Artificially Increasing Cortical Tension Improves Mouse Oocytes Development by Attenuating Meiotic Defects During Vitrification

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Oocyte cryopreservation demonstrates great benefits in the conservation of animal germplasm resources and assisted reproductive technology. However, vitrification causes damages in oocytes, which would lead to the decrease of oocyte quality, and embryonic development post fertilization. Cytoskeleton plays an important role in regulating cell shape, organelle migration, cell division and mechanical signal transduction. Cortical tension is a reflection of the physiological state and contractile ability of cortical cytoskeleton. Appropriate cortical tension is prerequisite for normal oocyte meiosis. In the present study, oocyte cortical tension was examined by evaluating the levels of cortical tension-related protein pERM (Phospho-Ezrin/Radixin/Moesin) and pMRLC (Phospho-Myosin Light Chain 2). We found that the cortical tension of vitrified oocytes was decreased. Increasing cortical tension of vitrified oocytes by adding 10 μg/ml ConA during in vitro culture could significantly improve the polar body extrusion rate and embryo development. Furthermore, increasing the cortical tension could improve spindle positioning, maintain kinetochore-microtubule (KT-MT) attachment, strengthen spindle assembly checkpoint (SAC) activity, and reduce the aneuploidy rate in vitrified oocytes. In conclusion, vitrification induced a remarkable decrease in cortical tension, and increasing the cortical tension could rescue the meiosis defect and improve oocyte quality.

Keywords: oocyte vitrification, cortical tension, meiosis, spindle assembly checkpoint, aneuploidy

INTRODUCTION

In oocyte meiosis, errors in chromosomes segregation generate eggs with an abnormal number of chromosomes. When fertilized, these eggs lead to aneuploid embryos. It is well known that aneuploidy would induce severe cellular dysfunction since each chromosome encodes thousands of genes (Webster and Schuh, 2017). Oocyte chromosome (or bivalent) segregation errors are usually classified to chromatids...
Cortical tension in vitrified oocytes

**Materials and Methods**

**Ethics Statement**

All chemicals were purchased from Sigma Chemical Co. (St Louis, MO, United States) unless otherwise stated. All animal manipulations were performed according to the guidelines of the Animal Care and Use Committee. The present study was approved by the Institutional Animal Care and Use Committee of China Agricultural University (AW01040202-1).

**Mouse**

Studies were performed using 8-week-old female mouse (CD-1® (ICR)) (Vital River Laboratory Animal Technology Co., Ltd. Beijing, China). Mouse was housed in ventilated cages on a controlled temperature (22 ± 2°C) with food and water freely available. Mouse was allowed to adapt to conditions for 7 days before the initiation of experiments.

**Oocyte Collection**

To collect immature oocytes, mouse was sacrificed by cervical dislocation 48 h after they were injected with 10 IU pregnant mare serum gonadotropin (PMSG). Germinal vesicle (GV) stage oocytes were collected by removing cumulus cells in a drop of M2 medium supplemented with dbcAMP (100 ng/ml) through repeatedly pipetting. Then GV oocytes were in vitro matured in the M16 medium at 37°C with 5% CO₂. To collect in vivo matured oocytes, mouse was superovulated using 10 IU PMSG, followed by injection with 10 IU human chorionic gonadotrophin (hCG) 48 h later to induce superovulation. At 13–14 h post-hCG injection, cumulus-oocyte complexes (COCs) were retrieved from the ampulla. Oocytes were collected in M2 medium, the cumulus cells were removed enzymatically using 0.1% (w/v) hyaluronidase.

**Oocytes Vitrification and Warming**

Vitrification and warming procedures were conducted as described previously (Huang et al., 2019). For vitrification, pretreatment solution was PBS medium contained 10% (v/v) dimethylsulfoxide (DMSO) and 10% (v/v) ethylene glycol while vitrification solution...
In vitro Maturation of Oocytes

GV oocytes were matured in M16 medium under mineral oil, maintaining in 5% CO2 with maximum humidity at 37°C. 10 μg/ml Concanavalin A (ConA) (Sigma, C7642) was added during the entire IVM (in vitro maturation) process. A previous study has shown ConA could penetrate zona pellucida intact oocytes (Chaigne et al., 2013). Polar body extrusion (PBE) rate was recorded at 10 h after GVBD.

