O-8(임상) Comparative Study of Embryo Development and Apoptosis: Vitrification Versus Slow Freezing of Biopsied Mouse Embryo

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Objectives: The availability of an efficient cryopreservation program is especially important in the case of embryos that have undergone blastomere biopsy for PGD. We compared cryosurvival rate and development of biopsied mouse embryos using slow freezing and vitrification.

Methods: We used mouse 2-cell embryos from 6 week-old ICR mice. Single-cell biopsy was performed on 64~68 h post-hCG using Tyrode's acid. Biopsied embryos were grown to blastocysts, cryopreserved and thawed. In slow freezing method, blastocysts were first exposed in 5% glycerol and then 9% glycerol + 0.2 M sucrose for 10 min each. In vitrification group, blastocysts were equilibrated in 7.5% ethylene glycol (EG) + 7.5% DMSO for 15min and then vitrified in 15% EG + 15% DMSO + 0.5 M sucrose within 1 min. The vitrification solution containing blastocysts were placed onto the pull and cut (PNC) straw, which was designed and made by pulling and cutting of 0.25 ml straw, and immediately plunged into the liquid nitrogen. In thawing procedure of slow-freezing, the embryos were thawed in 0.5 M, 0.2 M and 0 M sucrose solution sequentially for 5 min in each step. Vitrified blastocysts were warmed in 1 M sucrose at 37℃ for 1min, washed in 0.5 M sucrose for 3min and then washed twice in PBS for 5 min. Twenty-four hours later, blastocoel formation and total cell number of blastocysts were assessed in thawed blastocysts. Apoptotic cells in blastocyst were determined using DeadEnd Fluorometric TUNEL System (Promega).

Results: The survival rate of vitrification and slow freezing of biopsied embryo group (91.8% vs. 85.1%) was not significantly different non-biopsied embryo group (85.3% vs. 85.1%). Total cell number was 87.2±19.0 in slow freezing of non-biopsied embryos, 83.8±18.0 in vitrfication of non biopsied embryos, 74.2±22.2 in slow freezing of biopsied embryos and 72.1±20.0 in vitrification of biopsied embryos, respectively. The total cell number of biopsied embryo group was lower than non-biopsied embryo group, but it was not statistically significant. The apoptotic index of vitrification and slow freezing of non-biopsied embryo group (11.5±7.0 vs. 12.9±8.1) was not significantly different when compared to biopsied embryo group (12.5±7.9 vs. 15.6±7.5).

Conclusion: Both vitrification and slow freezing of biopsied mouse embryo is useful method for cryopreservation. These results showed that the vitrification and slow freezing methods would be a highly efficient method in clinical application of cryopreservation of supernumery blastocyst in PGD program.