

Permeability of the plasma membrane to water and cryoprotectants in mammalian oocytes and embryos: Its relevance to vitrification

Keisuke Edashige

College of Agriculture, Kochi University,
Nankoku, Kochi, Japan

Correspondence

Keisuke Edashige, College of Agriculture,
Kochi University, Nankoku, Kochi, Japan.
Email: keisuke@kochi-u.ac.jp

Abstract

The permeability of the plasma membrane to water and cryoprotectants is one of the important factors for determining the suitable condition for the vitrification of mammalian oocytes and embryos. Water and cryoprotectants move slowly through oocytes and early embryos, principally by simple diffusion, in the mouse, bovine, pig, and human. In contrast, water, glycerol, and ethylene glycerol move rapidly through morulae and blastocysts, principally by facilitated diffusion via aquaporin 3, in the mouse and bovine; whereas, in the pig, the permeability to water and these cryoprotectants increases not at the morula stage but at the blastocyst stage and further increases at the expanded blastocyst stage. Dimethyl sulfoxide also moves rapidly via channels other than aquaporin 3 in the mouse. In contrast, propylene glycol moves through morulae and blastocysts principally by simple diffusion in the mouse, bovine, and pig, as through oocytes. Therefore, the permeability of mammalian oocytes and embryos at early stages to water and cryoprotectants is low, but that of embryos at later stages to water and some cryoprotectants is markedly high by channel processes, although species specificity exists in some cases.

KEYWORDS

aquaporin, cryoprotectant, membrane permeability, vitrification, water

1 | INTRODUCTION

During cryopreservation of the cell, the cell can be damaged by a number of types of cell injury: chilling, extracellular ice formation during cooling, intracellular ice formation during cooling and warming, the chemical toxicity of cryoprotectants during exposure to cryoprotectant solutions, fracture damage, and osmotic swelling or shrinkage during the removal of the cryoprotectants after warming.¹ In order to succeed in cryopreservation, the cell must circumvent all of the injuries. The cryobiological property of the cell affects the susceptibility to each injury. The suitable conditions for the cryopreservation of mammalian oocytes and embryos are different among the developmental stages

and species. This specificity could be caused by the difference in their cryobiological properties of oocytes and embryos among the developmental stages and species, such as the sensitivity to chilling, the sensitivity to the chemical toxicity of cryoprotectants, the permeability of the plasma membrane to water and cryoprotectants, and the tolerance to osmotic swelling and shrinkage. Among them, the permeability of the plasma membrane of oocytes and embryos to water and cryoprotectants is the most important property because this property is markedly related to major causes of cell injury: the formation of intracellular ice, the chemical toxicity of cryoprotectants, and osmotic swelling of the cell volume. Vitrification has been used widely in the cryopreservation of mammalian oocytes and embryos.² As the vitrification solution

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contains a high concentration of cryoprotectant and thus has high toxicity to the cell, the permeability of the plasma membrane would be more important than with slow freezing for successful cryopreservation of the oocytes and embryos. The exposure time to vitrification solutions must be limited because of their high toxicity, but a short exposure time results in insufficient permeation, which produces intracellular ice formation. Therefore, the permeability of the plasma membrane markedly affects whether the conditions are suitable for vitrification.

2 | MOVEMENT OF WATER ACROSS THE PLASMA MEMBRANE OF MOUSE OOCYTES AND EMBRYOS

In most types of cells, water moves slowly through the plasma membrane by simple diffusion via the lipid bilayer. However, in some types of cells, such as red blood cells, water moves rapidly through the plasma membrane by facilitated diffusion via intrinsic membrane proteins that act as water channels called "aquaporins."³ Therefore, water moves across the plasma membrane by simple diffusion or by facilitated diffusion via aquaporins.

The major pathway for the movement of water across the plasma membrane can be deduced from the permeability to water (L_p) and its dependence on temperature (Arrhenius activation energy, E_a). In general, a L_p -value of $>4.5 \mu\text{m}/\text{min}/\text{atm}$ with an E_a -value of $<6 \text{ kcal}/\text{mol}$ for the permeability is suggestive of the movement of water principally by facilitated diffusion via aquaporins.⁴ In contrast, a low L_p -value with an E_a -value that is $>10 \text{ kcal}/\text{mol}$ for the L_p is suggestive of movement principally by simple diffusion across the plasma membrane.⁴ However, there is no theoretical background for the values.

