normal male, normal female and from a female with two well-differentiated alleles of 29 and 52 CGG repeats. PCR amplification of the FMRI CGG triplet repeat region is problematic due to “slippage” mechanism. We therefore developed a modified single-cell nested PCR protocol aiming to reduce the formation of secondary structure and facilitate the exposure of the DNA strands. This enable the determination of CGG repeat number. The analysis is performed by amplification of CGG repeats; Sry sex determining gene and one of five FMRI flanking polymorphic markers (AC1, AC2 and DXS548, DX998/DX1193). The sensitivity and accuracy of these tests in single cells exceeded 95%.

CONCLUSION: Using one of these various triplex nested PCR protocols, it is now possible to perform reliable PGD for fragile X syndrome for most of fragile X carriers.

Supported by: None.

P-315


OBJECTIVE: Blastomeres biopsied from cleavage-stage human embryos have been used to diagnose aneuploidy for chromosomes 13, 16, 18, 21, and 22. We assessed: (a) accuracy of fluorescent in-situ hybridization (FISH) to establish the chromosome complement for 90 embryos using a five probe mixture of DNA probe sequences homologous to specific regions on chromosomes 13, 16, 18, 21, and 22; (b) effectiveness of single cell biopsy for diagnostic accuracy and subsequent embryo development; (c) feasibility of simulated PGD using cohorts of surplus frozen embryos before clinical use.

METHODS: Prospective data analysis using simulated preimplantation genetic diagnosis.

MATERIALS AND METHODS: Pronucleate stage (2PN) embryos donated to research were thawed and cultured before embryo biopsy on ‘day 3’. A single cell was analyzed to determine chromosomal status for each embryo using Fluorescence In S itu Hybridization (FISH) for chromosomes 13, 16, 18, 21, and 22. We assessed: (a) accuracy of fluorescent in-situ hybridization (FISH) to establish the chromosome complement for 90 embryos using a five probe mixture of DNA probe sequences homologous to specific regions on chromosomes 13, 16, 18, 21, and 22; (b) effectiveness of single cell biopsy for diagnostic accuracy and subsequent embryo development; (c) feasibility of simulated PGD using cohorts of surplus frozen embryos before clinical use.

RESULTS: In 7 consecutive experiments, 90 embryos were successfully biopsied to provide 85 single blastomeres for FISH analysis. FISH efficiency per nucleated biopsied blastomere was 92% (80/87) and for all nuclei examined from whole embryos 86% (2980/3480). In 93% (77/83) of embryos, an informative FISH result on a single blastomere and the corresponding whole embryo was successfully obtained. FISH genotypic results were concordant between the biopsied blastomere and the whole embryo in 39% (30/77) of cases. FISH results (abnormal versus normal) were concordant between the biopsied blastomere and the whole embryo in 66% (51/77) of cases. Overall 81% (62/77) of embryos showed signs of active cell division post-biopsy, while 43% (33/77) of biopsied embryos developed into blastocysts by day 6. Based on the results from the single blastomere alone, a total of 37 normal (13,13,16,18,18,18,21,21,22,22) embryos (of the original 77, or 48%) would have been eligible for transfer, of which 73% (27/37) had a corresponding normal whole embryo result based on the modal (most frequently observed) genotype. FISH analysis of both the single cell and whole embryo resulted in a rate of 13% (10/77) false negatives, and 19% (15/77) false positives.

CONCLUSION: FISH results in single blastomeres are highly concordant with the whole embryo (as measured at the most advanced embryonic stage obtained in vitro), suggesting that a single blastomere result can accurately determine which embryos have a euploid complement for the chromosomes studied. The use of frozen embryo cohorts donated to re-search allows one to simulate the experience of clinical PGD and thus develop and validate new protocols under case conditions. The possibility of false negatives (leading to possible miscarriage) and false positive results (leading to disposal of ‘normal’ embryos) should be discussed with patients opting for preimplantation genetic diagnosis for common aneuploidies using FISH.

Supported by: None.

P-316

Preimplantation genetic diagnosis (PGD) for B Thalassemia with or without HLA typing. H. Karadayi, S. Kahraman, Y. Saglam, B. Umay, S. Sertyel, F. Fiorentino. Istanbul Memorial Hospital, Istanbul, Turkey; EmbryoGen, Rome, Italy.

OBJECTIVE: Preimplantation Genetic Diagnosis (PGD) for β Thalassemia reveals a option for the prevention of the birth of an affected child. Preimplantation Genetic Diagnosis is also developed as a therapeutic tool for the care of an affected child when combined with HLA typing. The purpose of the present report is to document our experience on PGD and PGD+HLA typing for β Thalassemia cases applied to our center.

DESIGN: A retrospective study.