Parthenogenesis Activation of Oocytes

The denuded oocytes were transferred first into (Ca2+)-free human tubal fluid (HTF) medium supplemented with 10 mM strontium chloride (SrCl2) and 5 μg/ml cytochalasin B, incubated at 37°C with 5% CO2 for 2.5 h. Then oocytes were transferred into HTF with 5 μg/ml cytochalasin B, incubated at 37°C with 5% CO2 for 3.5 h. Activated oocytes were then cultured in a KSOM medium at 37°C with 5% CO2 for early embryo development. Cleavage and blastocyst rates were recorded at 24 and 96 h after activation, respectively.

Immunofluorescence Staining and Chromosome Spread

Oocytes were fixed with 4% (w/v) paraformaldehyde (PFA) for 40 min at room temperature, followed by permeabilization with 0.5% Triton X-100 at room temperature for 1 h. After being blocked in 3% BSA for 1 h at room temperature, oocytes were incubated with different primary antibodies overnight at 4°C: Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) (48G2) Rabbit mAb (Cell Signaling Technology, #4688S), Phospho-Moslin Light Chain 2 (Ser19) Mouse mAb (Cell Signaling Technology, #3017S), Rabbit polyclonal anti-Mad2 (Biologend, #PRB-452C, 1:200); Purified Mouse anti-human BubR1 (BD Transduction Laboratories, 612502, 1:100); human anti-centromere (Immunovision, HCT-0100, 1:200), mouse monoclonal anti-alpha-tubulin with FITC (Sigma, F2168, 1:400). Oocytes were further incubated with FITC-conjugated Affinitpure Goat Anti-Rabbit IgG (H + L) (protein tech, SA00003-2, 1:200), FITC-conjugated Affinitpure Goat Anti-Mouse IgG (H + L) (protein tech, SA00003-1, 1:200) or anti-human-Cy3 (Jackson ImmunoResearch, AB-2340538, 1:200) for 1 h at room temperature. For F-actin staining, oocytes were incubated with Phalloidin-TRITC (Sigma, P1951, 5 μg/ml) at 4°C for overnight. Finally, oocytes were stained with 4',6-diamidino-2-phenylindole (DAPI) for 5 min at room temperature. To conduct chromosome spread, the zona pellucida was removed by 0.5% pronase. Then oocytes were fixed in a medium of 1% paraformaldehyde in distilled H2O containing 0.15% Triton X-100 and 3 mM dithiothreitol. After air drying, the chromosome was stained with DAPI for 10 min. Fluorescent images were taken with laser scanning confocal microscopy (A1 Cell Imaging System; Nikon) on single planes under the same staining procedure and confocal microscopy parameters. Then, fluorescence intensity was assessed using NIS-Elements AR software (Nikon Instruments, Tokyo, Japan). Measurements of vitrified and vitrified + ConA were standardized with fresh group data. Specifically, the relative fluorescence intensity is the ratio of the experimental group fluorescence intensity to the average fluorescence intensity of the fresh group.

Quantification of Mad2 and BubR1 Fluorescence Signal

The fluorescence intensity of Mad2 and BubR1 was quantified according to the previous study (Zhou et al., 2020). Fluorescence intensity was randomly measured and was quantified by drawing a circle of the dot-like CREST staining that includes SAC protein staining. The intensity of SAC proteins was normalized against the CREST fluorescence intensity.

Quantitative Reverse Transcription PCR

Quantitative reverse transcription PCR (qRT-PCR) was performed on at least three repeated experiments with expression data as mean ± SEM. Data were analyzed by unpaired-samples t-test, provided by GraphPad Prism eight statistical software. The level of significance was accepted as p < 0.05.

Statistical Analysis

All percentages or values from at least three repeated experiments were expressed as mean ± SEM. Data were analyzed by unpaired-samples t-test, provided by GraphPad Prism eight statistical software. The level of significance was accepted as p < 0.05.

RESULTS

Vitrification Induced a Decline in Cortical Tension in Oocytes

To verify whether cortical tension was altered in vitrified oocytes, pERM and pMRLC were examined (Figures 1A,B, 2A,B). pERM was significantly decreased in GV vitrified oocytes (Fresh: 1 ± 0.07 vs. Vitrified: 0.70 ± 0.03, p < 0.001;
Figure 1C). pERM was also significantly decreased in MII vitrified oocytes (Fresh: 1 ± 0.04 vs. Vitrified: 0.68 ± 0.04, \( p < 0.001 \); Figure 2C). Moreover, the cortical enrichment of pMRLC was significantly decreased in GV vitrified group (Fresh: 1.05 ± 0.03 vs. Vitrified: 0.93 ± 0.02, \( p < 0.01 \); Figure 1D), and the distribution of pMRLC in cytoplasm was significantly increased in MII vitrified group (Fresh: 1 ± 0.03 vs. Vitrified: 1.59 ± 0.04, \( p < 0.001 \); Figure 2D). These results indicated that cortical tension was decreased in vitrified oocytes.

