O-203 Infertility outcome improves in normal infertile women by early diagnosis of endometrial tuberculosis by polymerase chain reaction and treatment

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Introduction: Female genital tuberculosis (GTB) is a common cause of infertility in India. Conventional tests for tuberculosis (TB) have low sensitivity and specificity for diagnosis of GTB. We have previously shown a high probability of diagnosis of GTB with TB Polymerase chain reaction (PCR) in countries with high prevalence of TB (Int J Tubal & Lung Dis. 2010; 14: 1629-34).

Endometrial TB PCR positivity in the absence of tubal damage raises the possibility of a false positive test or the detection of sub-clinical or latent disease, with doubtful benefits of treatment. This study was undertaken to assess whether early treatment of GTB based on TB PCR positivity improves infertility outcome.

Material and Methods:
Design: Intervention study
Setting: An IVF center in Northern India
Period of Study: Jan 06 to December 08

All infertile women, of less than 40 years of age with infertility were considered for inclusion in the study. All of them had endometrial sampling done for assessment of GTB. Follow up assessment was done at least for 2 years. Women with history of TB and infertility due to tubo-peritoneal, endometrial factor or severe oligo-asthenospermia were excluded.

Endometrial curettings were obtained by aspiration (EA) or curettage as part of routine work up of infertility. Samples for MTB-PCR were sent to Reliance Life Sciences Pvt. Ltd. an accredited laboratory providing services in India. Nested PCR targeted against most conserved region IS 6110 of Mycobacterium tuberculosis (MTB PCR) was performed on these samples.

Of the total 3108 infertility cases 443 women fulfilled inclusion criteria. Of them 169(38.15%) women were found to have positive MTB-PCR (Group I) while 274 (61.85%) had negative MTB-PCR (Group II). All the MTB-PCR positive women were administered standard six month anti tubercular chemotherapy with 2 months of rifampicin, isoniazid, pyrazinamide and ethambutol followed by 4 months of maintenance therapy with rifampicin and isoniazid (2HRZE,4HR) in appropriate dosages. There was no significant difference between the two groups with respect to age, primary or secondary infertility, menstrual pattern, obstetric history and etiology of infertility. However, the study GTB-PCR positive group had significantly more women with lower education and socio-economic status and longer duration of infertility.

Results: There were no statistical differences in the two groups in pregnancy rate 101(59.8%) vs 167(60.9%), time to conception (≤12 months = 66.4% vs 66.5%; 13-24 months = 23.8% vs 19.2% and >24 months = 9.9% vs 14.4%) and live births or ongoing pregnancies (80.2% vs 80.2%) respectively.

There was a clear trend (not significant p = 0.154) towards higher spontaneous conceptions (51.5% vs 40.1%), lower pregnancy rate with super ovulation and intrauterine insemination (SO + IUI) (29.7% vs 40.1) and equal conception rate in in-vitro fertilization (IVF) (18.8% vs 19.8%) in the two groups respectively. When the mode and time to conception together were compared between the two groups, the difference became highly significant (P < 0.0001). In group I, 48(92.3%) spontaneous conceptions occurred within first 12 months i.e. during ATT or within 6 months of treatment completion, while in the group II, occurrence of spontaneous conceptions was distributed more evenly in relation to time i.e 36(53.7%) in less than 12 months as compared to 31; 46.3% after first year.

Conclusion: Early detection of GTB by endometrial MTB PCR improves chances of spontaneous conception in infertile women with no demonstrable evidence of tubal or endometrial involvement. Subtle tubal damage is one possible factor in women failing to conceive spontaneously or with SO + IUI. On the other hand, the success of IVF which does not depend upon tubal factor had equal success rate in the two groups.

O-204 Differential methylation of imprinting genes in first-trimester spontaneous abortions and recurrent pregnancy loss

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Introduction: Previously we have reported a tissue-specific loss of methylation in KCNQ1OT1 (11p15) and PLAGL1 (6q24) in first-trimester spontaneous abortions. An addition we have shown that errors of imprinting maintaining mechanisms on maternal chromosomes during embryo development may be among molecular processes responsible for recurrent pregnancy loss. The aim of the present investigation was identify differential methylation of imprinting genes in samples of first-trimester spontaneous abortions from women who have recurrent pregnancy loss.

