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REVIEW

Behaviour of cytoplasmic organelles and cytoskeleton during oocyte maturation

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Abstract Assisted reproduction technology (ART) has become an attractive option for infertility treatment and holds tremendous promise. However, at present, there is still room for improvement in its success rates. Oocyte maturation is a process by which the oocyte becomes competent for fertilization and subsequent embryo development. To better understand the mechanism underlying oocyte maturation and for the future improvement of assisted reproduction technology, this review focuses on the complex processes of cytoplasmic organelles and the dynamic alterations of the cytoskeleton that occur during oocyte maturation. Ovarian stimulation and in-vitro maturation are the major techniques used in assisted reproduction technology and their influence on the organelles of oocytes is also discussed.

KEYWORDS: assisted reproduction technology, cytoplasmic organelles, cytoskeleton, oocyte maturation, oocyte quality, fertilization

Introduction

In recent decades, assisted reproduction technologies such as in-vitro maturation (IVM), in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) have become attractive options for infertility treatment. However, at present, further studies are needed to improve the success rates of assisted reproduction technology. According to the 2010 assisted reproduction technology success rates report by the Centers for Disease Control and Prevention (Centers for Disease Control and Prevention et al., 2012), 147,260 assisted cycles were performed at 443 reporting clinics in the USA during 2010, resulting in only 47,090 live births (deliveries of one or more living infants) and 61,564 infants. Therefore, a great challenge for researchers is to find ways to improve assisted reproduction technology success rates.

Previous studies have indicated that reduced oocyte developmental competence is a primary reason for the

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reduced potential of in-vitro-produced embryos (Sirard et al., 2006; Watson, 2007). One hypothesis is that the process of cytoplasmic maturation is disturbed by ovarian stimulation or oocyte maturation *in vitro*, leading to the abnormal behaviour of cell organelles (De los Reyes et al., 2011; Lee et al., 2006a; Zeng et al., 2009). Therefore, improved knowledge of the mechanism of both nuclear and cytoplasmic maturation in the oocyte is necessary for the advancement of assisted reproduction technology.

Oocyte maturation is a long process that includes both nuclear and cytoplasmic maturation. Nuclear maturation mainly involves chromosome segregation, which has been well studied. Additionally, cytoplasmic maturation involves organelles that reorganize and store the mRNA, proteins and transcription factors required for oocyte maturation, fertilization and early embryogenesis (Watson, 2007). Cytoplasmic maturation is complex, and there are still many parts of this process that remain controversial. The proper spatial and temporal dynamics of organelles and the cytoskeleton ensures that the oocyte acquires the high developmental potency required for fertilization and subsequent embryo development (Sirard et al., 2006). Proper modification of the localization, morphology and biochemical properties of organelles must occur for the oocyte to acquire high developmental potency. Due to advances in modern experimental techniques, such as electron microscopy and immunofluorescence with different fluorescent dyes, the redistribution and morphological changes of organelles have been extensively studied.

Reorganization of cytoplasmic organelles

Mitochondria

Redistribution

Cytoplasmic maturation involves a series of complex events, including protein synthesis and transcription of cytoplasmic RNA, which consume energy. The primary function of mitochondria is to synthesize ATP. Therefore, mitochondria play an extremely important role in supplying the energy that is consumed during the maturation process (Krisher and Bavister, 1998; Stojkovic et al., 2001).

The movement of mitochondria to areas of high energy consumption is crucial for oocyte maturation (**Figures 1 and 2**). In the immature mouse oocyte, mitochondria are aggregated around the germinal vesicle (GV). The mitochondria move away from the perinuclear region at germinal vesicle breakdown (GVBD) and occupy most of the egg volume in a mature meiosis-II (MII) oocyte (Dumollard et al., 2006). An additional change in the mitochondrial distribution, involving a change in the size of mitochondrial clusters, was observed in maturing mouse oocytes. Upon GVBD, only 5% of oocytes show small mitochondrial clusters, whereas most mitochondrial clusters are larger. In contrast, at 2 h and 8 h after GVBD, a large proportion of oocytes contain mitochondria that form smaller clusters throughout the cytoplasm (Yu et al., 2010).