**Cortical F-Actin Level was Not Affected After Vitrification**

In order to explore the level of F-actin, GV + 9 h oocytes were stained with Phalloidin-TRITC (Figure 3A). We found that cortical F-actin level was not affected after vitrification (Fresh: 1 ± 0.07 vs. Vitrified: 0.95 ± 0.05, \( p > 0.05 \); Figure 3B). In addition, the mRNA levels of Mos and Arpc2 were detected by qPCR, and the result showed that vitrification did not affect the expression of
Mos (Fresh: 1 vs. Vitrified: 1.08 ± 0.10, p > 0.05; Figure 3C) and Arpc2 (Fresh: 1 vs. Vitrified: 0.90 ± 0.04, p > 0.05; Figure 3D).

**ConA Effectively Elevated Cortical Tension in Vitrified Oocytes**

In order to increase the cortical tension of vitrified oocytes, GV vitrified oocytes were cultured in medium with 10 μg/ml ConA to metaphase I stage (GV + 8 h). We found that the pERM level in vitrified group cannot be recovered spontaneously during culture (Fresh: 1 ± 0.07 vs. Vitrified: 0.70 ± 0.03, p < 0.001), while it could be rescued after ConA treatment (Vitrified: 0.70 ± 0.03 vs. Vitrified + ConA: 1.03 ± 0.08, p < 0.01; Figures 4A,C). Similarly, the cytoplasmic pMRLC level in vitrified group also cannot be resumed to the level of fresh group (Fresh: 1 ± 0.04 vs. Vitrified: 1.16 ± 0.05, p < 0.05), while it could be reversed after ConA treatment (Vitrified: 1.16 ± 0.05 vs. Vitrified + ConA: 0.98 ± 0.03, p < 0.01; Figures 4B,D). These results indicated that ConA could increase the cortical tension of vitrified oocytes during in vitro culture.
Increasing Cortical Tension Improved the Developmental Capability of Vitriﬁed Oocyte

To investigate whether increasing cortical tension of vitriﬁed oocytes can improve oocyte and embryo development. GV vitriﬁed oocytes were cultured in medium without or with ConA. We found that the polar body extrusion (PBE) rate of vitriﬁed oocytes was signiﬁcantly reduced (Fresh: 96.29 ± 1.87% vs Vitriﬁed: 83.99 ± 1.38%, p = 0.0061), while increasing the cortical tension could signiﬁcantly increase the PBE rate (Vitriﬁed: 83.99 ± 1.38% vs Vitriﬁed + ConA: 91.94 ± 1.80%, p = 0.0248; Figures 5A,C). Then, vitriﬁed-thawed MII oocytes were cultured 1 h in medium without or with ConA before performing parthenogenetic activation. During the subsequent embryo development, both the cleavage rate (Fresh: 98.67 ± 1.33% vs. Vitrified: 86.72 ± 0.82%, p < 0.01) and blastocyst rate (Fresh: 72.86 ± 2.56% vs. Vitrified: 25.91 ± 2.96%, p < 0.001) were remarkable compromised in vitriﬁed oocytes, while ConA treatment would signiﬁcantly increase the cleavage rate (Vitrified: 86.72 ± 0.82% vs. Vitrified + ConA: 93.15 ± 2.15%, p < 0.05; Figures 5B,D) as well as the blastocyst formation rate (Vitrified: 25.91 ± 2.96% vs. Vitrified + ConA: 43.94 ± 2.14%, p < 0.01; Figures 5B,E).