Methods: Differential methylation of imprinting genes were examined in 8 first-trimester spontaneous abortions (9.9 ± 2.7 weeks) with normal karyotype from women who have recurrent pregnancy loss. Four induced abortions (7.8 ± 0.7 weeks) were investigated as a control group. We analyzed DNA from two different germinal layers are extraembryonic mesoderm (EM) and cytotrophoblast (CT). We used GoldenGate Methylation Cancer Panel I (Illumina, USA) for the DNA methylation analysis. This panel includes 115 CpG sites from 52 genes imprinted in human. Two criteria were established to classify a CpG as differentially methylated in these groups:

a) Level of average Beta for CpGs from imprinting genes in control group should be 0.5 ± 0.17;

b) An average Beta value below 0.17 for hypomethylation and above 0.83 for hypermethylation.

Results: Eighteen of 115 (15.7%) CpGs from 16 of 52 (30.8%) imprinting genes were found to be disrupted methylation in analyzing group miscarriages as compared with the control group. Ten of these CpGs (PEG10_P978_R, GRB10_P486_R, CPA4_E20_F, PHLDPA2_P622_F, WT1_E32_F, WT1_P853_F, ZNF215_P71_R, HTR2A_P853_F, DLK1_E227_R, GABRB3_P92_F) from 9 genes were hypomethylated and 8 from 7 genes (DCN_P1320_R, TRPM5_P721_F, H19_P1411_R, INS_P804_R, ZIM3_P718_R, GABRA5_P682_R, GABRA5_P1016_F, PWCR1_P811_F) were hypermethylated. Differential methylation was found in 9 genes from paternally (5 hypomethylated, 4 hypermethylated) and 7 genes from maternally (4 hypomethylated, 3 hypermethylated) expressed alleles. Significantly, epimutations were found in both tissues or confined by EM or CT.

Conclusions: Our results provide evidence multiple epimutations in CpGs from imprinting genes on maternal or paternal chromosomes may be among molecular processes, which are responsible for dysfunction of imprinted loci and recurrent pregnancy loss. Presence methylation abnormalities in these genes, indicating an increased susceptibility of some genes to epigenetic alterations. It is important, that epimutations were presented by hypomethylation or hypermethylation CpGs. Presence of epimutations in both tissues indicate about mistake reprogramming in the primordial germ cells during gametogenesis: hypermethylation is a mistake in erasure while hypomethylation is a mistake in reestablishment of CpGs methylation in imprinting genes. Whereas, tissue-specific restriction of epimutations allows suggesting independent sporadic epigenetic events in various embryonic germ layers after its differentiation.
O-205 Altered expression of cyclooxygenase-2 gene results in unreceptive endometrium in women with idiopathic recurrent spontaneous miscarriage

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Objective: It is well established that the endometrium undergoes an ordered process of differentiation making it receptive to embryonic implantation. A recentive endometrium is vital for implantation of blastocyst and provides a continuous support for the development of embryo. Unsupportive endometrium is a major factor contributing to idiopathic recurrent spontaneous miscarriage (IRSM) due to abnormal implantation. Cyclooxygenase-2 (COX-2) is suggested to play an important regulatory role in successful blastocyst implantation. Since the process of implantation shares similarities with the proinflammatory response, involvement of COX-2 gene, whose expression is induced by inflammatory stimuli, has generated considerable interest amongst researchers attempting to understand implantation. However, little is known about the role of COX-2 in IRSM. Expression of COX-2 gene, which is reported to be induced by inflammatory cytokine interleukin-1β (IL-1β), is explored in women with IRSM during window of implantation.

Material and Methods: Blood and endometrial samples were collected during the mid secretory phase of the menstrual cycle (D21) from 56 women (age <35 years) reporting for treatment at the Institute of Reproductive Medicine, Salt Lake, Kolkata, India. The present study was approved by the Institute Committee and written informed consent obtained from all couples enrolled. The women were divided into two groups: one group consisted of 36 women with IRSM who did not receive any kind of medication and had no known causes of miscarriage or other gynecological disorders, while the other group included 20 fertile women undergoing sterilization and were considered as controls for the comparison purposes. Serum was separated from the collected blood and level of IL-1β measured in serum by ELISA. The endometrial tissue collected, was divided into two parts: mRNA were isolated from the first part and expression of COX-2 gene was assessed. The second part of the tissue was fixed to examine the surface characteristics using scanning electron microscopy (SEM). Data were compared using independent two sample t test and chi-square test, as applicable. All analyses were performed with KX Plot version 2.0 beta 13 (Koichi Yoshioka, 1997–2000) and Graphpad Prism software. Statistical significance was defined as p <0.05.

Result: Clinical characteristics of the women in the two groups showed no significant differences in terms of age, BMI, serum estrogen and progesterone levels. A 7 fold increased expression of COX-2 gene was observed in the endometrium of women with IRSM when compared to controls. The serum level of proinflammatory cytokine IL-1β was also found to be significantly higher in women with IRSM as compared to controls (p ≤0.05). Further, the endometrium of women with IRSM showed poorly developed pinopodes, which are well accepted morphological markers of receptivity. In contrast, well formed pinopodes were observed in the cases in the control group.

