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Effects of sperm insemination on the final meiotic maturation of mouse oocytes arrested at metaphase I after *in vitro* maturation

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Objective: The aims of this study were to investigate whether fertilization could induce the resumption of meiosis in mouse oocytes arrested at metaphase I (MI) after *in vitro* maturation (IVM), and to investigate the effect of Ca^{2+} chelator treatment at the time of fertilization on the transition from MI to metaphase II (MII).

Methods: MII-stage and arrested MI-stage mouse oocytes after IVM were fertilized, and then embryonic development was monitored. Blastocysts from each group were transferred into 2.5 days post-coitum pseudo-pregnant ICR mice. MI oocytes after IVM were treated with a Ca^{2+} chelator to investigate the effect of Ca^{2+} oscillations on their maturation.

Results: As insemination time increased, the number of oocytes in the MI group that reached the MII stage also increased. The blastocyst rates and total cell numbers in the MII group were significantly higher than in the MI group. No pregnancy occurred in the MI group, but 10 pregnancies were achieved (10 of 12) in the MII group. The proportion of MI oocytes that matured to MII oocytes after fertilization was significantly higher in the non-treated group than in the Ca^{2+} chelator-treated group.

Conclusion: The findings that a higher proportion of MI-arrested oocytes progressed to MII after fertilization and that the MI-to-MII transition was blocked by Ca^{2+} chelator treatments before fertilization indicate that the maturation of MI oocytes to MII oocytes is associated with intracellular Ca^{2+} oscillations driven by fertilization.

Keywords: Arrested metaphase I oocyte; Calcium oscillations; Fertilization; *In vitro* oocyte maturation

Introduction

In vitro maturation (IVM) of human oocytes is a feasible alternative to controlled ovarian hyperstimulation cycles, but the pregnancy rates of IVM cycles are still low because of the low number of mature oocytes [1]. In addition, 15% to 20% of the oocytes collected in controlled ovarian hyperstimulation cycles are immature, and some of these oocytes are arrested at the germinal vesicle (GV) and meta-

phase I (MI) stages, even after prolonged culture [2]. In the normal IVM and *in vitro* fertilization cycles, oocytes are exposed to spermatozoa after reaching metaphase II (MII), and immature oocytes (GV and MI) are discarded. The potential of using the immature oocytes in these cycles is important because doing so would allow more embryos to be obtained in each cycle, which may lead to an increased number of embryos being available for transfer or cryopreservation and, therefore, to an increased probability of achieving pregnancy.

Oocyte maturation is divided into nuclear maturation and cytoplasmic modification [3]. Nuclear maturation starts with GV breakdown (GVBD), and progresses to MI and then to MII. Cytoplasmic maturation is a more complex process, and includes the accumulation of essential maternal factors, changes in the expression profile of cell cycle control proteins, modification of plasma membrane permeability, and differentiation of the Ca^{2+} signaling machinery [3,4]. In most mammals, oocytes are arrested in MII after nuclear and cytoplasmic maturation and

are then exposed to spermatozoa. However, it has been reported that the maturation of immature oocytes is enhanced by co-incubation with spermatozoa, even before fertilization [5]. Niwa et al. [6] found that the maturation of bovine oocytes was not required for sperm penetration and Chian et al. [7] reported that bovine immature oocytes could promote oocyte maturation as a result of sperm penetration. Moreover, Van Blerkom et al. [8] reported that human oocytes fertilized at the GV stage resumed meiosis until the pronucleus (PN) stage and appeared to develop to the 2-cell stage. These reports suggest that sperm can penetrate immature oocytes, and then assist in the completion of the final step of maturation. However, no study has confirmed whether the final maturation of arrested oocytes at the time of insemination occurs spontaneously without sperm penetration or is initiated by sperm penetration into the cytoplasm. Moreover, Ca^{2+} oscillations derived by spermatozoa may influence the maturation process in which MI arrest is overcome. Therefore, this study was designed to examine whether MI oocytes were completely arrested after IVM, whether sperm insemination was capable of completing meiotic maturation in MI-arrested oocytes, and the developmental potential of oocytes fertilized at the MII or MI stage after IVM.

