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ARTICLE

Impact of phase transition on the mouse oocyte spindle during vitrification

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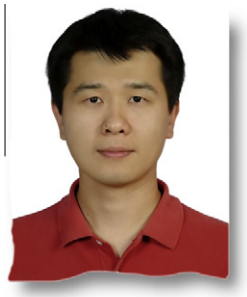

Ching-Chien (Jeremy) Chang obtained his BSc (1994) and MS degree (1996) at the National Chung-Hsing University, Taiwan. He was an embryologist at Lee Women's Hospital, Taiwan from 1998 to 1999. Then, he joined the xenotransplantation project of porcine cloning at the Animal Technology Institute, Taiwan from 1999 to 2001. He joined the Center for Regenerative Biology at the University of Connecticut in 2001 and obtained his PhD in 2005. Since 2005, he has been an embryologist and research coordinator at Reproductive Biology Associates, Atlanta, USA. His main research interests lie in the areas of oocyte and epigenetic reprogramming.

Abstract During vitrification, the glass-like solidification is the phase-transition process from liquid to solid. Phase transition is one of the major factors suspected to affect the physiology of the oocyte, such as the structure of the meiotic spindle. Therefore, it is very important to investigate the systematic and morphological alterations of the metaphase-II spindle and chromosome arrangement during complete course of a vitrification and warming process. B6D2F1 (C57BL/6 X DBA/2) mouse oocytes were cryopreserved by minimum volume cooling (MVC) method of vitrification in a solution with 15% ethylene glycol, 15% dimethylsulphoxide and 0.5 mol/l sucrose. To examine the spindle, oocytes were fixed before, during and after vitrification and were analysed by immunocytochemistry and confocal microscopy. It was shown that spindles in all oocytes could be maintained through the vitrification and warming process, even though they were exposed to extreme temperature and two rounds of phase transition. According to the sequential observations, chromosome alignment was maintained throughout the complete course of vitrification, warming and post-warming stage. The impact of phase transition was barely detectable when the oocyte was exposed to the vitrification and warming process. The oocyte spindle was able to recover immediately after warming.

KEYWORDS: meiotic spindle, oocyte, vitrification

Introduction

The meiotic spindle is extremely susceptible to low temperature. During oocyte cryopreservation, the oocyte spindle is exposed not only to low temperature, but it can also be damaged by the formation of ice crystals. Vitrification is a process that produces a glass-like solidification by which water is prevented from forming ice crystals due to the

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viscosity of highly concentrated cryoprotectant cooled at an extremely rapid rate (Rall and Fahy, 1985; Taylor, 1987). The glass-like solidification during vitrification is the phase-transition process from liquid to solid. Without ice crystal formation, it is not clear whether the phase transition of vitrification and warming process may impact the spindle structure. The influence of vitrification on metaphase-II (MII) oocyte spindle dynamics has gained a lot of attention and been investigated in recent studies (Chen et al., 2004; Ciotti et al., 2009; Gomes et al., 2008; Larman et al., 2007). However, these studies involving the structure of oocyte spindle during vitrification have been complicated by observations among laboratories and protocols that have been used. Thus far, the whole picture of the structural change of the spindle during vitrification remains uncertain. Chen et al. (2004) reported that only 48% of mouse oocyte spindles remained detectable by using Pol-scope after 15 min of warming. Larman et al. (2007) and Ciotti et al. (2009) reported that human oocyte spindles were maintained during vitrification immediately after warming according to Pol-scope observations. In contrast, Gomes et al. (2008) demonstrated that depolymerization of the mouse oocyte spindle occurs during vitrification—warming in response to the cooling and warming process in the observations of Pol-scope and immunocytochemistry. In order to clarify this subject, it is necessary to demonstrate a sequential and detailed observation which can reveal the oocyte spindle before, during and after vitrification.

During oocyte vitrification, phase transition is one of the major factors suspected to affect the physiology of the oocyte, such as the structure of the meiotic spindle. Although the efficiency of human oocyte vitrification has been improved dramatically in recent years (Antinori et al., 2007; Chang et al., 2008; Cobo et al., 2008; Chian et al., 2009a,b; Kuwayama et al., 2005; Kim et al., 2009, in press; Lucena et al., 2006; Nagy et al., 2009; Rienzi et al., 2010), it is still pertinent to know whether the process of vitrification could damage the oocyte spindle. To shed light on the whole process of vitrification, the present study employed a novel technique and sequential observations, which might allow the comprehension of the detailed alterations of the spindle. The information presented here will be invaluable to understanding in cryobiology as well as further the improvement of oocyte cryopreservation.

Materials and methods

Chemicals and culture media

Unless otherwise indicated, all chemicals purchased were from Sigma Chemical (St Louis, MO, USA).

Animals and recovery of metaphase-II oocytes

The B6D2F1 (C57BL/6 X DBA/2) mice used were from Charles River Laboratories (Wilmington, MA, USA). All animal treatments were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Connecticut, Storrs.

In-vivo matured MII-stage oocytes were collected from B6D2F1 female mice subjected to the following hormone priming protocol: superovulation was induced with 7.5 IU of equine chorionic gonadotrophin followed 48 h later with 7.5 IU of human chorionic gonadotrophin (hCG). Oocytes at MII stage were harvested after 14 h and freed of cumulus cells by brief exposure to 100 IU/ml of hyaluronidase at 37°C and gentle pipetting.

