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Optimal blastocyst stage for trophectoderm biopsy in array comparative genomic hybridization

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Introduction: A single blastomere is usually obtained from day 3 embryos for preimplantation genetic diagnosis (PGD) of chromosomal abnormalities using fluorescence in situ hybridization (FISH). Recently, with advances in molecular-biologic techniques, array comparative genomic hybridization (aCGH), which can provide more accurate and informative results on chromosomal abnormalities, has been developed for PGD. The aCGH method requires a larger number of cells to reduce the risk of diagnostic error. Therefore, blastocyst biopsy is believed to be better than embryo biopsy. The aim of the present study was to assess the blastocyst stage that would be optimal for trophectoderm biopsy, from the standpoint of the biopsied cell number and the survival rate of the blastocysts after biopsy.

Material and methods: Surplus blastocysts intended for disposal were used in this study after obtaining informed consent from the patients. A hole measuring 20-30 um in diameter was made in the zona pellucida using the ZIROS-tk laser system in unhatched blastocysts, including early (n = 17), full (n = 25) and expanded blastocysts (n = 15). At three to five hours after formation of the hole, herniating trophectoderm cells were biopsied using the laser system. On the other hand, fully hatched blastocysts (n = 11) were held gently so as to keep them from shrinking; trophectoderm cells on the opposite side to the inner cell mass were biopsied using the laser system. The numbers of dead and live biopsied cells were counted after staining with propidium iodide and Hoechst 33342. The viability of the blastocysts after biopsy was assessed on the following day. Moreover, aCGH was performed in biopsy samples obtained from blastocysts that developed to a larger size than the full blastocyst stage (n = 5).

Results: The average numbers of biopsied cells from early, full, expanded and fully hatched blastocysts were 3.5 ± 0.5, 6.3 ± 0.5, 7.2 ± 0.8 and 8.7 ± 1.0, respectively. Moreover, the average numbers of live cells were 2.1 ± 0.4, 4.7 ± 0.3, 4.7 ± 0.7 and 6.4 ± 0.9, respectively. The numbers of live cells in the early blastocysts were significantly fewer than those in the other blastocyst stages. There were no significant differences in the survival rates of the blastocysts after biopsy among the blastocyst stages ($P > 0.05$, 73 - 94%). Accurate results of aCGH were obtained from all the samples.

Conclusions: In the present study, live trophectoderm cells could be obtained from all blastocyst stages, despite biopsy being technically difficult in early and fully hatched blastocysts. However, the number of trophectoderm cells that can be biopsied from early blastocysts is fewer than that from other blastocyst stages, because the number of herniating cells is few. Therefore, for the purpose of reducing the risk of misdiagnosis in PGD using aCGH, trophectoderm biopsy of early blastocysts should be extended in time at least until they develop to the full blastocyst stage.

Key wards: preimplantation genetic diagnosis, blastocyst stage, trophectoderm biopsy, array comparative genomic hybridization