In GV human oocytes (**Figure 1**), mitochondria are predominantly spherical to oval with dense matrices and few arch-like or transverse cristae presenting an inert appearance (Sathananthan et al., 2006). At this stage, mitochondria are usually absent from the cortical part of the cytoplasm (Familiari et al., 2006; Sathananthan and Trounson, 2000). Mitochondria in MI and MII oocytes become even more numerous and are dispersed in the ooplasm (Motta et al., 2000; Sathananthan and Trounson, 2000). Unlike other species, mitochondria of human oocytes form voluminous aggregates with smooth endoplasmic reticulum (SER) tubules and vesicles at the end of the maturation process (Familiari et al., 2006; Motta et al., 2000; Sathananthan and Trounson, 2000. These mitochondrial-SER aggregates and the mitochondrial-vesicle complexes could be involved in the production of a reservoir of substances or membranes anticipating subsequent fertilization and early embryogenesis (Motta et al., 2000).

ATP content and oocyte quality

The distribution and organization of mitochondria are dynamic during oocyte maturation, and these changes may be related to the function of mitochondria. The main function of mitochondria is to provide ATP. Therefore, many research groups are interested in the relationship between the reorganization of mitochondria and ATP concentration. Indeed, previous studies have indicated that the reorganization of mitochondria is associated with ATP content in bovine oocytes (Stojkovic et al., 2001).

Yu et al. (2010) demonstrated, for the first time as far as is known, the ATP dynamics during maturation in a living mouse oocyte, thus providing additional evidence of the relationship between mitochondrial organization and ATP concentration. The rate of mitochondrial ATP production changes during spontaneous mouse oocyte maturation. Compared with GV-arrested oocytes, there are three distinct increases in cytosolic and mitochondrial ATP concentrations during oocyte maturation, including three phases of higher ATP production and two phases of lower ATP production. The first phase of increased ATP production occurs around the time of GVBD. The second phase occurs during the longer phase of spindle migration and the third phase occurs during the meiosis I (MI) to MII transition. This study also demonstrated that the clustering pattern correlates with the timing of ATP pulses. Specifically, it appeared that the formation of large clusters of mitochondria is associated with increased ATP production. In addition, disruption of the mitochondrial clusters by cytochalasin B treatment results in an inhibition of the bursts of ATP production (Yu et al., 2010). These results suggest that the clustering pattern correlates with bursts of ATP production.

Due to the critical role of energy metabolism in oocyte maturation, ATP content has been proposed as an indicator for the developmental potential of human (Slotte et al., 1990; Van Blerkom et al., 1995) and mouse oocytes (Leese et al., 1984). ATP is extremely important for nuclear and cytoplasmic maturation events. Spindle formation and chromosome movements depend on the expression and activity of motor proteins, which use ATP as their energy source. Previous studies demonstrated that an increase in ATP concentration is necessary for both bovine and human oocyte maturation (Duran et al., 2011; Nagano et al., 2006). Oocytes with higher concentrations of ATP have significantly higher fertilization and blastocyst rates (Nagano et al., 2006; Stojkovic et al., 2001). Lower ATP concentrations of