Oocyte vitrification

The basal medium used for oocyte cryopreservation was HEPES-buffered embryo culture medium (Cooper/Sage, Bedminster, NJ, USA) supplemented with 20% (v/v) fetal calf serum (FCS; Hyclone, Logan, UT, USA). The denuded oocytes were vitrified by minimum volume cooling (MVC) method, as described by Kuwayama et al. (2005). Briefly, the oocytes were equilibrated in equilibration medium (basal medium with 7.5% (v/v) EG (EG) and 7.5% (v/v) dimethylsulphoxide (DMSO)) at room temperature for 5 min. Oocytes were transferred into the vitrification medium (basal medium with 15% (v/v) EG, 15% (v/v) DMSO and 0.5 mol/l sucrose) at room temperature for 45—60 s. The cryoprotectant-treated oocytes were placed onto a fine polypropylene strip (Cryotop; Kitazato Bio Pharma, Japan). Then the polypropylene strip carrying the oocytes was submerged into liquid nitrogen and ready for storage.

Oocyte warming

The polypropylene strip with vitrified oocytes was immersed directly into 5.0 ml of warming solution (HEPES-buffered embryo culture medium with 20% (v/v) FCS and 1.0 mol/l sucrose) at 37°C for 1 min. Oocytes were then picked up and transferred into 1.0 ml of the dilution solution (HEPES-buffered embryo culture medium with 20% (v/v) FCS and 0.5 mol/l sucrose) for 3 min at room temperature. The oocytes were subsequently washed in 1.0 ml washing solution (HEPES-buffered embryo culture medium with 20% (v/v) FCS) for 10 min at room temperature. Finally, the oocytes were incubated in KSOM + AA medium (Specialty Media, Phillipsburg, NJ, USA) before oocyte fixation.

Timing of oocyte fixation

To thoroughly investigate the impact of phase transition on oocyte spindle during vitrification and warming, oocytes were fixed at different time points of the vitrification and warming process: (i) 0 h before the start of vitrification (non-treated control); (ii) 5 min exposure to the equilibration solution at room temperature; (iii) 1 min exposure to the vitrification solution at room temperature; (iv) 0 min of warming (warming oocytes directly into fixative); (v) control oocytes after treatment of room temperature (23°C) for 13 min; (vi) 0 min after warming procedures; (vii) 15 min at 37°C after warming procedures; (viii) 30 min at 37°C after warming procedures; (ix) 1 h at 37°C after warming procedures; (x) 2 h at 37°C after warming procedures; and (xi) 4 h at 37°C after warming procedures.

Immunohistochemistry and laser-scanning confocal microscopy

For examination of microtubules, oocytes were fixed in a microtubule stabilizing buffer containing 2% formaldehyde,

0.5% Triton X-100, 1 μmol/l taxol, 10 U/ml aprotinin and 50% deuterium oxide at 37°C for at least 30 min. They were then washed in washing buffer (PBS containing 3 mmol/l NaN_3, 0.01% Triton X-100, 0.2% non-fat dried milk, 2% normal goat serum, 0.1 mol/l glycine and 2% bovine serum albumin) three times and left in the washing buffer overnight at 4°C for blocking and permeabilization (Carabatsos et al., 2000). Oocytes were then double-stained to visualize microtubules and DNA. Briefly, samples were incubated in mouse anti-α-tubulin antibody (1:200) for 4 h at 37°C or overnight at 4°C. After three washes in washing buffer, the oocytes were incubated in fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (1:200) for 1 h at 37°C. Finally, the oocytes were washed, stained for DNA with 7.5 μmol/l propidium iodide, mounted in PBS containing 50% glycerol as an anti-fading reagent and 25 mg/ml NaN_3 and examined with a laser-scanning confocal microscope (TCS SP2; Leica, Exton, PA, USA).

Results

Before vitrification: the effect of cryoprotectants on the MII spindle

To evaluate the effect of cryoprotectants on the oocyte spindle, mouse oocytes were subjected to the cryoprotectants for vitrification. The organization of the MII spindle were compared before and after the treatment of cryoprotectants. First, oocytes were treated with equilibration solution containing permeable cryoprotectant 7.5% EG and 7.5% DMSO for 5 min at room temperature. In the presence of permeable cryoprotectants, bipolar spindle and chromosome alignment were maintained at room temperature for 5 min in all oocytes examined (**Figure 1**) and a few microtubule asters were also found to be established sparsely in the cytoplasm (**Figure 1D—F**; **Table 1**). Then, oocytes were subsequently treated with vitrification solution containing permeable cryoprotectant 15% EG and 15% DMSO and non-permeable cryoprotectant sucrose (0.5 mol/l) for 1 min at room temperature. Although diminished (a visual observation of a weaker fluorescent signal without quantitative assessment) microtubule apparatus was observed, the bipolar spindle and aligned chromosomes were still largely maintained (**Figure 1G—I**, **Table 1**) Interestingly, microtubule asters developed into bigger clusters when water was expelled out of the oocyte cytoplasm (**Figure 1G**).

After vitrification: the effect of phase transition on the MII spindle

The effect of the glass transition on spindle structure during oocyte vitrification has not been clarified, because it is not possible to check the oocyte spindle status when the oocyte is still in the vitrified (solid) state. In order to observe the oocyte spindle structure before the resulting damage of vitrification and warming, the cryopreserved oocytes were warmed directly into the fixative, which allowed the phase transition to occur in the fixative. In this condition, the resulting spindle structure represents the effect of two phase transitions (from liquid to solid and from solid to liquid) (**Figure 1J—L**). It was observed that the structure of oocyte spindle highly resembled the one before two rounds of phase transition in all examined oocytes.