Conclusion: Higher expression of COX-2 in the endometrial tissue of the women with IRSM during implantation window may be attributed to the increased expression of proinflammatory cytokines IL-1β. Since expression of COX-2 genes regulates endometrial development for receptivity during implantation, it is hypothesized that higher expression of this gene in these women may impair the process of endometrial differentiation for acquisition of a receptive state. This is evidenced by poorly developed pinopodes in the endometrium of women with IRSM, thereby adversely affecting the process of implantation of the embryo.

O-206 First trimester screening for Down syndrome in natural versus assisted conceptions

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Introduction: The reliability of first trimester Down syndrome screening in pregnancies achieved by assisted reproduction techniques and the need for specific adjustments is controversial. The aim of the present study is to compare the screening between natural and assisted conceptions.

Material and Methods: Retrospective study. Comparison of nuchal translucency (NT), free ß fraction of human chorionic gonadotropin (ß-hCG) and pregnancy-associated plasma protein A (PAPP-A) MoM (multiple of median) values, as well as false positive screening (FP) tests, in normal singleton pregnancies naturally conceived (control group, N = 2023) versus those achieved by assisted reproduction (N = 2226). The following subgroups were also compared with natural conceptions: intrauterine insemination (IUI) (N = 351), in vitro fertilization (IVF, N = 96), intracytoplasmic sperm injection (ICSI, N = 811) and ovum donation (N = 563) with IVF (N = 192) or ICSI (N = 353). Only fresh embryos were considered in the subgroup comparisons.

Results: In assisted conception free ß-hCG MoM values were higher -1.27 (1.22-1.31) - and PAPP-A MoM values were lower -1.27 (1.23-1.31) - than in natural conceptions - 1.17 (1.13-1.21) and 1.37 (1.32-1.41), respectively; p <0.05. No differences appeared in NT MoM values or in the FP rate. In the remaining subgroup comparisons ICSI PAPP-A MoM values were significantly reduced- 1.19 (1.13-1.23) - compared to natural conceptions - 1.37 (1.32-1.41), leading to an increased FP screening rate; 8.3% (6.40-10.20) versus 5.2% (4.23-6.17) in natural conceptions. Free ß-hCG MoM values were significantly higher in pregnancies achieved by ovum donation, regardless the mode of insemination (IVF or ICSI), but the final FP rates were similar to natural conceptions.

Conclusions: This study suggests that ICSI procedures would need adjustments in the first trimester Down syndrome screening to decrease the FP rates obtained.

O-207 Immature myeloid cells accumulate in the mouse placenta and actively promote angiogenesis

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Introduction: CD11b+ Gr1+ immature myeloid cells (IMCs), first described as myeloid derived suppressor cells (MDSCs), normally differentiate into mature granulocytes, macrophages or dendritic cells. Under pathological conditions, such as cancer, a partial block in the differentiation of IMCs into mature myeloid cells results in the expansion of this population. The IMCs/MDSCs have been identified in most cancer patients and in experimental mice with tumors based on their ability to suppress T cell activation. Importantly, IMCs have been shown to actively promote tumor growth and metastasis by modulating the cytokine environment, and by directly promoting angiogenesis within the tumor. Many of the proliferative, invasive, and immune tolerance mechanisms that are used to support normal pregnancies have been shown to be similarly exploited by tumors to establish a nutrient supply. We thus hypothesized that angiogenesis within the placenta might be supported by IMCs in analogy to their function in malignancies. The goal of our study was to test for the presence of CD11b+ Gr1+ IMCs in mouse placentas, and whether they share similar proangiogenic properties as those found in tumors.

Materials and Methods: Experiments were performed on 6-8 week old C57Bl/6 female mice. Placentas from pregnant mice or B16F10 tumors that were subcutaneously implanted were analyzed by flow cytometry and confocal microscopy. To determine the proangiogenic potential of FACS sorted IMCs, Matrigel plug assays were performed. RTPCR was used to detect the expression of key angiogenic genes in IMCs.

Results: In this study we show for the first time that mouse placentas are populated by CD11b+ Gr1+ IMCs. These cells consist up to 20-30% of bone marrow derived cells infiltrating the placenta with peak abundance at mid-pregnancy. Cytological examination of IMCs revealed a repertoire of differentiating myeloid cells, the majority (68%) being myelocytes and metamyelocytes. This population shows striking similarity to that observed in tumors, in terms of their relative abundance, their morphology and by expressing the same surface markers (CD11b and Gr1). Examination of placental sections by confocal microscopy
O-208 Assisted reproductive technology in Europe, 2008: results generated from European registers by ESHRE. Preliminary results

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Introduction: This is the twelfth report of the European IVF-monitoring (EIM), the ESHRE register on assisted reproductive techniques (ART) organisation. This report deals with the results of treatments initiated during 2008.

