

COMBINING ANALYSIS OF BOTH SINGLE GENE DISORDERS AND COMPREHENSIVE CHROMOSOME SCREENING ON A SINGLE BIOPSY OF EMBRYOS IMPROVES PATIENT OUTCOMES

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Abstract Body

Pre-implantation genetic diagnosis (PGD) prevents patients passing a known familial genetic problem to their children. Traditionally using PCR techniques and single tandem repeat markers (STR), and recently using next generation sequencing (NGS) technique of karyomapping, latter requiring more sophisticated equipment and relatively more expensive.

Chromosomal aneuploidy is significant in early embryos. Advances comprehensive chromosome screening (CCS) using array comparative genome hybridization (aCGH), and more recently NGS, significantly increased pregnancy rates and decreased miscarriage rates.

In 2012 we began combining PGD-PCR and aCGH, and recently NGS, for the simultaneous detection of single gene disorders and CCS from a single biopsy of embryos.

However using standard whole genome amplification (WGA) had high levels of allele drop out (ADO) in STR markers. Replacing WGA with multiple displacement amplification (MDA), known to have longer product size and more uniform coverage across the genome, dropped ADO rates to comparable to standard PGD-PCR, however NGS chromosome analysis noise increased.

Adjustments to protocols removed this adverse noise effect.

In this study we first validated use of MDA instead of WGA in NGS/PCR, and then used MDA and NGS/PCR for 6 different single gene disorders in 9 cycles. NGS results detected aneuploidy of human blastocysts accurately and with a 100 % 24-chromosome diagnosis concurrence with WGA. STR profiles using MDA on purified genomic DNA from blood samples showed low profile bias, comparable with much higher bias from WGA product. Using MDA, ADO rates on both genomic DNA from blood or from embryo cells was decreased to the same range as with standard PCR techniques. No specific additional amplification products were observed.

Pregnancy rates also appeared to have significantly increased.

In conclusion, PCR in combination with NGS using MDA is a successful single powerful procedure and appears to be the significant implications for successful cycles for patients.