Methods

1. Retrieval of immature oocytes and IVM

All animal procedures were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animal studies were also approved by the Animal Research Ethics Committee of the Maria Research Center. The B6D2-F1 female mice were given an intraperitoneal treatment of 10 IU of pregnant mare serum gonadotropin (Intervet, Boxmeer, the Netherlands) and were euthanized by cervical dislocation 48 hours later. Their ovaries were excised and immediately placed in pre-warmed MRC#D01 medium (Maria Medical Foundation, Seoul, Korea) buffered with 3-(N-Morpholino) propanesulfonic acid (MOPS) [9]. Cumulus-enclosed oocytes at the GV stage were collected by puncturing the large antral follicles and then cultured for 18 hours in maturation medium in an incubator with 6% CO_2, 5% O_2, and 89% N_2 at 37℃. The medium used for IVM was MRC#D46 (Maria Medical Foundation) with 20% serum substitute supplement (Irvine Scientific, Santa Ana, CA, USA), supplemented with 10 ng/mL of human recombinant epidermal growth factor (Invitrogen, Carlsbad, CA, USA), 0.7 IU/mL of recombinant follicle stimulating hormone (Fostimon; Institut Biochimique SA, Lugano, Switzerland), and 0.1 IU/mL of recombinant luteinizing hormone (Luveris; Merck Serono, Darmstadt, Germany).

2. Maturity assessment and *in vitro* fertilization

After IVM, adherent cumulus cells were removed from oocytes me-

chanically by gentle micropipetting using a fine-drawn glass pipette. The *in vitro* matured oocytes were divided into two groups according to their maturity: oocytes that displayed a distinct first polar body (PB) were classified as the MII group, while those without a PB and a visible GV nucleus were classified as the MI-arrested group. All oocytes from the MII group and some from the MI group were inseminated using sperm collected from the cauda epididymis of B6D2-F1 males in MRC#D01 medium. The remaining MI oocytes were cultured for a further 4 hours to investigate the spontaneous maturation rate in insemination medium. Throughout the fertilization period, the maturity of MI oocytes was re-evaluated by monitoring the appearance of the first PB every hour for up to 4 hours. After insemination, oocytes were washed and placed in insemination medium. The fertilization rate was assessed by the presence of the 2-cell stage at 20 hours following insemination. The cleaved embryos were cultured sequentially in cleavage and blastocyst medium (MRC#D13 and 46, Maria Medical Foundation) for 96 hours.

3. Embryo transfer

CD-1 females were used as embryo recipients. Females were mated with vasectomized males to induce pseudo-pregnancy, and successful mating was confirmed by a copulation plug check on the following morning. The day on which a vaginal plug was found was defined as day 1 of pregnancy. Pseudo-pregnant mice at 2.5 days postcoitum were randomly assigned to the MII and MI groups. Prior to embryo transfer (ET), recipients were anesthetized by being placed in a chamber filled with 2% to 3% isoflurane for 2 to 3 minutes (Hana Pharm, Seoul, Korea) in oxygen. ET using a non-surgical embryo transfer device (NSET) (ParaTechs Co., Lexington, KY, USA) was carried out according to the manufacturers' instructions. Briefly, small and large specula were placed sequentially into the vagina to open and expose the cervix. The NSET device tip, loaded with eight blastocysts, was inserted through the cervix and the blastocysts were transferred into the uterus. The recipients were allowed to deliver at term to evaluate the pregnancy and live pup birth rates.

4. Differential staining

Differential staining of the inner cell mass and trophectoderm cells was carried out as described previously [10]. Briefly, zona-intact or partially hatching blastocysts were incubated in 1 mL of MOPS-buffered mouse tubal fluid (MTF) medium with 1% Triton X-100 and 100 μg/mL of propidium iodide (Sigma, St. Louis, MO, USA) for up to 10 seconds. Then, the blastocysts were immediately transferred to 1 mL of fixative solution (100% ethanol with 25 μg/mL bisbenzimide [Hoechst 33258, Sigma]) and stored at 4℃ overnight. The blastocysts were then mounted onto a glass microscope slide in a drop of glycerol, gently flattened with a coverslip, and visualized for cell counting.

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Cell counts were determined from digital photographs of images obtained using an inverted microscope (Nikon, Otawara, Japan) with an ultraviolet lamp.

5. Ca²⁺ chelator treatment

Arrested MI oocytes after IVM were treated with 1 or 10 μg of a Ca^{2+} chelator (1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetra-acetic acid acetoxymethyl ester [BAPTA-AM; Molecular Probes, Eugene, OR, USA]) to investigate the effects of Ca^{2+} oscillations on the MI-to-MII transition. The oocytes were incubated in Ca^{2+}-free MTF medium containing 5 mg/mL of human serum albumin and BAPTA-AM for 30 minutes in the presence of Pluronic F-127 (Molecular Probes) to aid in loading. Control oocytes were incubated without BAPTA-AM in medium containing an equivalent volume of dimethyl sulfoxide and Pluronic F-127. After BAPTA-AM treatment, those oocytes were inseminated as described above and then their maturity was evaluated by monitoring the appearance of the first PB.