After warming: the recovery of the MII spindle

To clarify the timing of MII spindle disassembly, the detailed changes of spindle and chromosome alignment post warming were followed. Although the bipolar spindle and aligned chromosomes were maintained, the signal of microtubule was faint due to its temperature-sensitive nature when the oocytes were maintained at room temperature (23°C) for 13 min (**Figure 2A—C**) in the control group. The MII spindle was also examined immediately after the complete warming process (0 min after warming process) and it was found that the diminished bipolar spindle and aligned chromosomes were maintained in the cytoplasm in all examined oocytes (**Figure 2D—F**). After warming, the warmed oocytes were further examined at 15 min (**Figure 2G—I**), 30 min (**Figure 2J—L**), 1 h (**Figure 3A—C**), 2 h (**Figure 3D—F**) and 4 h (**Figure 3G—I**) incubation at 37°C. A high degree of consistency of MII spindle structure and chromosome alignment was observed in the sequential observation post warming. Other observations were that the spindles were completely recovered from the diminished state as early as 15 min after warming at 37°C, all the MII spindles were well maintained in a barrel-shaped structure and chromosomes were also aligned at the metaphase plate (**Table 1**).

Discussion

To date, few studies have investigated the systematic details of meiotic spindle changes during the vitrification and warming process. In the present study, the results describe the morphological changes of mouse MII spindle and chromosome arrangement during the complete course of a vitrification and warming process. It has been shown that all mouse oocyte spindles could be maintained through the vitrification and warming process, even though they had been exposed to extreme low temperature and two rounds of phase transition. According to the sequential observations, the results also indicate that the chromosome alignment was maintained throughout the complete course of the vitrification, warming and post-warming stage. Therefore, the impact of phase transition was barely detectable when the mouse oocyte was exposed to the vitrification and warming process. Moreover, the mouse oocyte spindle was able to recover immediately after warming.

Cryodamage after oocyte cryopreservation (i.e., organelle, mitochondria, cortical granules and oolema) has been reported in previous ultrastructural studies (Nottola et al., 2007, 2009). However, there are some technical difficulties in completely visualizing the microtubular scaffolding of the spindle and associated chromosomes by electron microscopy (Nottola et al., 2007). Therefore, instead of electron microscopy, other techniques (like Pol-scope and immunocytochemistry with confocal microscopy) were often chosen to investigate the spindle during oocyte cryopreservation. The introduction of Pol-scope has allowed real time investigation of the meiotic spindle in living oocytes. Noninvasive detection and assessment of the meiotic spindle using the Pol-scope has been proposed as a potential means to

