Permeability of Mouse Oocytes and Embryos at Various Developmental Stages to Five Cryoprotectants

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Abstract. To assess the permeability of mouse oocytes and embryos, matured oocytes and embryos at various stages of development were placed in five cryoprotectant solutions at 25°C for 25 min. From the cross-sectional areas of the oocytes/embryos, the relative change in volume was analyzed. In oocytes, shrinkage was least extensive and recovery was quickest in the propylene glycol solution, showing that propylene glycol permeates the oocytes most rapidly. Dimethyl sulfoxide, acetamide, and ethylene glycol permeated the oocytes slightly more slowly than propylene glycol. The oocytes in glycerol shrunk extensively and then expanded marginally, indicating slow permeation. The volume changes of 1-cell and 2-cell embryos were similar to those of oocytes, showing little change in permeability. In 8-cell embryos, the volume recovered much faster than in the earlier stages especially in glycerol and acetamide. In morulae, the volume recovery was much faster in glycerol and in ethylene glycol; in ethylene glycol, the extent of shrinkage was small and the recovery was fast, indicating an extremely rapid permeation. Although the permeability of oocytes/embryos generally increased as embryo development proceeded, the degree of increase varied greatly among the cryoprotectants. Interestingly, the volume change in propylene glycol was virtually unaffected by the stage of development. Such information will be valuable for determining a suitable protocol for the cryopreservation of oocytes/embryos at different stages of development.

Key words: Mouse, Embryo, Oocyte, Permeability, Cryoprotectant

The survival of mammalian oocytes and embryos after cryopreservation varies with the stage of maturation and development. In general, oocytes/embryos at earlier stages appear to be more sensitive to cryopreservation. In the mouse, the rate of survival after cryopreservation is lower for unfertilized oocytes than embryos and increases as development proceeds up to the 8-cell or morula stage, although it may decrease as the blastocoel enlarges [1–3].

Several cryoprotectants have been shown to be effective for the preservation of mammalian oocytes and embryos, including dimethyl sulfoxide (DMSO) [1], glycerol [4], ethylene glycol [5], propylene glycol [6] and acetamide [7]. Cryoprotectants for mammalian oocytes and embryos are virtually limited to these five agents both in slow freezing and in vitrification [8]. Although the protective action of cryoprotectants is considered colligative [9], each agent has its own specific properties. Among them, the permeating property is of great importance, because permeation of the cell with a cryoprotectant is critical for the successful cryopreservation of
mammalian oocytes/embryos. To prevent intracellular ice from forming, which is lethal to cryopreserved cells, permeation by a cryoprotectant and condensation are considered essential both in slow freezing and in vitrification. If the permeation is too slow, cells have to be exposed to the cryoprotectant either for a long time, at a high concentration, or at a higher temperature, which might expose the cells to the chemical toxicity of the cryoprotectant. Furthermore, cells are likely to be injured by osmotic over-swelling during the removal of the cryoprotectant after warming, if diffusion of the permeated cryoprotectant out of the cell is too slow. Generally, therefore, more permeating agents would be preferable.

When an oocyte or embryo is placed in a hypertonic solution containing 1–2 M cryoprotectant, it initially shrinks rapidly in response to the high extracellular osmolality, because diffusion of intracellular water out of the oocyte/embryo is faster than permeation of the cryoprotectant into the cell. After shrinking, the oocyte/embryo starts to regain its volume slowly as the cryoprotectant permeates the cell with water at a fixed osmolality. Consequently, it is possible to estimate the permeability of the oocyte/embryo to a cryoprotectant by measuring its volume changes. Leibo et al. [10] first adopted the volumetric approach to assess the permeability of embryos; they suggested that the permeation by DMSO of 8-cell mouse embryos is quite slow at 0°C. Mazur et al. [11] and Jackowski et al. [12] analyzed the permeability of mouse oocytes and embryos to glycerol quantitatively using a volumetric method. In a review paper, Mazur [13] stated that the permeability increased after fertilization, and further increased as the development proceeded from the 1-cell to 8-cell stage. Thereafter, several attempts were made to evaluate the rate at which cryoprotectants permeate oocytes or embryos at various stages in several species, though most of the studies investigated the permeability of oocytes/embryos at only one or two stages of maturation/development and for only one or two cryoprotectants.