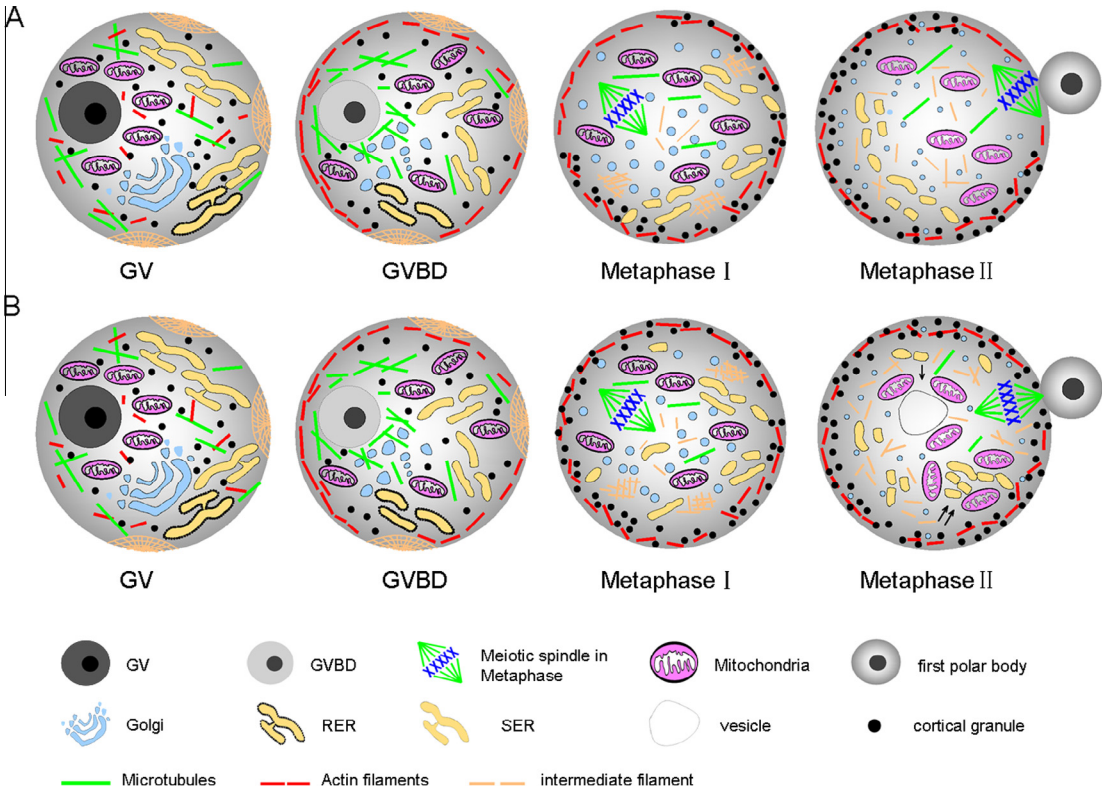

Figure 1 Overview of the distribution of cytoplasmic organelles and the cytoskeleton during oocyte maturation in mouse (A) and human (B). (A) In GV oocytes, mitochondria are aggregated around the GV, the Golgi apparatus is slightly more concentrated in the interior than at the cortex and the intermediate filaments are confined to large cortical aggregates, while the ER, cortical granules, microtubules, and microfilaments are uniformly distributed throughout the cytoplasm. Upon GVBD, mitochondria move away from the perinuclear region, the Golgi apparatus is fragmented and aggregated in the central part of the oocyte, the ER localizes in cortical regions and microtubules are condensed around the chromosomes, while microfilaments are densely accumulated in the subcortical region of the oocytes. At metaphase I, the Golgi apparatus is further fragmented and dispersed throughout the oocyte, the ER is concentrated in the vegetal half of the mature egg and cortical granules migrate towards the cortical cytoplasm and arrest in the cortex, while the large aggregates of intermediate filaments disperse into multiple small spots; in addition, microtubules are observed as fully organized meiotic spindles. At metaphase II, the first polar body has been extruded, spindles have formed below the first polar body and intermediate filaments are evenly distributed throughout the cytoplasm. (B) In metaphase-II oocytes, the spindle often orients perpendicular to the oocyte surfacem mitochondrial-SER aggregates (arrow) and mitochondrial-vesicle complexes (double arrow) are seen, ER clusters are present throughout the entire oocyte and cortical granules, which are usually absent in the mouse, are present over the spindle region. GV = germinal vesicle; GVBD = germinal vesicle breakdown; RER = rough endoplasmic reticulum; SER = smooth endoplasmic reticulum. (For interpretation of the references to colors in this figure legend, the reader is referred to the web version of this paper.)

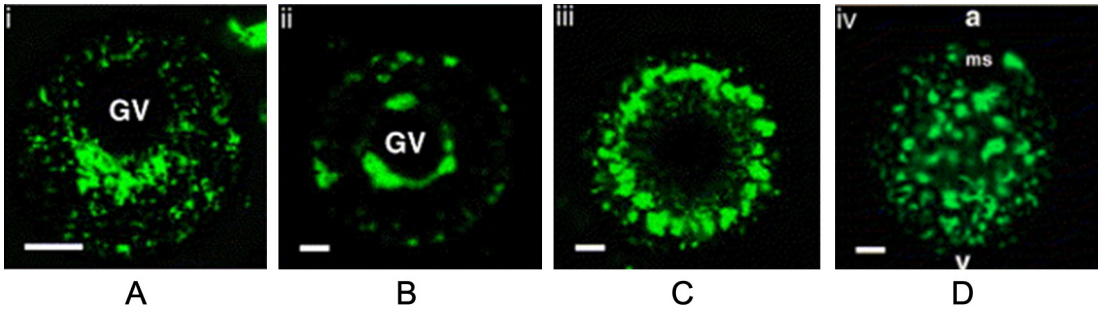

Figure 2 Mitochondrial distribution during oogenesis in the mouse: meiotically incompetent oocyte (A); fully grown GV-stage oocyte (B); maturing oocyte immediately following GV breakdown (C); and mature metaphase-II-arrested oocyte (D). Bars = 10 μm. GV = germinal vesicle; a = animal pole; ms = meiotic spindle; v = vegetal pole (reproduced from Dumollard et al., 2006, with permission of the publisher).

