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DETECTION OF CONTAMINATION BY QUANTITATIVE REAL-TIME (Q)PCR. C. L. Bohrer,[a] X. Tao,[a] E. L. Torpey,[a] D. Taylor,[a,b] R. T. Scott, Jr.,[a,b] N. R. Treff.[a,b] [a]Reproductive Medicine Associates of New Jersey, Basking Ridge, NJ; [b]Reproductive Endocrinology and Infertility, Robert Wood Johnson Medical School, Basking Ridge, NJ.

OBJECTIVE: DNA contamination is a critical factor to consider when performing PCR based methods of PGS, as it can result in misdiagnosis. Surprisingly very little has been published regarding the precision of predicting contamination in this setting despite growing and widespread use of PCR based methods (i.e. whole genome amplification). This study develops a new method of detection and investigates the sensitivity and specificity to varying levels and types of contamination using a novel method of qPCR based allelic discrimination.

DESIGN: Blinded.

MATERIALS AND METHODS: TaqMan qPCR based genotyping of 40 highly polymorphic SNPs was performed on mixtures of cells from cell lines with known relationships. In each case, 5 cells from one line were used to represent a trophectoderm biopsy and 1, 2, or 3 cells from another cell line were added to mimic a variety of possible sources of contamination (sibling embryo, sperm, cumulus cells, or unrelated DNA). Genotypes were assigned if they fell within the 95% CI of the Mehalanobis distance from the mean of known pure sample genotype clusters. ROC curves were used to define the number of SNPs which needed to fail to be given a genotype assignment in order to predict contamination. Performance was then evaluated on blinded mixtures and pure samples.

RESULTS: At a threshold of 5 SNPs there was 98% specificity for contamination (where 1 of 57 pure samples would have been predicted as contaminated). Overall sensitivity to contamination was 90% (where 4 of 24 single-cell, 3 of 24 two-cell, and 0 of 24 three-cell contaminated samples were predicted as uncontaminated).

CONCLUSION: This study has developed a new method for qPCR-based detection of contamination with excellent sensitivity and specificity. Future studies will investigate and define the level of contamination that may impact preimplantation genetic testing results such as aneuploidy and single gene disorder screening in order to determine the level of contamination that is clinically necessary to detect in these settings.

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A PILOT STUDY COMPARING FLUORESCENCE IN SITU HYBRIDIZATION (FISH) ANALYSIS IN PREIMPLANTATION GENETIC SCREENING (PGS) TO ARRAY COMPARATIVE GENOMIC HYBRIDIZATION (aCGH) TECHNIQUE. F. Balmir,[a] M. Hughes,[c] J. Jenkins,[b] J. R. Stelling.[a] [a]Obstetrics and Gynecology, Stony Brook University Hospital, Stony Brook, NY; [b]Reproductive Endocrinology, Reproductive Specialists of New York, Mineola, NY; [c]Genetics Lab, Genesis Genetics, LLC, Detroit, MI.

OBJECTIVE: To compare aCGH results on polar bodies and blastocysts with well performed day 3 blastomere FISH analysis for aneuploidy screening.

DESIGN: This was a prospective pilot study. We evaluated polar bodies (PB) and discarded blastocysts by aCGH in patients undergoing Day 3 blastomere biopsy for aneuploidy screening using FISH. We disaggregated blastocysts to analyze trophectoderm (TE) separately from inner cell mass (ICM).

MATERIALS AND METHODS: Patients undergoing Day 3 blastomere biopsy for aneuploidy screening using FISH were enrolled to allow concomitant polar body and blastocyst biopsies. There were a total of 9 participants between 2009-2011. PB biopsies were performed on the day of oocyte retrieval (Day 0) and after fertilization (Day 1), TE and the ICM were then sent for aCGH analysis. Results of aCGH and FISH were then compared.

RESULTS: A total of 10 embryos were analyzed with adequate informative PB, Day 3, and blastocyst results. Only one biopsy was completely concordant with FISH analysis and aCGH. Five embryos recorded as abnormal by FISH were found to be normal by aCGH on biopsy after being discarded. Three embryos were abnormal but had different types of aneuploidies. There were no mosaics found on TE biopsy or ICM biopsy. TE biopsies were concordant with ICM biopsies. PB biopsies agreed with ICM biopsies.

