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The remaining cell-free DNA from noninvasive preimplantation genetic test (NIPGT-A) could be used to identify single nucleotide polymorphisms (SNPs) related to the implantation process

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Study question

Could the remaining cell-free DNA from the same sample used for NIPGT-A diagnosis also identify SNPs related to the embryonic implantation process?

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%.

Conclusion

Regardless of the cell-free DNA concentration after the amplification process used by NIPGT-A technology, the success rate of genotyping was 75%. This study showed a novel system (NIPGT-A/SNPs) for embryo tracking. 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. In the future, NIPGT-A/SNPs dual evaluation could be an additional tool for embryo selection.

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Table 1. Main results of SNPs genotyping

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