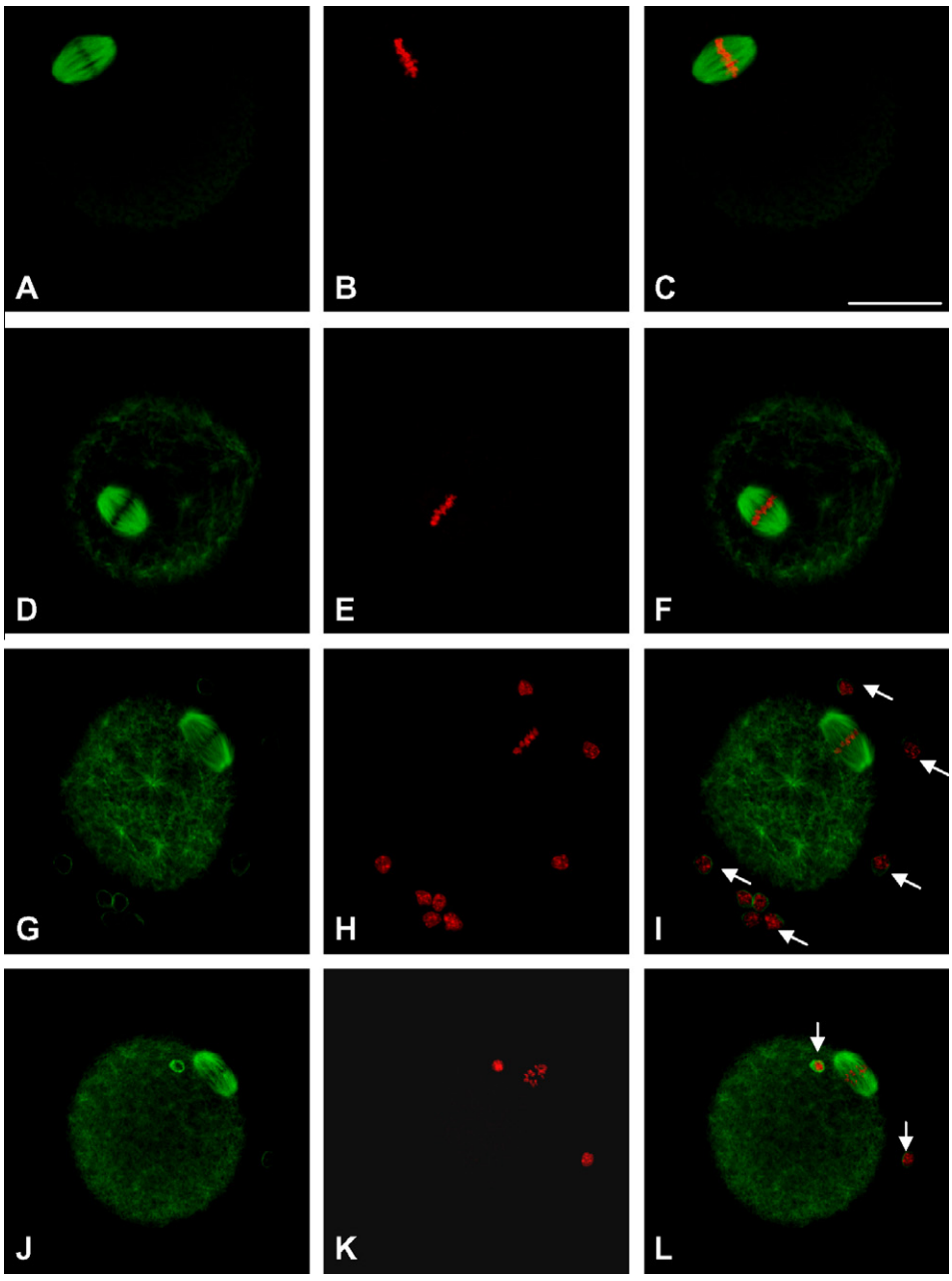

Figure 1 Influence of the vitrification process on the metaphase-II spindle. Confocal images of α-tubulin (**A**, **D**, **G** and **J**), chromatin (**B**, **E**, **H** and **K**) and both chromatin and α-tubulin (**C**, **F**, **I** and **L**) of representative oocytes. (**A**—**C**) Before cryoprotectant treatment ($n = 25$), (**D**—**F**) equilibrated with 7.5% ethylene glycol and 7.5% dimethylsulphoxide for 5 min ($n = 20$), (**G**—**I**) further exposed to 15% ethylene glycol, 15% dimethylsulphoxide and 0.5 mol/l sucrose for 1 min before vitrification ($n = 23$), and (**J**—**L**) warmed directly into the fixative ($n = 28$). Arrows = residual cumulus cells. Bar = 40 μm.

evaluate oocyte quality without injuring oocytes. In recent oocyte vitrification studies, most of the spindle observations were performed by Pol-scope (Chen et al., 2004; Ciotti et al., 2009; Gomes et al., 2008; Larman et al., 2007). These Pol-scope observations of oocyte spindle structure during vitrification were inconsistent between investigators, protocols and different species (human versus mouse). The detailed structural change of the spindle during vitrification also remains unclear, because technical limitations of polarized light microscopy do exist. For example, chromosome positioning cannot be detected in Pol-scope observations. Moreover, Pol-scope was unable to reveal the fine or minor spindle structural changes when it was compared with the immunocytochemistry result by applying high-performance confocal microscopy (Coticchio et al., 2010). In some cases, morphometric evaluation of the spindle through Pol-scope was not consistent with confocal analysis (Coticchio et al., 2010). It is believed that immunocytochemistry with confocal microscopy provides more sensitive and detailed information revealing the spindle structural changes and chromosome arrangement (Bromfield et al., 2009; Huang et al., 2008). In order to investigate the temperature-

Table 1 The dynamics of the meiotic spindle and chromosome alignment during oocyte vitrification.

Timing of fixation	*Spindle structure*				*Chromosome alignment*	
	Intact	*Diminished*	*Largely diminished*	*Completely disappeared*	*Metaphase plate*	*Misalignment of chromosomes*
Fresh control MII oocytes	100 (25/25)	0 (0/25)	0 (0/25)	0 (0/25)	100 (25/25)	0 (0/25)
Equilibration sol. 5 min RT	100 (20/20)	0 (0/20)	0 (0/20)	0 (0/20)	100 (20/20)	0 (0/20)
Vitrification sol. 1 min RT	0 (0/23)	100 (23/23)	0 (0/23)	0 (0/23)	100 (23/23)	0 (0/23)
Oocytes warmed directly into fixative	0 (0/28)	100 (28/28)	0 (0/28)	0 (0/28)	100 (28/28)	0 (0/28)
Control oocytes, 23°C 13 min	0 (0/26)	0 (0/26)	100 (26/26)	0 (0/26)	100 (26/26)	0 (0/26)
Post warming, 0 min	0 (0/28)	100 (28/28)	0 (0/28)	0 (0/28)	100 (28/28)	0 (0/28)
Post warming, 37°C 15 min	100 (28/28)	0 (0/28)	0 (0/28)	0 (0/28)	100 (28/28)	0 (0/28)
Post warming, 37°C 30 min	100 (39/39)	0 (0/39)	0 (0/39)	0 (0/39)	100 (39/39)	0 (0/39)
Post warming, 37°C 1 h	100 (19/19)	0 (0/19)	0 (0/19)	0 (0/19)	100 (19/19)	0 (0/19)
Post warming, 37°C 2 h	100 (13/13)	0 (0/13)	0 (0/13)	0 (0/13)	100 (13/13)	0 (0/13)
Post warming, 37°C 4 h	100 (28/28)	0 (0/28)	0 (0/28)	0 (0/28)	100 (28/28)	0 (0/28)

Values are % (*n*/total).
MII = metaphase II; RT = room temperature.

sensitive microtubule structure regarding the impact of phase transition during vitrification, the present study used immunocytochemistry with confocal microscopy and the sequential observations of the mouse oocyte spindle are consistent with previous Pol-scope observations reporting that oocyte spindles were maintained immediately after vitrification and warming procedures in the human model (Ciotti et al., 2009; Larman et al., 2007). However, further study of human oocyte observations by using immunocytochemistry and confocal microscopy will be necessary to verify the influences of phase transitions on human spindle dynamics.

