NONINVASIVE PREIMPLANTATION GENETICS TEST FOR ANEUPLOIDY (NIPGT-A): VALIDATION PROCESS OF FREE-DNA MEDIUM COLLECTION

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Introduction: This study aimed to validate the collection processes of free-DNA, released by the embryo to the culture media during their development, for future niPGT-A evaluation.

Material and Methods: This is a multicenter study performed by a total of 11 brazilian assisted reproduction centers (Bra-ARC). The validation process was performed into two steps; first step (frozen embryos): collection of the medium in which frozen/thawed embryos were cultured and second step (fresh embryos): collection of the medium in which fresh embryos were cultured.

For the first step: each center thawed at least 3 blastocysts (Day5/6), mean of 5.4 ± 3.1, previously biopsied for invasive PGT-A (iPGT-A) and diagnosed as genetically abnormal with consent for disposal. A total of 44 frozen-thawed biopsied blastocyst embryos were cultured in individual wells of 15 µl of culture medium in GPS dishware (MGPS010 Global) until they reached their expansion (4-24 hours). Expanded blastocysts were individually transferred to PCR tubes and their corresponding free-DNA spent medium were collected for niPGT-A evaluation. In this step, it was possible a comparison between the genetic diagnosis of the whole embryo (gold stander), with the free-DNA released by the embryo into the culture medium during their development and the diagnosis of previous trophectoderm biopsy results.

For the second step: each center cultured at least 3 fresh cleaved (D3) embryos, mean of 5.1 ± 2.9. A total of 40 embryos were transferred into individual well of 20 µl of culture medium in GPS dishware and were cultured from day 3 to day 5/6. Expanded blastocysts (grade 4) were vitrified and their respective spent culture medium were collected and transferred to PCR tubes for niPGT-A evaluation.

Results: A total of 84 samples of all Bra-ARC (from both first and second steps) were used for niPGT-A. From 74 sequenced samples, 26 embryos were euploids (35.1%), 38 embryos presented aneuploidy (51.4%) and 10 of them (13.5%) were mosaics. The sex rate was 55.4% for XY and 44.6% for XX. Ten samples (11.9%) failure sequencing for insufficient or poor DNA quality samples.
The mean of free-DNA concentration into the collected spent media was 29.8 ng/µl and 19.2 ng/µl for the first and second step, respectively.

From the first step (table1): From 46 Free-DNA samples and whole embryos, 44 (95.6%), were successfully amplified. Comparing the results of niPGT-A and whole embryos sequencing, the predictive value of the normal test was 100% (PVNT) and the false abnormal test (FAT) was 8.3%. On the other hand, when we compared the whole embryos and iPGT-A, false abnormal test was 25%, evidencing that the biopsy procedure increased 3 times the FAT rates compared with niPGT-A (no biopsy procedure).

Table 1. Results first step

niPGT-A	Whole embryo		iPGT-A	Whole embryo	
	Abnormal	Normal		Abnormal	Normal
Abnormal	33	3	Abnormal	33	11
Normal	0	8	Normal	0	0
PVNT=100%. / FAT= 8.3%			**FAT= 25%**		

From the second step: 73% of the medium was collected on D5 and only 27% of them were collected on D6, shortly thereafter the blastocyst reached grade 4. At the beginning of the validation process, 80% of all free-DNA spent medium evaluated presented enough free-DNA concentration to performed niPGT-A. Therefore, it increased for 96.9% after Bra-ARC validation process (data sent for publication). The mean DNA concentration collected by each center varied from 2.2 to 59.4 ng/µl, depending of blastocyst grade and day of collection. (D5/D6). From 32 spent free-DNA samples assessed, 40.6% (13/32) of them presented euploidy, 46.8% (15/32) aneuploidy, 12.5% (4/32) mosaicism.

Conclusion: The validation process showed to be a powerful procedure for future niPGT-A evaluation. All of the Bra-ARC has shown effective for the collection process of free-DNA for both protocols (fresh or frozen embryos culture), after protocol adjusting. The niPGT-A showed 3 times less false abnormal rates compared to iPGT-A.