6. Statistical analyses

Statistical analyses were conducted using the *t*-test to compare the fertilization rate, embryo development, total cell number, and pregnancy rate of the two groups. The effect of Ca^{2+} chelator treatment was evaluated by analysis of variance. Data were analyzed using SPSS ver. 17.0. (SPSS Inc., Chicago, IL, USA), and *p*-values < 0.05 were considered to indicate statistical significance.

Results

1. Effects of sperm insemination on meiotic maturation and embryo development

Schultz et al. [11] reported that the fertilization of GV oocytes was

Table 1. Comparison of the proportions of abnormal fertilization between MI-arrested oocytes and MII oocytes

Group	No. of oocytes	Normal fertilization	Abnormal fertilization	
		2 PN (%)	1 PN (%)	3 PN (%)
MII	94	76 (81.8 ± 5.1)	8 (8.5 ± 1.4)	10 (9.7 ± 5.5)
MI	101	81 (79.9 ± 4.7)	6 (6.0 ± 2.5)	14 (14.0 ± 3.0)

Values are presented as number (mean ± standard deviation).
MI, metaphase I; MII, metaphase II; PN, pronucleus.

Table 2. Maturation rates of GV oocytes after 18 hours of culture

No. of GV oocytes	Maturation stage (%)		
	MII	MI	GV
1,145	470 (42.4 ± 17.3)	664 (56.7 ± 17.5)	11 (0.9 ± 1.1)

Values are presented as number (mean ± standard deviation).
GV, germinal vesicle; MII, metaphase II; MI, metaphase I.

frequently associated with polyspermy. Therefore, a preliminary study was conducted to compare the proportion of abnormal fertilization between MI-arrested oocytes and MII oocytes. No significant difference in the rate of normal fertilization was found between the two groups (Table 1). To obtain MII and MI-arrested oocytes, 1,145 GV oocytes were cultured in maturation medium for 18 hours. The proportions of MII, MI, and GV oocytes were 42.4%, 56.7%, and 0.9%, respectively (Table 2). Additionally, the formation rate of 2PN embryos was similar to the 2-cell incidence rate presented in Table 3. Therefore, the formation rate of 2-cell embryos was regarded as the fertilization rate, and a separate investigation of 2PN formation was not performed in this experiment.

To evaluate the effect of sperm addition on oocyte maturation, MI oocytes in the presence or absence of sperm were monitored every hour for 4 hours during fertilization. Over time, the proportions of oocytes reaching the MII stage among the MI oocytes with spermatozoa were 3.7% at 2 hours, 75.5% at 3 hours, and 94.6% at 4 hours (Figure 1). However, the MI oocytes cultured in fertilization medium without spermatozoa did not proceed to the MII stage, but remained arrested in MI (1 of 146, 0.7%) (Table 3, Figure 1). Moreover, the spontaneous maturation rate (2.1%) of the MI oocytes further cultured for up to 20 hours was not significantly different from that of MI oocytes cultured for 4 hours. These results suggest that sperm induces the resumption of meiosis or the MI-to-MII transition in MI-arrested oocytes. The data comprise the pooled results of nine independent experiments.

There were no significant differences in the fertilization rate of oocytes between the MII (378 of 470, 80.0% ± 6.6%) and MI (377 of 489, 78.2% ± 9.1%) groups. However, the rate of blastocyst formation was significantly higher in the MII group (323 of 378, 84.6% ± 6.9%) than in the MI group (253 of 377, 66.5% ± 5.6%) (*p* < 0.01). Similarly, the numbers of total cells and trophectoderm cells in the MII group (101.2 ± 17.9 and 75.2 ± 16.8, respectively) were significantly higher than those in the MI group (90.2 ± 19.0 vs. 65.4 ± 14.6, respectively)
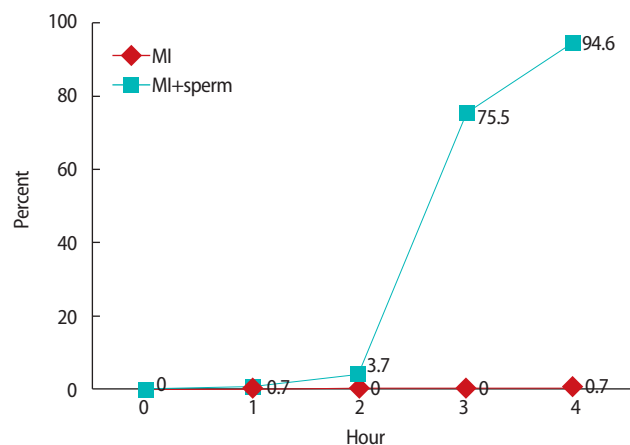

Figure 1. Maturation rates of MI oocytes after sperm addition.

