Multicenter concordance study of embryo cell-free DNA and trophectoderm biopsies: impact on clinical outcomes.

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Study question:

Are reproductive outcomes of transfers from euploid day-6 biopsied blastocysts different depending on results of the corresponding embryo cfDNA in the culture medium?

Introduction:

During development, embryos release cell-free DNA (cfDNA). There is, therefore, genetic material in the droplet of culture medium in which they develop. High concordance rates have been observed when analyzing, for the same embryo, the spent blastocyst medium containing cfDNA, trophectoderm (TE) and inner cell mass biopsies or the whole blastocyst. This high representativeness shown by cfDNA on the chromosomal content of the blastocysts opens a new era of possibilities for non-invasive preimplantation genetic testing for aneuploidies (niPGT-A).

Material and Methods:

Prospective, observational, multicenter study (ClinicalTrials.gov. ID NCT03520933) conducted in 10 assisted reproductive centers after approval by each local institutional review board.

From April 2018 to December 2022, 2539 embryos from 716 patients included in PGT-A cycles were cultured following a niPGT-A culture protocol. More in detail, embryos were cultured in routine conditions up to day 4, then washed and transferred to a new 10µl droplet. On day 6, 8-9µl of media were collected and frozen at -20°C; and blastocyst biopsy and vitrification were performed.

TE biopsy and media samples were analyzed by NGS (Ion ReproSeq PGS kit, ThermoFisher Scientific), and their results were processed with customized algorithms for TE/cfDNA and compared.

441 frozen single embryo transfers (SET) were performed, and their clinical data registered. Only euploid embryos, based on the TE biopsy result, were transferred. cfDNA results were blinded at the moment of embryo transfer.

Results:

Clinical outcomes were compared according to whether the TE and cfDNA result was euploid (euploid-euploid) or with euploid TE and aneuploid cfDNA (euploid-aneuploid). Transfers where cfDNA analysis had provided non-informative results were excluded.

- The euploid-euploid group included 288 transfers (mean female age = 35.3 ± 4.9 years), whereas the euploid-aneuploid group included 95 transfers (mean female age = 34.4 ± 5.2 years). The comparisons for the clinical outcome parameters between both groups did not reach statistical significance (Chi-square test), but miscarriage rate showed a two-fold increase in transfers with aneuploid cfDNA (Table 1a).
- A sub-analysis was performed considering only transfers from patients without endometrial factor history. Euploid-euploid and euploid-aneuploid groups included 231 and 73 transfers, respectively. The differences in ongoing pregnancy rate (49.4% vs 43.8%) and miscarriage rate (14.3% vs 25.6%) were slightly higher between both groups than in the global analysis but did not reach statistical significance (Chi-square test) (Table 1b).

a)	euploid-euploid		euploid-aneuploid	
	n	%	n	%
SETs	288	-	95	-
Biochemical pregnancy rate	172	59.7	61	64.2
Implantation rate	150	52.1	55	57.9
Miscarriage rate	22	14.7	14	25.5
Ongoing pregnancy rate	128	44.4	41	43.2

b)	euploid-euploid		euploid-aneuploid	
	n	%	n	%
	231	-	73	-
	145	62.8	47	64.4
	133	57.6	43	58.9
	19	14.3	11	25.6
	114	49.4	32	43.8

Table 1. Clinical outcome information for the euploid-euploid and euploid-aneuploid groups.
a) All transfers considered. b) Only transfers from patients without endometrial factor history were considered.

Conclusions:

Transfers with euploid trophectoderm biopsies but aneuploid cfDNA showed higher miscarriage rates than those with euploid trophectoderm biopsies and euploid cfDNA, although without reaching statistical significance. To better understand the trends observed, especially on the miscarriage rate, it would be necessary to collect clinical outcomes from more SET.

The analysis of embryo cfDNA could provide useful information to help select the best embryo for transfer and, therefore, decrease miscarriage rates and increase the chances of having a healthy newborn at home.

Some authors are employees of Igenomix, part of Vitrolife group

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