thrombophilia and having become pregnant during the study period was studied. The live births or miscarriage and the incidence of obstetric complications were reported.

Results: In the 164 women with a history of recurrent miscarriages, we found 114 heterozygous and 50 homozygous C677T MTHFR mutations. Eleven of the 50 women carrying a homozygous MTHFR mutation and none of those carrying a heterozygous MTHFR mutation displayed fasting blood homocysteine level (Hcy).

The obstetric antecedents observed in women carrying the MTHFR mutation were 627 first-trimester miscarriages, between 3 to 12 cases of miscarriage per woman, without difference between the groups 1 and 2. There were no differences between the two groups in terms of mean age at the start of pregnancy, body mass index (BMI) or tobacco or alcohol consumption.

In the first group 38 women gave birth (including four premature deliveries) after a mean term of 37.4 ± 5.2 weeks of amenorrhea. The mean birth weight was 2820 ± 585 g.

In the second group, 65 births occurred (including five premature deliveries), with a mean birth weight of 3372 ± 167 g and a mean term of 39.1 ± 3.7 weeks of amenorrhea. Hence, there was a significant difference ($p < 0.001$) in the delivery rate for women carrying a MTHFR mutation with the first (46.3%) and the second (79.2%) preventive treatment. The rate of recurrent miscarriage fell from 51.2% (group 1) to 21.2% (group 2). No heparin-induced thrombopenia or iatrogenic bleeding occurred during the course of treatment.

In the control group, 67 live births (including 5 premature deliveries) were recorded. The mean birth weight and term were respectively 3230 ± 565 g and 38.2 ± 4.3 weeks of amenorrhea. There was no significant difference in the delivery rate between women carrying a C677T MTHFR mutation with the second preventive treatment and those in the control group (79.2% and 85.8%, respectively).

Conclusion: Preventive treatment with aspirin, low-molecular-weight heparin and folic acid was the most effective therapy in women with recurrent miscarriage and carrying a C677 MTHFR mutation. The benefit of anticoagulation was not in relation with the thrombogenic effect of Hcy.

O-025 Homocysteine impaired endothelial function through compromised endometrial vascular endotelial growth factor: role in recurrent pregnancy loss

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Introduction: Hyperhomocysteinaemia, a risk factor for recurrent pregnancy loss, is related either to a hereditary defect within the methionine-homocysteine pathway or it might be acquired as a result of deficiencies of vitamin B_{12} and folate (B_9). However, the molecular mechanism by which hyperhomocysteinemia can lead to recurrent pregnancy loss has not been completely

described.On the other hand, homocysteine and vascular endothelial growth factor (VEGF) have been implicated in angiogenesis and in the development and progression of atherothrombotic vascular disease. In the present study, we hypothesized that hyperhomocysteinemia might be associated with a local endometrial VEGF disregulation. Therefore, we sought to determine whether homocysteine modulates local endometrial VEGF levels and their receptors expression in patients with homozygosity for the methylenetetrahydrofolate reductase (MTHFR) mutation.

Material and Methods: 15 patients with recurrent pregnancy loss and homozygosity for the MTHFR mutation were evaluated before and 3 mounths after oral administration of 5-methyltetrahydrofolate. Evaluation included measurements of plasma levels of homocysteine, and the local endometrial mRNA and protein expression of VEGF and its receptors (KDR and Flt-1) using RT-PCR and immunohistochemistry. The measurements were compared with baseline findings in 10 healthy fertile subjects.

Results: Basal homocysteine ($P < 0.05$) plasma levels were elevated in all patients versus healthy subjects. Moreover, basal endometrial VEGF mRNA and protein expression ($P < 0.05$) were also elevated in all patients versus healthy subjects. In patients with MTHFR mutation, folic acid (5-methyltetrahydrofolate) administration resulted in significant reduction ($P < 0.001$) of plasma levels of homocysteine, and endometrial mRNA and protein VEGF expression. On the other hand, no diferences were observed in VEGF receptors (KDR and Flt-1) mRNA and protein expression either before or after oral administration of folic acid (5-methyltetrahydrofolate).

Conclusions: Our findings demonstrate that lowering of homocysteine with folic acid (5-methyltetrahydrofolate) resulted in substantial reduction of endometrial levels of VEGF both at mRNA and protein level. These findings suggest that hyperhomocysteinemia-induced endothelial dysfunction could promote the development of infertility (recurrent pregnancy loss) through VEGF induction in the maternal endometrium, causing a chronic vascular damage. Moreover, this deleterious endometrial effect of hyperhomocysteinaemia is reverted with the administration of folic acid (5-methyltetrahydrofolate).

O-026 Maternal and perinatal outcomes following induced abortion

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Introduction: There is concern about the long term effects of induced abortion on future reproductive outcomes. In this project we used Scottish national data to investigate the effect of abortion on subsequent pregnancies.

Materials and Methods: Data were extracted on all women (aged 15-55) who had an induced abortion, a miscarriage, a live birth, or an ongoing pregnancy and live delivery in their first pregnancy recorded between 1981 and 2007 in the Scottish Morbidity Records databases. Obstetric and perinatal outcomes in a second ongoing pregnancy following an induced abortion were compared with those in primigravidae as well as those who had a miscarriage or live birth in their first pregnancy. Spontaneous preterm delivery rates were also compared in women following surgical and medical termination as well as after one or more consecutive induced abortions.