oocytes are at least partially responsible for decreased rates of positive spindle formation in IVM mammalian oocytes (Eichenlaub-Ritter et al., 2004; Zeng et al., 2007). Reducing ATP content by treatment with carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, an inhibitor of oxidative phosphorylation, results in a reduced percentage of mouse oocytes with nuclear maturation, normal spindle formation and chromosome alignment, evenly distributed mitochondria and an ability to form blastocysts (Ge et al., 2012b).

Copies of mitochondrial DNA during maturation

The number of mitochondria present in the cytoplasm of mammalian oocytes varies according to the developmental stage of the cell. During the pre-migratory stage of germ cells, the number of mitochondria is approximately 10, increasing to 200 in the oogonium stage. Primary oocytes contain approximately 6000 mitochondria and this number increases to more than 100,000 copies of mitochondrial DNA (mtDNA) during oocyte maturation, with one or two copies of mtDNA per organelle (Ferreira et al., 2009). However, the functional significance of high mtDNA copy number in oocytes remains unclear. According to previous studies, there are two possible explanations for the high mtDNA copy number.

First, it has been proposed that the increase in mitochondria and mtDNAs during oogenesis is a genetic mechanism to ensure that a sufficient number of organelles and genomes are present in the cells until implantation because mtDNA replication does not occur during the cleavage stages of embryogenesis in mammalian oocytes (Shoubridge and Wai, 2007). To investigate the effects of mtDNA copy number on fertility, Wai et al. (2010) genetically manipulated mtDNA copy number in a mouse model by deleting one copy of *Tfam*, an essential component of the mitochondrial nucleotide, at different stages of germ line development. They found that the mature oocyte, which has less than 4000 copies of mtDNA, can still be fertilized and progress normally from preimplantation development to the blastocyst stage, but there is a dramatic reduction in post-implantation embryonic viability in embryos with a low copy number of mtDNA ($<$50,000 copies of mtDNA). Inhibition of mtDNA replication with 2′,3′-dideoxycytidine, a nucleoside analogue that can inhibit mtDNA replication, has no detectable effect on mouse oocyte maturation and mitochondrial distribution. However, 2′,3′-dideoxycytidine does reduce blastocyst formation significantly (Ge et al., 2012b). Furthermore, in a porcine model, supplementation of neuregulin 1 stimulates mitochondrial replication, increases the number of mtDNA copies in MII oocytes compared with GV oocytes and increases the percentage of blastocysts in in-vitro-fertilized embryos (Mao et al., 2012). These findings suggest that the number of mtDNA copy mainly affects embryonic development potential but has little effect on oocyte maturation and IVF.

Second, the large number of mtDNA may decrease the accumulation of mutations in mtDNA (Cree et al., 2008), which is a small, maternally inherited genome. Variants segregate as early as the first mitotic division of germinal cell precursors and are then rapidly transmitted to future generations. Analysis of the distribution of pathogenic mtDNA mutations in the offspring of carrier mothers in mice shows that the chances of inheriting a pathogenic mutation increase based on the proportion of these mutations in the mother, but there is no strong selection against this type of mutation (Cree et al., 2008). Based on this concept, it was proposed that the large amount of mtDNA proportionately decreases the amount of mtDNA variants in the mother, thus effectively decreasing the chances that progeny will inherit a pathogenic mutation.

