lower LH levels in their last stimulation control than non-pregnant patients (6.5 versus 14.9 mIU/mL). A cut-off of 15 mIU/mL of LH could be calculated. The pregnancy rate was significantly lower in patients with a LH value > 15 mIU/mL (PR 3.2%) compared to those with a LH ≤ 15 mIU/mL (PR 17.2%, p < 0.01). After ovulation induction the pregnancy rate was almost as twice as high than after spontaneous ovulation (14.9 versus 7.4%, n.s.).

**Conclusions:** It can be concluded that ovulation induction improves the probability of conception after hormonal stimulation and IUI or timed intercourse. Awaiting occurrence of LH surge is associated with lower pregnancy rates probably due to an increased risk of post-ovulatory therapy.

**P-532 Clinical results and managements for natural cycle IVF**

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**Introduction:** Natural cycle invitro fertilization (IVF) was most ideal way utilized patient’s own hormonal secretion. However, control of spontaneous ovulation is sometimes difficult in the point of view to decide optimal oocyte collection, and usually only one oocyte is produced. We try to overcome these difficulties many years and this time report our protocol and ingenuity of natural cycle IVF.

**Materials and Methods:** We investigated 2,605 cycles of natural cycle IVF performed between January 2008 and December 2008 in our clinic. Their mean age was 38.3 ± 5.0 years old. All cases were informed consent and trans-vaginal ultrasound and hormonal assay (E2, LH, P4) were performed several days before expected ovulation. Dominant follicle reached 18mm in a diameter and E2 levels reached 250pg/ml, we administrated GnRHa agonist to trigger oocyte maturation.

Three protocols for the timing of maturation triggering and oocyte retrieval were used, depending on the LH levels. The first protocol involved triggering on midnight and retrieved 32 to 34hrs later (Type A), the second involved triggering during the day and retrieved following day (Type B), and the third involved no use of GnRHa agonist and retrieved on the same day or on the following day (Type C). In some cases, a single injection of a GnRHa antagonist (0.125 mg or 0.25 mg) was used to suppress a spontaneous LH surge. All cases divided into five groups dependent on their age (-30, 30-35, 36-40, 41-45, 46- years old) and six groups depending on their LH levels (≥9, 9.0-14.9, 15.0-19.9, 20.0-24.9, 25.0-29.9, 30.0- mIU/ml).

After isenmulated by conventionally or intracytoplasmic sperm injection (ICSI), normally fertilized pronucleus zygotes were cultured individually in 20μl of cleavage medium (SAGE, USA) from day1 to day3, and in Blasto cyst media (SAGE, USA) from day4 to day6. All embryo transfer performed single transfer and diagnosed for chemical pregnancy after serum β-hCG levels above 20mIU/ml and for clinical pregnancy after confirmed gestational sac.

**Results:** Spontaneous ovulation occurred in a total of 205 cases (7.9%), with no differences according to age. Oocytes were collected in 1674 cases (69.8%) in total, and significantly lower in the group of over 46 years old (57.1%, p < 0.01). Fertilization and cleavage rates were 84.7% and 91.0%, and no differences depending on age. Chemical and clinical pregnancy rates for fresh cleavage stage embryo per transfer were 39.2% and 35.4% in total, 43.5% and 43.8% (-30 years old), 59.1% and 48.1% (31-35 years old), 37.7% and 33.0% (36-40 years old), 20.0% and 15.0% (41-45 years old) and 0% and 0% (46- years old) respectively. Developmental rate to blastocyst were 41.8% in total, 50.0% (-30 years old), 54.7% (31-35 years old), 53.5% (36-40 years old), 31.4% (41-45 years old) and 21.2% (46- years old) respectively.

The percentage about the timing of Type A and Type B plus C in the groups of LH level were 94.4% and 5.6% (<9 mIU/ml), 53.4% and 46.6% (10.0-14.9 mIU/ml), 16.1% and 83.9% (15.0-19.9 mIU/ml), 6.4% and 93.6% (20.0-24.9 mIU/ml), 5.6% and 94.4% (25.0-29.9 mIU/ml), 10.5% and 89.5% (30.0 -mIU/ml) respectively. In the group of LH level was 10.0 to 14.9 mIU/ml, spontaneous ovulated rate was 14.8% and significantly higher than that in the other LH level groups (p < 0.01).

**Conclusions:** The rates of oocyte collection and fertilization, cleavage, and pregnancy after natural cycle IVF were acceptable. The rate of successful oocyte retrieval was increased by optimizing the retrieval time, but further insight is needed for the treatment of patients with slightly increased LH levels because of the high frequency of spontaneous ovulation.

**P-533 Relationship of antral follicle count with AMH and ovarian response is similar for 2D and 3D ultrasound techniques**

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**Introduction:** Antral follicle count (AFC) and anti-Mullerian hormone (AMH) are the most direct tests for quantitative ovarian reserve screening and appear equally predictive of ovarian response during IVF/ICSI treatment. Whilst AMH assays have recently been standardised there is no agreed technique to measure the AFC and several different ultrasound methods have been reported. The objective of this quasi-experimental study was to compare the relationship between AMH and antral follicle counts made using conventional 2D and 3D ultrasound in two different centres and to analyse their ability to predict ovarian response to controlled ovarian stimulation during assisted reproduction treatment (ART).

**Material and Methods:** A total of 316 subjects aged < 41 years with regular menstrual cycles of 21 to 35 days undergoing their first cycle of ART using a conventional long down-regulation protocol at two University-based assisted conception unit were prospectively recruited. Transvaginal ultrasound and venepuncture were performed in the early follicular phase (day 2-5) of the spontaneous menstrual cycle immediately prior to treatment. Subjects were excluded if they were found to have an ovarian cyst or follicle measuring ≥ 20 mm in diameter. The number of antral follicles measuring 2-10 mm was measured using a conventional 2D technique in one unit (Manchester) and by 3D technique in the other (Nottingham). The main outcome measures were the relationship between the AFC and early follicular phase AMH levels and the number of mature oocytes retrieved. Secondary measures included prediction of poor ovarian response. The distribution of the data was checked for normality using a normal probability plot. The relationship of each follicular cohort with AMH and ovarian response was evaluated using Spearman’s correlation coefficient (r). Regression analysis and receiver operative characteristic (ROC) curve analysis was used to evaluate the predictive value of AFC for the number of mature oocytes retrieved and poor response. Subsequently, comparison of two unpaired ROC curve areas were made.

**Results:** Analysis was performed on 296 subjects after excluding 20 subjects who did not meet the inclusion criteria. The median (range) age, BMI, basal FSH and antral follicle count were 33 (22-40) years, 24 (20-35) Kg/m², 7.1 (3.0-14.6) IU/L and 11 (0-42) respectively. The median (range) serum AMH level and number of mature oocytes retrieved were 12.1 (0.9-89.3) pmol/L and 11 (6-32) respectively.

The relationship of AFC with AMH and the number of mature oocytes retrieved was similar for the measurements made with conventional 2D (R² = 0.665 and 0.519 respectively) and manual 3D ultrasound (r = 0.641 and 0.618 respectively). Multiple linear regression analysis incorporating age, FSH, AMH and AFC revealed the better model for predicting the number of oocytes retrieved included AFC measurements made with the 3D technique (R² = 0.456) in comparison to the 2D (R² = 0.416) technique. Receiver operating characteristic curve analysis showed the predictive value of AFC for poor ovarian response was better for the 3D technique with significantly higher area under the curve (0.934; 0.892-0.976) compared to 2D (0.809; 0.721-0.897).

**Conclusions:** Antral follicle counts measured using 2D and 3D techniques demonstrated a similar relationship to serum AMH levels and predictive value for the number of mature oocytes retrieved in ART. However, the ability to discriminate poor responders was better when measurements were made with 3D ultrasound technique.
Introduction: Sex chromosome mosaicism is not uncommon and is often seen in individuals with ovarian dysgenesis. Data on the frequency of these aberrations in germ cells are lacking, but the incidence of sex chromosome mosaicism has been reported to be elevated in woman with recurrent spontaneous abortions. The aim of this study was to evaluate the frequency and type of chromosomal errors in the oocytes from patients with sex chromosome mosaicism, through the chromosomal analysis of the first polar body (PB1).

Materials and Methods: A total of 22 infertile couples underwent to 27 ICSI cycles with PB1 analysis. Sex chromosome mosaicism was diagnosed in 8 women (Group I, 13 cycles) with the following karyotype: 46,XX(94)/47,XXX(6); 46,XX(92)/45,X(8); 46,XX(92)/45,X(5)/47,XXX(3); 46,XX(91)/45,X(9); 46,XX(90)/47,XXX(5); 46,XX(86)/48,XXY(6); 47,XYY(2); and two cases 46,XX(96)/45,X(4). Fourteen couples with a normal karyotype and similar characteristics to Group I were treated in the same period and represented the control group (Group II). PB1s were tested for the chromosomes 13, 16, 18, 21, 22, 15 X. The maternal age in the two groups was, 39.7 ± 4.8 years in Group I and 38.0 ± 3.0 years in Group II (P = 0.273).

Results: The proportion of aneuploid oocytes was 45.4% in Group I and 43.8% in Group II.

The frequency of single chromosome aneuploidy in the oocytes from Group I and Group II were similar for chromosome 13 (3.5% vs. 7.7%, P = 0.255), 18 (9.5% vs. 16.2%, P = 0.187), 21 (10.0% vs. 15.7%, P = 0.175), and X (16.1% vs. 14.1%, P = 0.668), whereas it was significantly higher in Group I for chromosomes 16 (19.4% vs. 5.4%, P = 2.31E-6) and 22 (15.9% vs. 6.7%, P = 0.0176). In three patients performing 6 cycles, the sex chromosome aberrations in the female karyotype were significantly lower compared to the frequency of aneuploidy for chromosome X detected in the analysed oocytes: in 46,XX(94)/47,XXX(6) (2 cycles) the frequency was 25% (P = 0.007); in 46,XX(92)/45,X(9) (2 cycles) the frequency was 30% (P = 0.002) and in 46,XX(96)/45,X(4) (2 cycles) the frequency was 25% (P = 0.003). There were no differences in 46,XX(92)/45,X(5)/47,XXX(3) (3 cycles) where the frequency of aneuploidy of the chromosome X was 17.6% (P = 0.160). In the 46,XX(91)/45,X(9) (2 cycles), 46,XX(40)/47,XXX(60) (1 cycle) and in 46,XX(68)/45,XX/47,XXX(2) (1 cycle), the analysed oocytes (2-5 oocyte per couple) did not present aneuploidy for chromosome X.