CONCLUSION: In our small study, we found that FISH analysis was often incorrect. Several embryos were labeled as aneuploid by Day 3 FISH when polar body and blastocyst results by aCGH reported them to be normal leading to unwarranted discarding of normal embryos. We did not find mosaicism in our disaggregated embryos in the TE or ICM. Also our PB results did agree with our blastocysts results. aCGH should be preferred over FISH testing for aneuploidy screening.

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PREIMPLANTATION GENETIC DIAGNOSIS FOR MUCOPOLYSACCHARIDOSE TYPE I: ANALYSIS OF A NOVEL INDEL MUTATION. V. Baltaci,[a] C. Demirel,[b] A. Baltaci,[c] Ö. Ayvaz,[d] E. Ünsal,[e] T. Duman.[f] [a]Istanbul Bilim University, Istanbul, Sisli, Turkey; [b]Atasehir Memorial Hospital, Istanbul, Atasehir, Turkey; [c]Genart Women Health Center, Ankara, Çankaya, Turkey.

OBJECTIVE: Mucopolysaccharidosis type I (MPSI) is an autosomal recessive disorder caused by a deficiency in alpha-L iduronidase (IDUA), which leads to lysosomal accumulation of large sugar molecules called glycosaminoglycans (GAGs) dermatan and heparan sulfate. Severe MPS I occurs in approximately 1 in 100,000 newborns. Attenuated MPS I is less common and occurs in about 1 in 500,000 newborns. The use of in vitro fertilization (IVF) and preimplantation genetic diagnosis (PGD) may help couples at risk to avoid pregnancies with known genetic diseases; in this case to achieve pregnancy without MPSI. A novel indel mutation (c.956_972+9delinsTA) that was firstly identified in a Turkish family was analysed.

DESIGN: The deletion of 26 nucleotides and insertion of a dinucleotide in IDUA gene was firstly monitorized via sequence analysis in family members. Multiplex nested PCR tecnique was performed to detect the mutation in embryos. By means of fragment analysis, six informative STR markers were used to confirm that there were no allelic drop out (ADO-the random non amplification of one of the alleles).

RESULTS: PGD cycle resulted in 10 embryos of which three were found to be heterozygous and four mutant following single blastomere biopsy on day 3. Two of three normal embryos were transferred resulting in a healthy baby born at term.

CONCLUSION: The related indel mutation was recently released by Bertoli (Hum. Mut. 2011) in a Turkish family. Analysis of novel mutations using PGD contributes to successful clinical applications in assisted reproductive technology especially in populations with high consanguineousy. The experience provided by this study encourages the development of standardized molecular PGD protocols for many rare diseases.

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CASE REPORT: BIRTH OF HEALTHY BABY AFTER PREIMPLANTATION GENETIC DIAGNOSIS OF JUNCTIONAL EPIDERMOLYSIS BULLOSA. C. Demirel,[a] V. Baltaci,[b] A. Baltaci,[c] T. Duman,[d] E. Ünsal,[e] Ö. Ayvaz.[f] [a]Atasehir Memorial Hospital ivf Center, Istanbul, Atasehir, Turkey; [b]Istanbul Bilim University School of Medicine, Istanbul, Sisli, Turkey; [c]Genart IVF Center, Ankara, Çankaya, Turkey.

OBJECTIVE: Junctional epidermolysis bullosa (JEB) which is an autosomal recessive rare disease is one of the major form of epidermolysis bullosa, a group of genetic conditions that cause the skin to be very fragile and to blister easily. Blisters and skin erosions form in response to minor injury or friction, such as rubbing or scratching. Mutations in LAMA3, LAMB3, LAMC2, and COL17A1 genes may result with the disease. In this study we perform Preimplantation Genetic Diagnosis (PGD) for a Romanian family in which both parents are carriers of JEB. Woman carries c.1594C>T, p.R532X, in exon 13. and the man carries c.3061_3063delins AAAAGCTG, in exon 21 of the LAMB3 gene.

DESIGN: Case report.

MATERIALS AND METHODS: The couple underwent PGD using multiplex nested polymerase chain reaction. c.1594C>T mutation was detected with Restriction Fragment Lenght Polymorphism and sequence analysis was perfomed for the second indel mutation. Allelic drop out and false positive and negative results were controlled by using four informative STR markers.

RESULTS: Nine embryos were biopsied on day 3 and four of them were diagnosed as being normal. Two embryos were transferred and a singleton pregnancy was achieved, resulting in the birth of a healthy boy.