Impact of blastocyst quality and post-thaw embryo culture factors in non-invasive PGT-A informativity on vitrified blastocysts.

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Introduction:

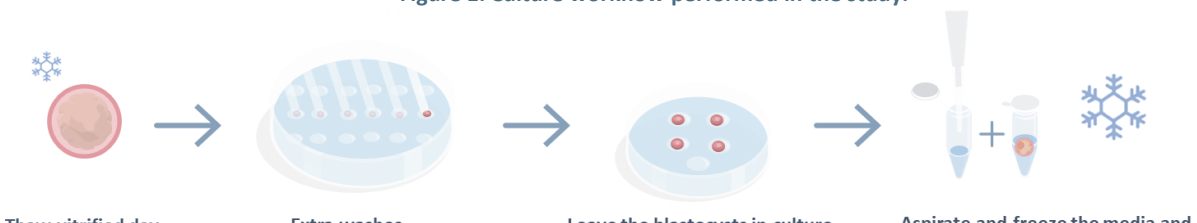
Non-invasive PGT-A (niPGT-A) is a technique that allows the study of the chromosomal content of blastocysts without the need of a biopsy. The informativity and concordance rates for fresh and frozen-thawed embryos have been related with the time in culture and the day of media collection. However, understanding the optimal conditions to obtain the highest values in both parameters is still necessary.

Objective: to identify which are the most important factor/s involved in the informativity and concordance rates of niPGT-A applied to previously vitrified day-5/6 blastocysts.

Material and Methods:

This is a prospective study including 135 spent blastocyst media (SBM) and the corresponding blastocyst samples from thawed day-5 and day-6 blastocysts from PGT-A (73) and non-PGT-A (62) cycles collected from January 2021 until March 2022. Blastocysts were discarded under IRB approval. Media and blastocysts were obtained from IVF patients (29-42 years old) at two different reproductive centers. Carriers of structural abnormalities or monogenic diseases were excluded. The culture workflow is detailed in Figure 1.

Figure 1. Culture workflow performed in the study.



Thaw vitrified day-5/6 blastocysts

Extra washes
To remove remaining cumulus cells.

Leave the blastocysts in culture
Individual embryo culture on 10µL of fresh media for conventional incubator or 20µL for EmbryoScope.

Aspirate and freeze the media and the blastocysts (-20°C)
Samples were analysed by Next Generation Sequencing (Thermo Scientific, Waltham, MA, USA).

The embryos were divided into 3 groups:
- **Day-5 Short**, day-5 blastocysts cultured for 8 hours (n=42),
- **Day-5 Long**, day-5 blastocysts cultured for 24 hours (n=50),
- **Day-6 Short**, day-6 blastocysts cultured for 8 hours (n=43).

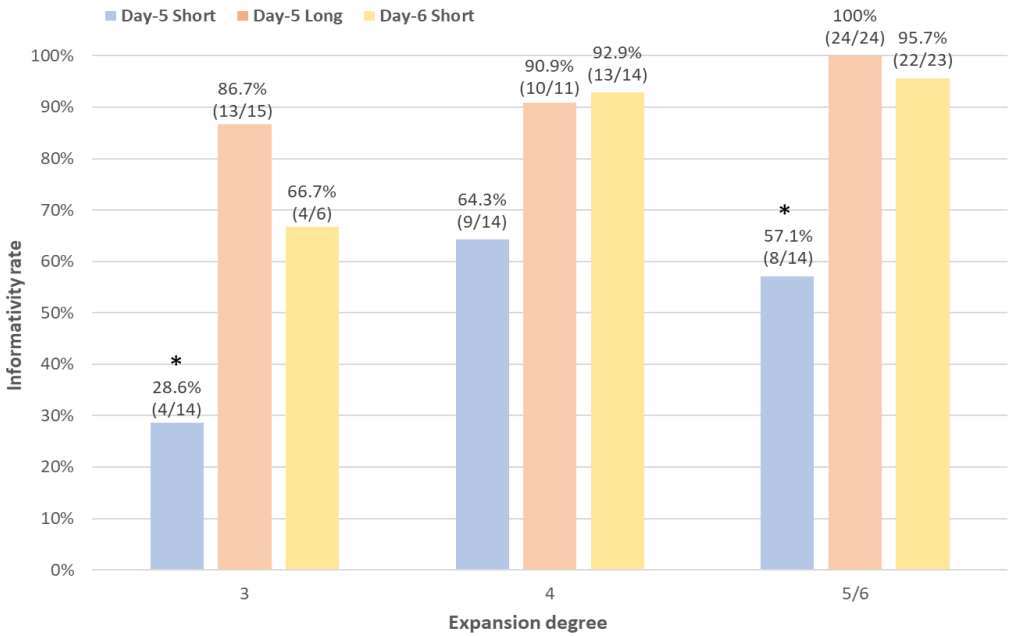
Correlation between concordance rates (overall agreement between SBM and blastocyst samples, i.e., being both euploid or aneuploid), informativity, embryo quality (considered before vitrification) and time in culture was evaluated.

Conclusions:

The data suggests that the day of vitrification and post-thaw time in culture of vitrified blastocysts are the most important factors for niPGT-A informativity rate, without correlation with blastocyst quality.
Regarding the concordance rate, there is no impact of the day of vitrification, the time in culture nor the expansion degree on the results.

Results:

All the embryos had a good embryo quality (BB or greater using Gardner Criteria), but different expansion degree. Informativity rate was significantly lower when day-5 embryos were cultured for only 8 hours, regardless of the expansion degree. See results on Graph 1.



Graph 1. Informativity rate vs Expansion degree.
* represents statistically significant differences (p-values < 0.05; Fisher's Exact Test).

The concordance rates between SBM and whole blastocysts were high (90.5% for day-5 Short, 93.6% for day-5 Long, 92.3% for day-6 Short) and similar between the 3 groups. They were not affected by the expansion degree or the time in culture.

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