Increasing Cortical Tension Attenuated Aneuploidy Rate in Vitriﬁed Oocytes

To investigate whether increasing cortical tension of vitriﬁed oocytes can decrease the aneuploidy rate, chromosome numbers in different groups was recorded by chromosome spread (Figure 6A). The aneuploidy rate was signiﬁcant higher in vitriﬁed oocytes (Fresh: 14.75 ± 2.63% vs. Vitrified: 41.82 ± 1.82%, p < 0.01), while increasing the cortical tension could correct it (Vitrified: 25.91 ± 2.96% vs. Vitrified + ConA: 15.87 ± 2.08%, p < 0.01; Figure 6B).
Increasing Cortical Tension Promoted Spindle Positioning in Vitrified Oocytes

In order to investigate whether the spindle positioning was affected in vitrified oocyte, spindle positioning was detected at GV + 9 h (Figure 7A). Then, spindle positioning was quantified according to the previous study (Pan et al., 2021). We found that the relative distance between the spindle leading pole and cortex of vitrified oocytes was significantly increased (Fresh: 0.11 ± 0.01 vs. Vitrified: 0.15 ± 0.01, p < 0.01), while increasing cortical tension could significantly decrease the distance (Vitrified: 0.15 ± 0.01 vs. Vitrified + ConA: 0.11 ± 0.01, p < 0.01; Figure 7B).
These results suggest that increasing cortical tension of vitrified oocytes could promote spindle positioning.

**Increasing Cortical Tension Regulated Microtubule Dynamics During Chromosome Segregation**

In order to investigate whether increasing cortical tension of vitrified oocytes can affect microtubule dynamics, kinetochores, microtubules and chromosomes were stained at BD (breakdown) + 6 h (Figure 8A). We found that vitrification induced significant higher proportion of unattached KT-MT (Fresh: 6.68 ± 0.62% vs. Vitrified: 12.08 ± 1.15%, p < 0.001), whereas increasing cortical tension could rescue this phenomenon (Vitrified + ConA: 6.26 ± 0.93%, p < 0.001; Figure 8C). Moreover, the rate of misaligned chromosome (Fresh: 18.37 ± 1.41% vs. Vitrified: 45.35 ± 3.61%, p < 0.001) and aberrant spindle proportion (Fresh: 6.33 ± 0.40% vs. Vitrified: 28.33 ± 1.67%, p < 0.001) were dramatically elevated in vitrified oocytes. Notably, ConA treatment could alleviate chromosome misalignment (Vitrified: 45.35 ± 3.61% vs. Vitrified + ConA: 18.67 ± 1.68%, p < 0.001; Figures 8B,D) and also reduce aberrant spindle rate (Vitrified: 28.33 ± 1.67% vs. Vitrified + ConA: 11.20 ± 0.72%, p < 0.001; Figures 8B,E) in vitrified oocytes. These results showed
that increasing cortical tension could improve the KT-MT attachment, chromosome alignment and spindle morphology of vitrified oocytes.

**Increasing Cortical Tension Strengthened SAC Activity**

Then, two essential SAC proteins [mitotic arrest deficient 2 (Mad2) and Bub1-related protein 1 (BubR1)] were stained and intensities were measured with CREST as a reference indicator (Figures 9A,B). Compared with the fresh group, the signal intensities of Mad2 (Fresh: 1.63 ± 0.05 vs. Vitrified: 0.90 ± 0.04, p < 0.001) and BubR1 (Fresh: 0.56 ± 0.01 vs. Vitrified: 0.31 ± 0.01, p < 0.001) were remarkably decreased after vitrification. When treated with ConA, the fluorescence intensities of Mad2 (Vitrified: 0.90 ± 0.04 vs. Vitrified + ConA: 1.21 ± 0.04, p < 0.001; Figure 9C) and BubR1 (Vitrified: 0.31 ± 0.01 vs. Vitrified + ConA: 0.62 ± 0.02, p < 0.001; Figure 9D) were significantly increased in vitrified oocytes. The results indicated that increasing the cortical tension could enhance SAC activity.

**DISCUSSION**

Oocyte underwent a dramatic osmotic pressure change during the vitrification/thawing process, which resulted in a drastic change in the morphology of the oocyte (Gallardo et al., 2019; Wang et al., 2020). To investigate whether this severe deformation could lead to changes in oocyte cortical tension, we examined the expression of two proteins, pERM and pMRLC, which play an important role in oocyte cortical tension regulation. It has been confirmed that the level of pERM protein in oocytes is consistent with the change of cortical tension (Larson et al., 2010). In this study, pERM protein levels of vitrified GV and MII oocytes were decreased (Figures 1C, 2C), which indicated that cortical tension of frozen oocytes were decreased. The distribution of pMRLC during meiosis is more complicated than pERM. pERM is consistently distributed in the cortex during meiosis, whereas pMRLC is gradually expelled from the cortex into the cytoplasm. Therefore, the cortical tension change was reflected by cortical enrichment of pMRLC in GV oocytes, while in regard to MII stage oocytes, it was reflected by cytoplasmic pMRLC. Specifically, cortical pMRLC has a positive correlation with cortical tension while cytoplasmic pMRLC has a negative correlation with cortical tension (Chaigne et al., 2013; Bennabi et al., 2020). The cortical enrichment of pMRLC in vitrified GV oocytes was decreased (Figure 1D), and the cytoplasmic pMRLC in vitrified MII oocytes was increased (Figure 2D). These change patterns of pMRLC are consistent with the decreased cortical tension model. Thus, vitrification could decrease the cortical tension of oocytes.

