for time and ease of use, ambiguity and comprehension. Between October 1st
and November 30th 2008, the survey was mailed twice to 26 Canadian fertility
clinics and to 8 individual Canadian MDs, satellite with these clinics. A
modified survey was distributed online, to 392 American SART-member
clinics. Results were tabulated and summarized using SPSS.
**Results:** 28 Canadian and 125 American surveys were completed (78% and
32% response rates). For Canada, the largest proportion of surveys (50%) was from Ontario. Respondents reported offering a total of 6927 stimulated
IVF cycles per year, equivalent to 77% of the total cycles provided in Canada for 2007 (n = 9019). The most common out-of-country treatment
sought by Canadians was IVF with anonymous donor-eggs: 363 of 452 patients
treated (80%). Canadian respondents provided satellite monitoring for 431 women undergoing out-of-country IVF. For patients entering Canada in order to receive fertility treatment (n = 146), the largest demand was for IVF (73% of patients treated), 52% of respondents recommended specific destination
countries to their patients, but not specific providers. Confidence in safety,
effectiveness and ethicality were considered very important by 71–
80% of respondents. Respondents felt that patients were most concerned
with effectiveness (88%) and safety (80%). 88% of Canadian respondents always provide the information requested by the destination clinic. Canadian
clinics were most interested in receiving information about complications of
treatment, number of embryos transferred and frozen.

For the United States survey, the largest proportion of responses came from
the Southern US (31%). Respondents reported offering 35,387 stimulated IVF cycles per year, equivalent to 41% of the total 85,326 stimulated cycles reported to SART for 2006. Responding US clinics reported treating 927 out-of-country patients, 51% of them with standard IVF. 36% of incoming patients were from Latin America and 23% from Europe. The largest proportion of the 220 patients leaving the US in order to receive IVF or donor egg IVF, traveled to India / Asia: 41% and 52% respectively. Respondents reported that confidence in treatment effectiveness and safety, as well as information from other patients, were very important factors in patients’ decisions to come to their clinics. The majority of respondents felt that recent laboratory results and track sheets from previous cycles should always be sent with out-
country patients. Good concurrence was seen between Canadian and Amer-
ocan clinics’ ratings of key data that should be provided along with returning
patients.

**Conclusions:** The number of Canadians traveling to the United States for ART is equivalent to approximately 5% of the total cycles performed in Canada. Eighty percent of these women seek anonymous donor egg IVF. Less than 1% of US patients leave the country for fertility care and for them, the most popular destination is India / Asia, for standard or donor egg IVF. In the USA, approximately 3% of the total ART volume is made up of women coming into the country for care. US clinicians stress the need for recent lab data and previous stimulation track sheets. All parties surveyed rated effectiveness
and safety of care as paramount in patient choice of destination.

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**Abstracts of the 25th Annual Meeting of ESHRE, Amsterdam, the Netherlands, 28 June - 1 July, 2009**

**O-127 Oral**  
PCR-based detection of chromosomal unbalances on embryos: a possible future (r)evolution of PGD for chromosomal translocations

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**Introduction:** Preimplantation genetic diagnosis (PGD) has been offered to carriers of balanced translocations as an alternative to prenatal diagnosis. Fluorescence in-situ hybridisation (FISH) is the method of choice for detecting chromosome rearrangements. The FISH strategy involves the simultaneous use of telomeric probes in combination with centromeric probes (reciprocal translocations), or alpha-satellite/locus-specific enumerator probes (Robertsonian translocations).

Here we present the development of a polymerase chain reaction (PCR)-based PGD approach for detection of chromosomal imbalances on embryos derived from both reciprocal and Robertsonian translocation carriers. The procedure involves testing of single blastomeres by fluorescent multiplex PCR analysis of polymorphic short tandem repeat (STR) markers located along the chromosomes involved by translocation.

**Material & methods:** STR markers were selected to be located at either side of each breakpoint (reciprocal translocations) or at any point of the chromosomes involved (Robertsonian translocation). STR markers were also included to determine the copy number of chromosomes 13, 14, 15, 16, 18, 21, 22, X, Y in patients of advanced maternal age. Informativity testing of STR markers was performed for both partners of each couple. Only fully informative markers presenting alleles not shared by the partners were selected. In order to avoid misdiagnosis due to possible allele drop-out (ADO) occurrences, at least three STR for each chromosome were included in the protocol. Embryos were diagnosed as “normal-balanced” if PCR results indicated two signals (peaks) for each chromosome tested. Embryos were diagnosed as “unbalanced” if the PCR results showed a deviation from the “normal-balanced” signal pattern, such as trisomies (three peaks), monosomies (one peak) and nullisomies (no PCR signals).