MATERIALS AND METHODS: Overall, twenty nine ovarian controlled hyperstimulation cycles (COH) were performed in 26 couples for β Thalassemia. In seven cycles PGD for β Thalassemia (Group I) was applied, where as in 22 cycles PGD for β Thalassemia combined with HLA typing (PGD+HLA typing; Group II) was performed. Totally 261 embryos were screened for β Thalassemia mutations including IVS-I -110 G/A, IVS-I -130 G/C, IVS-I -1 G/A, IVS-I -6 T/C, IVS II -1 G/A, IVS II -745 C/G, C195T, C282Y, C384T, CD8 -AC, CD8 -AG, CD248 -A, CD248 -C, CD71 -C, CD71 -G and -30 T/A. 215 embryos were further analysed for HLA compatibility after multiplex PCR by Minisequencing method and STR analysis. According to the results embryo transfer was performed with the disease-free and/or HLA compatible embryos.

RESULTS: 77 cycles in Group I, 14/22 cycles in Group II reached the embryo transfer stage. Unaffected embryos for transfer were detected in all PGD cycles in Group I, however in Group II, 8 transfers were cancelled due to the lack of HLA matched embryos. Overall, transfer of 37 disease free embryos resulted in 7 (2 birth, 3 term and 2 ongoing) pregnancies (33.3%) in 21 embryo transfers.

CONCLUSION: The results demonstrate that PGD is an efficient method for the prevention of the birth of affected children with β Thalassemia, providing an acceptable number of mutation free embryos for transfer. However, in case of a combined HLA typing, successful pregnancy is further limited by the number of HLA compatible embryos.

Supported by: None.

P-317

Preimplantation genetic diagnosis for single gene disorders combined with HLA matching. S. Kahraman, G. Karlikaya, H. Karadayi, B. Umay, Y. Saglam, F. Fiorentino. Istanbul Memorial Hospital, Istanbul, Turkey; EmbryoGen, Rome, Italy.

OBJECTIVE: The purpose of this study is to evaluate the clinical aspects and the efficiency of Preimplantation Genetic Diagnosis (PGD) for single gene disorders combined with HLA matching in order to obtain an unaffected child who can be an HLA donor for its sibling.

DESIGN: A retrospective study from a tertiary clinic.

MATERIALS AND METHODS: Overall 21 couples with 26 cycles were included with single gene disorders and a history of an affected child. 23 couples with β Thalassemia, 1 cycle with Wiscott Aldrich Syndrome and additional 2 cycles for only HLA matching for Acute Lymphoblastic Leukemia (ALL). A total of 206 embryos were biopsied, 31.5% of embryos were found to be mutation free, 42.7% were heterozygous embryos, 15.3% of embryos were found to be HLA compatible but G and -30 T/A. 126 embryos were found to be unaffected and HLA matched. Mutation analysis was carried out using Minisequencing method. HLA typing was realized with STR analysis after fluorescent PCR. The cases were evaluated according to the female age and ovarian reserve.

RESULTS: Out of 22 females aged below 35 years, 16 had embryo match. The others were cancelled due to the lack of HLA compatible embryos. A total of 7 pregnancies were achieved (43.7%). The rate of implantation was 27.7%. One pregnancy resulted in miscarriage (14.3%). However for females aged above 35 years, all 4 embryo transfers were cancelled due to the limited number of embryos suitable for HLA testing. In re-
Results of aneuploidy testing in ART cases with repeated spontaneous abortio

Supported by: None.

P-318

Overall 818 embryos were biopsied and 740 blastomeres were analyzed by FISH. 270 embryos analyzed in RSA group showed 46.7% chromosomal abnormalities. In RIF group 470 embryos analyzed giving the abnormality rate of 48%. Among the abnormalities 20.6 / 20.7 percent of embryos were found to be trisomic, 38 / 39.3 embryos have complex aneuploidy, and 11.9 / 11.5 embryos were found to be either haploid or polyplid for RSA and RIF group respectively.

CONCLUSION: The result of this study shows that increased chromo

Supported by: None.

P-320

Establishing an expanded fluorescence in situ hybridization (FISH) probe panel for preimplantation genetic diagnosis (PGD). R. Habibian, J. Knops, A. Hajianpour, J. Joyce, J.-C. Wang. Genzyme Genetics, Pasadena, CA; Genzyme Genetics, Santa Fe, NM.

OBJECTIVE: To design a 10 probe PGD panel with two sequential hybridization reactions that can screen for the most common aneuploidies of clinical significance.

MATERIALS AND METHODS: Data analysis of cytogenetic studies on products of conception specimens on 9377 unselected cases in our laborato

RESULTS: The relative percentages of individual chromosomal aneu

Supported by: WIH Faculty Research Fund.