Basic information of the human oocyte spindle is still lacking, such as the rate of microtubule assembly/disassembly, molecular structures of the human spindle and mechanisms for human oocyte spindle assembly. Since it is extremely difficult to get human oocytes for those fundamental studies, little is known about the differences in meiotic spindle organization and stability between human and mouse oocytes. However, extensive studies have shown that both mouse and human oocyte spindles are very sensitive to low temperatures (Keefe et al., 2003; Magistrini and Szöllösi, 1980; Pickering and Johnson, 1987; Sathananthan et al., 1992; Van der Elst et al., 1988; Wang et al., 2001). Mouse models played a critical role in oocyte cryopreservation studies due to their accessibility. Today, the mouse model still continuously provides data that facilitates further advancements in oocyte cryobiology.

In the second meiosis, timing of chromosome attachment and loss of cohesion is essential to faithful chromosome segregation. The cohesion between sister chromatids is important for two reasons: firstly, it assures pairwise alignment of sister chromatids on the spindle at MII stage. Secondly, it is necessary for generating tension across centromeres when spindle microtubules have made bipolar attachment to the sister chromatids (reviewed by Maresca and Salmon, 2010; Vogt et al., 2008). If pairwise alignment and tension of sister chromatids is not achieved, it might cause aneuploid embryos when the second meiosis is spontaneously triggered by a penetrating spermatozoon at the same time. Therefore, chromosome alignment of the MII oocyte spindle is very important for faithful chromosome segregation in subsequent embryo development. In the present study, phase transition was not found to have an impact on chromosome alignment as there was a high degree of consistency of chromosome alignment from before vitrification to post warming (**Table 1**).

The oocyte spindle has shown rapid depolymerization when exposed to sub-physiological temperatures (Magistrini et al., 1980; Sathananthan et al., 1992). In mouse oocytes, even reduction to room temperature has a dramatic effect on the spindle, possibly causing abnormal spindle configuration (Pickering et al., 1987; Van der Elst et al., 1988). However, addition of cryoprotectants has been shown to have a protective effect against the temperature-dependent depolymerization of the spindle (Chang et al., 2010; George and Johnson, 1993; Joly et al., 1992; Van der Elst et al., 1988), because cryoprotectants can actually stabilize the structure of spindle and prevent the disassembly of microtubule to