In mature mouse oocytes, the L_p -value in a hypertonic solution containing sucrose is low at 20–25°C ($0.4\text{--}1.0 \mu\text{m}/\text{min}/\text{atm}$) and the E_a -value is high ($11\text{--}15 \text{ kcal}/\text{mol}$).^{5–12} It is suggested that water moves through the oocytes principally by simple diffusion. In mouse embryos in the early stages (one-to-four cell stage, early embryos), the L_p -value remains low ($0.4\text{--}0.7 \mu\text{m}/\text{min}/\text{atm}$) and the E_a -value is high ($12\text{--}13 \text{ kcal}/\text{mol}$),^{5,10,12} as in the oocytes. Therefore, water also would move through the early embryos principally by simple diffusion. In mouse morulae and blastocysts, in contrast, the L_p -value is high at 25°C ($3.1\text{--}4.5 \mu\text{m}/\text{min}/\text{atm}$) and the E_a -value is low ($5.1\text{--}6.3 \text{ kcal}/\text{mol}$),¹² suggesting that water moves through the embryos principally by facilitated diffusion via water channels. In mouse morulae and blastocysts, aquaporin 3 is expressed exceedingly, compared to the other aquaporins.^{13,14} Furthermore, by suppressing the expression of aquaporin 3 in morulae by injecting aquaporin 3 double-stranded RNA into one-cell zygotes, the L_p -value markedly decreases.¹⁵ Therefore, water moves through the mouse morulae (and probably blastocysts) principally by facilitated diffusion via aquaporin 3. Aquaporins occur in two groups: one is highly selective for water and the other can transport not only water but also neutral solutes, including cryoprotectants.³ Aquaporin 3 can transport both water and cryoprotectants.¹⁶

3 | MOVEMENT OF CRYOPROTECTANTS ACROSS THE PLASMA MEMBRANE OF MOUSE OOCYTES AND EMBRYOS

Although there is no quantitative value for evaluating the movement of cryoprotectants through the plasma membrane, it would be reasonable to deduce that a low level of permeability to cryoprotectants (P_s), with a high E_a -value for the permeability, is suggestive of the movement of cryoprotectants principally by simple diffusion across the plasma membrane and that a high P_s -value with a low E_a -value for the permeability is suggestive of the movement of cryoprotectants principally by facilitated diffusion via channels.

In mouse oocytes and early embryos, the permeability to glycerol (P_{Gly}) is quite low at 20–25°C ($0.01\text{--}0.02 \times 10^{-3} \text{ cm}/\text{min}$)^{12,17,18} and the E_a -value is remarkably high ($42 \text{ kcal}/\text{mol}$).^{12,15} Therefore, glycerol moves through the oocytes and early embryos principally by simple diffusion. In contrast, the P_{Gly} -value of morulae is high at 25°C ($4\text{--}5 \times 10^{-3} \text{ cm}/\text{min}$) and the E_a -value is low ($10 \text{ kcal}/\text{mol}$),^{12,15} suggesting that glycerol moves through the morulae principally by facilitated diffusion. In addition, the high P_{Gly} -value of the morulae markedly decreases by suppressing the expression of aquaporin 3.¹⁵ Therefore, glycerol moves through morulae (and probably blastocysts) principally via aquaporin 3.

The permeability to ethylene glycol (P_{EG}) of oocytes is low at 25°C ($0.6 \times 10^{-3} \text{ cm}/\text{min}$) and the E_a -value is high ($17 \text{ kcal}/\text{mol}$).¹⁵ Therefore, ethylene glycol would move through the oocytes (and probably early embryos) principally by simple diffusion. In contrast, the P_{EG} -value of morulae is quite high at 25°C ($10 \times 10^{-3} \text{ cm}/\text{min}$) and the E_a -value is low ($9 \text{ kcal}/\text{mol}$).¹⁵ In addition, the high P_{EG} -value of morulae markedly decreases by suppressing the expression of aquaporin 3.¹⁵ Therefore, ethylene glycol moves through the morulae (and probably blastocysts) principally by facilitated diffusion via aquaporin 3.

The permeability to dimethyl sulfoxide (DMSO; P_{DMSO}) of oocytes is low at 25°C ($1.0 \times 10^{-3} \text{ cm}/\text{min}$) and the E_a -value is high ($18 \text{ kcal}/\text{mol}$).¹⁵ Therefore, DMSO would move through the oocytes (and probably early embryos) principally by simple diffusion. In contrast, the P_{DMSO} -value of morulae is higher at 25°C ($3.0 \times 10^{-3} \text{ cm}/\text{min}$) and the E_a -value is lower ($12 \text{ kcal}/\text{mol}$), compared to those of the oocytes.¹⁵ Therefore, DMSO would move through the morulae principally by facilitated diffusion via channels. However, the suppression of aquaporin 3 in the morulae does not decrease the P_{DMSO} -value,¹⁵ suggesting that channels other than aquaporin 3 are involved in the facilitated diffusion of DMSO.

