COMPARISON OF THE EFFECT OF CYTOCHALASIN B AND PACLITAXEL (TAXOL™) ON CRYOPRESERVATION OF ICR MOUSE OOCYTES

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**Summary**

**Objective:** To investigate the benefits of the cytoskeleton stabilizers paclitaxel and cytochalasin B (CCB) in the oocyte vitrification procedure.

**Materials and Methods:** ICR mice aged 4–6 weeks were superovulated with pregnant mare serum gonadotropin and human chorionic gonadotropin. In Experiment 1, all retrieved eggs were randomly assigned to group A or B for the cumulus cell effect test and went through the vitrification protocol. In Experiment 2, the oocytes were allocated randomly to one of two subgroups for the cytoskeleton stabilizer tests: subgroup B, paclitaxel, and subgroup C, CCB. Group B in Experiment 1 served as the control (i.e. subgroup A) in Experiment 2.

**Results:** In Experiment 1, denuded oocytes had a higher fertilization rate after thawing than cumulus-enclosed ones. Accordingly, cumulus-free oocytes were chosen for Experiment 2. In Experiment 2, there were significant differences in post-thaw survival rates and fertilization rates between subgroups A and B. There was also a significant difference in fertilization rates between subgroups A and C. However, there were no differences in cleavage rates of 2–4- and 6–8-cell embryos between the subgroups.

**Conclusion:** Cumulus-free oocytes have greater developmental competence than cumulus-enclosed ones. Adding paclitaxel significantly improves the survival rate and fertilization rates of post-thaw cumulus-free ICR mouse oocytes. The addition of CCB also improves the fertilization rate of post-thaw oocytes. [Taiwanese J Obstet Gynecol 2005;44(1):48–51]

**Key Words:** cumulus cell, cytochalasin B, mouse, oocyte, paclitaxel, vitrification

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**Introduction**

Based on clinical necessity, much effort has been invested in developing new methods of oocyte cryopreservation. For instance, oocytes can be stored in advance for patients undergoing chemotherapy, surgery, or radiotherapy, and used to build up a gamete bank. Recently, higher survival rates have been achieved with efficient vitrification using the newly developed closed pulled straw (CPS) method compared with those following the traditional slow freezing method [1]. However, there is still room in the vitrification procedure for enhancement allowing complete retrieval of developmental competence after thawing. This possibility is mostly available through protection of the oocyte cytoskeletal system, which is involved in meiotic division and embryo development, from injury [2,3].

The use of cytoskeleton stabilizers, such as paclitaxel (TAXOL™, Bristol-Myers Squibb Co, New York, NY, USA) and cytochalasin B (CCB), has been studied by other authors [3,4]. Paclitaxel appears to offer significant benefit in the blastocyst formation rate (58.6% vs 24% without treatment). Microfilament alterations during...
the freezing process can also be stabilized using CCB, to achieve a pregnancy rate up to 60%. With the intention of exploring the capability of these stabilizers, paclitaxel and CCB were examined using the CPS method. In addition, we determined which one had a greater effect on post-thaw survival rates and subsequent development of ICR mouse embryos.

Materials and Methods

Oocytes were collected from superovulated, 4–6-week-old, female ICR mice maintained on a constant light-dark cycle (5 A.M. to 7 P.M. light; 7 P.M. to 5 A.M. dark). Superovulation was induced by intraperitoneal injections of 7.5 IU pregnant mare serum gonadotropin (Sigma, St. Louis, MO, USA) and 7.5 IU human chorionic gonadotropin (hCG; Serono Inc, Norwell, MA, USA) administered 48–50 hours apart. The oviducts were excised 14 hours after hCG injection and immediately placed in human tubal fluid (HTF) medium. The freshly ovulated oocytes were collected under a stereomicroscope.

**Experiment 1: Effectiveness of cumulus cells after vitrification**

The collected oocytes were divided into two groups. Group A contained cumulus oocyte complexes (COCs). Group B oocytes were treated with brief exposure to HTF medium with 80 IU/mL hyaluronidase (Sigma). Immediately after dispersal of the cumulus, the oocytes were washed three times and cultured in HTF medium containing 0.5% bovine serum albumin (BSA; Sigma) in an atmosphere of 5% CO2 in air at 37°C to give denuded oocytes.

