## P25 Influence of basic medium and slush nitrogen ($SN_2$) on the survival of mouse blastocysts after vitrification/warming

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Objective: Sodium salt is main contributor to the solution effect during cooling and thawing of embryos. Also, it was recently suggested that introducing slush nitrogen ($SN_2$) into vitrification for higher cooling rate improved the integrity and subsequent development of oocytes after thawing. Although vitrification has been successfully introduced into oocyte and embryo freezing, the effect of different buffer system as a basic solution for dehydration/rehydration on the survival and developmental rates after vitrification are still unclear. So, the aims of this study were to examine the effect of different basic solutions and cooling rates on the recovery of mouse blastocysts after vitrification.

Materials and Methods: Mouse blastocysts derived from 2-cell embryos were collected and used for the study. Survival and apoptosis of mouse blastocysts were analyzed after vitrification according to application of basic solutions with a different buffer system or choline chloride (ChCl) and/or $SN_2$. In experiment I, blastocysts were loaded on electron microscopy (EM)-grids and then vitrified with NaCl-based DPBS (group 1), ChCl-based DPBS (group 2), and Mops-buffered solution (group 3), as basic solution. In experiment II, blastocysts loaded on grids were divided into two groups and then vitrified using $SN_2$ (group 4) or not (group 5). All blastocysts were pre-equilibrated with 1.5M ethylene glycol (EG) for 2.5 min and equilibrated with 5.5M EG and 1.0 M sucrose for 20 sec, and then stored in liquid nitrogen ($LN_2$). The warmed blastocysts in all groups were re-hydrated and cultured in Preimplantion-1 (P-1) medium with 10% SSS. Their survival and apoptosis after recovery were examined under microscopic observation and TUNEL assay. Statistical analysis was carried out by use of Chi-square and Student's *t*-tests.

Results: In experiment I, survival rate of mouse blastocysts in group 2 (78.5% (73/93)) after vitrified-thawed was higher than those in the group 1(61.4% (54/88)), and 3 (63.8% (60/94)), respectively ($p<0.05$). And in group 2, apoptosis in re-expanded blastocysts after thawing was decreased compared to those in others groups. In experiment II, survival and apoptotic rates of blastocysts in vitrified group with high cooling rate (group 4) were not different compared to those in conventional $LN_2$ (group 5).

Conclusion: The use of the high concentration of sodium in vitrification media is detrimental to survival of mouse blstocysts. Reducing or eliminating sodium in basic solution for vitrification allows mouse blastocysts to be frozen more efficiently.

**Key words:** vitrification, blastocyst, slush nitrogen, choline cloride, cryopreservation

## P26 Super-Rapid Cooling Using Slush Nitrogen ($SN_2$) Increases the Efficacy of Human Oocytes Vitrification and Subsequent Clinical Applications

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Objective: The establishment of the oocyte bank using freezing methods has a number of benefits in the field of human assisted reproductive technology (ART). Although relatively successful outcomes from thawed oocytes have been reported, the clinical applications are still limited because of poor viability and quality of oocytes after thawing. To improve survival rate of surplus oocytes stored from previous stimulation cycle and pregnancy rate in additional IVF-ET using those oocytes after failing of fresh cycles, we applied super-rapid cooling by using $SN_2$ into our oocytes vitrification protocol.

Material and Methods: Before this clinical application, we tested the efficiency of $SN_2$ on the survival of fertilization-failed oocytes and the anti-apoptotic effect in CC of GV oocytes after vitrifying/warming. From December 2003 to August 2005, 76 patients agreed to participate in an oocytes cryopreservation protocol using the $SN_2$-vitrification method for the extra oocytes while they were undergoing IVF cycles. After failing the fresh-IVF cycles, 28 patients (30 cycles) returned for ET using vitrified-thawed oocytes. All oocytes were pre-equilibrated with 1.5 M ethylene glycol (EG) for 2.5 min and then equilibrated with 5.5 M EG and 1.0 M sucrose for 20 sec. The oocytes were loaded onto electron microscope copper/gold grids and plunged into $SN_2$, and then stored in $LN_2$ for next use. After failing of the fresh cycle, stored oocytes were thawed by a five-step method, and then their survival was analyzed. Apoposis of cumulus cell (CC) after thawing were examined under microscopic observation and TUNE assay. Intact mature oocytes were fertilized by ICSI using husband's sperm and cultured for 3days, and then transferred into uterus of patients.

Results: Survival rate after thawing of fertilization-failed oocytes vitrified using $SN_2$ was increased compared with those of oocytes using conventional $LN_2$. And, after 3 hours of thawing, the degeneration and apoptosis in CC obtained from $SN_2$-vitrification was significantly lower than those in CC from in conventional $LN_2$-vitrification. In 30 cycles of clinical application, total of 364 surplus oocytes from 28 patients were vitrified by using $SN_2$. 302 of those were survived after liquefying and showed normality morphologically. Fertilization and cleavage rate were 77.1% (168/218) and 94.0% (158/168), respectively. Twelve pregnancies were resulted from 30 uterine transfers of 120 embryos and implantation rate was 12.5% (15/120).

Conclusion(s) : Super-rapid cooling using $SN_2$ improves the clinical efficiency of human oocyte vitrification.

**Key Words:** oocyte freezing, super-rapid freezing, Slush nitrogen (SN2), IVF-ET, pregnancy