Golgi apparatus

Fragmentation

The Golgi apparatus plays a central role in many intracellular trafficking events related to protein modification and delivery (**Figure 1**). In GV mouse oocytes, the Golgi apparatus is dispersed throughout the ooplasm in the form of a continuous membranous system, but it is slightly more concentrated in the interior than at the cortex (Moreno et al., 2002. Upon GVBD, the nuclear envelope is dismantled, the chromosomes condense and the Golgi apparatus undergoes fragmentation, as shown by an accumulation of dotted structures in the central part of the oocyte (Moreno et al., 2002). The Golgi apparatus is further fragmented and dispersed throughout the oocyte in MI, and this distribution is maintained following extrusion of the first polar body in MII oocytes (Moreno et al., 2002).

Electron microscopy studies show typical Golgi complexes consisting of smooth-surfaced cisternae, and vesicles are usually seen at the GV stage in both human and mouse oocytes, but they are rarely found in MII oocytes and cortical granules have been shown to originate from Golgi mem-

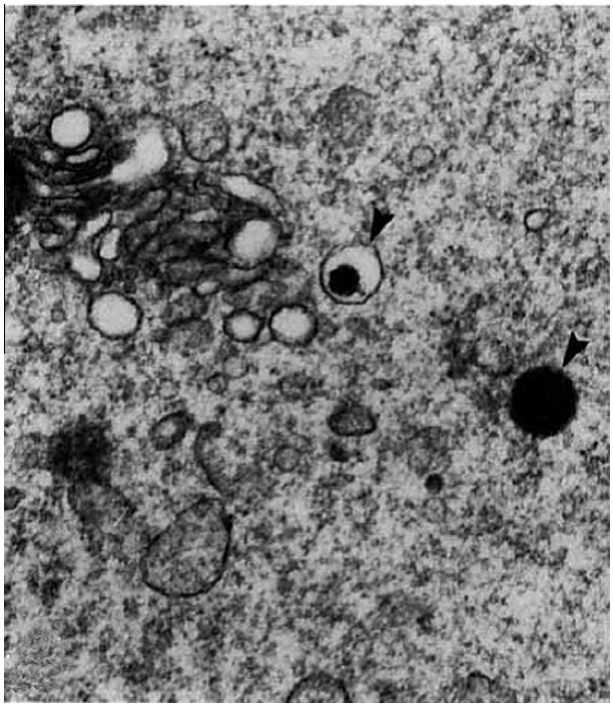

Figure 3 Golgi complex associated with developing cortical granules (arrowheads) in a human GV oocyte (transmission electron microscopy, 35,700×) (reproduced from Sathananthan 1994, with permission of the publisher).

branes in human oocytes (**Figure 3**; Sathananthan and Trounson, 1985), where two peaks of Golgi activity are reported in oocytes recovered from small antral follicles and in GV oocytes during cortical granule formation.

Incubation with specific inhibitors revealed that microtubules plays an active role in the final localization at GVBD, while cyclin-dependent kinase 2A is essential for Golgi fragmentation at GVBD stage in bovine oocytes (Racedo et al., 2012). Notably, GM130, a marker of the Golgi apparatus, colocalizes with Sec23, a marker for endoplasmic reticulum (ER) vesicle export sites, suggesting that the Golgi apparatus is not only fragmented, but it is also translocalized into the ER during bovine oocyte maturation (Payne and Schatten, 2003). Contrary to multiple reports, the dynamics of the Golgi membranes, the mechanism of Golgi membrane reorganization into the ER and the significance of Golgi disassembly during oocyte maturation in mammals remain controversial.

Function

To investigate the function of Golgi apparatus, brefeldin A, a membrane-trafficking inhibitor, was employed in GV oocytes. Brefeldin A blocks membrane trafficking from the ER to the Golgi apparatus, leading to Golgi disassembly and recycling to the ER. However, the function of the Golgi apparatus in oocyte maturation varies during different stages. In the GV stage, mouse oocytes cultured with brefeldin A are blocked at maturation upon GVBD and before the assembly of the MI spindle (Moreno et al., 2002). In contrast to what was observed in mouse GV oocytes, bovine zygotes cultured in the presence of brefeldin A are able to complete cytokinesis and divide up to the 8-cell stage (Payne and Schatten, 2003). These results indicated that intact, functional Golgi membranes are required for GVBD, and after that, the Golgi apparatus is fragmented and is no longer essential for oocyte maturation and zygotic cleavage until the 8-cell stage.