**Results:** Twelve PGD cycles were carried out for 12 couples carrying six different reciprocal translocations and two Robertsonian translocations. The mean maternal age was 36.4 ± 4.6 years. A total of 204 oocytes were collected. 158 (77.5%) were MII, 126 (79.7%) fertilized and 110 embryos were biopsied on day 3. PCR was successful in 102/110 (92.7%) blastomeres, accounting a positive amplification on a total of 1048/1128 (92.9%) loci. Overall, 102 (92.7%) embryos were successfully diagnosed, 52 of which resulted normal/ balanced, 44 were unbalanced and 6 resulted to be haploid. PGS was included in the PGD protocol of five couples, involving testing of 45 embryos, 40 (88.9%) of which were successfully diagnosed and 24 (60.0%) showed aneuploidies. Embryos suitable for transfer where identified in 10 cycles. Following transfer of 23 embryos (mean 1.9 ± 1.1), 7 women had a clinical pregnancy confirmed with fetal sacs and heart beat (70.0% pregnancy rate per embryo transfer). A total of 13 embryos implanted (56.5% implantation rate per embryo transferred), for 10 of which heart beat was also detected. Only 2 couples accepted to undergo to prenatal diagnosis, performed by chorion villus sampling (CVS) or amniocentesis, which confirmed the PGD results. All pregnancies are still ongoing.

**Conclusions:** The above results demonstrate the feasibility and reliability of our PCR-based PGD protocol for detection of chromosomal imbalances. The present technique has the potential to overcome to several inherent limitation of the FISH procedure, such as suboptimal fixation, overlapping signals, split signals, lack of signals, cross-hybridization, polymorphisms, limited availability of the probes, combination of colours, decreasing of the accuracy with re-probing. This approach has the advantage to be rapid, low expensive, amenable to automation, involving an easy procedure and data interpretation. Unlike FISH, with the presented protocol is also possible to distinguish the parental origin of chromosomoses, allowing detection of uniparental disomies and the achievement of a DNA fingerprint for each embryo, useful for identification of embryos that have implanted. Finally, because cell fixation is not necessary, the PCR-based protocol represents an easier procedure for management of transport PGD. Considering the encouraging preliminary clinical outcome obtained, this approach has the potential to represent a valuable alternative to FISH-based PGD.

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**O-128 Oral**  
IVM with non-elevated E2 levels and PGD for BRCA1 mutation in a breast cancer patient. First report on mutation-free twin’s pregnancy

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**Introduction:** Young breast cancer patients frequently wish to have biologic children but reproductive decisions are difficult especially when fertility...
treatments are indicated. When hereditary breast and ovarian cancer gene (BRCA) mutations are present there is 50% chance of transmission of the mutation to the offspring which causes anxiety and concern. Ovarian stimulation using fertility drugs frequently results in high E2 levels that is potentially risky in hormone sensitive breast cancer. However, IVF introduced for women with polycystic ovarian syndrome (PCOS) can avoid this risk. This is the first report of a pregnancy after combined in-vitro maturation (IVM) and pre-implantation genetic diagnosis (PGD) for BRCA1 in breast cancer patient.

**Materials and methods:** A 26 years old female was diagnosed with breast cancer estrogen receptor positive. The patient and her mother were positive for BRCA1 gene mutation. IVF cycle initiated prior to chemotherapy in purpose of preserving future fertility was canceled due to ovarian hyperstimulation syndrome. Following chemo/radiotherapy and adjuvant Tamoxifen therapy the patient was 3 years disease free, married and had irregular menstrual. She requested to stop therapy and attempt to conceive which was approved by the oncologic team.

Fertility workout indicated good ovarian reserve FSH 3.9, AMH-5.15, AFC >40, however, due to PCOS she was infertile. Stimulation protocol resulted in ovarian hyperstimulation and E2 levels were above 10,100pmole/l. IVF yielded 8 embryos but the patient did not conceive. To avoid high E2 levels in hormone sensitive breast cancer patient who suffered from PCOS, IVM technique was offered. As the patient was BRCA1 gene mutation carrier and since infertility treatments were indicated PGD for BRCA1 was offered. Institutional review board (IRB) approval was obtained.