The permeability to propylene glycol (P_{PG}) of oocytes is relatively low at 25°C ($1.7 \times 10^{-3} \text{ cm}/\text{min}$) and the E_a -value is high ($20 \text{ kcal}/\text{mol}$).¹⁵ Therefore, propylene glycol would move through the oocytes (and probably early embryos) principally by simple diffusion. However, the P_{PG} -value is higher than the permeability values of other cryoprotectants in the oocytes. This might be related to its higher hydrophobicity than other cryoprotectants. In the morulae, the P_{PG} -value is more than twice higher than that of the oocytes at 25°C ($3.8 \times 10^{-3} \text{ cm}/\text{min}$).¹⁵ This value is comparable to the P_{Gly} -value and P_{DMSO} -value

in morulae; glycerol and DMSO are suggested to move through the morulae principally by facilitated diffusion. However, the E_a -value for propylene glycol remains high (20 kcal/mol) in morulae.¹⁵ Therefore, propylene glycol would move through the morulae principally by simple diffusion.

4 | MOVEMENT OF WATER AND CRYOPROTECTANTS ACROSS THE PLASMA MEMBRANE OF OTHER MAMMALIAN OOCYTES AND EMBRYOS

4.1 | Bovine oocytes and embryos

In the bovine, the L_p -value of oocytes is low at 25°C (1.8 $\mu\text{m}/\text{min}/\text{atm}$) and the E_a -value is high (9 kcal/mol).¹⁹ Therefore, water would move through the oocytes principally by simple diffusion. However, the L_p -value is higher than that of mouse oocytes (0.4–1.0 $\mu\text{m}/\text{min}/\text{atm}$) and the E_a -value is slightly lower than 10 kcal/mol.¹⁹ Moreover, the L_p -value is higher than that of bovine morulae, in which the expression of aquaporin 3 was suppressed (0.6 $\mu\text{m}/\text{min}/\text{atm}$).¹⁹ Therefore, water channels would be partially involved in the movement of water in the oocytes. In the morulae and blastocysts, the L_p -value is high at 25°C (3 $\mu\text{m}/\text{min}/\text{atm}$) and the E_a -value is low (3 kcal/mol).¹⁹ The suppression of aquaporin 3 in the morulae markedly decreases the L_p -value.¹⁹ Therefore, water moves through bovine morulae (and probably blastocysts) principally by facilitated diffusion via aquaporin 3, as in mouse morulae.

The P_{Gly} , P_{DMSO} , and P_{PG} -values of bovine oocytes are low at 25°C and the E_a -values are high,¹⁹ essentially similar to mouse oocytes. Therefore, the cryoprotectants would move through the bovine oocytes principally by simple diffusion. However, the P_{EG} -value (3.5×10^{-3} cm/min) is higher than that of the mouse oocytes (0.6×10^{-3} cm/min) and the E_a -value (14 kcal/mol) is slightly lower than that of the mouse oocytes (17 kcal/mol).¹⁹ As the bovine oocytes would express water channels to some extent and the channels would be partially involved in the movement of water, as described above, ethylene glycol would move through the bovine oocytes mainly by simple diffusion and partially via water channels. In bovine morulae, the P_{Gly} , P_{EG} , and P_{PG} -values and the pathway for movement are essentially similar to those of the mouse morulae: glycerol and ethylene glycol move rapidly through the morulae principally by facilitated diffusion via aquaporin 3 because the suppression of aquaporin 3 expression markedly decreases the P_{Gly} and P_{EG} -values and propylene glycol would move principally by simple diffusion, although the P_{PG} -value is higher than that of the oocytes.¹⁹ The P_{DMSO} -value of bovine oocytes is relatively low at 25°C (1.5×10^{-3} cm/min) and the E_a -value is high (13 kcal/mol),¹⁹ suggesting that DMSO moves through the bovine oocytes principally by simple diffusion. In the bovine morulae, however, the P_{DMSO} -value remains low at 25°C (1.7×10^{-3} cm/min) and the E_a -value is higher (21 kcal/mol) than that of the bovine oocytes.¹⁹ Therefore, DMSO would move through the bovine morulae principally by simple diffusion, regardless of the developmental stage.