Methods: Data were collected from existing national registries in 36 countries (3 more than in 2007) and directly entered by each national coordinator into the EIM database through software developed by ESHRE. Data were analysed at ESHRE headquarters.

Results: In total, 1069 (+37) clinics participated (84.2% of registered clinics in the participating countries). They reported 528 040 treatment cycles: IVF (124 431), ICSI (186 735), frozen embryo replacement (FER, 97 633), egg donation (ED, 12 541), preimplantation genetic diagnosis/screening (PGD/PGS, 2859), in-vitro maturation (IVM, 1062) and frozen oocytes replacements (FOR, 3735).

Overall this represented a 7.0% increase in activity in the participating countries since 2007. Data on intruterine insemination using husband/partner’s (IUI-H) and donor (IUI-D) semen were reported from 25 countries (+2). A total of 146 741 IUI-H and 24 895 IUI-D cycles were included (+2.9% and -4.5%). When interpreting the results it is important to note that delivery rates may be underestimated due to lack of follow-up and incomplete reporting.

For IVF, the clinical pregnancy rates (PR) per aspiration and per transfer were 28.5% (-2.4% compared to 2007) and 32.5% (0.3%) respectively. The delivery rate (DR) per aspiration was at 22.1% (+1.3%). For ICSI, the corresponding rates were 29.4% (+0.8%), 32.0% (+0.0%), and 21.1% (+0.9%). For frozen embryos replacements, PR was 19.0% per thawing and 21.0% per transfer. The corresponding delivery rates were 13.4% and 14.5%. In oocyte donation cycles, PR and DR were 41.3% and 28.6% per transfer, respectively. For PGD/PGS, PR was 25.6% per aspiration and 33.1% per transfer. For in vitro maturation, PR and DR were 16.2% and 8.3% per aspiration respectively. Finally, 3340 replacements after oocyte freezing were reported, mainly from Italy. They resulted in 14.8% PR and 6.3% DR per thawing, respectively.

Following IUI-H the pregnancy rate and delivery rate was 12.4% and 10.1%, reaching 13.0% and 10.1% in women below 40.

Women’s age strongly influenced ART outcomes with delivery rates of 25.0%, 17.9% and 8.3% per aspiration in age groups < 35, 35-39 and ≥ 40 years respectively.

The transfer of 1, 2, 3 and 4 or more embryos following IVF or ICSI occurred in 22.5%, 52.8%, 22.5% and 2.2%, cycles respectively. The corresponding figures for 2006 were 22.8%, 57.6%, 18.4% and 1.2%, thus showing a slight increase in the number of transferred embryos. There were significant national differences in practice. The proportion of single embryos transfers varied from 3.0% in Lithuania to 69.5% in Sweden, whereas the proportion of transfers with 4 or more embryos varied from 0% in 13 countries to 41.8%. The proportions of singleton, twin and triplet deliveries after IVF and ICSI showed marginal differences compared to those in 2007, at 77.7% (-1.0%), 21.1%, and 1.1% respectively. The proportion of very preterm deliveries (< 33 weeks) increased from 2.8% for singleton pregnancies to 13.9% for twin pregnancies and 39.7% for triplet pregnancies, justifying a transfer policy aimed at decreasing the risk of multiples.

IUI-H in women below 40 years of age resulted in 11.1% twin and 0.8% triplet pregnancies, thus not higher than in IVF-ICSI.

Seven countries were able to report data on cross border reproductive care. They reported, in total, 2529 ART cycles (4.6% of all cycles performed in those countries), of which 1040 were performed for oocyte donation and 60 for sperm donation. The reasons for crossing borders were not sufficiently detailed to allow any firm conclusions.

Conclusions: In comparison with previous years, 2008 saw an increase in the number of reported ART cycles in Europe. However, delivery rates remained relatively stable, as did the numbers of embryos transferred. No significant change in the multiple birth rate was observed.

O-209 IVF World Data for 2007

O-210 Is world IVF going in one direction?


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Introduction: Cultural, socioeconomic and other differences make it necessary to assess whether access to care and the balance between effectiveness and safety are understood in similar ways in different regions of the world. There is generally worldwide agreement that markers include the birth of a healthy baby, low high order multiple births, low incidence of ovarian hyper stimulation syndrome (OHSS) and adequate access for patients needing treatment. Our