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Biopsied cells from frozen embryos in preimplantation genetic testing for monogenic are inferior to fresh embryos

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Introduction:

Preimplantation genetic testing for monogenic (PGT-M) is carried out by using both direct and indirect methods. However, due to the limited number of facilities where PGT-M can be implemented, its execution sometimes involves an embryo biopsy using frozen embryos transported from other infertility facilities as well as fresh embryos obtained by *in vitro* fertilization and embryo culture at the facility.
In the present study, the effect of freezing on the embryos was examined by comparing the frozen and fresh embryos on the basis of number of biopsied cells, amount of amplified DNA, and undiagnosed embryo rates in biopsied cells.

Material & methods:

The subjects used in the present study comprised 38 blastocysts obtained from 13 cycles of 7 couples who underwent PGT-M at our clinic between 2017 and 2018. Of the 38 embryos, 28 were fresh, obtained from 9 cycles of 5 couples, and 10 were frozen, obtained from 3 cycles of 3 couples. The diseases to be diagnosed were: Duchenne muscular dystrophy (1 case), myotonic dystrophy (3 cases), adrenoleukodystrophy (2 cases), and peroxisomal disease (1 case). Trophectoderm biopsy was performed using a laser, and a light microscope was used to count the number of biopsied cells. REPLI-g Single Cell Kit (QIAGEN) was used for whole genome amplification of the biopsied cells. Further, in order to enhance the accuracy of the analysis, haplotyping by short tandem repeat markers along with direct mutation detection were performed. This also ensured minimal potential errors caused by the undetected allele dropout and/or contamination.

Results:

The age of the females from whom fresh and frozen embryos were obtained was 34.7 and 32.7 years, respectively. Furthermore, the average number of biopsied cells between the fresh and frozen embryos did not differ much (7.8 and 8.6 respectively). However, the amount of amplified DNA from the frozen embryos (485.3 ng/μl) was noted to be significantly smaller than that from the fresh embryos (696.5 ng/μl). In addition to this, the incidence of undiagnosed embryos in case of frozen embryos was much higher than that in the case of fresh embryos (40.0% vs. 3.6%, $p < 0.05$).

Conclusions:

The results revealed that the biopsied cells from the same number of frozen embryos as the fresh embryos generated a relatively smaller amount of amplified DNA and higher rate of undiagnosed embryos. This difference can be attributed to the possibility of cell damage due to embryo freezing. The result of the current study suggests that biopsies should be performed on fresh embryos rather than on frozen ones.

Keywords:

preimplantation genetic testing for monogenic, biopsy, frozen embryo, whole genome amplification