Conclusions: The frequency of chromosome abnormalities in infertile couples is increased compared with the population baseline. In this study, the proportion of aneuploid oocytes was more of the 40% in study and control groups, but only two chromosomes, 16 and 22, showed a significantly higher incidence of aneuploidy in Group I compared to Group II. More important, the frequency of chromosome X aneuploidy was exactly the same in the patients with sex chromosome mosaicism as in the group with a normal karyotype. Furthermore, in Group I the proportion of oocytes with aneuploidy for chromosome X was significantly higher when compared to the frequency of sex chromosomal abnormalities in the karyotypes, where the aberrant cell lines constitute <10% of all analysed metaphase. These preliminary data on the oocytes confirm previous results on embryos demonstrating that when sex chromosome mosaicism in blood lymphocytes is low (<10% of all analysed metaphase), this condition is not predictive of higher risk of chromosome X nondisjunction in oocytes. Validation of these data would be especially interesting in cases where the incidence of sex chromosome mosaicism in blood lymphocytes is high.

P-535 ESX1 gene transcript as a spermatogenesis marker in infertile men
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Introduction: Azospermic patients, of both the OA (obstructive azospermia) and the NOA (non-obstructive azospermia) class, benefit from intracytoplasmic sperm injection (ICSI), because testicular sperm extraction (TESE) or its variant involving multiple surgical micro-sampling of one or both patient’s testes (microTESE) can retrieve the rare spermatozoon even in selected NOA cases. Identifying ongoing spermatogenesis molecular markers, predictive of a successful sperm recovery, is therefore a valuable goal. ESXI is a X-linked human homeobox gene expressed in testis, placenta, brain and lung. The mouse homologue, Esx1, is expressed in placenta and testis, specifically in pre- and post-meiotic cells. In humans, ESXI role has not been defined as yet.

Material and Methods: We have investigated ESX1 genomic sequence, expression (by RT-PCR), and epigenetic status (by promoter pyrosequencing) in testicular tissue samples from 81 infertile subjects, to check a possible association between ESX1 alterations and impaired spermatogenesis.

Results: ESX1 gene transcript was detected in 95% of samples showing varied levels of spermatogenesis at histological analysis: in 5/6 patients with incomplete Sertoli cell-only syndrome (ICOS), in 4/6 subjects with complete maturation arrest (cMA), and in 100% of cases diagnosed as OA (33), hypospermato genesis (18) and incomplete maturation arrest (iMA) (2). By contrast, it was detected in only in 3/16 (19%) of cases histologically classified as complete Sertoli cell-only syndrome (cSOS). In the same patient cohort, successful sperm recovery was obtained in 100% of OA, hypospermato genesis and iMA cases. In patients affected by a more severe impairment of spermatogenesis, sperm recovery was achieved in: cSOC (5/16), iSOC (4/6) and cMA (2/6). TESE was carried out in 19 of these 30 cases, and micro TESE in the remaining 11 cases. In this subset of patients, ESX1 expression and sperm extraction data were concordant in 14/19 cases subjected to TESE (74%, p < 0.04 applying one-tailed exact Fisher’s test), but only in 3/11 men who had undergone micro-TESE (27%). Concordance between ESX1 expression and sperm recovery was therefore much higher when the twins samples subjected to ESX1 expression analysis and sperm extraction originated from adjacent testicular areas (as it happens in TESE). The pyrosequencing of ESXI CpG island revealed methylation levels that were significantly lower in ESXI expressors as compared to non-expressors.

Conclusions: ESX1 was proven to be a spermatogenesis-related gene in the human. Crossing of histological, ESX1 expression and sperm retrieval data suggested that ESXI transcript is a sensitive indicator of spermatogenesis across all patients, and that, in NOA patients affected by testicular heterogeneity in respect to the presence/distribution of spermatogenesis foci, discordance in ESXI transcript detection and sperm recovery data mostly reflected the sampling procedure. Thus, ESXI transcript emerges as a reliable spermatogenesis molecular marker and as a predictor of gamete recovery from infertile men.

P-536 Preliminary validation of SNP genotyping and karyomapping for preimplantation genetic diagnosis of fifty eight autosomal single gene defects
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Introduction: Genome wide single nucleotide polymorphism (SNP) genotyping of parents and appropriate family member(s) allows the parental origin of chromosomes and chromosome segments to be identified in preimplantation embryos by Mendelian analysis, or karyomapping, providing a universal method for the combined detection of chromosome aneuploidy and linkage based preimplantation genetic diagnosis (PGD) of single gene defects and other inherited conditions. To date, about 125 inherited conditions, including 101 SGDs, have been licensed by the Human Fertilisation and Embryology Authority (HFEA) for PGD in the UK. Of the SGDs, 58 are caused by mutations in 57 different autosomal genes distributed across all 22 autosomes. Here, we examine the efficacy and accuracy of karyomapping for all 57 of these autosomal SGDs in a couple who had PGD for a structural chromosome abnormality.

Materials and Methods: A white Caucasian couple having PGD for a structural chromosome abnormality using conventional methods consented to follow up analysis of seven biopsied unbalanced embryos by SNP genotyping and karyomapping. After zona removal, each embryo was transferred into 2 ul of PBS and stored at -20°C on day 4 post insemination. Following whole genome amplification (WGA) (Genomplex, Sigma), parental genomic DNA and the WGA products were genotyped at 299,174 genome wide SNP loci using a bead array (Illumina CytoSNP-12, Illumina) and the SNP genotype data analysed by karyomapping using a custom macro in Microsoft Excel. (The SNP data were
only analysed as a set of anonymous markers for Mendelian analysis purposes and no other features such as known associations with genetic conditions or copy number variation were analysed.) For each SGP, informative SNP loci within 3 Mb regions flanking either side of the gene and any intragenic loci were examined in both of the parental chromosomes inherited by six of the embryos. The genotype of the remaining embryo was used as a reference to establish phase.

**Results:** Excluding the parental translocation, 3/7 embryos had maternal aneuploidies (trisomy 16, monosomy 16, 21 and 22 and monosomy 16). Of the 57 gene loci examined, only two -FANC A (Fanconi’s Anemia A) and ARSK (Metachromatic Leukodystrophy) on chromosomes 16 and 22 - are outside the region covered by the SNP loci genotyped using the chosen array. For the other 55 SGDs, an average of 217 (range 71 to 374) informative SNPs were identified in the flanking regions of both parental chromosomes combined. Furthermore, for 38 autosomal SGDs (69%), there were 1-27 informative intragenic SNPs. Karyomap analysis of each locus (excluding regions of imbalance) in the six embryos (n = 641) unambiguously identified parental haplotypes in almost all cases (97.8%), including a significant proportion (36%) with one or more concordant intragenic informative SNPs. Of the few cases where the gene could not be assigned to a parental haplotype, most involved a crossover between flanking informative SNPs and in two cases an intragenic crossover. The incidence of non-concordant informative SNPs was low (80/26,755; 0.3%). The average interval between flanking informative markers for all the loci examined was 861.2 Kb (range 52.3-5052.4 Kb). The only exception was CFTF (cystic fibrosis) on chromosome 7, which, in this couple required analysis of SNP loci further distal to the gene to be fully informative.

**Conclusions:** Genome wide SNP genotyping and karyomap unambiguously identified almost all of the parental haplotypes flanking all 55 autosomal SGDs examined (located within the regions covered by the array). Furthermore, the relatively short distance between flanking informative SNPs and the presence of one or more intragenic informative SNPs in many cases, minimises the risk of misdiagnosis resulting from double recombination, eliminating the need for mutation analysis.

**P-537** FSH-R polymorphism in severe types of OHSS (type III and IV/V): results of a Czech pilot study

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**Introduction:** The Asn680Ser polymorphism influences the FSH-R sensitivity to FSH and thus the risk of ovarian hyperstimulation syndrome (OHSS). Genotype Asn680/Asn680 is associated with highest, while Ser680/Ser680 genotype fell from 15.5% to 0%. In type IV/V Asn680/Ser680 (37.2%), Asn680/Ser680 (47.3%) and Ser680/Ser680 (15.5%). In type III OHSS real-time PCR on ABI Prism 7000.

**Results:** Controls were characterized by the prevalence of the Asn680/Asn680 (37.2%), Asn680/Ser680 (47.3%) and Ser680/Ser680 (15.5%). In type III OHSS the prevalence of Asn680/Ser680 genotype increased from 47.3% to 72.3%, while Ser680/Ser680 genotype fell from 15.5% to 0%. In type IV/V Asn680/Asn680 increased from 37.2% to 60.0%. Due to small numbers of tested cases compared to the higher number of controls statistics was not appropriate.

**Conclusions:** The results of our pilot study indicate that risk of less severe type III of OHSS is associated with Asn680/Ser680 heterozygosity, whereas the most severe types IV/V are associated with the highest sensitivity genotype Asn680/Asn680. These results support the hypothesis that the FSH-R genotype predicts not only the increased risk of OHSS, but also its clinical severity in Czech females. Systematic study of larger cohorts is necessary to verify these risks in other populations with different ethnicity and lifestyle.

**P-538** The FMR1 gene in human granulosa cells: expression and functional insights

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**Introduction:** The FMR1 (Fragile X Mental Retardation 1 gene) premutation is the most frequent genetic factor associated with premature ovarian failure (FX-POI). Fragile X-associated primary ovarian insufficiency. FX-POI is present in about a quarter of premutation carriers. FMR1 is normally expressed in germ cells of human female fetuses. Fmr1 mRNA is highly concentrated in mouse ovaries at the stage of oogonia cell proliferation, during development and, in adult female mice, Fmrp levels are higher in growing follicles. The size of the FMR1 CGG repeat, the pattern of X-chromosome inactivation, transcription and translation alterations, and RNA toxic gain-of-function are factors that may be involved in the risk for FX-POI.

**Material and Methods:** To investigate the FMR1 gene functioning in the human ovary, we evaluated the expression FMR1 mRNA and FMRP and sub-cellular FMRP distribution in human granulosa cells obtained from follicular aspirates of women undergoing in vitro fertilization.

**Results:** FMR1 mRNA was detected in these cells by RT-PCR and FMRP, by Western blotting. Various isoforms of the protein were observed, the molecular weight of which did not differ substantially from those observed for Fmrp from rat brain. Immunofluorescence of cells cultured for six days revealed a diffuse, dotted, cytoplasmic distribution of FMRP. After induction of oxidative stress by treating cell culture with sodium arsenite, FMRP was shifted to coarse perinuclear granules, colocalizing with TIA1, a stress granule marker.

