Successfully Clinical Outcomes using Combination of Preimplantation Genetic Testing for Aneuploidy (PGT-A) and Preimplantation Genetic Testing for Monogenic Disorders (PGT-M)

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Preimplantation genetic testing for aneuploidy (PGT-A) is significantly for early embryos. Using recent technology of next generation sequencing (NGS), significantly increased pregnancy rates and decreased miscarriage rates. In 2016, Superior A.R.T. began combining NGS and preimplantation genetic testing for monogenic disorders (PGT-M) by PCR for the simultaneous detection 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. It has been shown to significantly increased pregnancy to 80% with 62% of implantation rate.

METHOD

In this study we used MDA and NGS/PCR for 26 different single gene disorders in 35 cycles. Couple having NGS/PCR cycle were worked-up before they underwent routine IVF procedures. The embryos were cultured to blastocyst stage on Day 5 or Day 6 when 3-5 trophectoderm cells were biopsied. NGS/PCR process was performed and the result were came out within 10 days after biopsy.

Genetic Disorder	Inheritance pattern
Blood disorder	
Beta-Thalassemia	Autosomal recessive
Beta-Thalassemia and HLA matching	Autosomal recessive
Alpha-Thalassemia	Autosomal recessive
Alpha-Thalassemia and HLA matching	Autosomal recessive
Neurodegenerative disorder	
Tuberous Sclerosis (TSC2)	Autosomal dominant
Spinal Muscular Atrophy 1 (SMA1)	Autosomal recessive
Charcot-Marie-Tooth disease (CMTX)	X-linked dominant
Cancer predisposition	
Neurofibromatosis type 1 (NF1)	Autosomal dominant
Hereditary multiple osteochondromas (HMO)	Autosomal dominant
Multisystem disorder	
Polycystic Kidney Disease type 1 (PKD1)	Autosomal dominant
Polycystic Kidney Disease type 2 (PKD2)	Autosomal dominant
Polycystic kidney disease with hepatic disease (PKHD1)	Autosomal recessive
Skin disease	
Oculocutaneous Albinism Type 1 (OCA1)	Autosomal recessive
Metabolic disease	
Phenylpyruvic	Autosomal recessive
Pantothenate kinase 2 associate with Neurodegeneration with brain iron accumulation	Autosomal recessive
G6PD	X-linked
Other	
DNA polymerase gamma (POLG)	Autosomal recessive

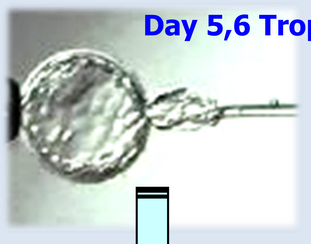


Figure 1. Diagram illustrating the NGS/PCR process

Multiple Displacement Amplification (MDA) and Next Generation Sequencing (NGS)

ABNORMAL embryo(s) by NGS → ***Discard***

NAD embryo(s) by NGS → ***PCR for single gene disorder using MDA product***

RESULT

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.

