EMBRYO CELL-FREE DNA HIGHLY REPRESENTS THE CHROMOSOMAL CONSTITUTION OF BOTH THE INNER CELL MASS AND THE TROPHECTODERM OF HUMAN BLASTOCYSTS

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Objective

Recent studies have shown the existence of embryo cell-free DNA (cfDNA) in spent blastocyst medium (SBM), opening a new era of possibilities for non-invasive preimplantation genetic testing for aneuploidy (niPGT-A). High concordance rates of cfDNA with trophectoderm (TE) biopsies and with whole blastocysts have been reported. The objective of this study was to evaluate the concordance rate of cfDNA in SBM with the chromosomal constitution of inner cell mass (ICM) and TE biopsies.

Materials and Methods

We carried out a prospective study to investigate the concordance of cfDNA with the corresponding TE and ICM biopsies (ClinicalTrials.gov. ID NCT03520933). A total of 117 day-6/7 blastocysts underwent TE biopsy and SBM collection in the same PGT-A cycle. These blastocysts were donated for research as part of a clinical trial, and ICM biopsy was performed afterwards. Media, TE and ICM biopsies were analyzed from January 2019 to March 2021. Embryos were cultured in routine conditions up to day 4, when embryos were washed, transferred to a new 10µl culture medium droplet, and cultured for at least a further 44 hours. Culture media were collected before TE biopsy and frozen at -20°C. Assisted hatching, blastocyst biopsy and vitrification were performed after media collection. All samples were analyzed by NGS using the Ion ReproSeq PGS Kit (ThermoFisher Scientific) using Ion Chef™, plus the Ion S5 XL Sequencer™, with modifications in the amplification protocol for the embryo cfDNA. Customized algorithms were applied to the interpretation of results in TE, ICM and embryo cfDNA.

Results

Informative results were obtained in 98.3% of the TE biopsies (115/117), 92.3% of the cfDNA samples from SBM (108/117), and 93.2% of the ICM (109/117). In combination, the three sample types were informative in 84.6% of the blastocysts (99/117). Considering ICM as those cells that will become the fetus, ploidy concordance with cfDNA was 85.9% (85/99) and with TE was 89.9% (89/99), without statistical differences. False positive rates were similar for cfDNA and TE biopsy (6.1% and 9.1%, respectively), and false negative rates were not significantly different, but higher in cfDNA (8.1%) than in TE (1.0%), due to potential contamination with maternal DNA. Embryo cfDNA ploidy concordance with TE biopsies was 89.9% (89/99) (Table 1). Considering the 14 non-concordant cases of the cfDNA with the ICM, 6 of them corresponded to aneuploid cfDNA and euploid ICM, that could be attributed to the presence of mosaicism in the blastocyst (TE biopsies were also aneuploid in the 6 cases). The remaining 8 non-concordant embryos corresponded to euploid cfDNA and aneuploid ICM, in most of the cases due to the presence of maternal contamination.

Figure 1. Example of ploidy (and full) concordance between SBM, TE and ICM.

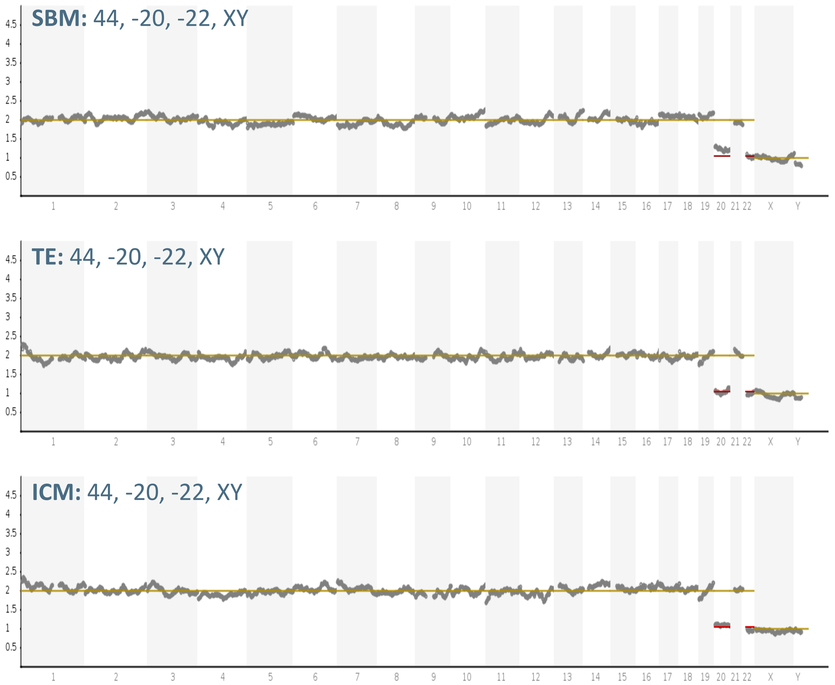

Table 1. Detailed results for the comparison between SBM, TE and ICM.

	ploidy concordance*	full concordance	partial concordance	false negative	false positive	PPV**	NPV	specificity	sensitivity
SBM-TE (N)	89.9% (89)	68.7% (68)	21.2% (21)	10.1% (10)	0% (0)	1.000	0.444	1.000	0.890
SBM-ICM (N)	85.9% (85)	68.7% (68)	17.2% (17)	8.1% (8)	6.1% (6)	0.928	0.500	0.571	0.906
ICM-TE (N)	89.9% (89)	77.8% (77)	12.1% (12)	1.0% (1)	9.1% (9)	0.900	0.889	0.471	0.988

*There is ploidy concordance between two samples when they are both euploid or aneuploid. Ploidy concordance includes both full concordance (when the chromosomal status for all the chromosomes in both samples is the same) (Figure 1) 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.

Conclusions

Embryo cfDNA detected in SBM correlates with both ICM in 85.9% of the blastocysts, and TE in 89.9% of the blastocysts.

Impact Statement

We have shown high concordance rates of embryo cfDNA, not only with TE biopsies, as in previous studies, but also with ICM biopsies, which represent the true chromosomal content of the embryo. The niPGT-A approach would avoid the need of invasive biopsy techniques for embryo aneuploidy testing.