Table 3. Effects of sperm insemination of MI-arrested oocytes on embryonic development and cell numbers

Group	No. of oocytes	No. of MII oocytes (%, mean ± SD)	No. of embryos developed to		No. of cells (n = 65)		
			Two cells (%, mean ± SD)	Blastocysts (%, mean ± SD)	Total cells	ICM cells	TE cells
MII+sperm	470	470	378 (80.0 ± 6.6)	323 (84.6 ± 6.9)[a)]	101.2 ± 17.9[b)]	26 ± 10.6	75.2 ± 16.8[b)]
MI+sperm	518	489 (94.6 ± 4.1)[a)]	377 (78.2 ± 9.1)	253 (66.5 ± 5.6)[c)]	90.2 ± 19.0[d)]	24.9 ± 10.5	65.4 ± 14.6[d)]
MI	146	1 (0.7 ± 2.1)[c)]	-	-	-	-	-

Values are presented as mean ± standard error of the mean unless otherwise indicated.
MI, metaphase I; MII, metaphase II; SD, standard deviation; ICM, inner cell mass; TE, trophectoderm.
[a,c)]Significant differences within each column (p < 0.01); [b,d)]Significant differences within each column (p < 0.05).

Table 4. Live pups produced following the transfer of blastocysts derived from MII or MI oocytes

Group	No. of recipients	No. of pregnant recipients (%)	No. of embryos transferred	No. of live pups	Live pups per transferred embryos (%)
MII	12	10 (83.3)[a)]	96	31	32.3[a)]
MI	12	0[b)]	96	0	0.0[b)]

MII, metaphase II; MI, metaphase I.
[a,b)]Significant differences within each column (p < 0.01).

Table 5. Effects of BAPTA-AM treatment on the MI-to-MII transition

BAPTA-AM (μg)	No. of MI Oocytes	No. of MII oocytes after fertilization (%, mean ± SD)	No. of 2-cell embryos (%, mean ± SD)
0	177	165 (93.2 ± 5.7)[a)]	117 (72.1 ± 12.6)[a)]
1	166	5 (3.3 ± 3.2)[b)]	0[b)]
10	166	2 (1.3 ± 1.8)[b)]	0[b)]

BAPTA-AM, 1,2-bis(2-aminophenoxy)ethane-N,N,N´,N´-tetra-acetic acid acetoxymethyl ester; MI, metaphase I; MII, metaphase II; SD, standard deviation.
[a,b)]Significant differences within each column (p < 0.01).

(p < 0.05). However, the numbers of inner cell mass cells did not significantly differ between the MII and MI groups (26.0 ± 10.6 vs. 24.9 ± 10.5, respectively).

Ninety-six blastocysts of each group were transferred to 12 pseudopregnant recipients. The rate of live pups in the MII group was significantly higher than in the MI group (p < 0.01) (Table 4). No pregnancy was obtained from the MI group. In contrast, 10 pregnancies were achieved (10 of 12, 83.3%) in the MII group, and the rate of live pups per transferred embryo was 32.3% (31/96). All live pups were morphologically normal.

2. Effects of Ca²⁺ oscillations on overcoming MI arrest

MI oocytes after IVM were treated with 1 or 10 μg of BAPTA-AM to investigate the role of Ca^{2+} derived from sperm during the MI-to-MII transition. Following incubation with or without BAPTA-AM, the oocytes were fertilized with sperm. The vast majority of oocytes in the BAPTA-AM-treated groups remained at MI without formation of the first PB (Table 5). The proportion of MI oocytes that matured to MII oocytes after fertilization was significantly higher in the non-treated

group (165 of 177, 93.2% ± 5.7%) than in the 1 or 10 μg BAPTA-AM-treated group (5 of 166, 3.3% ± 3.2%; and 2 of 166, 1.3% ± 1.8%, respectively) (p < 0.01). Moreover, those oocytes were unable to form 2 cells (Table 5). This result indicates that induction with BAPTA-AM was sufficient to block the intracellular Ca^{2+} oscillations, and that the maturation of MI oocytes to MII after fertilization was related to the intracellular Ca^{2+} oscillations driven by fertilization. The data comprise the pooled results of five independent experiments.

