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

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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.

Objective:

To validate a new procedure that combining PGD-PCR and NGS using MDA instead of WGA in order to increase pregnancy outcome for patient.

Methods:

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. STR profiles using MDA on purified genomic.

Day 5,6 Trophectoderm biopsy

Multiple Displacement Amplification (MDA) and freeze all biopsied embryo

Sample to Next Generation Sequencing (NGS)

NAD embryo(s) by NGS

PGD-PCR for single gene disorder using MDA product

ABNORMAL embryo(s) by NGS

Discard

Results:

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.

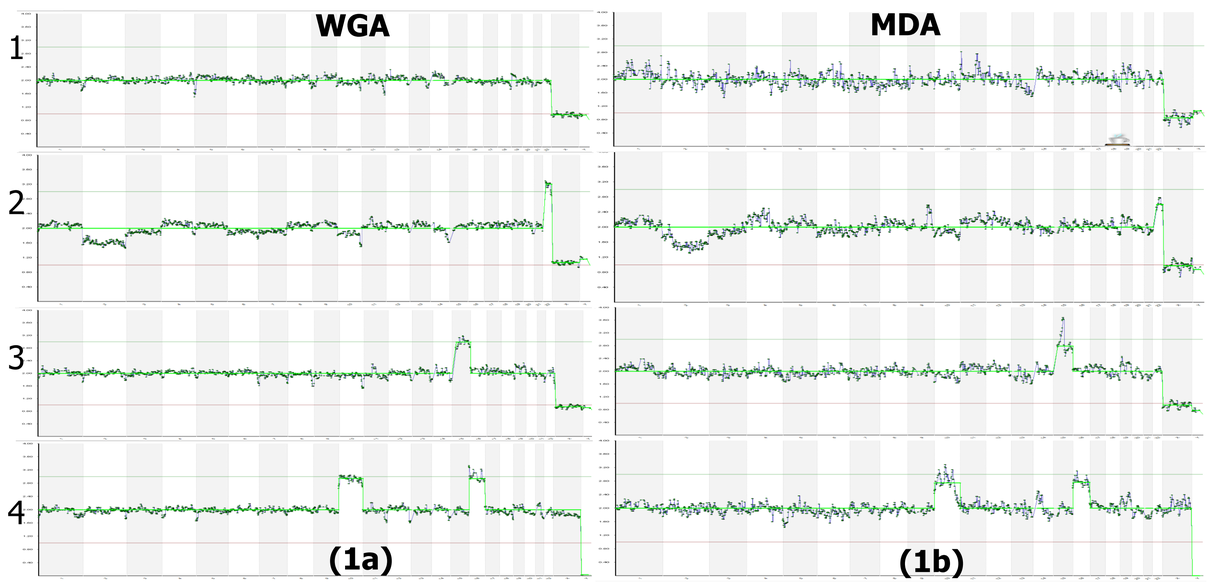
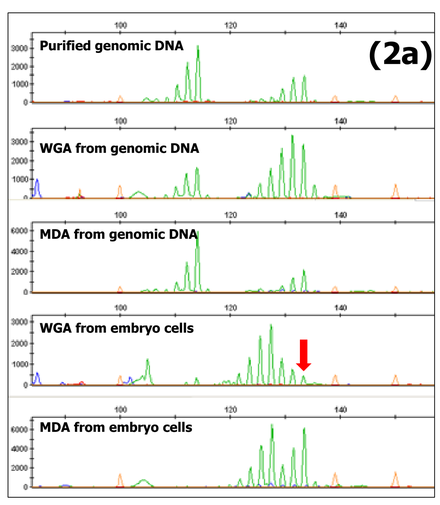
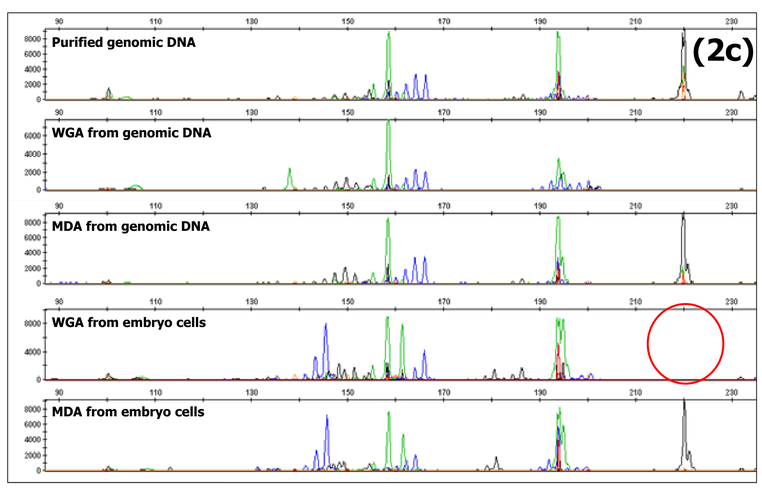


Figure 1. NGS result of 24-chromosome aneuploidy screening using WGA (1a; 1-4) compare to MDA (1b; 1-4). NGS with MDA show accurate result and with a 100% concurrence with WGA.





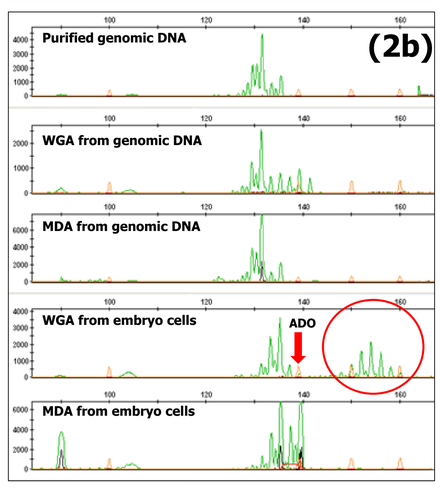


Figure 2. Result of using MDA on purified genomic DNA from blood samples showed low profile bias (2a-2c), comparable with much higher bias from WGA product. ADO observed in the WGA (2b), while MDA from both genomic DNA and embryo cell show the same profile without ADO.

Conclusions: PCR in combination with NGS using MDA is a single powerful procedure that would be clinically used in PGD cycle. Pregnancy rates also appeared to have significantly increased with NGS/PCR. Moreover, this is also an advantage for patient in order to transfer embryo with free from both a single gene disorder and chromosome abnormalities.

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