Single cell molecular diagnosis protocol used non-direct informative familial marker analysis. Genomic DNA samples of the patient and her parents were screened for informative single nucleotide polymorphism markers located in or adjacent to the BRCA1 gene on 17q21. Two markers, D17S951 and D17S1185, located 700 Kb upstream and downstream, respectively, from the gene sequence were found to be highly informative. The patient inherited a 93 bp allele linked to the mutated BRCA1 allele from her affected mother.

**Results:** On 2nd day of spontaneous cycle E2 was 122 pmole/l and multiple follicles (5–6 mm) were present. On the 8th day E2 was 210 pmole/l and maximal follicle diameter was 8 mm. FSH 75 IU/d was added for 4 days, on the 11th day E2 was 600 pmole/l, leading follicle was 16 mm and 20 antral follicles were visualized. Following HCG administration 14 immature oocytes were collected. Two metaphase I (MI) stage and 12 germinal vesicle stage (GV) were cultured in IVM medium supplemented with FSH and LH. Within 2 days 5 eggs matured in-vitro (MII) and ICSI was performed only on these oocytes. Two good quality embryos underwent biopsy on the 5th day post aspiration and blastomers were sent for single cell evaluation. The 93 bp maternal allele linked to the mutated BRCA1 gene was not inherited in one blastomere suggesting an unaffected embryo which was transferred on the same day. The patient conceived carrying a monochorionic bimniotic twin pregnancy. Second trimester amniocentesis showed two 46 XY normal embryos that were negative for the BRCA1 gene mutation. At present the patient is at her 33w of pregnancy.

**Discussion:** This breast cancer patient suffering from infertility due to PCO completed successful assisted reproduction cycle using IVM technique without elevation of E2 levels. Eggs that matured in-vitro were suitable for single cell PGD analysis for BRCA gene. The pregnancy following the IVM-PGD procedures solved patient’s infertility with no added risk and avoided passing mutated BRCA gene to patient’s progeny in an ethically accepted approach.

**O-129 Oral** Use of polarized light microscopy to assess meiotic spindle configuration prior to first polar body biopsy for preimplantation genetic diagnosis

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**Introduction:** Preimplantation genetic diagnosis (PGD) for chromosomal alteration and single-gene disorders (of maternal origin) using the first polar body (IPB) is the only approach possible in Italy where by Law inseminated/fertilized oocytes and embryos cannot be manipulated. Only after have obtained the genetic diagnosis of each single oocyte (which takes between 4 to 8 hours), insemination can be performed. Thus, to avoid oocyte ageing the biopsy is normally performed soon after pick-up, which means between 35 to 37 hours post hCG administration (Gianaroli et al., 2007). In this study we investigated the usefulness of polarized microscopy to evaluate oocyte maturation stage (by assessing meiotic spindle configuration) prior to IPB biopsy.

**Materials & Methods:** All patients undergoing an ICSI-PGD cycle on IPB for single-gene disorder in our centre between September and December 2008 were enrolled in this study. Oocyte retrievals were performed 35 hours post-hCG administration and oocyte demadenation one hour later. Meiotic spindles were assessed immediately after demadenation with Oosight system (Cri, Woburn, USA) followed by IPB biopsy. ICSI was finally performed on selected oocytes (maximum of 3 per patients according to the Law), between 6–8 hours post-biopsy, when diagnosis were available.

**Results:** A total of 60 cumulus-oocyte complexes have been obtained, of which 39 displayed a IPB and were thus suitable for biopsy. When observed at polarized light microscopy 15 (38.4%) were at Metaphase II (MII) stage with a clear meiotic spindle in the oocyte cytoplasm, 13 (33.3%) were at Telophase I (TI) stage with a meiotic spindle in between the IPB and oocyte cytoplasm and in 11 oocytes (28.2%) no spindle was detectable. Polar body biopsy was successful in all MII stage oocytes (15/15) and all oocytes without meiotic spindle detectable (11/11), while in the TI group 3/13 oocytes degenerated (23.1%) due to cytoplasmic continuity and rupture during biopsy (P = 0.04). Successful DNA amplification was obtained in 12/15 MII oocytes, 4/10 TI oocytes and 4/11 oocytes without MS detectable (P = 0.04). The fertilization rate with the selected MII oocytes was 80.0%. In one case two TI oocytes were however used for ICSI (due to the lack of availability of healthy MII) and both developed to IPI oocytes.