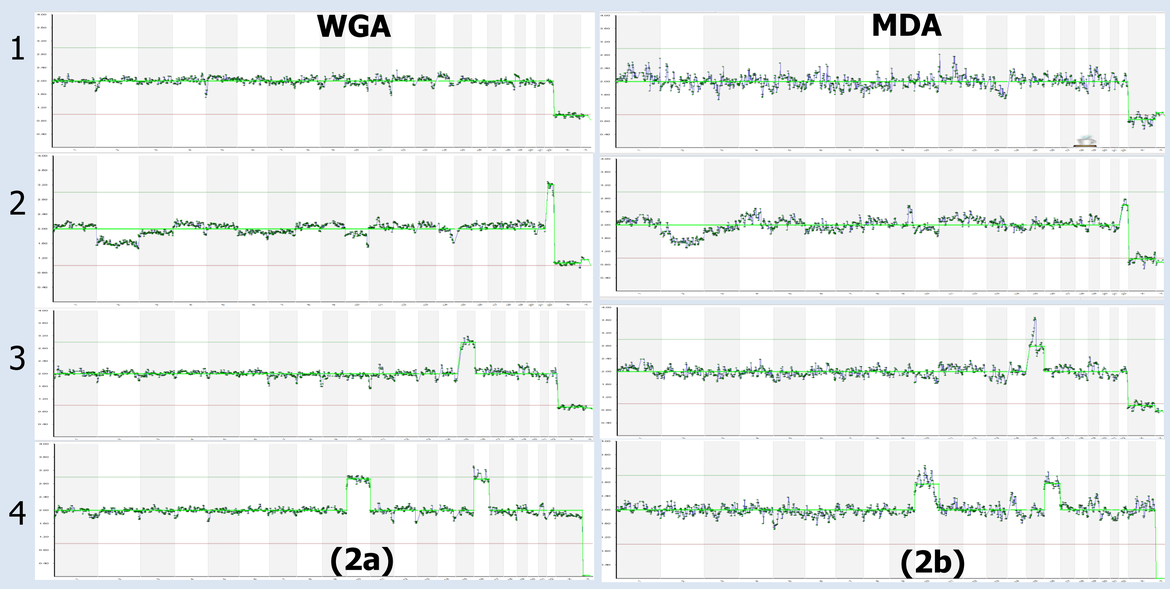
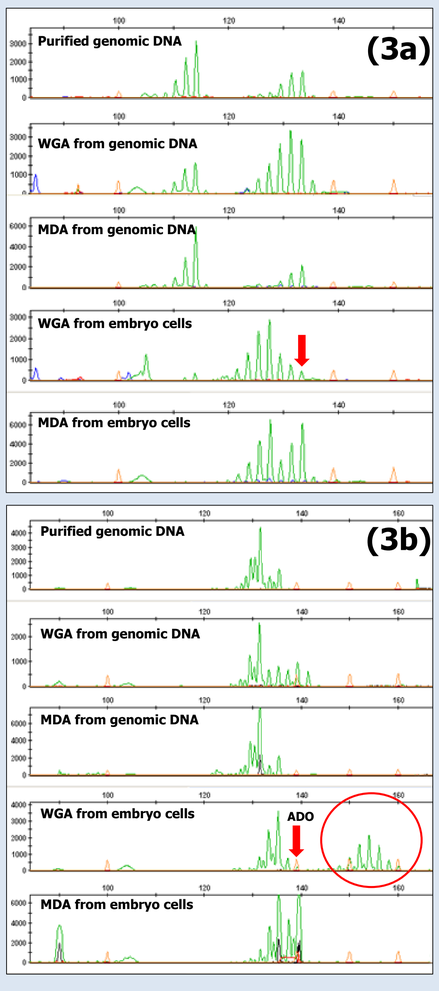


Figure 2. NGS result of 24-chromosome aneuploidy screening using WGA (2a; 1-4) compare to MDA (2b; 1-4).



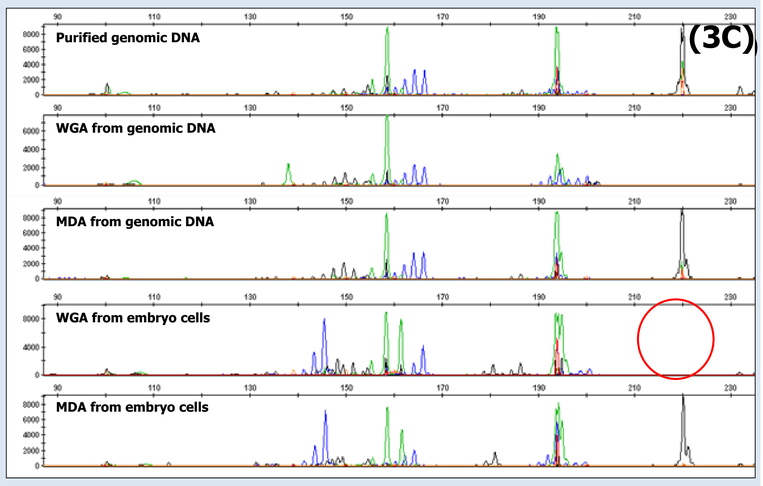


Figure 3. Result of using MDA on purified genomic DNA from blood samples (3a-3b), while MDA from both genomic DNA and embryo cell show the same profile without ADO (3C).

No. of cycle	35
No. of embryo transfer	45
Average age	32.05±3.23
% Pregnancy rate	80
% Implantation rate	62

CONCLUSION

In conclusion, PGT in combination of PGT-A and PGT-M can be applied to virtually whole chromosome aneuploidy and any genetic condition and is capable of improving single gene disorder preimplantation genetic testing in a patient-tailored manner thus increasing pregnancy rates, saving costs/time and increasing patient reliability.