The Golgi apparatus is also known to be a platform for numerous cytoplasmic proteins with diverse cellular roles. Many proteins have been proven to have unexpected roles in the control of cell polarization, migration and division (Colanzi and Corda, 2007). However, most of the previous studies have focused primarily on mitotic cells, and the role of such proteins in mammalian meiosis remains unknown. GM130, a *cis*-Golgi protein, plays key roles in various mitotic events, but its function in mammalian oocyte meiosis remains unknown. A recent study found that GM130 localizes to the spindle poles at both MI and MII stages and is associated with the midbody at telophase I (Zhang et al., 2011). Their results also suggested that GM130 regulates microtubule organization and plays a role in spindle organization, migration and asymmetric division during mouse oocyte maturation (Zhang et al., 2011).

Endoplasmic reticulum

Redistribution and structural changes

The ER functions in protein folding and degradation and lipid metabolism, and it is the major internal store of calcium ions. The ER undergoes redistribution and structural changes during oocyte maturation (**Figures 1 and 4**). In-vivo

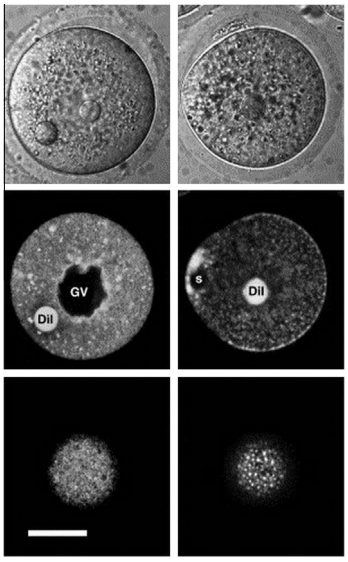

Figure 4 DiI-labelled endoplasmic reticulum in mouse GV and MII oocytes. Oocytes were microinjected with DiI as a solution in soyabean oil and examined using confocal microscopy. Typical examples of an oocyte at GV (left) and MII (right). Bright-field (top), equatorial (middle) and cortical (bottom) fluorescence confocal slices are shown. At GV stage (left) the ER is arranged as a network of small accumulations throughout the cytoplasm. At MII (right) the ER extends throughout the cytoplasm in a more uniformly reticular manner, but there is no labelling in the area where the spindle resides in the animal cortex. In the cortical confocal slices (bottom), distinctive clusters of ER 1–2 μm in diameter present in MII oocytes, but not in GV oocytes. The presence of ER clusters in the oocyte cortex causes the outer edge of the equatorial slice to appear more strongly labelled in MII oocytes (middle). Note that the DiI-containing oil droplet is visible in both images (DiI). The germinal vesicle (GV) and regions containing the MII spindle(s) are also indicated. Bar = 30 μm. DiI = dialkyl indocarbocyanine (reproduced from FitzHarris et al., 2007, with permission of the publisher).

analyses of mouse oocytes at the GV stage showed that the ER is uniformly distributed throughout the ooplasm. However, following maturation to MII, the ER localizes in the cortical regions and accumulates in small 1–2-μm-wide clusters throughout the cytoplasm. Such accumulations are concentrated in the vegetal half of the mature egg, but they are noticeably absent in the region of the animal hemisphere directly surrounding the meiotic apparatus (**Figures 1 and 4**; FitzHarris et al., 2007; Kline, 2000; Mehlmann et al., 1995).

Monitoring ER structure after mouse oocyte activation has demonstrated that ER clusters disappear at the completion of MII and do not return in the first mitotic division (FitzHarris et al., 2007). The distribution of ER in human GV-stage oocytes is similar to that in mouse oocytes. However, in MII human oocytes, ER clusters are present not only in the cortex but also throughout the entire oocyte, with no apparent polarity in relation to the meiotic spindle (Mann et al., 2010). The SER of human oocytes exists in two forms: the isolated vesicular SER, which is evenly distributed in the

ooplasm, and large peripheral aggregates of smaller elements of SER, which are tubular or irregular in shape at the electron microscopy level (Motta et al., 2003; Sathananthan et al., 1988). These aggregates increase in content during preovulatory maturation (Sathananthan et al., 1988). Unlike the SER, the rough endoplasmic reticulum is absent in mature oocytes because ribosomes are very sparse (Motta et al., 2003; Sathananthan et al., 1988).