**Conclusions:** When IPB biopsy has to be performed, the use of polarized light microscopy is useful to assess oocyte nuclear maturity which influences the outcome of the procedure in terms of oocyte survival and successful DNA amplification. In particular, TI oocytes may be damaged during biopsy resulting in oocyte degeneration. It can also be supposed that, in case of survival, the chromosomal arrangement of TI oocytes may be disrupted during aspiration. It is suggested that timing of IPB biopsy should be adapted according to oocyte nuclear maturation stage assessed by polarized light microscopy.

**O-130 Oral** Genome-wide karyomapping for pgd of cystic fibrosis combines accurate linkage based testing with 24 chromosome aneuploidy screening

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**Introduction:** Karyomapping uses genome wide analysis of single nucleotide polymorphism (SNP) genotyping of parents and offspring to ascertain the haplotypes of each parental chromosome. When applied to one or more cells biopsied from a preimplantation embryo Karyomapping thereby identifies the parental and grandparental origin of each chromosome or chromosome segment presen. For preimplantation genetic diagnosis (PGD), Karyomapping has the advantage that there is no requirement to develop a patient or disease specific test for any single gene defect within the regions of the genome covered by the SNPs, in addition, chromosomal aneuploidies, including meiotic trisomies and uniform monosomies, are identified simultaneously. Here we demonstrate that Karyomapping not only provides highly accurate linkage based diagnosis for cystic fibrosis (CF) but also enabled two autosomal aneuploidies to be identified in embryos at the blastocyst stage.

**Materials and Methods:** PGD for CF was performed in a couple where both parents carry the common ∆F508 deletion by mechanical zona dissection and cleavage stage biopsy of one or two cells in embryos with 6 or more cells. Genetic analysis of the single cells by PCR and gel electrophoresis to identified four homozygous unaffected, nine carriers and 3 homozygous affected embryos. Two hatching blastocysts were transferred on day 5 resulting in a healthy liveborn girl. Five other embryos cryopreserved at the time of transfer were subsequently donated for research with the patients’ informed consent. These embryos were thawed and three embryos at the blastocyst stage biopsied and 3–10 cells removed from the trophoderm. Lysis and whole genome amplification (WGA) was then carried out essentially according
Results: Karyomapping confirmed the CF status of all five embryos (2 homozygous unaffected, 2 carriers and 1 homozygous affected) with multiple flanking and intragenic SNP markers across the region of 7q which includes the CFTR locus. Furthermore, unlike the original analysis which could not distinguish the parental origin of the deletion, the parental allele present in each of the carriers was identified. In one embryo, a recombination event is located immediately distal to the CFTR raising the possibility that conventional STR markers flanking the gene may only have detected the recombination event and failed to give a definitive diagnosis. Karyomap analysis also revealed two autosomal aneuploidies in two blastocyst stage embryos: one maternal monosomy 6 and one maternal trisomy 9, which was identified as a meiosis II error.

Conclusions: This preliminary data demonstrates that Karyomapping following WGA and SNP analysis from small numbers of embryo or trophoectoderm cells biopsied from embryos at the blastocyst stage can provide highly accurate linkage based analysis for CF combined with detection of chromosomal aneuploidy. With improved embryo survival following vitrification, therefore, it should now be possible to vitrify embryos at the blastocyst stage after biopsy and analyse the embryos for virtually any genetic disease and screen for aneuploidy of all 24 chromosomes simultaneously. This approach should make PGD by Karyomapping less expensive than conventional monogenic disease PGD because fewer embryos will be biopsied, more embryos will be euploid following growth to the blastocyst stage, and there is no need to custom develop tests for each disease or couple interested in PGD.

O-132 Oral Contribution of cryopreservation of blastocysts biopsied at the cleavage-stage to the success of a pre-implantation genetic diagnosis programme
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Introduction: An elective single embryo transfer (SET) policy has not been applied to pre-implantation genetic diagnosis (PGD) for inherited genetic disorders because of concerns regarding post-thaw survival of biopsied embryos. Our objective was to evaluate the survival and pregnancy potential of embryos biopsied for PGD at the cleavage-stage and cryopreserved at the blastocyst stage and its contribution to the overall success of an elective SET policy in a PGD programme.