**Conclusions:** These data indicate that, human ovaries at ovulation/ hyperovulation present high expression levels of FMR1 mRNA and FMRP and, similar to the rat and mouse central nervous system submitted to stress, human FMRP function should be regulating translation in ovarian granulosa cells, shifting between polyribosomes and stress granules, in specific physiological conditions. Biochemical fractionation experiments will help to validate the morphological data reported here.

**P-539** Ploidy of spermatogenic cells in testicular suspensions of non-mosaic Klinefelter’s syndrome cases, using a computerized cell scanning system

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**Introduction:** Klinefelter’s Syndrome (KS) is the most frequent chromosomal abnormality in infertile men. Meiotic completion of spermatogenic cells and the origin of euploid spermatozoa in these patients remain controversial, especially due to difficulties in the identification of these cells. Our aim was to study the ploidy of spermatogenic cells from non-mosaic KS patients, based on corresponding morphology and FISH images.

**Material and Methods:** Testicular tissue specimens were obtained from eleven men with non-mosaic KS, six of whom had spermatozoa in their testicular tissue (positive) and the remaining five had none (negative). Three autopsy specimens from accident victims were used as a control group. Testicular tissue was minced and enzymatically digested. Cells obtained were affixed using a Cytospin centrifuge and alcohol. The cells were multi-sequentially stained by Giemsa-May Grunwald, then by FISH for centromeric probes X, Y and 18. We used a computerized automated cell scanning system (Duet16, BioView Ltd, Nes-Ziona, Israel) which enables simultaneous view of morphology and FISH.
in the same cell. Coordinates and images of all cells found on the slide were stored digitally for subsequent analysis.

**Results:** A total number of 11,991 cells (mean 2,398.2 ± 1126.30 nuclei per case) from negative KS patients, 12,387 cells (mean 2,064 ± 1380.59 nuclei per case) from positive cases and 1,711 cells (mean 570.3 ± 187.28 nuclei per case) from the three controls were analyzed. The rate of secondary spermatocyte and post meiotic cells (round, elongating spermatids and sperm cells) was 1.1 ± 1.39% in the negative KS patients, 2.9 ± 3.33% in the positive ones and 66.6 ± 6.70% in the control. Aneuploidy in the control group was equally distributed between mitotic (4.3 ± 7.45%) and meiotic cells (4.62 ± 5.79% for primary spermatocytes, and 4.39 ± 7.03% for secondary spermatocytes), with a non significant decline in the post meiotic cells (1.5 ± 1.71%). In both negative and positive KS patients the majority of the spermatogonia were aneuploid (94.9 ± 2.22 and 91.7 ± 4.54%, respectively). Similarly, primary spermatocytes showed 90.4 ± 2.14% and 85.4 ± 9.30% aneuploidy. The prevalence of aneuploidy in the secondary and post meiotic cells was significantly lower than in the primary spermatocytes, resulting in 13.5 ± 14.51% (P < 0.002) and 24.0 ± 35.65 (P < 0.008) aneuploid cells in the positive and in the negative KS patients respectively.

The presence of both autosomal (18) and sex chromosomes bivalents (Sex Vesicle body) in primary diploid spermatocytes was 11.1 ± 6.84% compared to 29.3 ± 8.43% in the control group (P < 0.03). Pairing of both 18 and XY chromosomes was identified also in the aneuploid 47XXY primary spermatocytes (17.5 ± 9.45%). There was no difference in the percentage of pairing between the two KS subgroups.

**Conclusions:** The computerized cell scanning system enables to precise identification of spermatogentic cells. In both, the study and control groups, decline in the percentage of aneuploids was observed after the first meiotic division. 46XY and 47XXY spermatogonia and primary spermatocytes were present in KS patients, with and without sperm. Pairing of XY could be detected among 47XXY primary spermatocytes. Spermatogenesis in the KS patients with and without sperm seems to be similar.

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**P-540 Assessment of male genome competence**


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**Introduction:** Motile Sperm Morphology examination (MSOME) is often requested by patients, and entails the live scanning of magnified spermatooza for irregularities linked to a compromised ability to fertilize and support embryo development. It has also been claimed that genomic integrity is impaired in spermatooza displaying vacuoles and other surface irregularities. However, as the role of chromatin fragmentation on the health of the conceptus and its ability to implant has not been confirmed, so the link between sperm head surface defects and genomic competence has not been proven.

Here we aim to assess genomic complement and epigenetic information of male gametes carrying surface irregularities and vacuoles.

**Material and Methods:** Consenting men undergoing infertility screening were recruited. Following standard semen analysis, the sperm suspension was adjusted to 1 x 10⁹/ml and diluted in 1:8l PVP solution. With a micromanipulator 100 motile spermatooza showing head dysmorphisms were selected at 6000X, retrieved individually, and placed on pre-coated slides. These dysmorphisms included acrosomal abnormalities as well as single or multiple vacuoles occupying at least 4% of the sperm head. Normally appearing motile spermatooza served as controls. Sperm DNA fragmentation was assessed by placing on a glass slide two droplets of 3μl prewarmed agarose each containing 50 spermatozoa served as controls. Sperm DNA fragmentation was assessed by placing on a glass slide two droplets of 3μl prewarmed agarose each containing 50 spermatooza, and hybridized with two sets of probe mixture, which contain locus specific probes for chromosomes X, Y, 13, 15, 16, 17, 18, 21, 22 (MultiVysion™ PB probe mixture, Abbott; OligoFISH™ probe kit, One Cell System).

TUNEL and FISH slides were analyzed at 1000X under a fluorescent microscope. The scoring technician was blinded for SCD, TUNEL, and FISH.

**Results:** A total of 8 men (35.9 ± 4 yrs) were included in the study. The mean sperm concentration was 79.5 ± 56.7 x 10⁹/ml, a motility of 52.9 ± 5%, and normal morphology of 4.0 ± 2%. The overall incidence of sperm head defect was 57.0%. A total of 3260 spermatooza evaluated for DNA fragmentation and ploidy status, revealed 1936 with surface defects and 1684 without. The total fragmentation rate detected by SCD assay was 3.9% (22/576) for vacuolated spermatooza and 4.5% (22/486) for the controls.

Assessment of sperm ploidy indicated a comparable proportion of chromosomal abnormalities between the two groups (1.5% vacuolated vs 1.1% control, respectively).

**Conclusions:** The presence of sperm nuclear defects assessed by high magnification microscopy does not appear to correlate with the ploidy or state of the sperm nuclear chromatin. Thus, the presence of spermiogenetic defects such as nuclear vacuoles and other head abnormalities do not directly translate to chromosomal imbalances or presence of DNA breakage.

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**P-541 Outcomes of 303 cycles on preimplantation human leukocyte antigen typing with or without mutation analysis**

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**Introduction:** Genetically inherited hemopoietic disorders and some acquired disorders need Hematopoietic Stem Cell Transplantation (HSCT) or bone marrow transplantation for the cure of the disease. Because of the difficulty to find a suitable donor, Preimplantation Human Leukocyte Antigen (HLA) typing with or without a single gene defect has become a promising option for the couples having an affected child. The objective of this study is to present our seven year experience between 2003 and 2010 on preimplantation HLA typing with or without a single gene disorder.

**Materials and Methods:** Overall 303 cycles were performed for 157 couples. 249 of these were for single gene disorders (SGD) (B-Thalassaemia, Fanconi anemia, eg.) combined with HLA typing applied to 127 couples, 54 of them were for only HLA typing for acquired diseases (ALL,AML, eg.) applied to 30 couples. Cycles performed for single gene disorders in combination with HLA typing were B-Thalassaemia (n = 224), Wiscott Aldrich Syndrome (n = 4), Gaucher Disease (n = 4), Alpha Mannosidosis (n = 4), X-ALD (n = 3), Fanconi Anemia (n = 3), Hurler Syndrome (n = 3), Glanzman Disease (n = 2), Hyper IgD (n = 1) and Sickle Cell Anemia (n = 1). One blastomere was removed from day 3 embryos by laser technique. Mutation analysis was done using either minisequencing or restriction fragment length polymorphism (RFLP) method combined with linked short tandem repeat (STR) marker analysis, while HLA typing was performed using polymorphic STR markers scattered through the HLA gene cluster.

**Results:** Totally 2834 blastomeres were biopsied and in 2506 (88.4%) a complete diagnosis was achieved. Embryo transfer was performed in 155 cycles (62.2%) with SGD + HLA typing, and 39 cycles (72.2%) with HLA typing only. Totally 68 clinical pregnancies (35%) were obtained out of 194 ET cycles. To date, with 48 deliveries, 57 healthy and HLA compatible children were born. 18 sick children were already cured with cord blood cell and/or bone marrow transplantation. 23 children are waiting for their newborn siblings to gain weight and grown up more to be ready for donation of stem cells.

**Conclusions:** The demand for the preimplantation HLA typing is increasing tremendously since this method is an effective therapeutic tool for the cure of an affected child. PGD for HLA typing technique allows the birth of a healthy child who is at the same time the savior of her or his sibling. Despite the lower probability of finding suitable embryos for embryo transfer, the data
presented in this report shows the feasibility and the practicability of PGD-HLA application.

**P-542** PGD for translocations: should we continue to biopsy two blastomeres?

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Introduction: The effects of embryo biopsy on subsequent development of embryos are still a matter of discussion. It has been shown that biopsy of single blastomere may have a positive effect on the likelihood of blastocyst formation, with keeping the same efficiency for the FISH diagnosis in case of PGD for aneuploidy screening.

In our centre, two blastomeres are usually biopsied. In the aim to investigate the feasibility of biopsying a single blastomere in case of translocations, we have analyzed retrospectively chromosomal results obtained for each blastomere (N = 1014) in the course of 122 PGD. We have compared the diagnosis efficiency currently obtained with two cells to the hypothetic one obtained with a single cell.

Material and Methods: From January 2006 to December 2008, 80 couples underwent PGD for Robertsonian or reciprocal translocations, resulting in 122 oocyte retrievals with 507 embryos available for biopsy. We have established the number of embryos for whom: (i) one or two blastomeres were successfully analyzed (ii) two blastomeres were discordant (a balanced and unbalanced (iii) blastomeres failed to be analyzed. Diagnosis efficiency (E) was calculated according to (Goossens et al. 2008) i.e. E = (D1 + D2) (1-d/d). D1 and D2 (number of embryos for whom diagnosis was successfully obtained from one or two cells); d (rate of discordant embryos); n (total number of biopsied embryos). The diagnostic efficiency if a single blastomere was biopsied has been calculated dividing by two D1 value. A Mac Nemar test was used to compare diagnosis efficiencies.