In the mouse, several studies have examined the permeability of matured oocytes [14–20], whereas only a few studies have examined the permeability of embryos at the 1-cell [21], 8-cell [22], and 1-cell and morula [23] stages. One study compared the permeability of 1, 2, 4, and 8-cell embryos, but only for one cryoprotectant [24]. In bovine, permeability has been examined in oocytes [18, 25] or embryos at the morula and/or blastocyst stage [26–29]. In some species (e.g., human [14, 30–33], monkey [34], and goat [35, 36]), only oocytes have been assessed for permeability to cryoprotectants. Oocytes/embryos of other species in which the permeability has been assessed include rabbit morulae [37], equine blastocysts [38], sheep morulae [39], rat oocytes [40] and rat 1-cell embryos [41]. Therefore, the literatures contain no systematic comparison of the permeating properties of various cryoprotectants in relation to the developmental stages of oocytes/embryos.

In the present study, the permeating properties of five major cryoprotectants were examined using mouse oocytes at metaphase II and embryos at five developmental stages ranging from the 1-cell to the expanded blastocyst stage at room temperature. The change in volume of the oocytes and embryos was measured by precision method using a micromanipulator system, because their changes in volume are rapid.

**Materials and Methods**

**Oocytes and embryos**

Mouse oocytes at the metaphase II stage and embryos at the 1-cell, 2-cell, 8-cell, morula, and expanded blastocyst stages were used in the study. Mature female mice of the ICR strain (CLEA Japan Inc., Tokyo) were induced to superovulate with an intraperitoneal (i.p.) injection of 5 I.U. equine chorionic gonadotrophin (Serotropin, Teikokuzoki, Tokyo) followed by an i.p. injection of human chorionic gonadotrophin (hCG) (Puberogen, SankyoZoki, Tokyo) given 48 h later. For the collection of embryos, females were mated with mature males of the same strain. The females were sacrificed by cervical dislocation, and oviducts or uteri were removed. For the collection of oocytes, matured oocytes surrounded by cumulus cells were removed from the ampullar portion of the oviducts at 14 h after the injection of hCG from unmated animals. The oocytes were freed from cumulus cells by suspending them in PBI medium [42] containing 0.5 mg/ml hyaluronidase (H-3506, Sigma, St Louis, MO, USA) followed by washing in fresh PBI medium in a culture dish (Ø 35 × 10 mm).
under paraffin oil. For the collection of 1-cell, 2-cell and 8-cell embryos, oviducts of mated animals were flushed with PB1 medium at 25, 44, and 68 h, respectively, after the injection of hCG. For the collection of morulae, the uteri of mated animals were flushed with PB1 medium at 78 h after the injection of hCG. Some of the morulae were cultured in a modified Krebs-Ringer bicarbonate medium [43] under paraffin oil in a culture dish in a CO$_2$ incubator, and expanded blastocysts were collected after 18–24 h of culture.

Only oocytes and embryos of normal morphology were used in the experiments. Oocytes had a polar body, 1-cell embryos had polar bodies and pronuclei, and 2-cell embryos had two equal sized blastomeres. Eight-cell embryos were carefully examined for the presence of 8 distinct blastomeres, while morulae were examined for compaction. Expanded blastocysts had an apparent expanded blastocoeI and zona pellucida, but were not hatching from the zona pellucida. The oocytes and embryos were washed and pooled in fresh PB1 medium in a culture dish under paraffin oil until each suite of experiments.

Measurement of volume change

The cryoprotectant solutions used were 10% v/v ethylene glycol, 10% v/v glycerol, 10% v/v DMSO, 10% v/v propylene glycol, and 1.5 M acetamide, all dissolved in PB1 medium. The osmolalities of the solutions were measured with osmometers based on the depression of the freezing point (OM 801, Vogel, Germany) and on the vapor pressure (Vapro 5520, Wescor Inc., Logan UT, USA), and are shown in Table 1.