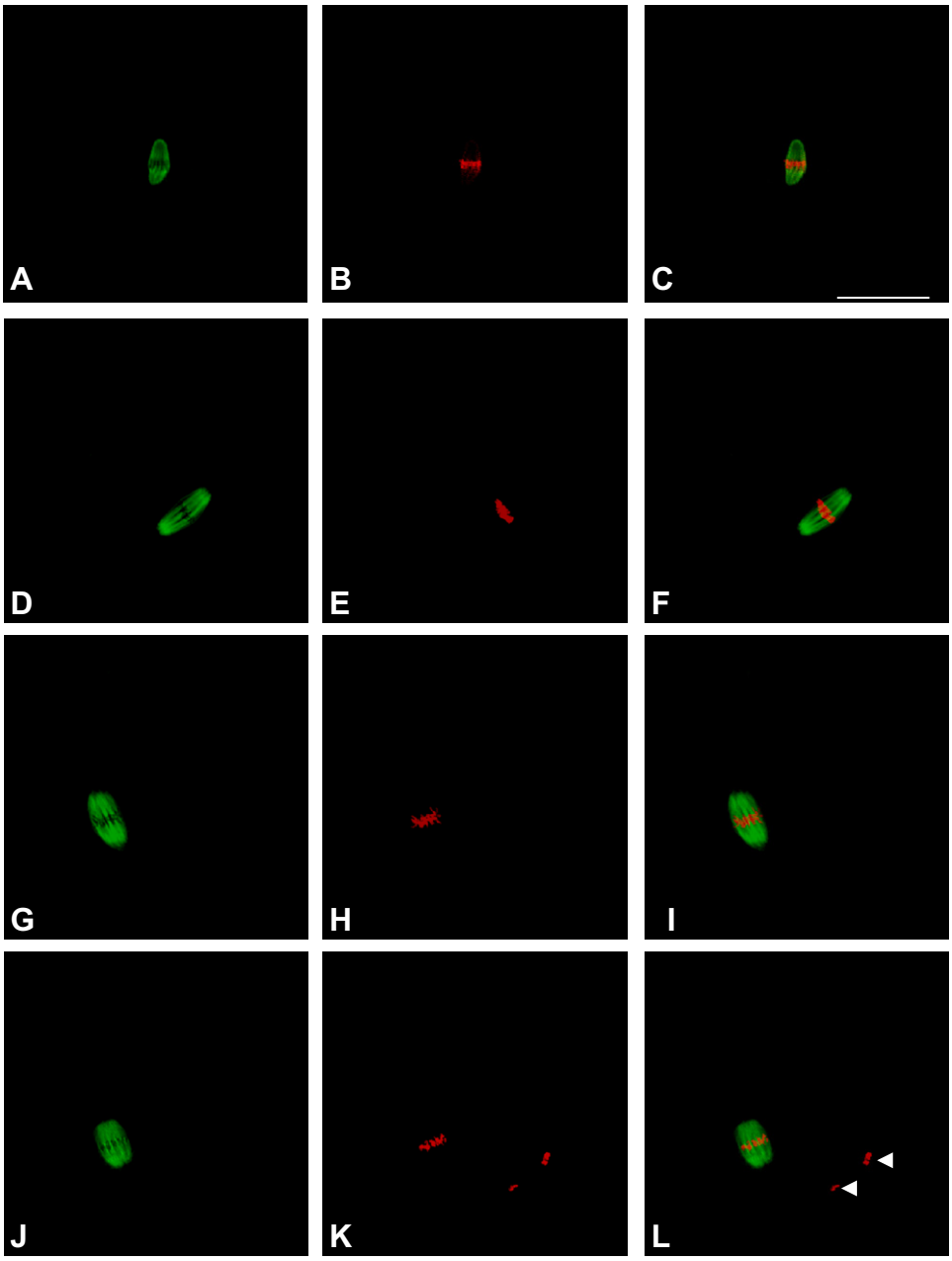

Figure 2 Metaphase-II spindle organization immediately after the warming process. Confocal images of α-tubulin (**A**, **D**, **G** and **J**), chromatin (**B**, **E**, **H** and **K**) and both chromatin and α-tubulin (**C**, **F**, **I** and **L**) of representative oocytes. (**A**–**C**) Control oocytes maintained at 23°C for 13 min ($n = 26$), (**D**–**F**) 0 min after the warming process ($n = 28$), (**G**–**I**) 15 min of culture after the warming process ($n = 28$), (**J**–**L**) 30 min of culture after the warming process ($n = 39$). Arrows = residual polar bodies. Bar = 40 μm.

resist low temperature. In the present observations, after exposure to the cryoprotectants (EG and DMSO, and sucrose) in equilibration and vitrification solutions for 5–6 min at room temperature, the oocyte spindle structure was largely maintained and some microtubule asters were also induced by the cryoprotectants (Figure 1). However, it is still not clear whether those induced cytoskeleton structures may enhance survivability against the challenges from temperature, osmotic stress and phase transitions.

Ideally, if the oocyte spindle status can be checked when the oocyte is in and after the vitrified (solid) state, then it will be possible to evaluate whether the glass transition would damage the spindle structure during oocyte vitrification. However, it is impossible to investigate the spindle structure when the oocyte is still in the solid phase, because the oocyte has to be in the liquid phase to observe the oocyte spindle using Pol-scope or immunocytochemistry methods. Accordingly, the vitrified oocytes was warmed directly into the fixative, where the oocyte spindle would be fixed immediately when phase transition was occurring. Intriguingly, the spindle change was undetectable when comparing the status before and immediately after the impact of phase transition (Figure 1). However, it is likely that the microtubule disassembly and reassembly might also

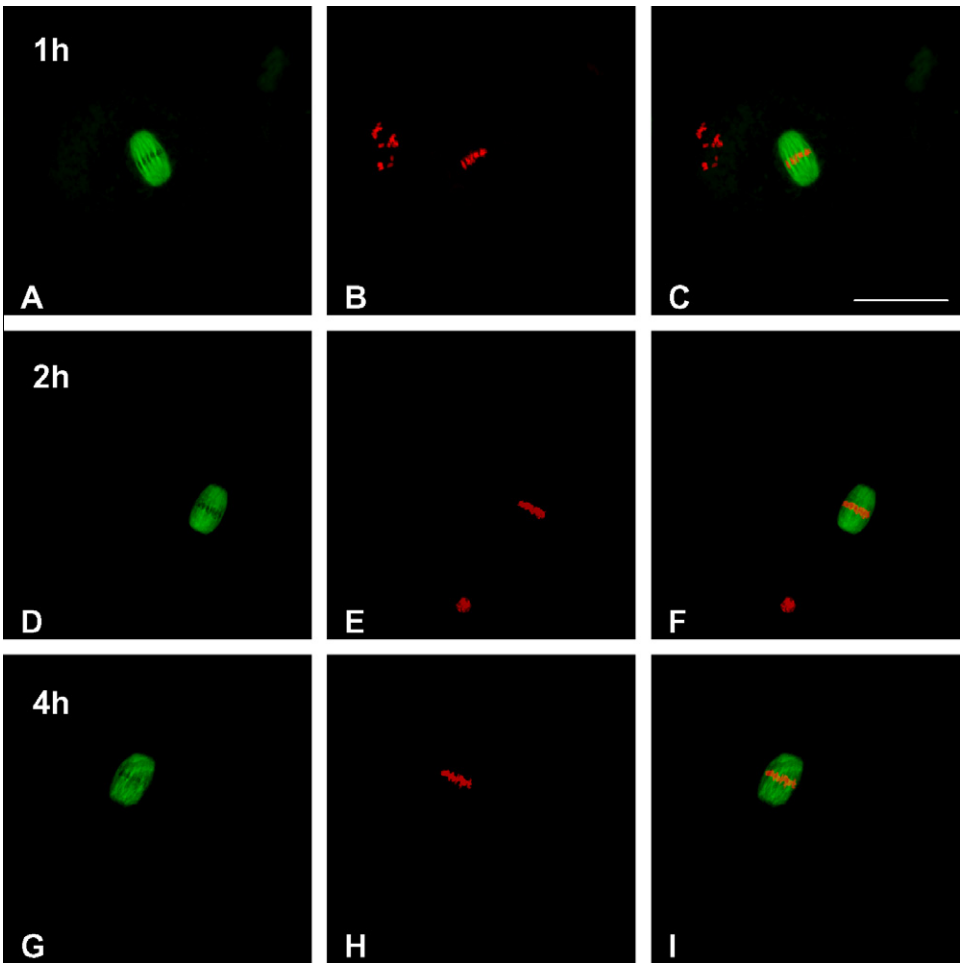

Figure 3 Recovery of the MII spindle after warming. Confocal images of α-tubulin (**A**, **D** and **G**), chromatin (**B**, **E** and **H**) and both chromatin and α-tubulin (**C**, **F** and **I**) of representative oocytes. (**A**–**C**) 1 h of culture after the warming process (n = 19), (**D**–**F**) 2 h of culture after the warming process (n = 13), and (**G**–**I**) 4 h of culture after the warming process (n = 28). Bar = 40 μm.