Results: For Robertsonian translocations, among 237 biopsied embryos, two blastomeres were successfully analyzed for 155 (65.4%) and one for 57 (24.1%). 15 embryos (6.3%) were discordant (4 monosomies, 8 trisomies, 2 haploidies and 1 tetrasomy). 10 embryos failed to be analyzed (4.2%). The current efficiency found was 83.8% and would significantly decreased to 72.5% if only a single cell was available for genetic analysis (p < 0.001).

For reciprocal translocations, among 270 biopsied embryos, two blastomeres were successfully analyzed for 170 (70.4%) and one for 56 (20.7%). 9 embryos (3.3%) were discordant (partial monosomies associated with partial trisomies) and 15 (5.5%) failed to be analyzed. The current efficiency found was 88.1% and would significantly decreased to 78% if only a single cell was available for genetic analysis (p < 0.001).

Conclusion: The present investigation showed first that the biopsy of a single blastomere would lead to an efficiency loss of 10%.

Knowing that 6.3% and 3.3% of biopsied embryos were discordant respectively in case of Robertsonian and reciprocal translocation, the risk of transferring an unbalanced embryo in these cases would be of 3.15% and 1.65%. In view of these results we decide to maintain the biopsy of two blastomeres in case of translocations.

**P-543** p27Kip1 mutant mice exhibit increase in number of maturing follicles and profound increase in multioocyte follicles (MOFs)

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Introduction: p27Kip1 is an inhibitor of cell proliferation which belongs to CKIs group (Cyclin-dependent Kinase Inhibitors or CdK Inhibitors). p27Kip1-/- female mice are sterile but the exact mechanism by which p27Kip1 affects ovary function is not yet totally established. The aim of this research was: a. To look for possible structural differences between oocytes from p27Kip1-/- and wt mice. b. If differences are found, to find out the molecular mechanisms determining the role of p27Kip1 in ovary development and function.

Material and Methods: Ovaries from wt and p27Kip1-/- juvenile (4 weeks) and young adult (12 weeks) mice were fixed in paraformaldehyde, paraffin embedded and serially sectioned. Immunohistochemical staining with VASA antibody was performed. Subsequently, the slides were scanned and digital images were analysed using Aperio scanner and analysis software. The analysis determined: a. Number of VASA-positive germ cells, which corresponds to the total number of oocytes. b. Number of follicles at different stages of growth: “small” (primordial and monolayered primary), “large” (multilayered primary, secondary and Graafian) c. Number of multioocyte follicles (MOFs).

Results: Our results showed an increase in the number of oocytes per ovary (twofold greater) and in the number of “large follicles”, i.e. multilayered primary and secondary (5.4 times greater) in p27Kip1-/- mice compared to wt. The ratio “small follicles” vs. “large follicles” was also increased in p27Kip1 mutants (twofold greater) and is an important parameter to distinguish them from wt mice. A most striking finding was the presence of an increased number of MOFs (multioocyte follicles) in mutant mice (.32.2 times greater).

Conclusions: Our results indicate a role for p27Kip1-/- in oogenesis and ovary development. This protein is involved in proliferation, differentiation and apoptosis, major events occurring during ovarian development. Multioocyte follicles (MOFs) are extremely rare in normal ovaries. The strikingly increased presence of MOFs in the mutants suggests that p27 is involved in the formation and assembly of ova.

Further studies are needed to better understand the ovarian cellular dynamics and might be helpful to find more effective treatments for ovarian-related diseases.

**P-544** Fragile sites and chromosome breakage in embryos from carriers of structural chromosomal abnormalities determined by preimplantation genetic diagnosis

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Introduction: Fragile sites are sites on particular chromosomes which are more prone to chromosome breakage when exposed to conditions of DNA replication stress. Cellular mechanisms such as cell cycle checkpoints and DNA repair monitor and determine the stability of these sites. Under conditions of stress, instability at these regions can predispose to de novo heritable chromosome rearrangements.

Fragile sites are expressed as a chromosome gap, where breakage can lead to the separation of the two parts of the chromosome divided by the gap. Aneuploidy of fragile sites are then generated, where the part of the chromosome remaining at the gap could be lost at subsequent cell cycle stages. Carriers of structural chromosomal abnormalities may opt to have Preimplantation Genetic Diagnosis (PGD), whereby one or two blastomeres are removed and tested from cleavage stage preimplantation embryos generated through in vitro fertilization (IVF), in order to select those embryos which are balanced or normal, aiming to establish an unaffected pregnancy.

This study looks for evidence of chromosome breakage in embryos from such couples undergoing PGD at the UCL Centre for PGD. The aim of the study is to assess whether the level of breakage is higher or more frequent in cases where the breakpoints of the aberration are on or near reported fragile sites, or on chromosomes where no common fragile sites have been reported.

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Materials and Methods: A total of 21 couples that were referred for PGD for structural chromosomal abnormalities; 18 reciprocal translocations, one inversion, one intrachromosomal insertion, were divided into three groups: a) one of the breakpoints was on a fragile site, b) one of the breakpoints was near (at a position distal) a fragile site and c) no fragile sites have been reported on the two chromosomes involved in the aberration tested for.

Each group included 7 couples in total, treated in 9, 10 and 8 cycles respectively. In general 2 blastomeres were biopsyed per embryo and tested using Fluorescent in Situ Hybridization (FISH). Either two centromeric and one subtelomeric probe or one centromeric and two subtelomeric probes were used for the diagnosis. Full follow up of the untransferred embryos was also performed.

Results: For the chromosomes where two probes were used it was possible to determine whether there was chromosome breakage in the embryos where at least 2 or more of the nuclei tested (from the biopsied cells and the untransferred embryo) showed evidence for breakage.

A total of 37 embryos were analysed in group A, 74 in group B and 31 in group C. From group A, 22/37 embryos (59.5%) showed evidence for chromosome breakage, compared to 35/74 (47.3%) and 9/31 (29%) for groups B and C. Chromosome breakage has been reported as being a fairly widespread phenomenon in human preimplantation embryos, which would explain why chromosome breakage was still seen in embryos from group C. It seems however that in embryos from groups B and A the frequency of chromosome breaks increases, with group A exhibiting breaks in more than half of the embryos studied. Since at the preimplantation stages of development the cell cycle checkpoints are not fully functional, it could be that the conditions in the culture media where the embryos are kept cause replication stress which then cannot be fully repaired, resulting in embryos with chromosome breaks.

Conclusion: It seems that couples carrying translocations where the breakpoints are on or near fragile sites are more likely to generate embryos with aberrant chromosomes due to chromosome breakage and fragment loss. Consequently these couples are at a greater risk of producing unbalanced embryos, a new element that should be considered when counseling patients.

P-545 Meiotic segregation analysis in reciprocal translocation carriers with embryos from preimplantation genetic diagnosis cycles

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Introduction: Preimplantation genetic diagnosis (PGD) has been offered to carriers of reciprocal translocations (RT) to reduce the frequency of recurrent abortions or birth of chromosomally abnormal children pregnancy loss. However, the pregnancy rate of PGD is highly related to the number of chromosomally normal embryos available for replacement and the proportion of balanced embryos varies from different type of reciprocal translocations. Thus, it is important to assess the risk of chromosomally normal embryos for individual patient before PGD and provide the patient informative genetic counseling. The purpose of the present study is to explore whether the segregation patterns of the embryos from carriers of RT are related to chromosomes involved, position of the breakpoints, or gender of the carrier.

Materials and Methods: A total of 34 RT carrier couples underwent 41 PGD cycles in a university-based centers for reproductive medicine. Meiotic segregation patterns of the 278 embryos were analyzed by fluorescence in situ hybridization (FISH) in their PGD cycles. For each translocation, both centric segments (CS, arms not involved in the translocation plus interstitial segments situated between the centromeres and the breakpoints) and the translocated segments (TS) were measured.

Results: The ratio of alternate embryos among all the embryos is 12.6% (35 of 278). There was no significant difference between the male RT subgroup and the female RT subgroup (12.5%) vs. 12.7%) regarding the proportion of the alternate embryos. And different from previous study, we found that the incidence of alternative embryos from PGD cycles in reciprocal translocations was not associated with acrocentric chromosomes. The alternate embryo percentage in the pregnancy subgroup and pregnancy failure subgroup was 16.7% and 11.4%, respectively. The proportion of alternative segregation in preimplantation embryos from PGD cycles with TS/CS ratio < 0.2 was lower than that with TS/CS ratio > 0.6 (8.0% vs. 16.7%).

Conclusion: The incidence of alternative segregation producing normal or balanced embryos was relatively low in reciprocal translocations associated with terminal breakpoints. The results may be helpful to predict the possibility of normal or balanced embryos for reciprocal translocation carriers before PGD-FISH cycles.

P-546 Validation and preliminary clinical results of array CGH for PGS

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Introduction: Chromosome abnormalities contribute to low implantation rates and high miscarriage rates with increasing maternal age, and thus their screening previous to transfer may increase implantation rates, reduce miscarriage rates and increase take-home baby rates. Unfortunately, differences in methodology have yielded conflicting results when day-3 biopsy and FISH analysis have been used. The use of Comparative Genome Hybridization (CGH) combined with blastocyst biopsy and vitrification seem to provide very high implantation rates (60%) and ongoing pregnancy rates (80%). However, patients still prefer fresh over a frozen cycle. A superior technique to FISH and CGH is array CGH (aCGH), which provides results in 24 hours for all chromosomes per multiple loci. The purpose of this study is to provide validation results for this technique as well as preliminary clinical data.

Materials and Methods: aCGH was performed on 74 cycles from 17 fertility centers. Average maternal age was 37.3. Indications were AMA (≥ 38, n = 41), recurrent pregnancy loss (RPL) (n = 22) or other < 38 years old (n = 11). A single cell was biopsyed per embryo on day 3 of development. 605 embryos were biopsyed and the cells sent to a PGD reference lab for analysis. Normal embryos were replaced on day 5. To determine the error rate of the technique some abnormal embryos were fully fixed and reanalyzed by FISH. To determine if pregnancy rates and miscarriage rates were improved, we compared the results of each center with those reported for that same center by ART controlling it by age group (< 35, 35-37, 38-40, 40-42).

Results: Only 2.4% of cells did not produce amplification. Of the cells that amplified 38.7% were euploid, and the rest were abnormal. Euploidy decreased with maternal age from 46.1% in < 35 years, to 44.8% in 35-39 years, to 28.1% in the 40-42 years old group (p < 0.001). After reanalyzing 84 abnormal embryos by FISH 6 were normal by FISH (7% error rate). Of the 74 cycles, we obtained follow up data from 40 cycles past first trimester. Of those 40 cycles 20 (50%) became pregnant, compared to an expected pregnancy rate for those centers and ages of 39.3%. In addition, of those 20 pregnancies only one miscarried (5%) compared with a 20.4% expected miscarriage rate for those centers and ages.

