# NONINVASIVE PREIMPLANTATION GENETIC TEST (niPGT-A): THE REMAINING CELL-FREE DNA COULD BE USED TO IDENTIFY SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) RELATED TO THE IMPLANTATION PROCESS

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**Introduction:** niPGT-A is a new technology that uses cell-free DNA present in the spent culture medium of human blastocyst, which reflects its ploidy status. This DNA is an important source of information for the embryo. Since there is a gene pattern signature of endometrial receptivity, several SNPs such as those found in the LIF, VEGF, TP53 and MMP9 genes have been used as markers of the implantation process. However, there are no reports on the possibility of identifying SNPs in embryos using remaining cell-free DNA in culture medium. This study aimed to observe if the remaining cell-free DNA from the same sample used for niPGT-A diagnosis could also identify SNPs related to the embryonic implantation process.

**Material and Methods:** This prospective cohort study included 23 samples of remaining cell-free DNA obtained in culture medium on day 5 after embryo culture during niPGT-A technique (Yikon Genomics). A total of 13 patients participated in this study after the couple's informed consent. Cell-free DNA evaluation used the amplified DNA obtained after niPGT-A technique (Yikon Genomics) and quantified by Qubit fluorometer (Thermo Fisher Scientific). SNPs were evaluated by real-time polymerase chain reaction (PCR) using individual TaqMan. SNP genotyping assays (Thermo Fisher Scientific) for each SNP (LIF rs929271, TP53 rs1042522, VEGF rs3025010, MMP9 rs17576) and TaqPath™ ProAmp™ Master Mix (Thermo Fisher Scientific), following the manufacturer's instructions, on a StepOnePlus™ Realtime PCR System (Thermo Fisher Scientific).

**Results:** All cell-free DNA samples in the culture medium had at least one SNP identified regardless of their quantification (table 1). It was observed that of all 92 genotyping reactions performed, 69 were properly amplified, leading to an accuracy of 75%.

**Conclusions:** The studied SNPs involved in the implantation process were successfully amplified and genotyped with the remaining cell-free DNA of culture medium after niPGT-A. Regardless of the cell-free DNA concentration after the amplification process used by niPGT-A technology, the success rate of genotyping was 75%. In the future, niPGT-A/SNPs dual evaluation could be an additional tool for embryo selection.

**Table 1. Distribution of ploidy according to age rate**

| SAMPLE ID | TP53 (C>G) | LIF (G>T) | VEGF (C>T) | MMP9 (A>G) | DNA (ng/µL) |
|---|---|---|---|---|---|
| 1 | CC | * | CC | GG | 10.4 |
| 2 | GG | * | TT | * | 13.4 |
| 3 | * | GG | CC | * | 13.3 |
| 4 | * | TT | * | AA | 18.2 |
| 5 | CG | * | CC | GG | 10.2 |
| 6 | GG | TT | * | * | 9.7 |
| 7 | CG | TT | TT | AA | 47.0 |
| 8 | GG | GG | CT | GG | 16.8 |
| 9 | * | GG | TT | * | 11.7 |
| 10 | CC | TT | TT | AG | 37.0 |
| 11 | CC | TT | TT | AA | 24.0 |
| 12 | GG | TT | TT | GG | 27.0 |
| 13 | GG | TT | TT | GG | 31.0 |
| 14 | * | TT | TT | AA | 25.0 |
| 15 | CC | TT | CT | AA | 92.0 |
| 16 | CC | TT | TT | * | 32.0 |
| 17 | * | TT | TT | AA | 24.0 |
| 18 | CC | TT | CT | AA | 32.0 |
| 19 | * | * | CT | * | 15.8 |
| 20 | CG | * | * | * | 9.7 |
| 21 | GG | TT | TT | GG | 24.6 |
| 22 | GG | GG | CC | AA | 28.6 |
| 23 | * | GG | TT | * | 9.8 |

*genotyping failure