High concordance of the embryonic cell-free DNA with the inner cell mass: impact of blastocyst quality, patient age and mode of fertilization.

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The existence of embryonic cell-free DNA (cfDNA) in spent blastocyst medium has been confirmed in recent studies, opening a new era of possibilities for non-invasive preimplantation genetic testing for aneuploidies (niPGT-A). High concordance rates of cfDNA with trophectoderm (TE) biopsies and with whole blastocysts have been reported. However, the compartment(s) from where this DNA originates remain unclear. Both TE and inner cell mass (ICM) are potential sources, but, at the moment, the origin of this cfDNA is unknown as well as the mechanisms underlying its secretion into the medium.

Study question: Does the embryonic cfDNA in the culture medium represent the chromosomal content of the ICM? Which factors impact concordance rates?

Material and Methods

We carried out a prospective study (ClinicalTrials.gov. ID NCT03520933) to investigate the concordance of cfDNA with the corresponding TE and ICM biopsies. 141 embryos were donated for research after written informed consent signature for the project approved by the Ethics Committee. The culture workflow is detailed in Figure 1.
cfDNA, TE and ICM biopsies were analyzed from January 2019 to November 2021 by NGS (Ion ReproSeq PGS kit, ThermoFisher Scientific) and the results were analyzed with customized algorithms for cfDNA, TE and ICM biopsies.

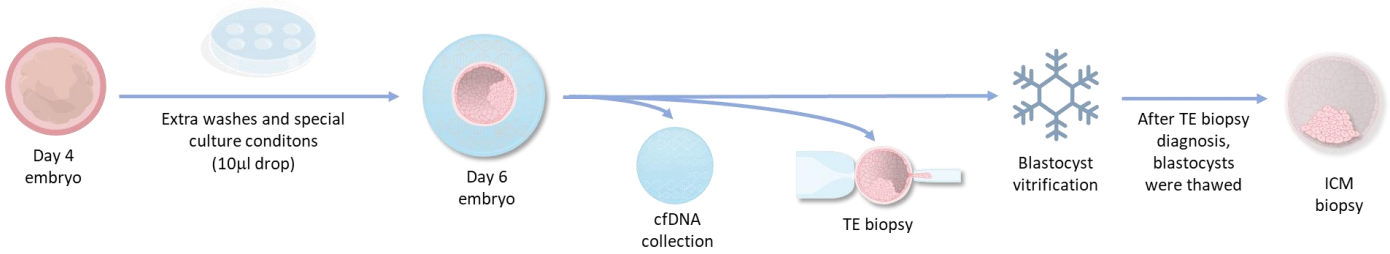

Figure 1. Culture workflow performed in the study.

Results

In combination, the three sample types (cfDNA, ICM and TE) were informative in 81.6% of blastocysts (115/141). Detailed results for the comparison between cfDNA, TE and ICM can be observed at Table 1.

Table 1. Results for the comparison between cfDNA, TE and ICM.

	ploidy concordance	*full concordance	*partial concordance	false negatives	false positives	**PPV	**NPV	specificity	sensitivity
†TE-cfDNA	89.6%	67.0%	22.6%	10.4%	0.0%	1.000	0.455	1.000	0.886
†ICM-cfDNA	86.1%	67.8%	18.3%	7.8%	6.1%	0.926	0.571	0.632	0.906
†ICM-TE	89.6%	77.4%	12.2%	0.9%	9.6%	0.896	0.889	0.421	0.990

†Sample reference for the comparison.
*Ploidy concordance includes both full concordance (when the chromosomal status for all the chromosomes in both samples is the same) and partial concordance (the chromosomal status for some chromosomes might differ between samples, but they are both aneuploid).
**PPV: positive predictive value; NPV: negative predictive value.

When the results were stratified by female age (≤37 or >37 years), insemination technique (ICSI or IVF), blastocyst expansion degree (expanded, hatching or fully hatched), and ICM/TE quality (A or B), the informativity of the cfDNA was very similar between the different groups and ranged from 83.7% to 100%. Nevertheless, there were subtle differences for ICM-cfDNA ploidy concordance. It was slightly increased for the older female age group (88.3% vs 83.6% female age ≤37) as well as for ICSI (89.7% vs 82.5% in IVF) and for ICM quality B (88.4% vs 80.0% for ICM A). None of those differences reached statistical significance.

Conclusions

The embryonic cfDNA released to the culture medium provides information of the overall blastocyst chromosomal constitution, as suggested by the high ploidy concordance rates reported between ICM biopsies and embryonic cfDNA. This value is independent of female age, insemination technique and embryo quality. This supports the use of niPGT-A as an alternative to other invasive aneuploidy detection methods that require a biopsy.

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