Conclusions: For a new technique to be validated, it should be compared to the standard one in use. We have found that aCGH provides results in one day turn-around, with very low frequency of no results (2%), and similar error rate than FISH (7%) even though it can analyze double number of chromosomes. This error rate is identical than the rate of midsization expected from mosaicism when a single cell is analyzed (Colls et al. 2007), and thus it cannot be reduced unless biopsy is performed on day 5. The tendency observed in pregnancy and miscarriage rates is towards an improvement of results, and thus justifies the performance of larger randomized trials using this technique.

P-547 Inheritability of multiple offspring in families with monozygotic twinning after art

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Introduction: ART is known to increase the number of multiple births. The increase in dizygotic twinning (DZT) is influenced by a higher number of transferred embryos, but the precise nature of the increased incidence of monozygotic twinning (MZT) is presently unclear. This study has focused on the inheritance of MZT as a possible cause of this phenomenon.

Material and Methods: In a sample of pregnant women after IVF/ICS1 between 2000–2008, patients with MZT (group A) were identified using US (the
number of gestational sacs was higher than the number of transferred embryos or monochoroidiody or monoamnioidly was clearly defined). Group A was compared with families that only had one child delivered (group B). All patients received a genetic questionnaire or they were personally interviewed on the history of MZ or DZ twins in their immediate family (parents, grandparents, all siblings and children). Statistical analysis was done by chi-square test.

**Results:** In group A, 17 of 20 couples with MZT responded to our questionnaire (85%).

A record of MZT in the immediate family was found in 7 couples (41.2% p < 0.001) and DZT in 4 families (23.5%, NS). MZT or DZT (in 4 of them the zygosity was not specified because of abortion, early child death or missing information) were reported in 13 families (76.5%, NS). In group B (n = 72), we found 9.7% MZT, 22% DZT and 31% incidence of all multiple pregnancies.

Seven families who reported MZT were tested in detail. In all seven pedigrees, 51 pairs were monitored. For these couples, we found 19 families having MZT (37%).

Three geological trees, describing female and male transfer will be presented. One of our male patients had MZT with a first and also later with a second woman.

**Discussion:** The frequency of MZT in families with a history of MZT is significantly higher than in families with just single child births. Autosomal dominant inheritance with low penetrance (38%), described by Hamamy et al. (2004), was also acknowledged in our study. Our results were in accordance with inheritance of the gene responsible for MZT, described through both maternal and paternal lines (Harvey et al., 1977).

**Conclusion:** Our data suggest that monozygotic twinning has autosomal dominant inheritance across both paternal and maternal lines with low penetrance. It is inheritance and not ART technologies that is responsible for the high frequency of MZT after IVF.

**P-548 Impact of oocyte morphology on chromosomal aberrations and ICSI outcomes**

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**Introduction:** The introduction of the intracytoplasmic sperm injection (ICSI), which requires the removal of cumulus cells, allowed the visualization of new information regarding oocyte morphology. However, it is still a matter of debate whether morphology is a good predictor of oocyte viability. With the advent of preimplantation genetic diagnosis (PGD) associated to techniques such as the polymerase chain reaction (PCR) and the FISH (Fluorescent “In situ” Hybridization), for gene and chromosomal abnormalities, respectively, the selection of the most competent oocyte or embryo became much more accurate. The FISH is a technique that uses fluorescent probes that bind to specific parts of the chromosome in order to detect and localize the presence or absence of specific DNA sequences on specific chromosomes. However, the practical and inexpensive application of morphological evaluation should not be discharged with no further investigation. Therefore, this study was designed to verify the relationship between oocyte morphology and chromosomal abnormalities evaluated by the FISH in patients submitted to ICSI.

**Material and Methods:** The population in the present study included 654 embryos from 219 patients submitted to PGD following ICSI. The blastomeres were evaluated using the FISH, which was performed by spreading the nuclei from the cells onto microscope slides, dehydrating and staining with specific fluorescent probes. Chromosomes 13, 18, 21, X and Y were tested for aberrations. Patients’ age, FSH administered for ovarian stimulation, serum estradiol levels, oocyte yield, oocyte morphology (i.e., cytoplasmic granularity, smooth endoplasmic reticulum cells, vacuoles, perivitelline space size, perivitelline space granularity and fragmented first polar body) and ICSI outcome were compared among groups showing normal (FISH-) or abnormal chromosomes (FISH +) using the Student t test. Regression analysis was performed to test the relationship between studied response variables and the incidence of chromosomal abnormalities. Results were described as mean ± standard error of the means, odds ratios (OR) and respective confidence limits (CL).

**Results:** Of the FISH analysis showed 107 cycles with chromosomal aberrations (FISH-) and 112 with normal chromosome organization (FISH +). The dose of FSH and serum estradiol level did not differ between the groups. However, the group of patients FISH + was older then the group FISH- (38.05 ± 0.38 vs. 35.86 ± 0.57, respectively; p = 0.001). Oocyte yield, reflected by the number of retrieved oocytes per number of aspired follicles, was higher in the FISH- group when compared to the FISH + group (62.3% vs 73.7%, p < 0.05). Furthermore, a higher rate of oocytes with large perivitelline space size (0.44 ± 0.05 vs. 0.33 ± 0.04, p < 0.05) and perivitelline space granularity (0.59 ± 0.05 vs 0.44 ± 0.05, respectively, p < 0.05) were found in the FISH + group when compared to the FISH- group. No significant differences on pregnancy, implantation and miscarriage rates were observed between the groups of patients FISH + and FISH-. Regression analysis showed a close relationship between the incidence of perivitelline space granularity (OR: 1.85, CL: 1.08-3.15, p < 0.05) and large perivitelline space size (OR: 1.58, CL: 0.92-2.73, p < 0.05) with the odds of chromosomal abnormalities.

**Conclusion:** Results indicate that, similarly to previous studies, the incidence of chromosomal abnormalities is higher in older women, indicating that PGD is an important tool in case of age-related infertility. Furthermore, oocyte yield as well as the evaluation of oocyte morphology in ICSI cycles, especially regarding perivitelline space, appears to be indicative of a higher incidence of chromosome abnormalities. A hypothesis to explain such results would be that these alterations on the perivitelline space would be related to the abnormal polar bodies which could reflect chromosomal abnormalities on the embryos. However, further studies are necessary to test this hypothesis.

**P-549 Diminished ovarian reserve affects embryo multiple abnormalities occurrence in ICSI cycles**

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**Introduction:** A reasonable percentage of women undergoing infertility treatment respond poorly to the ovarian stimulation protocol. Low follicular recruitment following ovarian stimulation may be observed in 9 to 24% of the in vitro fertilization (IVF) cycles. It is well known that ovarian poor response to stimulation protocols increases as women get older. Those patients are usually stimulated with high doses of gonadotropins; however, due to the low number of retrieved oocytes and transferred embryos, low pregnancy rates are expected. In addition, embryos originating from poor prognosis IVF patients were proposed to have higher aneuploidy rates. The aim in this study was to test the hypothesis that aged women with poor ovarian response express an increase on embryo chromosomal alterations when compared to aged women who presented normal response.

**Material and Methods:** The present study included couples undergoing intracytoplasmic sperm injection (ICSI) cycles associated to preimplantation genetic screening (PGS), as a result of advanced maternal age. Patients were subdivided into two groups according to the number of collected oocytes after ovarian stimulation: Poor Responder (PR group, n = 34), patients who produced four or less oocytes; and Normoresponder (NR group, n = 50), patients in which five or more oocytes were retrieved. After ICSI, all the biopsied embryos were analysed for chromosomes X, Y, 13, 16, 18, 21 and 22. Groups were compared regarding ICSI outcomes and aneuploidy frequency. The presence of two or more chromosomal abnormalities was characterized as multiple abnormalities. Influence of poor ovarian response on aneuploidy rates was assessed using logistic regression analysis, and the results were expressed as odds ratios (OR), confidence intervals (CI) and p-values.

**Results:** Cycle’s general characteristics were similar between the groups. There were no significantly differences on fertilization (73.9% vs 76.6%; P = 0.7861), implantation (22.6% vs 20.6%; P = 0.6863) and pregnancy rates (28.0% vs 29.4%; P = 0.9208) between NR and PR groups; however, a significantly increase on the mean number of transferred embryos (2.0 ± 1.0 vs 1.1 ± 0.8, P = 0.0047) was observed on NR group. Regression analysis showed no
significant influence of poor response on the percentage of embryos showing autosomal aneuploidy (OD: 1.23, CI: 0.74-2.05, P = 0.417), sexual aneuploidy (OD: 0.9, CI: 0.44-1.85, P = 0.769). On the other hand, poor response was associated with a significantly increased risk of embryo multiple abnormalities occurrence (OD: 2.15, CI: 1.15-4.04, P = 0.017). In addition, cycle cancellation rate was significantly higher on PR group (4.0% vs 23.5%, P = 0.0128). This finding was confirmed through the logistic regression model (OR: 7.38, CI: 3.25-23.24, P = 0.016).

Conclusion: In this study we attempted to associate ovarian poor response with increased occurrence of embryo chromosomal alterations in aged women. Results showed that embryo multiple abnormalities occurrence risk is more than two-fold higher in PR patients. This finding also makes plain the statistically lower number of transferred embryos on PR group, since a diminished number of viable embryos were available to be transferred. In addition, odds of cycle cancellation rate were more than seven-fold higher due to the decreased presence of normal embryos on PR group. Similar implantation and pregnancy rates in NR and PR groups could possibly be explained by equivalent embryo morphology and chromosomal status of transferred embryos after PGD performance. Despite the poor prognosis of the IVF outcome in aged patients with low response to ovarian stimulation, the present study shows that poor response is not constantly associated with a decreased implantation and pregnancy rate, however, poor ovarian response impairs embryo chromosomal integrity and percentage of viable embryos, reducing the chances of IVF cycles completion. Moreover, our results highlight the predictive value of aged women ovarian reserve for oocyte quality and competence.

P.550 Potential benefits of PGS in recurrent implantation failure: a prospective controlled trial in a properly characterized population


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Introduction: In recent years great controversy has emerged regarding the application of preimplantation genetic screening (PGS) for different indications. Methodological aspects and patients’ selection criteria could explain differences among authors. The aim of this study was to evaluate the usefulness of PGS in patients < 40 years with recurrent implantation failure. Patients were prospectively randomized to either of the following two groups: conventional IVF/ICSI or PGS, both treatments with day-5 embryo transfer.