The vitrification procedures used in our study were principally based on the method developed by Chen et al [1]. The solutions for vitrifying and thawing protocols were prepared using Dulbecco’s phosphate-buffered saline (Gibco, Grand Island, NY, USA) with 20% (v/v) fetal bovine serum (Gibco). The COCs (Group A) or denuded oocytes (Group B) were pretreated with 1.5 M ethylene glycol (Merck, Darmstadt, Germany) for 5 minutes. They were transferred to a drop of vitrification solution (200 μL) containing 5.5 M ethylene glycol and 1 M sucrose (Sigma). Twenty seconds later, they were transferred to another drop of the same medium for equilibration. These two steps and loading the oocytes into the pulled straw (I.V.M., I’aigle, France) took less than 1 minute. The tip of the straw was loaded with the diameter of 2 mm vitrification medium, 2 mm air, 2 mm vitrification medium holding oocytes, 2 mm of air, and 2 mm of vitrification medium in a syringe (Figure).

After storage for between 1 hour and 1 week, the oocytes were thawed into a washing drop (400 μL) of 0.5 M sucrose and then transferred sequentially into 0.5, 0.25, and 0.125 M sucrose solutions for 2.5 minutes each in a four-well dish at 37°C. The oocytes were washed, transferred into culture medium, and incubated at 37°C under a humidified atmosphere of 5% CO2 in air for 2 hours prior to insemination.

For *in vitro* fertilization (IVF), sperm were collected from mature male ICR mice. The vas deferens and cauda epididymides were dissected and one drop (0.5–1 mL) of pre-equilibrated Whittingham’s medium [5] with 3% BSA was added. The sperm were released into the medium and allowed to disperse for 15 minutes at 37°C. After dispersion, the sperm concentration was determined to achieve a final concentration of 1 x 10^6 spermatozoa/mL. The insemination dishes were then incubated for 1–2 hours before the addition of thawed or fresh oocytes. After 4 hours of incubation with sperm, the oocytes were washed and cultured in HTF medium with 0.4% BSA and 0.01 mM ethylenediaminetetraacetic acid (Sigma). The progression of embryonic development was monitored every day until 96 hours after culture and the number of embryos surviving were recorded.

**Experiment 2: Effectiveness of paclitaxel and cytochalasin B**

We used subgroup B from Experiment 1 as the control group (subgroup A) here. All normal metaphase II morphology oocytes were randomly allocated to two subgroups: B, vitrification medium with paclitaxel (Sigma) and C, pretreatment with CCB (Sigma).

In subgroup B, the pretreatment and vitrification ethylene glycol medium were both supplemented with 1 μM paclitaxel. In subgroup C, the oocytes were incubated at 37°C in 5% CO2, and air for 30 minutes in HTF medium containing 7.5 μg/mL CCB. The oocytes were then transferred to pretreatment and vitrification ethylene glycol medium containing 7.5 μg/mL CCB. The loading technique was the same as described above.
Morphologic survival was defined as the possession of an intact zona pellucida and plasma membrane and refractive cytoplasm. Surviving oocytes were counted and recorded.

Subsequent to IVF, the rates of damage and fertilization were calculated from the percentages of cleavage embryos and blastocysts formed among post-thaw oocytes. The Chi-squared test was used for statistical assessment.

**Results**

**Experiment 1: Effectiveness of cumulus cells in vitrification**

Denuded oocytes had a relatively higher fertilization rate after vitrification and thawing than cumulus-enclosed oocytes ($p < 0.05$) (Table 1). There were no significant differences in survival rate after thawing or cleavage rates of 2–4-cell and 6–8-cell stages.

**Experiment 2: Effectiveness of paclitaxel and cytochalasin B**

There were significant differences in the survival rate after thawing ($p < 0.05$) and the fertilization rate ($p < 0.05$) between subgroup B and the control subgroup A. However, there were no significant differences in cleavage rates of 2- to 4-cell and 6–8-cell stages. There was also a significant difference in the fertilization rate ($p < 0.05$) between subgroup C and the control subgroup A. There were no significant differences in survival rate after thawing or cleavage rates of 2- to 4-cell and 6–8-cell stages (Table 2).

**Discussion**

It is controversial whether cumulus cells are beneficial for pre-implantational development of oocytes that are vitrified and thawed at the mature stage. In our study, cumulus-free oocytes had a higher fertilization rate after thawing than intact cumulus-enclosed ones. Fabbri et al hypothesized that one of the major factors affecting the oocyte freezing process is the ratio of surface area to volume [6]. That is, the larger the oocyte, the lower its developmental competence after freezing and thawing. Logically speaking, a larger oocyte requires longer to reach osmotic balance in the cryoprotectant solution, leading to a worse result after freeze-thawing procedures [7].