Previous research found that cortical tension decreases during oocytes meiosis progression (Larson et al., 2010). The mechanism lies in that the up-regulated of Mos would activate Arp2/3 at BD + 3 h, and eventually result in cortical F-actin increase and spatially discharging Myosin II from cortex into cytoplasm (Nakanishi et al., 2007; Mendoza et al., 2011). To investigate whether the F-actin levels in vitrified oocytes were changed. We detected the level of F-actin at GV + 9 h, we found that the cortical F-actin density was not significantly different between vitrified and fresh groups (Figure 3B). Then, we examined the expression of Mos and Arpc2 at BD + 3 h, and the result showed that vitrification did not affect the mRNA levels of Mos and Arpc2 (Figures 3C,D). Furthermore, these results indicated that the cortical F-actin was not affected after vitrification.

Concanavalin A (ConA) is a tetavalent lectin that crosslinks the cell surface through binding to membrane glycosylated proteins (Chainge et al., 2013). Several studies have used ConA to increase oocyte cortical tension (Larson et al., 2010; Chainge et al., 2013), suggesting that the effect of ConA is stable. 100 μg/ml ConA treatment caused a 69% increase in cortical tension of fresh MII oocytes (Lee et al., 2009). However, excessive
cortical tension in fresh oocytes resulted in impaired spindle migration and increased aneuploidy rate (Chaigne et al., 2013). Therefore, we added 10 μg/ml ConA during in vitro culture of vitrified oocytes. A study reported that the localizations of pMRLC and phosphorylated ERM were not dramatically different between controls and ConA-treated eggs (Larson et al., 2010). But this study did not measure the fluorescence intensity of pERM and pMRLC. In our study, ConA did not change the localizations of these two proteins also, and their changes in fluorescence intensity could indicate that 10 μg/ml ConA increased cortical tension (Figure 4).

Then, we investigated the effect of cortical tension on oocyte development. Our results indicated that the polar body extrusion (PBE) rate of vitrified oocytes was decreased, while increasing the cortical tension could increase the PBE rate (Figure 5C). Fresh oocytes treated with 100 μg/ml ConA showed a decreased PBE rate, which could be rescued by succinyl-concanavalin A, an antagonist of ConA (Chaigne et al., 2013). Combined with our study, it can be speculated that there is a narrow window of cortical tension for normal polar body extrusion, too low or too high cortical tension will lead to disruptions in PBE. In addition, increasing the cortical tension could improve the subsequent development of vitrified oocytes (Figures 5D,E). The mechanical properties of zygote have an important impact on the developmental potential of human embryos (Yanez et al., 2016). What’s more, cell surface forces can influence the
FIGURE 8 | Increasing cortical tension regulated microtubule dynamics during chromosome segregation. (A) KT-MT attachment in different groups at 6 h after GVBD. White arrows indicate nonconnected kinetochores. Scale bar = 25 μm. (B) Representative images of spindle morphologies and chromosome alignment in fresh, vitrified, and V + ConA oocytes at 6 h after GVBD. Scale bar = 25 μm. (C) The rate of unattached KT-MT in the fresh (n = 450), vitrified (n = 444), and V + ConA (n = 381) groups. (D) The rate of misaligned chromosome in the fresh (n = 64), vitrified (n = 44), and V + ConA (n = 46) groups. (E) The rate of aberrant spindle in the fresh (n = 64), vitrified (n = 44), and V + ConA (n = 46) groups. Data were presented as mean percentage (mean ± SEM) of at least three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001.
differentiation of embryonic stem cells (Bergert et al., 2021; De Belly et al., 2021; Muncie and Weaver, 2021). We speculate that increasing cortical tension of vitrified oocytes could affect mechanical property of zygotes and cell fate determination, thus improved embryo development. Interestingly, the cortical tension of post-ovulatory aging oocytes was also decreased.