Material and Methods: Study design: Before starting the cycle, patients were allocated through computer-generated randomization into two groups: conventional IVF/ICSI cycle (group A) or PGS cycle with screening for chromosomes 13, 15, 16, 17, 18, 21, 22, X and Y (group B). All patients recruited underwent an exhaustive infertility work-up: vaginal ultrasound (hysterosonography or hysteroscopy when needed); blood karyotypes; Anti-cardiolipin and lupus anti-coagulant antibodies; Antithrombin III and APCR levels, factor V Leiden mutation. Inclusion criteria were:

- Presence of at least one of the previous infertility work-up: vaginal ultrasound (hysterosonography or hysteroscopy when needed); blood karyotypes; Anti-cardiolipin and lupus anti-coagulant antibodies; Antithrombin III and APCR levels, factor V Leiden mutation.
- Any type of infertility: primary or secondary infertility, previous uterine or uterine manipulations.
- Previous difficulty in embryo transfer and cycle cancellation.
- High percentage of embryos with morphologi- cal abnormalities and chromosomal abnormalities.

Conclusion: Our results showed that embryo multiple abnormalities occurrence risk is more than two-fold higher in PR patients. This finding also makes plain the statistically significant influence of poor response on the percentage of embryos showing autosomal aneuploidy (OD: 1.23, CI: 0.74-2.05, P = 0.417), sexual aneuploidy (OD: 0.9, CI: 0.44-1.85, P = 0.769). On the other hand, poor response was associated with a significantly increased risk of embryo multiple abnormalities occurrence (OD: 2.15, CI: 1.15-4.04, P = 0.017). In addition, cycle cancellation rate was significantly higher on PR group (4.0% vs 23.5%, P = 0.0128). This finding was confirmed through the logistic regression model (OR: 7.38, CI: 3.25-23.24, P = 0.016).

P.551 Spontaneous abortion and 44-bp insertion-deletion polymorphism of the SLC6A4 gene


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Introduction: Although the relationship between antidepressant use during pregnancy and its adverse effects has been widely investigated, very few studies have evaluated the impact of antidepressant use during pregnancy on the risk of spontaneous abortion (SA). Several studies suggest that gestational exposure to antidepressants, especially selective serotonin reuptake inhibitors (SSRI), can lead to spontaneous abortion.

On the basis of these reports we have analyzed the allele and genotypes frequencies of the 44-bp insertion-deletion polymorphism in 5-Hydroxytryptamine transporter gene promoter region (5-HTTLPR) in samples from SA.

Material and Methods: Parafine embded tissues samples, from SA of unknown etiology (n = 29), and a group of 89 fertiles women, were genotyped for the 44-bp insertion-deletion polymorphism located in the promoter region of the SLC6A4 gene. Parafine from tissues was discarded by Xylol washing. DNA was extracted from tissues and blood samples by salting-out method. PCR amplified samples were analysed by Snapshot (Applied Biosystem).

Results: The genotypes and allele frequencies of the groups were: SA: SS = 0.71, SL = 0.18 and LL = 0.11; control group, SS = 0.32, SL = 0.43, LL = 0.25 (p < 0.001). It has been reported that cells homozygous for the L form produced steady-state concentrations of SLC6A4 mRNA that were 1.4 to 1.7 times those in cells containing 1 or 2 copies of the S variante. In these studies, the data associated with the S/S and L/S genotypes were similar, whereas both differed from the L/L genotype, suggesting that the polymorphism has more of a dominant-recessive than a codominant-additive effect. Although L/S polymorphism of the SLC6A4 has been related to several mental diseases, and human behavior, and the short (S) form (functionally L/S or S/S) shows an increase in brain metabolism, very few of these studies are referred to the development of the brain embryo, and none has been related to foetal viability as the present report.
Conclusions: Although this is an ongoing study, with a low sample number by now, we observed a strong presence of SS genotype in samples of fetuses. These results permit to conclude that metabolism of serotonine has some influence on human fetal viability. Supported by Grants AF2008-03314 and PTQ 09-01-00496.

P-552 Validation of putative CNVs on the X chromosome in POF patients
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Introduction: Premature ovarian failure (POF) is the spontaneous cessation of the menstruation before age 40 accompanied with elevated FSH, low AMH and oestradiol levels. Copy number variants (CNVs) are submicroscopic deletions or duplications ranging from 1 kilobase to several megabases and are of increasing interest in explaining human genetic variation. Since microscopic X chromosomal aberrations identified by karyotyping are associated with POF, we hypothesized that submicroscopic CNVs also contribute to POF pathogenesis. These variants will go undetected by conventional karyotyping but can be readily detected with high-density SNP arrays. Using Illumina 370k SNP genotyping chips and Using PennCNV calling software, we identified putative deletions in the Xq21.3 locus in nearly 15% of the 110 patients that were analyzed and none in controls (ESHRE abstract 2009 - Hum. Reprod. 24:i12 O-029). Notably, karyotypic abnormalities have been reported in this locus to be associated with POF.

Material and Methods: To substantiate this finding we re-analyzed 13 of 15 subjects that suggested a deletion on the Xq21.3 locus using an 8-plex 60K array that was custom designed using eArray. The array (ID 023317, Agilent Technologies Inc., Santa Clara, CA, USA) contains 48,325 probes, evenly distributed over the X chromosome, with a medium distance between the probes of 1.9 Kb. All probes were selected from the probe groups present on the catalogue 1M and 400K arrays (ID 021529 and ID 021850, respectively, Agilent).

Results: We did not observe deviating intensity values for consecutive probes in the analyzed samples, indicating that the samples do not contain CNVs in this locus. Therefore, the putative CNVs identified are most likely false-positives. We hypothesize that batch or probe binding effects may have played a role in the earlier Illumina experiment.

Conclusions: - The earlier identified deletion locus on Xq21.3 could not be validated using a custom designed ultra high density X chromosome comparative genomic hybridization array.
- CNVs on the X chromosome do not play a major role in the etiology of POF.
- Careful validation is required when using high density genotyping chips for CNV detection.

P-553 Parthenogenetic activation of human oocytes as a model for polar bodies-PGD
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Introduction: Pre-implantation genetic diagnosis (PGD) has become an established clinical approach for prevention of genetic disorders and polar bodies analysis instead of, or together with, blastomeres analysis has gained increasing importance in recent years.

We evaluated a model of parthenogenetic activation of human oocytes in order to assess the feasibility of PGD for monogenic disorders on human oocytes in our laboratory. Aim of this model was to test an ethical experimental setting independent from fertilization, resembling the dynamics of chromosome segregation and crossover during meiosis. In this model, after the molecular analysis of the first polar body (PB1), the second polar body (PB2) can be obtained in order to have a complete genetic analysis and validate molecular results. Indeed, PB2 analysis is useful to confirm a diagnosis of homozygous PB1 or to predict which maternal allele will be present in the oocyte in case of heterozygous PB1.

Material and Methods: After partial zona pellucida dissection, PB1s were removed from spare donated metaphase-II oocytes and immediately transferred to lysis buffer for subsequent molecular analysis. Parthenogenetic activation was conducted on biopsied oocytes through exposure to 5 mM iomomycin in culture medium for 5 minutes at 37 °C, 5% CO2 in the dark. These were then washed three times in fresh cleavage medium, placed separately in 40-μL microdrops of the same medium under mineral oil, and cultured in standard conditions. After 18-20 hours, oocytes were evaluated for signs of activation. Oocytes showing one enlarged pronucleus and extrusion of PB2 were considered activated; PB2s were biopsied and treated as PB1s.

The molecular analysis involved a fluorescent multiplex polymerase chain reaction (PCR) of highly polymorphic short-tandem repeat (STR) markers, closely linked to the disease-causing gene. In this set of experiments, a panel of ten highly polymorphic STR markers flanking ‘Cystic Fibrosis Transmembrane Conductance Regulator’ (CFTR) gene was selected for haplotype analysis. Based on a number of informative markers, genetic analysis was performed on PB1 and PB2.

Results: Twenty-one oocytes were selected from seven women. After PB1 removal, twenty of them were subjected to parthenogenetic activation, and fifteen (75%) extruded the PB2. All of PB1s were successfully PCR-amplified but three out of 15 PB2 did not get any amplification product. Limiting the results to 12 oocytes with both PB1 and PB2, 10 out of 12 PB1s were heterozygous and 2 homozygous for the evaluated markers, thus resulting in a percentage of cross-over recombination of 93%. PB2s analysis consistently permitted the prediction of the oocyte’s genotype in case of heterozygous PB1s (n = 10), and confirmed PB1 results in homozygous cases (n = 2).

Among all evaluated markers, we observed a frequency of allele drop out (ADO) of 5.1% (3/59) with no more than one case of ADO for single PB.

Conclusions: The model of parthenogenetic activation confirms that polar bodies-PGD for single gene disorders is feasible and offers a valuable tool for setting up PGD procedures without wasting zygotes. Our results confirm a high recombination rate in CFTR gene suggesting a very limited possibility of selecting oocytes according to genetic analysis of PB1 only.

P-554 Application of mild stimulation and single blastocyst transfer with vitrification in preimplantation genetic diagnosis (PGD) cycles: case report
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Introduction: The efficacy of mild stimulation and single blastocyst transfer has been reported previously, however these techniques are not commonly applied in PGD cycles. We set out to develop a new approach for PGD.

Material and Methods: Nine couples consisting of subjects with a mean age of 35.1 years who were carriers of translocations, had experienced two or more consecutive pregnancy losses, and failed to experience a live birth in their entire reproductive history. Prior to the commencement of the study, approval from the ethical committee was received from the Japan Society of Obstetrics and Gynecology and our local Institutional ethics committee.

From January 2007 to December 2009, 9 patients underwent mild ovarian stimulation, which was carried out by administration of clomiphene citrate in combination with a minimum amount of urinary HMG (150IU X less than 3 times). Administration of clomiphene citrate was initiated from day 3 of the menstrual cycle at 50mg/day 4 times) or recombinant FSH (150IU X less than 3 times). Administration of clomiphene was continued, and embryos developing to the blastocyst stage were vitrified.

Results: Of the 17 patients, 9 patients underwent mild ovarian stimulation, which was carried out by administration of clomiphene citrate in combination with a minimum amount of urinary HMG (150IU X 3 times) or recombinant FSH (150IU X less than 3 times). Administration of clomiphene was continued, and embryos developing to the blastocyst stage were vitrified.