Discussion

MI oocytes that were cultured for a further 4 hours after IVM in fertilization medium without spermatozoa did not complete maturation, but when cultured with spermatozoa, a significant proportion completed maturation to MII (Table 3, Figure 1). The spontaneous maturation rate (2.1%) of the MI oocytes further cultured for up to 20 hours was not significantly different from that of MI oocytes cultured for 4 hours (0.7%). In addition, most MI oocytes incubated for a further 20 hours showed a tendency to degenerate when sperm was introduced (data not shown). Additionally, the oocytes arrested at the GV stage remained arrested after sperm insemination (data not shown). Chian et al. [7] reported similar results for bovine oocytes; only oocytes that reached MI or beyond were stimulated by sperm penetration to complete meiotic maturation, whereas oocytes arrested at the stages before MI remained arrested after sperm penetration. Overall, these results indicated that MI oocytes after IVM were completely arrested at the MI stage, and that sperm could induce the resumption of meiosis or the MI-to-MII transition in MI-arrested oocytes.

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Strassburger et al. [12] reported a higher fertilization rate in human IVM MII oocytes after intracytoplasmic sperm injection than in arrested IVM MI oocytes (44% vs. 33%). However, in the present study, the fertilization rate of oocytes arrested in MI was not significantly different from that of those in MII (80.0% vs. 78.2%). Likewise, although the data are not presented here, the fertilization rate in each group showed a tendency similar to the maturation rate from MI to MII. If the fertilization rate was low, the rate of maturation from MI to MII likewise tended to be low. This suggests that sperm penetration affected the progression of oocyte maturation from MI to MII. Despite similar fertilization rates, the rate of blastocyst formation and the number of total cells were significantly higher in the MII group than in the MI group (Table 3). Moreover, no pregnancy was obtained from the MI group (Table 4). This may have been because of their lower developmental competence than the MII group. The reduced developmental competence of MI-arrested oocytes can be further explained by their nuclear and cytoplasmic immaturity. Liu et al. [4] reported that cytoplasmic immaturity decreased the blastocyst rates and cell number by retarding cell proliferation, which may influence post-implantation development and the low efficiency of animal production after ET. Emery et al. [13] suggested that incomplete nuclear maturation and cytoplasmic maturation were associated with poor development and increased embryonic aneuploidy. Their results indicated that nuclear and cytoplasmic immaturities played a role in low developmental competence. Nevertheless, Strassburger et al. [12] demonstrated that human oocytes arrested in MI were fertilized and developed to a healthy neonate with a normal karyotype, although no pregnancy was obtained from MI-arrested oocytes in this study. Further studies are needed to fully understand the physiological and cellular mechanisms involved in the nuclear and cytoplasmic maturation of mammalian oocytes activated by spermatozoa. Additionally, the reason for the failure to achieve pregnancy should be determined. The causes may include problems with cytoplasmic immaturity, oocyte aneuploidy, or differences between humans and mice.

To generate the Ca^{2+} oscillations, a fertilizing spermatozoon introduces phospholipase C-zeta, a sperm-specific isoform of phospholipase C, which activates oocytes [14]. Diffusion of phospholipase C-zeta into the oocytes triggers oscillations of the cytoplasmic free Ca^{2+} concentration in the oocytes. Kline and Kline [15] reported that incubation of oocytes in >1.0 μg of BAPTA-AM prior to *in vitro* fertilization blocked the Ca^{2+} oscillations transiently, so that the oocytes remained at the MII stage and second PB formation did not occur. In the present study, to evaluate the role of Ca^{2+} oscillations derived from sperm during the MI-to-MII transition, fertilization profiles were analyzed after treating MI-arrested oocytes with 1 μg and 10 μg of a Ca^{2+} chelator. The vast majority of oocytes in the BAPTA-AM-treated groups remained at MI and did not show formation of the first PB. These findings indicate that treatment with BAPTA-AM was sufficient to block the intracellular Ca^{2+} oscillations. Moreover, Ca^{2+} oscillations derived from sperm play an important role in overcoming oocyte arrest after IVM.