**FIGURE 9** Increasing cortical tension strengthened SAC activity. (A) The localization of Mad2 at prometaphase I stage in fresh, vitrified, and V + ConA oocytes. At 3 h after germinal vesicle breakdown (GVBD), oocytes were immunostained for Mad2, CREST, and DNA (DAPI). Scale bar = 50 μm. (B) The localization of BubR1 at prometaphase I stage in the fresh, vitrified, and V + 10 μg/ml ConA oocytes. At 3 h after GVBD, oocytes were stained for BubR1, CREST, and DNA (DAPI). Scale bar = 50 μm. (C) The relative fluorescence intensity of Mad2 to CREST was measured in fresh (n = 240), vitrified (n = 180), and V + ConA (n = 163) groups. The signal intensity of Mad2 was normalized with that of CREST. (D) The relative fluorescence intensity of BubR1 to CREST was measured in the fresh (n = 159), vitrified (n = 154), and V + ConA (n = 123) groups. The signal intensity of BubR1 was normalized with that of CREST. Data were presented as mean percentage (mean ± SEM) of at least three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001.
Whether increasing the cortical tension of post-ovulatory aging oocytes could improve oocytes and subsequent embryos development deserves a further study. Our previous study showed that aneuploidy rate was significantly increased in vitrified oocytes (Cheng et al., 2014). In this study, we found that increasing the cortical tension of vitrified oocytes could decrease the aneuploidy rate (Figure 6B). It was reported that in mouse oocytes, reduction of cortical tension by the expression of cVAC (cortical verprolin-homology coflillin-homology acidic) or cFH1FH2 (cortical FH1FH2 nucleating domain of formin 2) would induce abnormal high aneuploidy rate (Bennabi et al., 2020). Our results further confirmed that cortical tension was involved in maintaining oocyte euploidy. In addition, we discovered that the distance between spindle leading pole and cortex was increased in vitrified oocyte, which could be shortened by increasing the cortical tension (Figure 7B). This indicated that the decreased cortical tension was contributed to the impaired spindle positioning in vitrified oocytes, and our data was consistent with the previous finding that proper cortical tension was essential for normal spindle positioning (Chaigne et al., 2013).

In our study, unconnected KT-MT was increased in vitrified oocytes, while increasing the cortical tension could rescue this phenomenon (Figure 8). The result implied that proper KT-MT was essential for normal meiosis progression, which was consistent with the previous finding that accurate chromosome separation depends on the correct attachment between microtubule and kinetochore (Nakagawa and FitzHarris, 2017). The decrease of SAC activity weakened checkpoint monitoring, resulting in unattached KT-MT or other attachment errors that cannot be corrected (Skoufias et al., 2001). Then we detected the levels of Mad2 and BubR1, which are two important components of SAC (Homer, 2006; Musacchio, 2015). Mad2 and BubR1 were both decreased in vitrified oocytes, indicating that the SAC activity was decreased. Nevertheless, increasing the cortical tension could strengthen the SAC activity in vitrified oocytes (Figure 9). A study reported that kinetochore stretching promoted SAC silencing by PP1 recruitment stimulation (Uchida et al., 2021). Whether cortical tension can affect SAC activity through kinetochore stretching needs to be further studied. To sum up, increasing cortical tension of vitrified oocytes could enhance SAC activity, and the activated checkpoint monitoring could facilitate proper KT-MT attachment, which in turn ensures correct chromosomes separation and maintains euploidy in oocytes. This is the first study revealed that vitrification decreased the oocyte cortical tension. Furthermore, increasing the cortical tension of mouse vitrified oocyte could decrease aneuploidy rate through improving SAC activity and KT-MT attachment.

CONCLUSION

In conclusion, vitrification decreased the cortical tension of mouse oocytes. Artificially increasing cortical tension in vitrified oocyte could increase oocyte and embryo development. Furthermore, it could improve spindle positioning, strengthen SAC activity and ensure KT-MT attachment. Importantly, cortical tension could regulate aneuploidy rate through influencing SAC activity and KT-MT attachment. Our results will provide an interdisciplinary perspective into understanding the cryoinjuries in mouse oocytes, and provide a reasonable theoretical basis for delineating the mechanism underlying cortical tension-regulated meiotic progression.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Institutional Animal Care and use Committee of China Agricultural University.

AUTHOR CONTRIBUTIONS

XD, XF and JL conceived and designed the study; XD and QZ performed experiments, collected data, and analyzed data; LZ, LM, PR, XH, JB participated in animal research; XD, XF and JL wrote the manuscript and YH, PW, WS, SZ revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2022.876259/full#supplementary-material
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