IFV was performed for all oocytes, and embryos reaching at least the 6-cell stage on day 3 of development were biopsied by mechanical and cleavage aspiration. Two blastomeres were removed and fixed by the Carnoy method. Culture of all the biopsied embryos was continued, and embryos developing to the blastocyst stage were vitrified.

FISH analysis was performed on all blastomeres using 3 to 4 appropriate FISH probes for several loci divided by the patient’s own
breakpoint. Cryopreserved blastocysts which showed alternate were thawed and intrauterine transfer of single blastocysts was performed.

Results: Sixty-eight oocytes were collected in 25 OR cycles from 9 patients, and 94% (60/64) were fertilized, 95% (57/60) of the embryos were biopsied, and 100% (57/57) of the embryos were successfully biopsied. Of the embryos that were successfully biopsied, 98% (56/57) yielded a diagnostic result of which only 30% (17/56) were transferable at the 8-cell stage. Culture of all the biopsied embryos was continued and 61% (33/57) of the embryos developed to the blastocyst stage. Of the 35 vitrified blastocysts, only 37% (13/35) resulted in allograft, and 11 of the blastocysts which showed alternate were thawed and intrauterine transfer was performed by single blastocyst transfer for 8 patients. Two blastocysts are still cryopreserved and kept for second children. A positive heart beat in 7 cycles was obtained in 7 patients, 64% per ET (7/11) and 28% per OR (7/25).

Finally, the delivery rate was 55% per ET (6/11) and 24% per OR (6/25), and one case is ongoing at 9 weeks; there were no spontaneous abortions and none of the clinical pregnancies was lost to follow up. There were 6 deliveries of 6 babies, consisting of 2 males and 4 females with a birth weight of 2198 to 3296 g and gestational age of 37.0 to 40.4 weeks, with no malformations at birth. Prenatal diagnosis revealed normal karyotype in 3 and inherited reciprocal translocation in 2 cases. Pre or post-natal diagnosis was not undertaken in the remaining one case, however, there were no reports of developmental or mental retardation in any of the children. Therefore, we assume that all of the babies were healthy.

Conclusions: Many researchers have reported that the most important factor for PGD is the risk of misdiagnosis. The PGD results of 9 patients in this study strongly suggest that not only the diagnosis was performed accurately, but also the mild stimulation and single blastocyst transfer with vitrification was effective for PGD.

P-555 Spindle and chromosome configuration of human in vitro matured oocytes after vitrification: effect of cytochalasin b pretreatment

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Introduction: Although many advances in oocyte cryopreservation have been made, unlike embryo and sperm cryopreservation, it is still under debate, mostly because the fertilization rate and implantation rates are still low; the subsequent developmental competence is poor. Now many reports have proved that the disappointment success rates of oocyte cryopreservation due to the damage on plasmic sperm injection (ICSI) cycles (include stage GV and MI) after ovarian stimulation, followed in vitro matured (IVM) for 24h-48h, 170 oocytes were matured (with the exclusion of first polarbody), were randomized into five groups to vitrification. Group A: 37 oocytes were treated with CCB for 20mins before vitrification; Group B: 38 oocytes with CCB for 30 mins. Group C: 33 oocytes with CCB for 40 mins. Group D: 36 oocytes without CCB before vitrification. Group E: 26 oocytes without vitrification and CCB as the controlled group.

Results: In group A1, B1, C1, D1 (27.3%, 30.0%, 45.5% and 33.3%, P > 0.05). There was no significant difference in the frequencies of normal spindle and chromosome in group A1, B1, C1, D1 (27.3%, 30.0%, 45.5% and 33.3%, P > 0.05). No statistical difference was found in survival rate and frequencies of normal spindle and chromosome between in vitro matured groups treated with CCB and in vivo matured groups after vitrification (P > 0.05).

Conclusions: Spindle and chromosome configuration of human in vitro matured oocytes was damaged in vitrification, which can not improved by CCB pre-treatment before vitrification. Oocytes in vitro matured or in vivo matured have the same tolerance to the cryoinjury.

P-556 Modulation of an imprinted gene network in placenta as a compensatory mechanism for normal development of in vitro manipulated mouse embryos

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Introduction: Genomic imprinting regulates the expression of several genes crucial for mammalian development, by parental-specific mechanisms such as allele-specific DNA methylation at differentially methylated regions (DMRs). We have previously shown that in vitro fertilization and embryo culture result in methylation defects at the imprinted H19-Igf2 locus in the mouse blastocyst (Fauque et al. 2007). The current study was designed to evaluate the consequences of the same manipulations on genomic imprinting after implantation using a mouse model.

Materials and Methods: Mouse blastocysts were produced following three different experimental conditions: (i) a control group with embryos obtained after in vivo fertilization and development and two groups of embryos maintained in different culture systems (either M16 or G1/G2 medium) after (ii) in vivo fertilization or (iii) in vitro fertilization. All blastocysts were transferred into pseudopregnant females. Embryos and placentas were then collected from recipient females at day 10.5 of development. DNA methylation patterns of the H19, Igf2, Igf2r and Dlk1-Dio3 DMRs were analyzed by quantitative pyrosequencing. Furthermore the expression levels of 19 genes belonging to the imprinted gene network (IGN) involved in embryonic growth (Gabory et al. 2009; Varrault et al. 2006) were analyzed in placental tissues using quantitative RT-PCR and genNorm quantification.

Results: No significant alteration was observed in the methylation profiles of four DMRs from the H19, Igf2, Igf2r and Dlk1 genes, neither in placentas nor in embryos of experimental groups compared to controls.

Interestingly, placentas displayed a correlated misregulation of several imprinted genes. Significantly disturbed levels of H19 and Igf2 mRNA were observed, as well as of most other genes from the IIGN. Both maternally and paternally expressed imprinted genes were modified and mostly up-regulated. In addition, we observed that the expression patterns were not significantly different between both culture systems.

Conclusions: In contrast to the methylation defects detected at the blastocyst stage, we found that after in vitro manipulations, retransplanted E10.5 embryos and placentas were phenotypically normal and showed no modification of the methylation profiles of several imprinted genes. We hypothesize that a completely restored epigenetic pattern could be required for normal foeto-placental development. Furthermore, the modulation of a coordinated network of imprinted genes could result in a compensatory process capable of correcting potential dysfunction of placenta and protecting the embryo. These data obtained at post-implantation stage show a moderate risk of epigenetic abnormalities in the mouse and appear more reassuring for the health of children born by ART compared to studies investigating preimplantation embryos.

References:
Abstracts of the 26th Annual Meeting of ESHRE, Rome, Italy, 27 June – 30 June, 2010

P-557 Application of A-CGH on blastocyst biopsy in patients with idiopathic recurrent abortions
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Introduction: Previous studies including ours showed the importance of A-CGH in assessing chromosomal abnormalities in Preimplantation Genetic Screening (PGS) suffering from recurrent IVF failure. The success rate was encouraging to offer such a screening to any patient with more than 7 recurrent IVF failures. The current report shows the application of A-CGH on patients suffering from idiopathic recurrent abortions. Biopsy was performed on embryos at blastocyst stage instead of day 3 stage.

Material and Methods: Patients with 3 consecutive recurrent abortions before the 9th week of pregnancy were recruited for PGS using A-CGH (agilent platform). Embryos are biopsied day 5 morning and the transfer is performed on day 6 early morning. The new protocol adopted will be detailed.

Result: Ten patients have been already screened and the result shows abnormalities in many chromosomes. Pregnancy rate is reaching 65%. Detailed results of the pregnancy duration will be detailed.

Conclusion: This result is the first to use A-CGH with agilent platform on blastocyst biopsy for patients with idiopathic recurrent abortion. Pregnancy rate and the type of chromosomal abnormalities detected would direct us to highlight which of the chromosomes to be screened in a less expensive technique like FISH. More patients will be screened in order to have a more reliable conclusion on the outcome of the protocol adopted.

P-558 Association of folate-pathway gene polymorphisms with pregnancy outcome in recipient women undergoing in vitro fertilization with donor eggs
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Introduction: Folate metabolism disorders have been related with poor fertility outcome, most frequent condition is hyperhomocysteinemia and altered DNA methylation patterns due to genetics variants and depleted dietary intake of folate and B12 related vitamins. Periconceptional folic acid and vitamin B12 supplementation has been largely extended during last decade and we have implemented in recipients undergoing our IVF oocyte donation programs. Genetic condition of recipients has lost interest because of their small contribution in embryo inheritance. However the association of single nucleotide polymorphisms (SNPs) in the one carbon methyl group pathway and in vitro fertilization (IVF) outcome is still unclear. Our aim is to analyze distribution and association of functional SNPs of enzymes related to methyl group metabolism in recipients undergoing IVF treatment in our oocyte donation program for fertile tested donors. For this purpose we decided to study distribution of polymorphic markers of key regulating enzyme of Folate and Homocysteine metabolism MTHFR677 (rs1801133), MTHFR1298 (rs1801131), MTR (rs12749581), CBS (rs5742905) and TCN2776 (rs1801198). Estrogenic metabolism of recipient was assessed for of two relevant SNPs ESR15P (rs2234693) and ESR13P (rs9340799).

Material and Methods: A total of 42 recipients without child undergoing IVF treatment in our oocyte donation program were included with average age of 37.8 ± 6.5 years) they were women with at least one previous attempt with own oocyte in IVF treatment. Donors were mothers with children that had provided at least two ongoing pregnancies in no more than 3 embryo transfer in different recipients. Genomic DNA extraction was carried out from buccal swab (Qiagen Mini KIT). Genotyping of SNPs was developed by PCR multiplex amplification and minisequencing (SNaPshot™ ABI PRISM 3130). Hardy-Weinberg equilibrium was assessed for markers in all groups. Chi-squared test was performed for compare differences between recipients and general population frequencies and also between pregnant and non pregnant recipients condition. Logistic regression models were calculated for odds ratio (OR), 95% confidence interval (CI), and corresponding p values performed for assess clinical pregnancy outcome and genotype distribution.

