
**TRACKING IMPLANTED EMBRYOS USING**
**CELL-FREE FETAL DNA FROM MATERNAL CIRCULATION AT 9 WEEKS GESTATION BY TARGETED**
**NGS.**

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**Abstract:**
**OBJECTIVE:** Embryonic DNA fingerprinting is a powerful tool that identifies the implanted embryo following multiple embryo transfer during IVF cycles and allows for elimination of all non-embryonic variables from studies design to identify and validate markers of embryonic reproductive competence. Cell-free fetal DNA (cfDNA) in the serum and plasma of pregnant women has been used widely in noninvasive parental diagnosis. To accommodate our current targeted NGS (tNGS) based comprehensive chromosome screening (CCS) platform, this study aims to establish a method for fingerprinting fetal DNA obtained from the maternal circulation at 9 weeks gestation using single nucleotide polymorphisms (SNPs) within the tNGS CCS amplicons
**DESIGN:** Prospective, blinded.
**MATERIALS AND METHODS:** 36 Patients with single embryo transfer and singleton implantation were included in this study. All the transferred embryos and untransferred sibling embryos were evaluated with tNGS. cfDNA was enriched from maternal plasma at 9 weeks gestation using QIAamp MinElute Virus Kit (Qiagen). Parental blood DNA was isolated using QIAsymphony DSP DNA midi kit (Qiagen). tNGS was performed on parental DNA and cfDNA. Allele frequencies were calculated for parental DNA, cfDNA, transferred embryos, and untransferred sibling embryos for each patient. The criteria for informative SNPs were as follows: maternal genotype was homozygous, paternal genotype was heterozygous, and embryo genotype was heterozygous. The paternal allele fraction for the informative SNPs in cfDNA was calculated for each embryo. cfDNA should contain a higher proportion of the paternal allele for the implanted embryo.
**RESULTS:** The average paternal allele fraction in the cfDNA was ~2.5%. The transferred embryos were successfully identified in 34 out of 36 patients. The 2 failed cfDNA samples showed less than 1% of the paternal DNA fraction.
**CONCLUSIONS:** This study demonstrates that cfDNA fingerprinting by tNGS can identify the implanted embryos at 9 weeks gestation, reducing the time by ~31 weeks for newborn fingerprinting. This may be integral to robust development of new markers of reproductive competence at an early stage of pregnancy.