Materials and Methods: From January 2006, all couples who had two or more transferable PGD blastocysts biopsied on day three of culture were offered single blastocyst transfer and cryopreservation of surplus blastocysts(s) using a slow freezing technique. We compared the outcome of 32 cryo-thawed PGD cycles with that of 191 cryo-thawed conventional IVF/ICSI cycles performed between January 2006 and July 2008. We also compared the outcome of all fresh PGD cycles performed before and after January 2006.

Results: The blastocyst survival rate was similar between the PGD and IVF/ICSI groups (87% vs 88%, P = 0.94). There was no significant difference in the implantation and clinical pregnancy rates between the two groups (35% vs 29%, p = 0.45 and 34% vs 36%, P = 0.77, respectively). During the same period, the multiple pregnancy rate in the fresh PGD programme dropped from 36% to 10% (OR = 0.20, 95% CI 0.08–0.48, P < 0.001) with no reduction in pregnancy rates.

Conclusion: The survival and implantation potential of biopsied PGD embryos cryopreserved at the blastocyst stage is comparable to that of non-biopsied IVF/ICSI cryopreserved blastocysts. Elective SBT and cryopreservation of surplus blastocyst(s) for later use should extend to include PGD for inherited genetic disorders.

O-133 Oral Two-year experience of double-factor preimplantation genetic diagnosis: first preliminary results
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Background: In couples affected by a monogenic disease, Double-Factor Preimplantation Genetic Diagnosis (DF-PGD) allows doubly selection of embryos, i.e. free of the monogenic disease and being potentially euploid. The aim of DF-PGD is to avoid the transfer of embryos diagnosed as being genetically normal for the couple’s causative mutation but without any information of chromosomal alterations, which may lead to the transfer of aneuploid embryos, indeed with little chance of viability. Hence, the main objective of DF-PGD is to increase the implantation rate in couples affected by monogenic diseases. The intention of this manuscript is to evaluate the feasibility and possible positive effect on implantation of the DF-PGD after two years of clinical application.

Methods: Eight couples affected by the recessive disease Cystic Fibrosis (CF), one by Angelman’s Syndrome (AS) and another by von Hippel-Lindau disease (VHL), both dominant illnesses, participated in our study. Six of the patients had no indication of PGD due to their maternal age being less than 35 (mean 32.3 years old), but the other four patients suffered AMA (mean 39.25 years old). After oocyte fecundation by ICSI the first polar bodies (1PB) were biopsied and were then analysed using Comparative Genomic Hybridization (1PB-CGH) in order to screen for aneuploidies in the whole-chromosome complement. On day 3, a blastomere was biopsied from the evolutive embryos that was immediately amplified using Multiple Displacement Amplification (MDA), following by Karyomapping less expensive than conventional monogenic disease PGD because fewer embryos will be biopsied, more embryos will be euploid following growth to the blastocyst stage, and there is no need to custom develop tests for each disease or couple interested in PGD.

Results: One-hundred-fifteen 1PBs were obtained, achieving a satisfactory CGH in ninety-nine of them (86.2%). During the clinical case, only 1PB-CGHs from developing embryos were analysed (77), 45.45% of them being non-affected by the respective disease (i.e. wild-type homozygotes or heterozygote carriers for CF and wild-type homozygotes for VHL and AS). In 35 of the genetically healthy blastomeres (66.7%) the 1PB-CGH was also available, 15 of them (42.9%) were potentially euploid, therefore they were classified as DF-PGD transferable embryos. The other 20 (57.1%) originated from an euploid oocyte. All of the other non-affected embryos with no informative CGH were tagged as being PGD transferable.

Nine out of the fifteen DF-PGD transferable embryos and eight out of the ten PGD transferable embryos were selected morphologically for transfer to 10 patients. Three out of the nine (33.3%) DF-PGD embryos and one out of the eight (12.5%) PGD embryos did implant and developed, resulting in the birth of four healthy babies. Even though the implantation rate of the DF-PGD group is almost three times higher than the PGD group, these differences were not significant.

Conclusion: Despite the reduced number of cycles performed using DF-PGD, it seems to be a good tool to increase the implantation rate in couples affected by a monogenic disease.

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Tuesday 30 June 2009
11:45–13:00
Merck-Serono Company Symposium