

only aneuploid embryos would have been selected based on morphologic criteria. In select patients, we advocate aneuploidy PGD. Additional cycles are being evaluated.

Supported by: None

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4:30 p.m.

O-239

Experience on Preimplantation Genetic Diagnosis Combined With HLA Matching. F. Fiorentino, S. Kahraman, H. Karadayi, A. Biricik, S. Sertyel, M. Baldi. "GENOMA" Molecular Genetics Laboratory, ROME, Italy; ART and Reproductive Genetics Unit, Istanbul Memorial Hospital, Istanbul, Turkey; Embryogen - Centre for Preimplantation Genetic Diagnosis, ROME, Italy.

OBJECTIVE: Preimplantation genetic diagnosis (PGD) has become an important option for couple at risk of conceiving affected children with single gene disorders. PGD has recently been used in combination with Human Leukocyte Antigen (HLA) matching, allowing selection and transfer of unaffected embryos also having a close HLA match with those of an existing affected child. In such cases, PGD was used not only to avoid the birth of affected children, but also to conceive healthy children who may be potential HLA-identical donors of haematopoietic stem cells (HSC) for transplantation in siblings with a life-threatening disorder.

DESIGN: This study includes 76 cycles, for 55 couples overall, performed in a 2-years period. In a total of 48 couples preimplantation HLA matching was performed in combination with PGD for a single gene disorder, from whom 66 cycles were performed, including 65 cycles for 47 couples for β -thalassaemia and 1 cycle for Wiscott-Aldrich syndrome. HLA matching procedure, not involving testing for a causative gene, was performed for 7 couples having a child affected by ALL (5 cycles) and sporadic DBA (5 cycles).

MATERIALS AND METHODS: An indirect single-cell HLA typing protocol based on a multiplex fluorescent polymerase chain reaction (PCR) of short tandem repeat (STR) markers scattered throughout the HLA complex was used. A nested multiplex PCR assay was used to co-amplify all the selected loci. The first round PCR contained the external primers for the amplification of the informative HLA STR markers, the gene regions involved by mutations, STR markers linked to these regions for ADO detection and STR markers used for detection of aneuploidies in patients of advanced reproductive age. The first round multiplex PCR was followed by separate second round PCR reactions for each locus. Mutation analysis was performed using the minisequencing method.

RESULTS: A total of 696 embryos were tested, involving analysis of 1178 blastomeres, in 1088 (92.4%) of which a successful amplification was obtained. A reliable HLA haplotype was obtained in 1088/1088 (100%) of the blastomeres with positive PCR results. Testing for chromosome 6 copy number revealed 53 (7.6%) embryos with aneuploidies, including a total of 5 (0.7%) trisomies, 48 (6.9%) monosomies, which affected the HLA matching diagnosis for these embryos, leading to a conclusive diagnosis in only 643/696 embryos (92.4%). Recombination was found in 34 (4.9%) embryos, 7 (1.0%) of which were originally unaffected and HLA compatible, but were not considered for transfer because of recombination. In total, 102 (15.9%) embryos revealed an HLA match with the affected siblings, 76 (11.8%) of which resulted unaffected and 64 (10.0%) have been transferred back to patients in 41 cycles. Twelve pregnancies were achieved (29.3% per transfer); 4 pregnancies resulted only biochemical, one spontaneously miscarried and one resulted ectopic, and was then terminated. Five healthy HLA matched children have been already delivered and cord blood stem cells were transplanted to 3 affected siblings, resulting in a successful hematopoietic reconstruction.

CONCLUSION: These preliminary results are very encouraging and, complemented by other similar experiences in the field, demonstrate that preimplantation HLA matching represent a valid alternative for the achievement of a successful treatment in children affected by severe congenital or acquired bone marrow disorders, in the absence of a compatible related donor.

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O-240

Clinical Outcome of PGD. I. Tur-Kaspa, A. Horwitz, N. Ginsberg, J. Cieslak, S. Rechitsky, Y. Verlinsky. Reproductive Genetics Institute, Chicago, IL.

OBJECTIVE: To determine the pregnancy, obstetrical, and newborns (n=480) outcome after IVF with PGD for aneuploidy, translocations and single gene disorders.

DESIGN: Prospective follow up of pregnancies, deliveries, and newborns after PGD.

MATERIALS AND METHODS: Information was obtained from patients during pregnancy and/or after the delivery. IVF was done at multiple sites but in most cases our embryologists performed the polar bodies and/or blastomere biopsy for PGD.

RESULTS: From all clinical pregnancies that were obtained by IVF-PGD until 2004, 79.3% were singletons, 18.5% twins, 2.2% triplets, and 0.6% were extra uterine pregnancies. While all couples were highly recommended to undergo prenatal diagnosis, it was done by only 30% of patients. Take home baby rates were 72.9% for PGD for aneuploidy and 81.4% for PGD for translocations, which were significantly better compared to these patient previous pregnancies outcome (p<0.001). Maternal complications occurred in 17.3% of pregnancies. 57% delivered vaginally and 43% by cesarean sections. Mean gestational age and newborn weight were 38.4 weeks and 3590 gr for singletons, 35.6 weeks and 2662 gr for twins, and 31.9 weeks and 2019 gr for triplets, respectively. Intrauterine growth retardation occurred in 8.5% of singletons and 8.1% were large for gestational age. Data for the condition of the newborn was obtained for 480 babies. 4.4% of babies (n=21) had minor birth anomalies (such as hemangiomas, birthmarks, heart murmurs, torticollis, toe syndactyly). 1.7% (n=8) were reported to have major birth defects (such as hip dysplasia, seizures, foot deformity, tetralogy of Fallot).

CONCLUSION: PGD may be offered safely to most patients who wish to improve their pregnancy outcome while transferring only 1-2 embryos to prevent multiples. The obstetrical outcome after PGD is similar to that of other IVF/ICSI pregnancies. Based on data obtained at birth by parents and the expected rates of anomalies in the North American population, PGD is not associated with an increase in birth defects.

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O-241

Preimplantation Genetic Diagnosis of Embryos Developing From Intracytoplasmic Sperm Injection of In Vitro Matured Metaphase II Human Oocytes. A. E. Jones, G. Wright, S. J. Colon, H. I. Kort, J. B. Massey, Z. P. Nagy. Reproductive Biology Associates, Atlanta, GA.

OBJECTIVE: The recovery of immature human oocytes is a common occurrence in routine IVF. While in vitro maturation (IVM) of germinal vesicle (GV) or Metaphase I (MI) stage oocytes can be accomplished in conventional culture medium, the viability of these oocytes is questionable. Cell cycle regulatory mechanisms could be altered, and although fertilization can be achieved, the downstream affects of IVM are largely unknown. Here, we assessed chromosomal status of the embryos that developed from in vitro matured MI oocytes which had reached the Metaphase II stage at the time of ICSI which was performed within four hours post egg retrieval.

DESIGN: A retrospective study involving 33 ICSI-IVF cycles. Embryo development and chromosome status of microinjected MI-MII oocytes was assessed by fluorescent in situ hybridization (FISH) and compared to results obtained from their sibling embryos (MII - MII) which did not require IVM.

MATERIALS AND METHODS: Oocytes were exposed to hyaluronidase for 1 minute immediately following egg retrieval and returned to culture. After thirty minutes coronal cells were removed, and oocytes were assessed for maturity. Intracytoplasmic sperm injection (ICSI) was carried out on all in vitro matured oocytes within 3-6 hours post egg retrieval, and all MI-MII injected and fertilized eggs were cultured separately. Seventy-