In the cover of a Petri dish (Ø 94 × 10 mm), 200-µl drops of isotonic PB1 medium and cryoprotectant solutions were prepared under paraffin oil and placed on an inverted microscope. The temperature of the solution was monitored and the temperature of the room was adjusted so that the solution was kept at 25 C (± 0.5). In a micromanipulator system fixed to the microscope, a holding pipette (70–100 µm) and a covering pipette (150–200 µm) were set on the left and right arms, respectively (Fig. 1). The inner diameter of the holding pipette to which the oocyte/embryo was attached was small enough to prevent distortion of the oocyte/embryo. The covering pipette covered the oocyte/embryo together with the holding pipette. An oocyte or embryo was placed in PB1 medium and was held by the holding pipette. In the case of 2-cell embryos and 8-cell embryos, the embryo was oriented so that the blastomeres were clearly visible (Fig. 1A). Then, by sliding the dish, the pipette assembly holding the oocyte/embryo was introduced into a drop of cryoprotectant solution. By removing the covering pipette, the oocyte/embryo was exposed to the cryoprotectant solution instantly (Fig. 1B) [44]. A microscopical image of the oocyte/embryo, before and during the exposure to the cryoprotectant solution, was recorded by a time-lapse video tape recorder (ETV-820, Sony, Tokyo) every 0.5 sec for 25 min. From the images, the cross-sectional area of the oocyte/embryo in isotonic PB1 medium (0 sec) and at 5, 10, 20, 30, and 45 sec, and 1, 2, 3, 4, 5, 6, 8, 10, 15, 20 and 25 min after exposure to the cryoprotectants was measured. Then, the area relative to that in isotonic PB1 medium was calculated, and converted into the relative volume, on the assumption that the volume changes proportionally, using the equation V=Sc$^{3/2}$ where S is relative cross-sectional area and V is the relative volume. For each treatment, 3–4 oocytes/embryos were examined. The average relative volume was

<table>
<thead>
<tr>
<th>Cryoprotectant solution</th>
<th>Osmol/kg</th>
<th>Osmometer A*</th>
<th>Osmometer B*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 M acetamide/PB1</td>
<td>1.98</td>
<td>1.70</td>
<td></td>
</tr>
<tr>
<td>10% v/v DMSO/PB1</td>
<td>2.09</td>
<td>1.73</td>
<td></td>
</tr>
<tr>
<td>10% v/v ethylene glycol/PB1</td>
<td>2.55</td>
<td>1.92</td>
<td></td>
</tr>
<tr>
<td>10% v/v glycerol/PB1</td>
<td>1.98</td>
<td>1.74</td>
<td></td>
</tr>
<tr>
<td>10% v/v propylene glycol/PB1</td>
<td>2.33</td>
<td>1.60</td>
<td></td>
</tr>
<tr>
<td>PB1 medium</td>
<td>0.291</td>
<td>0.294</td>
<td></td>
</tr>
</tbody>
</table>

* A: freezing point depression, B: vapor pressure.
Results

Upon their transfer to the cryoprotectant solutions, the oocytes/embryos began to shrink quickly, reaching a minimal volume within 1 min of exposure, at all stages of development and in all the solutions. Then, they began to recover their volume slowly. However, the relative minimal volume and the recovery rate differed markedly between the stages of development and the cryoprotectants.

The average volume changes for oocytes during 25 min and the initial 5 min of exposure to the five cryoprotectants at 25 C are shown in Fig. 2. The least shrinkage and the fastest recovery of oocyte volume were observed in the propylene glycol solution, suggesting that propylene glycol permeated the oocytes the most rapidly. Oocytes in DMSO, acetamide, and ethylene glycol recovered much the same as the oocytes in propylene glycol, but slightly more slowly. On the other hand, oocytes exposed to glycerol had the largest extent of shrinkage and regained their volume extremely slowly, indicating that the permeability of oocytes to glycerol is quite low.

In two of the oocytes exposed to the acetamide solution, blebs formed and the oocytes were damaged after 6–8 min of exposure, therefore the graph in Fig. 2A ends at 6 min of exposure. This suggests that acetamide is toxic to mouse oocytes if used for a long period at 25 C.

The characteristic volume change of oocytes/embryos was manifested during a short period after suspension in the cryoprotectants, thus the data on embryos from the 1-cell to morula stage cover only 5 min of exposure. As shown in Fig. 3, the change in volume of 1-cell embryos was quite similar to that of oocytes, indicating that the permeability does not change at fertilization. The pattern of change essentially remained the same in 2-cell embryos. In 8-cell embryos, however, the volume recovered faster, especially in acetamide, ethylene glycol and glycerol. Notably, embryos suspended in the glycerol solution became more permeable, their recovery almost matching those of embryos exposed to the other cryoprotectants. In morulae, the recovery was much faster than in the earlier stages in ethylene glycol and glycerol; in ethylene glycol, the shrinkage was only slight and the embryos recovered their initial volume within 2 min of exposure (Fig. 3). This shows that ethylene glycol permeates morulae extremely rapidly.

