Journal of Assisted Reproduction and Genetics, Vol. 21, No. 5, August 2004 (© 2004)

Freezing of Human Immature Oocytes Using Cryoloops with Taxol in the Vitrification Solution

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Submitted March 29, 2004; accepted July 16, 2004

***Purpose*:** In human frozen immature oocytes, there has been little successful delivery. We examined the feasibility of vitrification solution including Taxol, cytoskeltal stabilizer.
***Methods*:** We set four experimental groups that immature oocytes has cumulus cells or not, or including Taxol or not in the vitrification solution. Frozen–thawed oocytes have been performed IVM, ICSI, and IVC.
***Results*:** There were no significant differences in survival, maturation, and fertilization rate, respectively. However, in the group enveloped by cumulus cells and including Taxol in the vitrification solution, one embryo was developed to blastocyst.
***Conclusions*:** Our results showed that using vitrification solution with Taxol proved so effective.

KEY WORDS: Cryoloop; immature oocytes; Taxol; vitrification.

INTRODUCTION

Oocyte freezing is a very useful technique for assisted reproductive technology (ART), particularly in cases of malignant diseases, premature ovarian failure, and oocyte donation. Immature oocytes, particularly germinal vesicle (GV) oocytes, have less depolymerization of the microtubles, stray chromosomes, and aneuploidy than those of mature oocyte. Moreover, we can retrieve greater numbers of immature oocytes than those of mature oocytes without ovarian stimulation. Therefore, in the near future, immature oocyte freezing and thawing techniques will become more useful than the freezing techniques for mature oocytes.

The GV oocyte is less permeable to cryoprotectants than the mature oocyte and the junction between oocytes and cumulus cells is extremely susceptible to mechanical stress; thus, the oocyte has a tendency to be damaged by freezing. Furthermore, the GV oocyte requires an additional maturation procedure. A complete in vitro maturation (IVM) procedure for human GV oocytes has not yet been established and the embryo development of mature oocytes derived from IVM is low. It has been reported that frozen–thawed human GV oocytes develop poorly because aneuploidy and spindle abnormalities of metaphase II oocytes occur more frequently (1).

Only one successful delivery using frozen human immature oocytes has been reported (2), whereas many successful cases—including those of our clinic—have been reported after the freezing of human mature oocytes (3–6).

We are hopeful that vitrification will have a high survival rate of postthaw oocytes because it can avoid the cryoinjury of cells (cryohydrate is produced in the cryopreservation solution). Moreover, vitrification does not require a programmed freezer and the technique is quite simple. Vitrification has been applied to many animal species (7–9), as described in the report of Rall and Fahy (10); several reports describe successful pregnancy outcomes (11–13).

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Table I. Development of Frozen–Thawed Oocytes by Vitrification Method Using Cryoloops

Group	*N*	Survival	Maturation	Fertilization	Cleavage	Compacted morula	Blastocyst
C(+)T(+)	30	90.0 (27/30)	85.2 (23/27)[a]	73.9 (17/23)	82.4 (14/17)[ab]	11.8 (2/17)	5.9 (1/17)
C(+)T(−)	20	80.0 (16/20)	81.3 (13/16)	61.5 (8/13)	37.5 (3/8)[ac]	0 (0/8)	0 (0/8)
C(−)T(+)	34	91.2 (31/34)	58.1 (18/31)[ab]	77.8 (14/18)	42.9 (6/14)[bd]	0 (0/14)	0 (0/14)
C(−)T(−)	53	79.2 (42/53)	81.0 (34/42)[b]	64.7 (22/34)	77.3 (17/22)[cd]	0 (0/22)	0 (0/22)

Note. Values in the same row with the same superscripts are significantly different ($P < 0.05$).

To determine the best IVM–IVF–IVC techniques, we examined the feasibility of using human immature oocytes with either enclosed cumulus cells or free cumulus cells using vitrification methods. We also examined the effect of Taxol, one of the cytoskeleton stabilizers, using human immature oocytes.

