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The effectiveness of non-invasive preimplantation genetic testing using spent culture medium or blastocoel fluid.

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Introduction

This study investigates non-invasive preimplantation genetic testing (PGT) using cell-free DNA (cfDNA) found in spent culture medium (SCM) and blastocoel fluid (BF). The study measures cfDNA concentrations in BF and SCM to determine the amount of cfDNA that can be used for stable next-generation sequencing (NGS) analysis. Concordance is also evaluated by comparing cfDNA with DNA from trophectoderm and blastocyst obtained from the same embryos.

Material & methods

[Study 1] Discarded vitrificated blastocysts with patient consent were thawed and cultured in a 30μl culture medium drop for 24 hours. BF was aspirated from the blastocoel cavity with an ICSI needle and dissolved in 10μl of sterile water, and all SCM was collected in a PCR tube. The amount of cfDNA in the recovered BF and SCM was measured using Real-Time qPCR. [Study 2] After thawing the vitrificated blastocysts and opening the zona pellucida, a trophectoderm biopsy was performed from the expanded blastocysts. A biopsied blastocyst was collected after removed the zona pellucida. The recovered SCM, trophectoderm, and blastocyst were analyzed for chromosomes by NGS. Blastocysts diagnosed as aneuploid by PGT-SR were used for aneuploid blastocyst samples.

Results

[Study 1] The average total cfDNA content in BF and SCM was 7.9 ± 2.9 pg and 132.2 ± 18.0 pg, respectively, with significantly higher levels found in SCM. [Study 2] The mean

total cfDNA content was also significantly higher in SCM.　Furthermore, the WGA amplification rate of cfDNA samples from SCM collected from embryos cultured for 24 hours after thawing was 91.3% (21/23), and 24-chromosome ploidy information was successfully obtained for all samples in trophectoderm and blastocyst. The results of the chromosome analysis for the culture medium and trophectoderm corresponding to the blastocyst were compared. The complete concordance rate between blastocysts and SCMs was 47% (10/21), of which 66.7% (14/21) were consistent with aneuploidy determination results. The sex chromosome concordance rate was 96.2% (20/21), of which 100% (11/11) of the samples were concordant for XY. The concordance rate with normal blastocysts was 40.0% (4/10) for SCM, and 90.9% (10/11) with abnormal blastocysts (PGT-SR) for SCM. All trophectoderm samples were consistent with blastocysts. Upon closer examination of the SCM that were not in agreement with normal blastocysts, it was found that all six culture medium samples were discordant due to mosaicism.

Conclusions

The study revealed that the amount of cfDNA collected from BF was inadequate for precise chromosomal analysis, as it was only equivalent to that of a single cell. In contrast, the amount of cfDNA found in SCM was sufficient for analysis, as demonstrated by the high concordance rate observed in abnormal embryos from PGT-SR cases. Although it is possible to diagnose aneuploid embryos, which can lead to miscarriage, the concordance rate with normal blastocysts was only 40%. All instances of discordance were due to low-frequency mosaicism. It is believed that the presence of cell-free DNA from aneuploid and apoptotic cells in spontaneous miscarriage contributes to this. New criteria are needed for the diagnosis of mosaicism to improve accuracy.