

VITRIFICATION OF HUMAN ZYGOTES AND EMBRYOS

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Introduction: Slow-cooling (SC) cryopreservation of supernumary pronuclear stage oocytes during IVF/ICSI is well established and routinely implemented in the clinical IVF-programme. Recently, worldwide high survival and pregnancy rates with Cryo-Embryotransfer by vitrification using minimum volume cooling method have been reported. The radical strategy of vitrification is to result in a total elimination of ice crystal formation, both within the cells being vitrified (intracellular) and the surrounding solution (extracellular). In the present study, we examined the survival rate of vitrified and rewarmed human pronuclear stage oocytes that were cultured for additional 24 h before Cryo-ET as well as to evaluate the pregnancy rate. The results were compared to survival- and pregnancy rate using the slow-cooling cryopreservation method retrospectively.

Material and Methods: Between January 2000 and November 2005 a total of 752 patients had 3616 supernumary zygotes during IVF/ICSI treatment. These zygotes were cryopreserved using the slow-cooling method. A total of 1005 supernumary zygotes from 211 other patients were vitrified between April 2004 and January 2008 using the Cryotop (Kuwayama, RBM-online, 2005, pp 608-615). For vitrification, zygotes were placed into equilibration solution (7.5% Ethylenglycol; 7,5% DMSO) and incubated for 8 min. at room temperature (RT). Hereafter zygotes were incubated in vitrification solution (15% Ethylenglycol; 15% DMSO; 0,5M Saccharose) for 45-60 sec. at RT and placed on the Cryotop-strip and were plunged directly into the liquid nitrogen. After Vitrification a hard plastic cover is attached to protect the strip during storage in liquid nitrogen. In total 1438 zygotes were thawed according to the conventional Slow-cooling-protocol. 107 zygotes were rewarmed after being vitrified: the hard plastic cover was removed in liquid nitrogen and the Cryotop was plunged in thawing solution (1M Saccharose) at 37 C for 1 min. Zygotes were placed in diluent solution (0,5M and 0.25M Saccharose) at RT each for 3 min. Washing was done many times before culture. After both procedures, vitality of zygotes was evaluated under dissecting microscope one hour after rewarming. Embryo transfer was done 24 hours after culture in programed cycles. Clinical pregnancies per Cryo-ET were evaluated and compared for both methods.

Results: In total 1438 zygotes were thawed after being cryopreserved with the slow-cooling method. 848 zygotes seemed to be vital after thawing with a survival rate of 59%, while 381 zygotes were rewarmed after being vitrified corresponding to a survival rate of 96.3%.

583 patients underwent Cryo-ET after Slow-cooling procedure of zygotes. The clinical pregnancy rate per Cryo-ET was 10.2% (n=111). In contrast 115 patients underwent Cryo-ET after vitrification of zygotes. Pregnancy rate was 33.3% (n=69). Out of these 39 healthy babies were born.

Conclusion: These retrospective comparative results clearly demonstrate that the Cryotop vitrification method of supernumary zygotes showed a high post-thaw survival and pregnancy rates suggesting that the Vitrification-protocol may be preferable because of its simplicity, cost-effectiveness and time saving in a busy laboratory daily-work.