Ca^{2+} plays important roles in oocyte physiology, from oogenesis to maturation and fertilization [3,16]. Murnane and DeFelice [17] reported that plasma membrane Ca^{2+} currents selectively increased in growing oocytes and that intracellular or plasma membrane Ca^{2+} currents may mediate the onset of murine oocyte maturation. Lee et al. [18] also showed that GV and GVBD-arrested oocytes had some defects in Ca^{2+} channel expression or translation. These results suggest that oocytes develop the ability to generate sperm-induced Ca^{2+} oscillations during meiotic maturation. This requires several cytoplasmic changes, such as reorganization of the endoplasmic reticulum (ER), an increase in the quantity of Ca^{2+} ions stored in the ER, and the redistribution of ER Ca^{2+}-binding proteins [14].

The ER is the major Ca^{2+} storage organelle in oocytes. In GV oocytes, the ER forms a fine patch-like network within the cytoplasm [19,20]. At the time of transition from MI to MII, the patch-like ER structure disappears and a distinctive ER cluster forms in the cortical region of the oocyte. FitzHarris et al. [20] reported that ER clusters in the oocyte cortex formed independently of meiotic progression to MII, based on their finding that oocytes spontaneously arrested in MI displayed cortical ER clusters similar to those present in MII eggs. In addition, several ER proteins, such as calreticulin and calnexin, function as Ca^{2+}-binding molecules and as molecular chaperones. The distribution of these proteins changes during the maturation of mammalian oocytes. Balakier et al. [21] reported that calreticulin in human MI oocytes forms patch-like accumulations in the cortex, similar to those observed in MII oocytes. Additionally, calnexin has been found to form three distinct cortical zones in GV oocytes, whereas the three zones of cortical calnexin were redistributed into a single layer in MI/MII oocytes. Similarly, Eppig et al. [22] reported that MI-arrested oocytes exhibited a protein synthesis pattern similar to that of MII-arrested oocytes. Therefore, they concluded that some critical aspects of cytoplasmic maturation can occur in oocytes whose nuclear maturation is arrested at MI. Furthermore, Mehlmann and Kline [19] reported that, similar to mature oocytes, immature mouse oocytes were capable of producing repetitive Ca^{2+} transient oscillations when fertilized. Moreover, Jones et al. [23] found that oocytes spontaneously arrested in MI that were unable to achieve MII even after prolonged culture produced continuous Ca^{2+} oscillations similar to those generated in fertilized MII oocytes. These results suggest that the development of the ability of oocytes to generate long-lasing Ca^{2+} oscillations is more dependent on cytoplasmic changes than on the progression of meiosis.

These previous reports led us to hypothesize that some critical aspects of cytoplasmic maturation occur in oocytes whose nuclear maturation is arrested at MI. To confirm this, GV-arrested oocytes and MI-arrested oocytes after IVM were fertilized, and then their maturation rates were compared (data not shown). The GV-arrested oocytes did not proceed to the MII stage, and most remained arrested at GV, whereas higher proportions of the MI-arrested oocytes progressed to the MII stage. These findings suggest that some cytoplasmic changes, such as ER reorganization and the redistribution of ER Ca^{2+}-binding proteins, are completed in MI-arrested oocytes, similarly to MII oocytes, and can generate sperm-induced Ca^{2+} oscillations.

The ability of oocytes to undergo the exocytosis of cortical granules in response to Ca^{2+} occurs between MI and MII [24], and is critical for the establishment of the zona block to polyspermy [25]. The fertilization of GV oocytes is frequently associated with polyspermy, possibly due to their inability to undergo cortical granules exocytosis and to mount a zona block [11]. Therefore, a preliminary study was conducted to compare the proportions of abnormal fertilization between MI-arrested oocytes and MII oocytes. No significant difference in the rate of normal fertilization was found between the two groups (Table 1). Additionally, the rates of 1PN and 3PN formation were not significantly different from each other. These data are consistent with those obtained by Strassburger et al. [12] and Shu et al. [26], demonstrating that arrested human MI oocytes exhibited abnormal fertilization patterns similar to those of MII oocytes. It is suggested that the increase in Ca^{2+} after fertilization in MI-arrested oocytes is sufficient for cortical granule exocytosis, which is responsible for the alteration of the zona pellucida that establishes the zona block to polyspermy.

In conclusion, it was found in this study that oocytes arrested in MI after IVM were activated following fertilization and developed to blastocysts, but their developmental competence was lower than that of MII oocytes. Additionally, the maturation of MI to MII oocytes is associated with the intracellular Ca^{2+} oscillation driven by fertilization. Further studies are needed to fully understand the mechanisms of overcoming MI arrest by Ca^{2+}. Such studies will be helpful for overcoming oocyte arrest and thus for future IVM practice.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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