Ca^{2+} release and fertilization

Based on previous studies, in all animals, oocytes must undergo a proper calcium response during fertilization in order for fertilization and subsequent development to proceed normally (Bootman et al., 2002; Machaca, 2007). Because the ER is the major intracellular Ca^{2+} store, it plays an extremely important role in intracellular signalling by storing and releasing calcium. Oocytes of different species arrest at disparate stages of the cell cycle, and thus the fertilization-induced Ca^{2+} signal takes the form of single or multiple transients, depending on the species. For example, in jellyfish, sea urchin and *Xenopus*, a single Ca^{2+} wave is observed at fertilization (Stricker, 1999). In contrast, in annelids, ascidians and mammals, multiple Ca^{2+} transients can be detected (Stricker, 1999).

Regardless of differences in species, Ca^{2+} signals arise due to either Ca^{2+} release from the ER or Ca^{2+} influx from the extracellular milieu. The release of Ca^{2+} from the ER is mediated through either the inositol 1,4,5-trisphosphate (IP3) receptor or the ryanodine receptor, both of which are ligand-gated ion channels that localize to the ER membrane (**Figure 5**; Machaca, 2004). It has been well established in *Xenopus* eggs that the affinity of the IP3 receptor increases during egg maturation and that Ca^{2+} release via IP3 and its receptor is essential for oocyte activation during fertilization (El-Jouni et al., 2005; Machaca, 2004). A recent study revealed that phosphorylation of IP3 receptor increases its sensitivity, which releases Ca^{2+} to the cytoplasm, transiently increasing Ca^{2+} concentration during mouse oocyte maturation (Wakai et al., 2012). IP3 receptor is involved in the initiation and maintenance of Ca^{2+} spikes at fertilization (Malcuit et al., 2006; Whitaker, 2006).

However, the maintenance of Ca^{2+} spikes in mammalian oocytes also requires Ca^{2+} influx from the extracellular milieu. The regulation of the Ca^{2+} channels involved in this process is not well studied in oocytes. STIM1 is a single transmembrane protein with a Ca^{2+} sensor domain located in the luminal space of the ER (**Figure 5**). It senses imperceptible changes in Ca^{2+} concentration stored within the ER lumen (Williams et al., 2002). Upon ER depletion, STIM1 aggregates and activates a series of calcium channels in the plasma membrane (Zhang et al., 2005). A recent study found that there is an up-regulation of STIM1 expression at GVBD and that this expression remains steady during subsequent maturation stages of mouse oocytes (Gomez-Fernandez et al., 2012; Lee et al., 2012). Their findings suggest that STIM1 is essential for normal fertilization, as it is involved in the maintenance of the long-lasting repetitive Ca^{2+} signal (Gomez-Fernandez et al., 2012; Lee et al., 2012).