The results of this study indicate that adding the cytoskeleton stabilizers paclitaxel and CCB can significantly improve the fertilization rate of mature cumulus-free ICR mouse oocytes. Paclitaxel also improved the survival rate of mature oocytes after freezing and thawing. Neither stabilizer improved the cleavage rates of 2–4-cell and 6–8-cell stages after thawing compared with controls. There seemed to be a greater potential for paclitaxel to improve the fertilization rate compared with CCB. These results support the findings of other investigators. Park et al demonstrated that adding paclitaxel improved the post-thaw development of

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**Table 1. Comparison of pre-implantation development of cumulus-enclosed and cumulus-free oocytes vitrified and thawed at the mature stage**

<table>
<thead>
<tr>
<th>Status of oocytes</th>
<th>Total</th>
<th>Survived after thawing, %</th>
<th>Fertilized, %</th>
<th>2-4 cells, %</th>
<th>4-8 cells, %</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Survival</td>
<td>Death</td>
<td>Survival</td>
<td>Death</td>
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<tr>
<td>Cumulus-enclosed</td>
<td>45</td>
<td>28</td>
<td>17</td>
<td>17</td>
<td>11</td>
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<tr>
<td>Cumulus-free</td>
<td>48</td>
<td>29</td>
<td>19</td>
<td>23</td>
<td>7</td>
</tr>
<tr>
<td>$p$</td>
<td></td>
<td>0.62</td>
<td>0.05</td>
<td>0.21</td>
<td>0.20</td>
</tr>
</tbody>
</table>

**Table 2. Comparison of the effect of paclitaxel and cytochalasin B (CCB) on the development of post-thaw cumulus-enclosed oocytes and cumulus-free oocytes at the mature stage**

<table>
<thead>
<tr>
<th>Treatment method</th>
<th>Total</th>
<th>Survived after thawing, %</th>
<th>Fertilized, %</th>
<th>2-4 cells, %</th>
<th>4-8 cells, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Survival</td>
<td>Death</td>
<td>Survival</td>
<td>Death</td>
</tr>
<tr>
<td>Control</td>
<td>48</td>
<td>29</td>
<td>19</td>
<td>23</td>
<td>7</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>36</td>
<td>21</td>
<td>15</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>$p$</td>
<td></td>
<td>0.04</td>
<td>0.01</td>
<td>0.11</td>
<td>0.42</td>
</tr>
<tr>
<td>CCB</td>
<td>40</td>
<td>23</td>
<td>17</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>$p$</td>
<td></td>
<td>0.18</td>
<td>0.02</td>
<td>0.45</td>
<td>0.58</td>
</tr>
</tbody>
</table>
vitrified oocytes without decreasing blastocyst quality [4]. Isachenko et al suggested that pretreatment with CCB has a positive effect on vitrification of germinal- }

vesicle stage porcine oocytes [8].

Among the technical aspects of oocyte cryopreservation, a major concern is the disruption of the cytoskeletal structure, which may cause chromosomal anomalies and abnormal cytokinesis. The appropriate organization of spindle microtubules is essential for correct chromosome alignment and segregation [6]. Paclitaxel is generally used as an anticancer drug and acts by increasing the polymerization rate, thereby reducing the critical concentration of tubulin [9]. It can stabilize microtubules and interfere with the dynamic changes that occur during formation of the mitotic spindle and further arrest mitosis [10]. When oocytes are thawed and prepared for further treatment, they will undergo more frequent cytoskeletal rearrangement. Accordingly, we hypothesize that paclitaxel stabilizes the cytoskeleton and interferes with spindle formation during the cryopreservation procedure, ensuring that post-thaw oocytes can proceed to future development correctly.

CCB had the same protective effect as paclitaxel on fertilization rate in vitrified and thawed ICR mouse oocytes. CCB inhibits microfilament polymerization and has a specific and reversible effect on cytoskeletal elements because it makes them more flexible and less susceptible to cryodamage [11,12].

In this study, we used 7.5 μg/mL CCB and 1 μM paclitaxel to pretreat the oocytes. These protocols were chosen from the studies of Park et al [4] and Dobrinsky et al [3]. Optimal concentration of cytoskeleton stabilizers has been studied by many investigators, who have used them to pretreat different kinds of mammalian or human oocytes. However, the best condition needs to be investigated further using serial concentration experiments.

Cytoskeleton stabilizers such as paclitaxel and CCB have positive effects on protecting vitrified and thawed ICR mouse oocytes. On the other hand, using them in human oocyte cryopreservation is still controversial. They improve the developmental competence of thawed oocytes by interfering in microfilament or tubulin formation, further affecting spindle formation. More surveys should be carried out to investigate whether the interfering effect is reversible or not. Aneuploidies such as trisomy or monosomy can occur when spindle formation is out of order. In addition, paclitaxel is well known as an anticancer drug that may cause cell toxicity at specific concentrations. Consequently, the optimal concentration of these cytoskeleton stabilizers, which may improve the post-thaw survival rate without causing irreversible harm, should be further investigated.

References