Results: A total of 171208, 458337 and 6908 women with a documented second pregnancy following an initial induced abortion (IA), livebirth and miscarriage respectively between 1981 and 2007 were identified, as were 458,339 primigravid women. Women with a previous induced abortion were older, more socially deprived and more likely to be smokers than primigravidae or those who had a live birth or a miscarriage in a previous pregnancy ($p < 0.001$).

Women with an IA in a first pregnancy had a higher risk of spontaneous preterm birth in the next pregnancy than women in their first pregnancies [Adjusted odds ratio (Adj. OR) 1.33, 95% Confidence Intervals (CI) 1.28-1.39] or women who had a live birth in their first pregnancy [Adj. OR 1.87, 95% CI 1.80-1.95]. They were also more likely to be diagnosed with placenta praevia than either primigravidae [Adj. OR 1.47, 95% CI 1.29-1.67] or women with a previous livebirth [Adj. OR 1.31, 95% CI 1.16-1.48]. In comparison with the latter group, women with a history of IA had a higher risk of pre-eclampia [Adj. OR 1.96, 95% CI 1.88-2.05], still birth [Adj. OR 1.57, 95% CI 1.39-1.77] and induced abortion [Adj. OR 1.51, 95% CI 1.46-1.57].

A history of IA did not put women at higher risk of preterm birth in comparison with a previous miscarriage [Adj. OR 0.94, 95% CI 0.81-1.10]. In comparison with women who had an initial miscarriage, women with an IA in their first pregnancy were less likely to have a subsequent miscarriage [Adj. OR 0.28, 95% CI 0.24-0.32] or ectopic pregnancy [Adj. OR 0.57, 95% CI 0.46-0.71] but more likely to have a second induced abortion [Adj. OR 2.26, 95% CI 1.86-2.75]. They were also more prone to develop pre-eclampsia [Adj. OR 1.43, 95% CI 1.18-1.74] in their next ongoing pregnancy.

Surgical abortion was associated with a higher chance of spontaneous preterm birth in the next ongoing pregnancy than medical abortion [Adj. OR 1.27, 95% CI 1.11-1.45]. In comparison with a single IA, the adjusted odds ratios (95% CI) for spontaneous preterm birth in the next ongoing pregnancy following two, three and four consecutive IAs were 1.11 (0.99-1.24), 1.43 (1.15-1.77) and 1.87 (1.41-2.89) respectively.

Conclusions: Induced abortion in a first pregnancy is associated with a higher risk of spontaneous preterm birth in a subsequent pregnancy than that in primigravidae or women with a previous livebirth, but is not significantly higher than that observed in women with an initial miscarriage. This risk is increased in women who undergo more than two consecutive induced abortions. Surgical abortion appears to be associated with an increased risk of spontaneous preterm birth in comparison with medical termination of pregnancy

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SELECTED ORAL COMMUNICATION SESSION

SESSION 06: ENDOMETRIOSIS AND SURGERY

Monday 4 July 2011 **10:00 - 11:30**

O-027 Prospective assessment of the impact of laparoscopic excision of endometrioma on ovarian reserve; gentle surgery can benefit the ovary

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Introduction: Endometriotic cysts affect women of reproductive age. Surgery is the mainstay of treatment. Controversy exists about indications for surgery and the surgical method of choice. A major concern is possible detrimental effect of surgical excision on ovarian reserve. We aimed to prospectively assess whether laparoscopic excision of endometriomas causes a decrease in ovarian reserve as assessed with anti mullerian hormone (AMH) levels and antral follicle count (AFC).

Materials and Methods: Women with ≥ 1 endometrioma were prospectively recruited from a university gynecology clinic. Women with irregular periods, polycystic ovarian syndrome, history of ovarian surgery, and who used medication which could affect ovarian function, i.e. oral conraceptives, GnRH analogues etc. in six months before surgery were excluded. Indications for surgery were pain and/or infertility. Surgery was performed in early follicular phase. In the morning of surgery and one month after surgery all women underwent transvaginal ultrasound examinaton. Number and size of endometriomas and antral follicle count (AFC) were determined. The average of the largest diameters on three orthogonal planes were measured for size assessment. Sera were collected and frozen simultaneously. FSH and AMH levels were measured in frozen thawed sera. Laboratory personnel were blinded for timing of serum collection with regard to surgery. AFC included only ovaries which would be/were operated on.

All operations were performed by the same operator. Pneumoperitoneum was achieved with CO2 insufflation. One 10 mm and two 5 mm trocars were introduced. We tried to avoid cyst perforation to the possible extent. Cleavage plane between ovarian tissue and the cyst wall was identified with sharp and blunt dissection and the cyts wall was gently stripped off the ovary. Hemostasis was achived with minimal bipolar cauterisation.

Results are expressed as median (interquartile range). Pre- and postoperative serum FSH and AMH levels and AFC were compared with Wilcoxon signed rank test. Two sided p values are reported. Statistical significance was set at 0.05.