Figure 4 shows the volume changes of expanded blastocysts during 25 min and the initial 5 min of exposure to five cryoprotectants at 25 C. The volume changes of expanded blastocysts could not be measured as accurately as those of other

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**Fig. 1.** The device and the procedure for direct transfer of an oocyte/embryo to a cryoprotectant solution using a micromanipulator system. [A] A photograph of a 2-cell embryo held by a holding pipette separate from the covering pipette on the right. [B] Schematic presentation of the device and the procedure with an oocyte/embryo (solid circle), a cryoprotectant solution (shaded hemisphere), and isotonic PB1 medium (dotted hemisphere).
Fig. 2. The change in volume of mouse oocytes at the metaphase II stage during exposure to 1.5 M acetamide (AA), 10% DMSO, 10% ethylene glycol (EG), 10% glycerol (Gly), and 10% propylene glycol (PG), dissolved in PB1 medium, for [A] 25 min and [B] 5 min at 25 °C.

Fig. 3. The change in volume of mouse embryos at the [A] 1-cell, [B] 2-cell, [C] 8-cell and [D] morula stages during exposure to 1.5 M acetamide (AA, reversed triangles), 10% DMSO (diamonds), 10% ethylene glycol (EG, circles), 10% glycerol (Gly, squares), and 10% propylene glycol (PG, triangles), dissolved in PB1 medium, for 5 min at 25 °C.
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Because the blastocysts had a large blastocoel and shrank unproportionally. Furthermore, the degree of expansion before measurement was not exactly the same among blastocysts. Therefore, standard deviation values for blastocysts, especially in acetamide and glycerol, were large (Table 2). Nevertheless, the results suggest that the permeability of blastocysts to ethylene glycol is similar to that of morulae, and the permeability to glycerol increased.

Table 2. Standard deviations (SD) of the relative volume for each type of oocyte/embryo after exposure to cryoprotectants for 1, 3 and 5 min

<table>
<thead>
<tr>
<th>Time</th>
<th>Stage</th>
<th>Cryoprotectant*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AA</td>
</tr>
<tr>
<td>1 min</td>
<td>Oocyte</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td>1-cell</td>
<td>0.079</td>
</tr>
<tr>
<td></td>
<td>2-cell</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td>8-cell</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>Morula</td>
<td>0.066</td>
</tr>
<tr>
<td></td>
<td>Blastocyst</td>
<td>0.117</td>
</tr>
<tr>
<td>3 min</td>
<td>Oocyte</td>
<td>0.055</td>
</tr>
<tr>
<td></td>
<td>1-cell</td>
<td>0.073</td>
</tr>
<tr>
<td></td>
<td>2-cell</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>8-cell</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>Morula</td>
<td>0.077</td>
</tr>
<tr>
<td></td>
<td>Blastocyst</td>
<td>0.144</td>
</tr>
<tr>
<td>5 min</td>
<td>Oocyte</td>
<td>0.065</td>
</tr>
<tr>
<td></td>
<td>1-cell</td>
<td>0.067</td>
</tr>
<tr>
<td></td>
<td>2-cell</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>8-cell</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>Morula</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>Blastocyst</td>
<td>0.116</td>
</tr>
</tbody>
</table>


![Graph](image-url) Fig. 4. The change in volume of expanded mouse blastocysts during exposure to 1.5 M acetamide (AA), 10% DMSO, 10% ethylene glycol (EG), 10% glycerol (Gly), and 10% propylene glycol (PG), dissolved in PB1 medium, for [A] 25 min and [B] 5 min at 25°C.
Exceptionally, the recovery of volume ceased, and shrinkage occurred after 2–4 min of exposure in acetamide. In the propylene glycol solution, three of four blastocysts also stopped expanding and started to shrink at 5–8 min.

The average change in volume of oocytes and embryos, except expanded blastocysts, during 5 min of exposure was reconstructed for each cryoprotectant, and is shown in Fig. 5. It is clear that 1) the permeability of oocytes/embryos to
acetamide increases at the 8-cell stage; 2) the permeability of oocytes/embryos to ethylene glycol increases gradually until the 8-cell stage, and markedly at the morula stage; 3) glycerol is much less able to permeate oocytes up to the 2-cell stage, but becomes quite effective at the 8-cell stage and the morula stage; and 4) interestingly, the pattern of volume change of oocytes/embryos in propylene glycol was quite similar at all stages, showing that the permeability of oocytes and embryos to propylene glycol does not change during development.