be arrested in the solid phase when the oocyte was still vitrified. Hence, if there is detectable damage as a result of the phase changes during vitrification, it should be revealed by the observations after oocyte warming. Interestingly, the loss of spindle structure was not detected, even though vitrified–warmed oocytes were examined in a sequential and detailed timeframe after warming (**Figures 2** and **3**). Since the impact of phase transition was barely detectable when the oocyte was exposed to the vitrification and warming process, the oocyte spindle was able to recover immediately after warming. This has far-reaching implications for human oocyte cryopreservation, because technicians may consider shortening the post-vitrification recovery time of the regular oocyte warming procedure required for spindle reformation.

At 0-min post warming (**Figure 2D**–**F**), the warmed oocytes had been through the warming solution (containing 1.0 mol/l sucrose) at 37°C for 1 min, dilution solution (containing 0.5 mol/l sucrose) for 3 min at room temperature and washing solution for 10 min at room temperature. Therefore, the warmed oocytes were exposed to room temperature for a total of 13 min. A surprising result was observed when the warmed oocytes were compared to the control group, both of which were exposed to room temperature for 13 min. It was surprising to observe that the spindle signal was faint in the control group (**Figure 2A**–**C**), but not in the warming group (**Figure 2D**–**F**). It has been suggested that cryoprotectants can actually stabilize the structure of the spindle and prevent the disassembly of the microtubule to resist low temperature (Chang et al., 2010; George and Johnson, 1993; Joly et al., 1992; Van der Elst et al., 1988). Hence, the preservation of the meiotic spindle could be due to 2 possibilities: one is that the higher concentrations of cryoprotectants used in the vitrification may result in a prolonged protective effect compared with that in the slow freezing. Alternatively, sucrose, a non-permeable cryoprotectant, could stabilize the microtubule structure when oocytes were exposed to room temperature (23°C) during the warming procedures.

Unlike vitrification, slow freezing cannot prevent ice crystal formation during temperature depleting and phase transitions. During oocyte slow freezing, spindle depolymerization in all examined oocytes was observed during the thawing process (the removal of the cryoprotectant) (Chang et al., 2010; Rienzi et al., 2004). Besides, even with several hours of recovery after thawing, still about 15% of oocytes would have been activated by the oocyte slow freezing and thawing process (Chang et al., 2010). However, significant

alteration of the oocyte spindle was not found during and after the vitrification procedure. Therefore, this study also indicates that oocyte vitrification may have less impact on spindle structure than slow freezing method.

In conclusion, the present study suggests that phase transition and low temperature causes little impact on the mouse oocyte spindle during the vitrification and warming process. The observations herein may provide key insights to exploring enhanced oocyte cryopreservation methods that limit the impact on the cellular structure of the oocyte.

References

Antinori, M., Licata, E., Dani, G., Cerusico, F., Versaci, C., Antinori, S., 2007. Cryotop vitrification of human oocytes results in high survival rate and healthy deliveries. Reprod. Biomed. Online 4, 72–79.

Bromfield, J.J., Coticchio, G., Hutt, K., Sciajno, R., Borini, A., Albertini, D.F., 2009. Meiotic spindle dynamics in human oocytes following slow-cooling cryopreservation. Hum. Reprod. 24, 2114–2123.

Carabatsos, M.J., Combelles, C.M., Messinger, S.M., Albertini, D.F., 2000. Sorting and reorganization of centrosomes during oocyte maturation in the mouse. Microsc. Res. Tech. 49, 435–444.

Chang, C.C., Shapiro, D.B., Bernal, D.P., Wright, G., Kort, H.I., Nagy, Z.P., 2008. Human oocyte vitrification: in-vivo and in-vitro maturation outcomes. Reprod. Biomed. Online 17, 684–688.

Chang, C.C., Sung, L.Y., Lin, C.J., et al., 2010. The oocyte spindle is preserved by 1,2-propanediol during slow freezing. Fertil. Steril. 93, 1430–1439.

Chen, C.K., Wang, C.W., Tsai, W.J., Hsieh, L.L., Wang, H.S., Soong, Y.K., 2004. Evaluation of meiotic spindles in thawed oocytes after vitrification using polarized light microscopy. Fertil. Steril. 82, 666–672.

Chian, R.C., Huang, J.Y., Gilbert, L., et al., 2009a. Obstetric outcomes following vitrification of in vitro and in vivo matured oocytes. Fertil. Steril. 91, 2391–2398.

Chian, R.C., Gilbert, L., Huang, J.Y., et al., 2009b. Live birth after vitrification of in vitro matured human oocytes. Fertil. Steril. 91, 372–376.

Ciotti, P.M., Porcu, E., Notarangelo, L., Magrini, O., Bazzocchi, A., Venturoli, S., 2009. Meiotic spindle recovery is faster in vitrification of human oocytes compared to slow freezing. Fertil. Steril. 91, 2399–2407.