MATERIALS AND METHODS

The immature oocytes used in this study were recovered from patients undergoing ICSI. Cumulus cells were removed by hyaluronidase treatment and those having oocytes with a visible germinal vesicle were used for the study. Some oocytes which had two or three layers of cumulus cells and had visible germinal vesicle were used.

Freezing and thawing procedures involved using vitrification via a cryoloop. Modified human tubal fluid (m-HTF; Irvine Science) was used as a vitrification solution; the solution was supplemented with 20% serum substitute supplement (SSS; Irvine Science), 20% dimethyl sulfoxide (DMSO; Sigma), 20% ethylene glycol (EG; Sigma), 0.65 M sucrose (Sigma), 10 mg/mL Ficoll (Amersham Pharmasia Biotech), and with or without 1 μM Taxol (Paclitaxel; Bristol Parmaceuticals K. K.). Before freezing, the oocytes were equilibrated in m-HTF supplemented with 20% SSS, 10% DMSO, and 10% EG for 2 min at room temperature. After equilibration, oocytes were transferred to the vitrification solution and mounted on a cryoloop, which held a thin film of the vitrification solution. The cryoloops were plunged directly into liquid nitrogen within 20 s after equilibration. We set four experimental groups; cumulus(+)Taxol(+) (C(+)T(+)), C(+)T(−), C(−)T(+), and C(−)T(−).

Thawing was performed by direct immersion of the cryoloops into thawing solution 1 (m-HTF supplemented with 20% SSS and 1.0 M sucrose). After 2.5 min, the oocytes were transferred to thawing solution 2 (m-HTF supplemented with 20% SSS and 0.5 M sucrose). After 2.5 min, the oocytes were transferred to thawing solution 3 (m-HTF supplemented with 20% SSS and 0.25 M sucrose). After 2.5 min, the oocytes were transferred to thawing solution 4 (m-HTF supplemented with 20% SSS and 0.125 M sucrose). After 2.5 min, oocytes were transferred to m-HTF supplemented with 20% SSS.

The thawed oocytes were transferred to the IVM medium; TCM199 (Invitrogen Corporation) supplemented with 10 IU/mL FSH (Fertinorm P; Serono), 20 IU/mL HCG (Mochida Seiyaku), 10 ng/mL E2 (Sigma), and 20% human inactivated follicular fluid. After 24 and 48 h of IVM, the matured oocytes were subjected to ICSI. Fertilization confirmation was established 16–18 h after ICSI by the visualization of two PNs and two PBs. Fertilized embryos were initially cultured in K-SICM (Cook) and subsequently in K-SIBM (Cook).

RESULTS

Table I shows the embryo development after freezing, thawing, and ICSI. The C(+)T(+) group was found to have superior results for maturation, fertilization, and cleavage rate when compared to the cumulus free or nonsupplemented Taxol groups. However, in the C(−)T(+) group a low maturation and cleavage rate was found. Only the C(+)T(+) group produced embryonic development to the blastocyst stage.

DISCUSSIONS

Park *et al.* reported that supplementation of Taxol to cryoprotectant at freezing resulted in a high potential for development in mice (14). In our data, Taxol seems to have the same function as it does in human GV oocytes. However, C(−)T(+) group showed low maturation and cleavage rate. Perhaps this finding is due to the buffering of cumulus cells from Taxol. Excessive concentration of Taxol may be harmful for human GV oocytes. Similarly, C(+)T(−) group showed low cleavage rate. It indicated the possibility of Taxol that affected to cumulus cells. It may be that Taxol

stabilized the cytoskeleton of cumulus cells, and they promoted maturation and development of human GV oocytes.

In conclusion, this study has demonstrated that supplementation of Taxol could promote embryo development after thawing, but the function of Taxol is unclear. Further research is indicated to clarify the function of Taxol and its optimal concentration in order to improve the rate of embryo development.

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