Results: Ongoing pregnancy rate were of 52.4%. Distribution of all genotypes were in HW equilibrium except for markers MTHFR C677T (rs1801133) (p = 0.049) in non pregnant woman. Higher frequencies of mutant alleles were found in recipients respect general population in markers MTHFR677 (rs1801133) [X2 = 7.639; p = 0.0219], MTR (rs12749581) [X2 = 7.639; p = 0.0219]. However all mutant alleles of the other markers were overrepresented in recipients without statistical significant results. When comparing clinical pregnancy outcome between recipients, significant differences in genotype frequencies were also found in the MTHFR A1298C (rs1801131) [X2 = 6.615; p = 0.0366] in this case mutant allele was present in higher frequency in pregnant recipients. The results show that the recipient group is highly selective by confirmation of deviation of the genotypes frequencies for the folate gene polymorphisms studied. Supposedly, due to all women were treated with folate and Vit B12 supplementation, genetic factor should acquired less relevance. In fact 2 pregnant recipients presented 3 mutations for the C677T and A1298C MTHFR polymorphisms (CTTC). This genotype only normally found in fetal samples, are very rare in general populations (0.002). Strong association of the A1298C MTHFR mutated allele (OR = 3.2 p = 0.0077 (1.27-10.86) 95% CI) with the pregnancy outcome could be related with the low incidence of MZT in the MTHFR C677T SNP considered as functionally more relevant on enzymatic activity.

Conclusions: Folate gene variants present a high incidence in infertile women and could be involved in IVF outcome, although further analysis with higher population need to be completed to extract clinical conclusions. Supported by Grants SAF2008-03314 and PTO 09-01-00496.

P-559 Young women with good ovarian function and a family record of monzygotic twins are at a high risk of monzygotic twinning after ART
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Introduction: There is evidence for at least a 2-fold rise in the incidence of monzygotic twinning (MZT) after assisted reproductive technologies compared with natural conception. The aim of the study was to evaluate the influence of micromanipulation techniques (AH, ICSI), length of cultivation, type of cultivation media and the age of patients on the incidence of MZT in IVF/ICSI patients and to estimate the potential genetic risks for MZT.

Material and Methods: The study group included patients treated for infertility in our IVF centre between 2000–2008. We evaluated the micromanipulation technique used, length of embryo cultivation, type of cultivation media and the age of the patient. Ovarian function was determined by basal FSH, the estradiol (E2) level on the day of oocyte collection, the number of oocytes and consumption of gonadotropins. The parameters of ovarian function in one group of MZT pregnancies (A) were compared with the population of women with dizygotic pregnancies (B), single pregnancies (C) and abortions (D). A genetic survey on multiple pregnancies in the immediate family (up to the third generation) was done for groups A, B and C. Statistical analysis was done using multiple regression analysis.

Results: The percentage of monzygotic twins after assisted conception was 1.3% (n = 1353). We found no significant difference between groups A (20), B (355), C (669) and D (309) in terms of the micromanipulation technique used, length of embryo cultivation and the type of cultivation media. Patients in group A were significantly younger 28.6 (A) vs. 29.8 (B), 30.1 (C) and 30.9 (D). Basal FSH was 7.0 IU/l (A) vs. 7.4 IU/l (B), 7.4 IU/l (C) and 7.7 IU/l (D). E2 level was 3 321 pmol/l (A) vs. 2588 pmol/l (B), 2534 pmol/l (C) and 2575 pmol/l (D). The number of retrieved oocytes was 16.2 (A), 13.4 (B), 13.0 (C) and 13.1 (D). The total amount of FSH needed was 2 012 IU (A), 2 217 IU (B), 2222 IU (C) and 2313 IU (D). The number of embryos transferred was 2.2 (A) vs. 2.8 (B), 2.7 (C) and 2.8 (D). The families of patients in group A had a 41% incidence of MZT, families in group B had an MZT incidence of 14.1% with a 9.7% incidence of MZT in family group C (A vs. C p < 0.001).

Conclusions: The incidence of monzygotic twins in women treated for infertility was 3 times higher than in women conceiving spontaneously.
Micromanipulation techniques, the length of cultivation and type of cultivation media did not influence the incidence of MZT. Ovarian function was markedly better in women with MZT pregnancies compared to women with dizygotic and single pregnancies. Women with MZT were significantly younger and had a lower number of transferred embryos. MZT could be expected after IVF/ICSI in young women with good ovarian function and with a history of MZT in their families. These women should be indicated for elective single embryo transfer.

POSTERS:

REPRODUCTIVE SURGERY (FEMALE & MALE)

P-560 Diagnostic accuracy of three dimensional sonohysterography (SHG) for intrauterine abnormalities in infertility compared to two dimensional SHG

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Introduction: Evaluation of the uterine cavity prior to in vitro fertilization (IVF) is a key diagnostic step and sonohysterography (SHG) is often used for this purpose. This study was performed to compare the diagnostic accuracy of two-dimensional (2D) and three-dimensional (3D) SHG for the diagnosis of intrauterine lesions in infertility patients.

Materials and Methods: This is a prospective analysis of 209 consecutive 3D SHG procedures and 70 hysteroscopies performed from January 2009 to December 2009 in a private infertility practice. All patients underwent 3D SHG as part of the infertility evaluation. All procedures were performed using a Voluson 730 Pro or a Voluson E8 Expert (General Electric Medical Systems, Zipf, Austria). When an abnormality was present operative hysteroscopy was recommended. Data were analyzed comparing 2D and 3D SHG and hysteroscopy findings.

Results: Of the 209 3D SHGs performed, 110 were normal and 99 abnormal (47%). Of the normals, 70 underwent hysteroscopy. In every one of the normal SHGs some intrauterine pathology was found (PPV 100%). Abnormalities found at 3D SHG included 31 polyps, 10 polyps associated to other pathology, 8 myomas, 27 intrauterine adhesions, 9 uterine septum or subseptum, 12 subseptum associated with other pathology, 1 unicornuate uterus with synchiea and 1 arcuate uterus. The 3D coronal views of the uterus improved the diagnostic accuracy of the procedure in 64 cases (65% of normals, 31% of the total). A total of 27 patients with abnormal 3D SHG would have been considered normal by 2D SHG (27/99 = 27%). When only 1 abnormality was present, the diagnosis made at 3D SHG was confirmed by hysteroscopy in 100% of cases. When 2 abnormalities were associated, and one of them was a septum or subseptum, 1 of them was initially missed at 3D SHG in 8 cases. However, retrospective analysis of the SHG images allowed correct identification and localization of all lesions.

Conclusions: This study demonstrates that 3D SHG has a PPV of 100% for intrauterine lesions. The 3D coronal planes improves the diagnostic of additional lesions not visible in the 2D images and adds precision to the localization of the lesions. Experience of the operator is important and there is a learning curve, but the availability of stored images for later review makes 3D SHG an invaluable tool for the diagnosis of intrauterine pathology.

P-561 Incidence of uterine anomalies in normal and infertile women and the role of hysteroscopic metroplasty in improving the outcome of fertility treatment

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Introduction: Congenital uterine malformations have long been associated with poor obstetrical outcome (miscarriage and premature delivery), but their role in infertility as been proposed only recently. Three-dimensional ultrasound (3D-US) has introduced in clinical practice a novel, non-invasive instrument for the study of uterine morphology that allows evaluation of the incidence of uterine anomalies in both normal and infertile women. This study was designed (1) to detect the incidence of uterine anomalies in an infertility population in comparison with fertile women and (2) to assess the impact of hysteroscopic metroplasty on the outcome of fertility treatment.

Material and Methods: A retrospective review of our electronic medical records (EMR) (Viewpoint, GE, Zipf, Austria) evaluating all 3D-US studies performed from July 2008 to June 2009 was performed. 583 consecutive women seen in a single private gynecologic center either for infertility (n = 458, 79%) or for routine gynecologic care (n = 125, 21%) were included. US were performed using a Voluson 730 or a Voluson E8 expert; the 3D volumes of the uterus and patients’ medical histories stored in our EMR, were retrieved and the 4D images reviewed using the software 4D view (GE, Zipf, Austria). The diagnosis of uterine malformations was made by 3D-US in all cases and confirmed by 3D sonohysterography in 43 cases; 39 patients underwent hysteroscopy and hysteroscopic metroplasty as appropriate.

Results: The uterine morphology was normal in 489 women (83.9%) while 94 (16.1%) has some uterine anomaly: of these 79 (84%) were found in infertile patients and 15 (16%) in routine gynecologic patients. Of the 94 uterine anomalies 42 were subseptate uteri (45%), 41 were arcuate uteri (44%), 8 were septate uteri (8%), 2 were unicorionate (2%), 1 bicorionate uteri (1%). Of the 125 routine gynecologic cases 15 (12%) had a uterine anomaly; 13 were arcuate uteri (10.4% of the total) and 2 were subseptate uteri (1.6%). Of the 458 infertility women 79 (17%) had a uterine anomaly; 28 were arcuate uteri (6.1%), 40 subseptate (8.7%) and 8 septate (1.7%), 2 unicorionate (0.4%) and 1 bicorionate (0.2%). Hysteroscopic metroplasty was performed in 39 cases, all with infertility (2 arcuate uteri, 32 subseptate uteri and 5 septate uteri) and subsequent IVF treatment in these cases resulted in a pregnancy rate of 58%, as opposed to a PR of 24% in the group of patients in which the uterine anomaly was not corrected. Patients in the routine gynecologic group had no history of miscarriage or infertility. Of the 79 infertile women diagnosed with a uterine anomaly 21 (26.6%) patients subseptate uteri and 4 in arcuate uteri) had a history of one or more miscarriages.

Conclusions: This study shows that the incidence of uterine anomalies is significantly higher in patients with infertility, than in normal women, suggesting a role of uterine malformations in the pathogenesis of infertility. Based on these findings, hysteroscopic metroplasty may be indicated in these cases even in the absence of a history of recurrent pregnancy loss. The increased pregnancy rates reported after surgical correction of the uterine malformation further supports this conclusion.

P-562 Recovery rates and treatment outcome after testicular biopsy for male factor infertility

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Introduction: Severe male factor infertility and in particular azoospermia is a diagnosis made with increased frequency in today’s clinical ART practice. We aimed to review the outcome of testis biopsies and subsequent ICSI treatment outcomes in couples with the diagnosis of severe male factor.

Methods: This was a 2-year retrospective individual chart review of all cases where a testicular biopsy was performed at the Human Assisted Reproduction Ireland (HARI) Unit, a tertiary referral academic centre. The following variables were recorded: age of partners, male/female FSH, type of infertility, underlying cause of male infertility, male genetic profile, rate of freeze after biopsy, parameters of extracted sperm, type of stimulation protocol, number and grade of embryos, day of transfer and pregnancy outcome. Data was analysed using SPSS.

Results: We identified 49 cases of testicular biopsy (TB). Primary infertility was diagnosed in 33(65%) couples. The indication for TB was azoospermia in 44(90%) of cases while the rest had extreme oligozoospermia requiring biopsy. The cause of azoospermia was obstructive in 31(63%) men and non-obstructive in 17 (27%). With the exception of failed reversal of vasectomy, genetic profile was performed in all other cases. All had normal karyotype 46XY, 2 were

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