Cobo, A., Kuwayama, M., Pérez, S., Ruiz, A., Pellicer, A., Remohí, J., 2008. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. Fertil. Steril. 89, 1657–1664.

Coticchio, G., Sciajno, R., Hutt, K., Bromfield, J., Borini, A., Albertini, D.F., 2010. Comparative analysis of the metaphase II spindle of human oocytes through polarized light and high-performance confocal microscopy. Fertil. Steril. 93, 2056–2064.

George, M.A., Johnson, M.H., 1993. Cytoskeletal organization and zona sensitivity to digestion by chymotrypsin of frozen-thawed mouse oocytes. Hum. Reprod. 8, 612–620.

Gomes, C.M., Silva, C.A., Acevedo, N., Baracat, E., Serafini, P., Smith, G.D., 2008. Influence of vitrification on mouse metaphase II oocyte spindle dynamics and chromatin alignment. Fertil. Steril. 90 (4 Suppl), 1396–1404.

Huang, J.Y., Chen, H.Y., Park, J.Y., Tan, S.L., Chian, R.C., 2008. Comparison of spindle and chromosome configuration in in vitro- and in vivo-matured mouse oocytes after vitrification. Fertil. Steril. 90 (Suppl. 4), 1424–1432.

Joly, C., Bchini, O., Boulekbache, H., Testart, J., Maro, B., 1992. Effects of 1,2-propanediol on the cytoskeletal organization of the mouse oocyte. Hum. Reprod. 7, 374–378.

Keefe, D., Liu, L., Wang, W., Silva, C., 2003. Imaging meiotic spindles by polarization light microscopy: principles and applications to IVF. Reprod. Biomed. Online 7, 24–29.

Kim, T.J., Laufer, L.R., Hong, S.W., 2009. Vitrification of oocytes produces high pregnancy rates when carried out in fertile women. Fertil. Steril. [Epub ahead of print].

Kuwayama, M., Vajta, G., Kato, O., Leibo, S.P., 2005. Highly efficient vitrification method for cryopreservation of human oocytes. Reprod. Biomed. Online 11, 300–308.

Larman, M.G., Minasi, M.G., Rienzi, L., Gardner, D.K., 2007. Maintenance of the meiotic spindle during vitrification in human and mouse oocytes. Reprod. Biomed. Online 15, 692–700.

Lucena, E., Bernal, D.P., Lucena, C., Rojas, A., Moran, A., Lucena, A., 2006. Successful ongoing pregnancies after vitrification of oocytes. Fertil. Steril. 85, 108–111.

Magistrini, M., Szöllösi, D., 1980. Effects of cold and of isopropyl-N-phenylcarbamate on the second meiotic spindle of mouse oocytes. Eur. J. Cell Biol. 22, 699–707.

Maresca, T.J., Salmon, E.D., 2010. Welcome to a new kind of tension: translating kinetochore mechanics into a wait-anaphase signal. J. Cell Sci. 123, 825–835.

Nagy, Z.P., Chang, C.C., Shapiro, D.B., et al., 2009. Clinical evaluation of the efficiency of an oocyte donation program using egg cryo-banking. Fertil. Steril. 92, 520–526.

Nottola, S.A., Macchiarelli, G., Coticchio, G., et al., 2007. Ultrastructure of human mature oocytes after slow cooling cryopreservation using different sucrose concentrations. Hum. Reprod. 22, 1123–1133.

Nottola, S.A., Coticchio, G., Sciajno, R., et al., 2009. Ultrastructural markers of quality in human mature oocytes vitrified using cryoleaf and cryoloop. Reprod. Biomed. Online 19 (Suppl. 3), 17–27.

Pickering, S.J., Johnson, M.H., 1987. The influence of cooling on the organization of the meiotic spindle of the mouse oocyte. Hum. Reprod. 2, 207–216.

Rall, W.F., Fahy, G.M., 1985. Ice-free cryopreservation of mouse embryos at −196 degrees C by vitrification. Nature 313, 573–575.

Rienzi, L., Martinez, F., Ubaldi, F., et al., 2004. Polscope analysis of meiotic spindle changes in living metaphase II human oocytes during the freezing and thawing procedures. Hum. Reprod. 19, 655–659.

Rienzi, L., Romano, S., Albricci, L., et al., 2010. Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. Hum. Reprod. 25, 66–73.

Sathananthan, A.H., Kirby, C., Trounson, A., Philipatos, D., Shaw, J., 1992. The effects of cooling mouse oocytes. J. Assist. Reprod. Genet. 9, 139–148.

Taylor, M.J., 1987. Physico-chemical principles in low temperature biology. In: Grout, B.W.W., Morris, G.J. (Eds.), The Effects of Low Temperatures on Biological Systems. Edward Arnold, London, pp. 3–71.

Van der Elst, J., Van den Abbeel, E., Jacobs, R., Wisse, E., Van Steirteghem, A., 1988. Effect of 1,2-propanediol and dimethylsulphoxide on the meiotic spindle of the mouse oocyte. Hum. Reprod. 3, 960–967.

Vogt, E., Kirsch-Volders, M., Parry, J., Eichenlaub-Ritter, U., 2008. Spindle formation, chromosome segregation and the spindle checkpoint in mammalian oocytes and susceptibility to meiotic error. Mutat. Res. 651, 14–29.

Wang, W.H., Meng, L., Hackett, R.J., Odenbourg, R., Keefe, D.L., 2001. Limited recovery of meiotic spindles in living human oocytes after cooling-rewarming observed using polarized light microscopy. Hum. Reprod. 16, 2374–2378.

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