Discussion

When an oocyte or an embryo is suspended in a drop of cryoprotectant solution from an isotonic solution, it first floats to the surface, and then sinks to the bottom. It is therefore difficult to record a focused microscopical image until it sinks after 1 or 2 min [10, 25, 27, 28]. However, the oocyte/embryo undergoes considerable volume excursion during the first few minutes of exposure, because the diffusion of water out of the cell upon exposure is very rapid. McGrath [45] devised a microscope diffusion chamber for direct real-time observation of the dynamic osmotic response of an individual cell on a dialysis membrane, in which the solution around the cell can be replaced by a perfused solution through the membrane. However, when evaluating the rate at which a solute permeates the cell, the rate at which the solute diffuses across the dialysis membrane must be taken into account. Gao et al. [46] devised a microperfusion method, in which an oocyte was held with a minute pipette in a minimal volume of isotonic solution under oil, and then a large volume of test solution was added. In this case, the oocyte would be exposed to a fast flow of test solution. In the present study, an oocyte/embryo was held by a holding pipette attached to a micromanipulator system and introduced into the cryoprotectant solution. Occasionally, when the oocyte/embryo was first introduced directly, it dropped off or was distorted during its passage through the oil. Therefore, the oocyte/embryo held by the holding pipette was covered with a covering pipette during its passage. Upon removal of the covering pipette, the small amount of isotonic PB1 medium around the oocyte/embryo would flow up instantly.

Therefore, this method made it possible to expose an oocyte/embryo in an isotonic solution to a cryoprotectant solution instantly and smoothly in a fixed position for a microscope. This enabled accurate measurement of the volume change from the moment of exposure without any correction [44].

The results shown in Fig. 2 essentially reveal that oocytes are moderately permeable to propylene glycol, DMSO, acetamide and ethylene glycol, but less permeable to glycerol. This is consistent with reports on the permeability of mouse oocytes to propylene glycol [17], DMSO [15–17, 19, 24], ethylene glycol [19], and glycerol [12, 20].

However, it is not possible to compare the various results, because the conditions for volumetric assessment, e.g., the methods of measurement, the concentrations of the cryoprotectants, and the temperatures, vary among the reports. In some studies, the permeability coefficient for each cryoprotectant was calculated from a volume change curve. Therefore, it might be possible to compare the permeabilities in different reports quantitatively using the coefficient. However, several theoretical models have been devised to calculate the coefficient based on different assumptions [12, 15, 47], thus coefficients differ with the model even when the volume change is the same [24]. In the present study, therefore, we examined apparent volume changes of oocytes/embryos at six developmental stages in five cryoprotectants to enable direct comparison between stages and between cryoprotectants.

In comparison of the permeating properties of cryoprotectants based on the apparent volume change, the concentration of the cryoprotectants is an important factor. It would be preferable to use solutions with the same osmolality. Measurements of osmolality made with two types of osmometers using different measurement principles gave values which were roughly similar in all solutions. However, we decided they were not sufficiently similar (Table 1), so we used the same percentage by volume in the five cryoprotectant solutions (1.5 M acetamide approximately corresponded to 10% v/v). For more precise comparisons, a more accurate consideration of the cryoprotectant concentration will be necessary.

From the assessment of the volume change of mouse embryos at various developmental stages (Fig. 3), it was found that the permeability
remained virtually the same up to the 2-cell stage, but changed considerably at the 8-cell stage, especially for glycerol and acetamide, and at the morula stage for ethylene glycol and glycerol. Some reported data on the permeability of mouse embryos essentially agree with the present results; e.g., for 1-cell embryos exposed to ethylene glycol [21] and to DMSO [24], and for 8-cell embryos exposed to glycerol [13, 22]. Notably, Kasai [23] presented volume changes of 1-cell embryos and morulae for five cryoprotectants, which were quite similar to the present results, although the temperature was different (20 C). However, the results of a few reports are not consistent with the present results; e.g., Jackowski et al. [12] reported that the permeability of oocytes to glycerol increased after fertilization, and Pfaff et al. [24] reported that the permeability of embryos to DMSO decreased from the 4-cell stage to the 8-cell stage. No other comparative data are available.