4.2 | Pig oocytes and embryos

In the pig, the L_p -value of oocytes is low at 25°C (1.0 $\mu\text{m}/\text{min}/\text{atm}$) and the E_a -value is high (19 kcal/mol), as in the mouse oocytes.²⁰ In pig morulae, the L_p -value is low, unlike in the mouse and bovine morulae,²⁰ probably because pig embryos become compacted at the four-cell stage. Therefore, water would move through the pig oocytes and morulae principally by simple diffusion. In pig blastocysts, the L_p -value increases significantly and further increases in the expanded blastocysts at 25°C (3.4 $\mu\text{m}/\text{min}/\text{atm}$).²⁰ The E_a -value for the permeability of the expanded blastocysts (7 kcal/mol) is close to 6 kcal/mol. Therefore, water would move through the pig expanded blastocysts principally by facilitated diffusion via water channels. As pig expanded blastocysts express the mRNA of aquaporin 3 abundantly, aquaporin 3 would be involved in the facilitated diffusion of water.²⁰

In pig oocytes, the P_{Gly} , P_{EG} , P_{DMSO} , and P_{PG} -values are low at 25°C and the E_a -values are high, suggesting that these cryoprotectants move through the pig oocytes principally by simple diffusion, similarly to those of the mouse oocytes.²⁰ In pig morulae, they remain low, as does the L_p -value. Therefore, these cryoprotectants would move through the morulae principally by simple diffusion. In the blastocysts, the P_{Gly} and P_{EG} -values increase and further increase in the expanded blastocysts.²⁰ The E_a -values for the permeability of the expanded blastocysts are much lower than those of the oocytes. Therefore, glycerol and ethylene glycol would move through the pig expanded blastocysts principally by facilitated diffusion. As aquaporin 3 can transport these cryoprotectants, aquaporin 3 would be involved in the facilitated diffusion. The P_{DMSO} -value increases slightly in the expanded blastocysts, compared to those of the oocytes and morulae.²⁰ The E_a -value for the permeability decreases at the expanded blastocyst stage. Therefore, DMSO would move through the pig expanded blastocysts partially by facilitated diffusion by channels. In contrast, the P_{PG} -value is higher but the E_a -value remains high at the expanded blastocyst stage.²⁰ Therefore, propylene glycol would move principally by simple diffusion, regardless of the developmental stage, like in the mouse and bovine.

4.3 | Human oocytes

In human oocytes, the L_p -value in hypertonic solutions containing a non-permeating solute is low at 20–22°C (0.4–1.0 $\mu\text{m}/\text{min}/\text{atm}$)^{6,21,22} and the E_a -value is high (9–11 kcal/mol).^{6,22} Therefore, water would move through the human oocytes principally by simple diffusion, as in the mouse oocytes. The P_{EG} -value of human oocytes is also low at 22°C (1.2×10^{-3} cm/min) and the E_a -value is high (15 kcal/mol),²² as in the mouse oocytes. The P_{DMSO} -value is also low at 22–24°C (1.5 – 1.6×10^{-3} cm/min),^{22,23} as in the mouse oocytes, and the E_a -value is high (21 kcal/mol).²³ The P_{PG} -value at 22–24°C (1.7 – 2.1×10^{-3} cm/min) is similar to that of the mouse oocytes^{22,24} and the E_a -value is high (16 kcal/mol).²⁴ These results suggest that water and cryoprotectants move slowly through human oocytes, principally by simple diffusion, as in mouse oocytes.

5 | CONCLUSION

This assessment of the movement of water and cryoprotectants in oocytes and embryos shows that the pattern of the movement is more stage-specific than species-specific, although species specificity exists in some cases. Therefore, the protocols that have been developed for the cryopreservation of oocytes and embryos in one species generally would be applicable to other species at a similar stage, in terms of permeability.

In order to design protocols for the vitrification of mammalian oocytes and embryos, it would be important to consider the pathway of the movement of water and cryoprotectants for each stage. In vitrification, the time of exposure and temperature of the vitrification solution are important because the vitrification solution contains a high concentration of cryoprotectant and thus is highly toxic to oocytes and embryos. When water and cryoprotectants move through oocytes and embryos principally by simple diffusion, the temperature and time of exposure to the vitrification solution are important because temperature affects the permeability to water and cryoprotectants. When water and cryoprotectants move through embryos principally by facilitated diffusion via channels, the amount of time of exposure to the vitrification solution is more important because the permeability is less affected by temperature. However, the exposure of oocytes and embryos to the vitrification solution at a high temperature should be avoided because cryoprotectants are more toxic at higher temperatures.

DISCLOSURES

Conflict of interest: The author declares no conflict of interest. **Human rights statements and informed consent:** This article does not contain any study with humans that was performed by the author. **Animal studies:** All the institutional and national guidelines for the care and use of laboratory animals were followed.

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