Permeability generally increased as the development proceeded, although the degree of the increase and the transition stage differed between cryoprotectants (Fig. 5). At the 8-cell and morula stages, the permeation by ethylene glycol and glycerol increased dramatically, whereas the increase in permeability to DMSO was small. Furthermore, and interestingly, the permeating rate of propylene glycol was virtually unaffected by the stage of development. These characteristics definitely show that there exists a selective mechanism for the permeation by different agents.

The change in the pattern of permeation at the 8-cell and morula stages must be a reflection of certain physiological changes in the cell. Although the mechanism of change is not clear, the compaction of blastomeres might be involved because preparation for the following compaction would start at the 8-cell stage.

One of the major factors determining the permeating rate of a cryoprotectant is molecular weight (MW). Actually, the slower permeation by glycerol (MW=92.1) than ethylene glycol (MW=62.1) of oocytes and embryos is reflected by the higher molecular weight, because these two agents belong to the same group of polyhydric alcohols. However, the similar or slightly higher permeability of mouse oocytes and 1-cell and 2-cell embryos to propylene glycol (MW=76.1) than to acetamide (MW=59.1) and ethylene glycol (MW=62.1) is incompatible with the molecular weight being a determining factor in permeability. The molecular configuration might also affect the affinity for the membrane.

As a possible mechanism for the permeability, recent studies have suggested that water channels on the plasma membrane might be involved, because certain types of water channels, e.g., aquaporin 3 and aquaporin 7, can transport not only water but also glycerol, a representative cryoprotectant [44]. Actually, it has been shown that mRNAs of aquaporin 3 and aquaporin 7 are expressed in mouse oocytes and embryos [48], although the level of protein is not known. If such a channel actually affects permeation by glycerol, it might be reasonable to assume that the channel also transports ethylene glycol, a smaller polyhydric alcohol. However, it is not possible to account for the different patterns of permeation for other cryoprotectants with one type of water channel. Notably, the mechanism for the constant permeating pattern of propylene glycol throughout embryo development is difficult to explain. It might be that unknown channels which can transport propylene glycol selectively are expressed throughout the stages examined. Alternatively, propylene glycol might permeate the lipid bilayer rapidly.

Oocytes in acetamide formed blebs, and expanded blastocysts in acetamide and propylene glycol stopped expanding and began to shrink during exposure (Fig. 4). This unpredicted behavior of oocytes/embryos suggests that these cryoprotectants are more toxic than others. In toxicity tests for the same five cryoprotectants in mouse morulae, acetamide was the most toxic, followed by propylene glycol and then DMSO, whereas glycerol and ethylene glycol were much less toxic [49, 50].

There have been several reports on the permeability of mammalian oocytes and embryos to cryoprotectants. In matured oocytes, glycerol permeated more slowly than propylene glycol and DMSO in bovine [25], and glycerol permeated more slowly than ethylene glycol and propylene glycol in the goat [36], whereas the permeation by DMSO and ethylene glycol was similar in bovine oocytes [18]. In matured oocytes of humans and monkeys, DMSO [31, 32], propylene glycol [33], and ethylene glycol [34] permeated at moderate rates. In rat 1-cell embryos, propylene glycol permeated more rapidly than ethylene glycol and DMSO [41]. In
sheep morulae, ethylene glycol permeated more rapidly than propylene glycol, DMSO and glycerol [39]. In bovine blastocysts, glycerol permeated more rapidly than DMSO [26]. All these observations are essentially consistent with the present results in mouse oocytes/embryos. Therefore, permeability may be affected more by the stage of development than the species, although a precise comparative study has not been conducted, except for our preliminary study on bovine oocytes and embryos [51]. We have examined the permeability of bovine oocytes and embryos to five cryoprotectants, as in the present study, and found that the pattern of permeation did not change from matured oocytes up to the 16-cell stage, but at the compaction stage (morula), the permeability to glycerol and ethylene glycol increased markedly. Although the pattern is not exactly the same as that of mouse oocytes/embryos, the results support the idea that permeability is affected more by the developmental stage in mammalian oocytes/embryos.

Although the mechanism of the change in the permeability to cryoprotectants is not known, the present study on five major cryoprotectants provides practical information for adopting a suitable cryoprotectant and for determining suitable conditions for impregnation and removal of the cryoprotectant at each stage of development in oocytes/embryos.

References

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