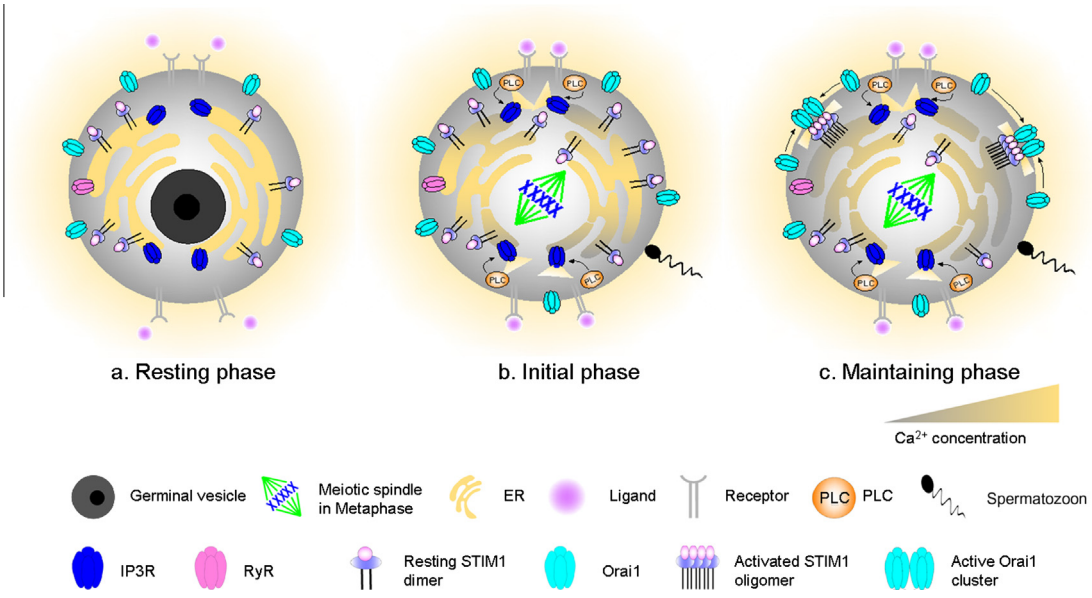

Figure 5 Regulation of Ca^{2+} channels during fertilization. (a) Resting phase: before fertilization, the cytosolic Ca^{2+} of the oocytes is in homeostasis and immature oocytes show no ligand binding to PLC-coupled receptors and no activation of STIM1 and Orai1. (b) Initial phase: the initial Ca^{2+} spike is triggered by fertilization and, after spermatozoon stimulation, the ligand binds to PLC-coupled receptors, and this activity initiates the phosphoinositide signalling pathway; consequently, the released IP3 diffuses rapidly within the cytosol to interact with ER-localized IP3R, which is a channel that releases Ca^{2+} from the ER lumen to generate the initial Ca^{2+} signalling phase. (c) Maintenance phase: Ca^{2+} influx from the extracellular milieu is involved in the maintenance phase, and, following depletion of ER Ca^{2+} (b), STIM1 is activated and translocates by diffusion into the ER–plasma membrane junctions where it interacts with the plasma membrane; here, STIM1 activates plasma membrane-located Orai1, which is a channel that releases Ca^{2+} from the extracellular milieu to maintain the Ca^{2+} signal. ER = endoplasmic reticulum; IP3R = inositol 1,4,5-trisphosphate receptor; PLC = phospholipase C; RyR = ryanodine receptor.

Orai1, a plasma-membrane-resident Ca^{2+} channel, activated by STIM1, has recently been identified in porcine oocytes and is another important molecule responsible for mediating Ca^{2+} entry from the extracellular milieu following the mobilization of intracellular Ca^{2+} (**Figure 5**; Wang et al., 2012). Orai1 is also required to maintain Ca^{2+} oscillations at fertilization and to support proper embryo development in mammalian oocytes (Wang et al., 2012).

Biochemical and structural changes in the ER during maturation are crucial for proper functioning of intracellular calcium regulation. One of the functions of the Ca^{2+} signal is to block polyspermy through a fast and a slow block, each of which is critically dependent on Ca^{2+} (Machaca, 2007). The initial Ca^{2+} rise at fertilization gates Ca^{2+}-activated chlorine (Cl) channels, which depolarize the egg membrane (fertilization potential) and block further spermatozoon entry, as shown in *Xenopus*, in which spermatozoon fusion is voltage-dependent (Jaffe et al., 1983). The slow block is a Ca^{2+}-signal-induced fusion of cortical granules that provides a long-term blockage of polyspermy (Wolf, 1974). As already mentioned, the ER localizes in cortical regions and accumulates in small 1–2-μm-wide clusters throughout the cytoplasm during oocyte maturation. This reorganization of the ER brings the Ca^{2+} source close to its primary targets in terms of the block to polyspermy, with Ca^{2+}-activated Cl channels at the cell membrane and cortical granules in the egg's cortex. Clearly, because IP3 receptor localizes to the ER membrane, the translocation of the ER will directly alter the localization of Ca^{2+}. However, the contribution of ER reorganization to Ca^{2+} signalling differentiation during maturation requires further study.

Cortical granules

Mammalian cortical granules are derived from Golgi complexes during oocyte growth (Austin, 1956; Gulyas, 1980). During early follicular growth, Golgi complexes undergo hypertrophy and proliferation, and the formation of cortical granules from Golgi complexes occurs at this stage (Abbott and Ducibella, 2001; Gulyas, 1980; Szollosi, 1967).

At first, small vesicles are formed from hypertrophied Golgi complexes that migrate towards the subcortical region of the oocytes (**Figure 1**). These vesicles then coalesce to form mature cortical granules that eventually separate from the Golgi complexes (Gulyas, 1980). Mammalian cortical granules range in diameter from 0.2 μm to 0.6 μm and appear morphologically similar to each other at the ultrastructural level (Gulyas, 1980). Cortical granules are randomly dispersed throughout the cytoplasm of immature oocytes and migrate towards the cortical cytoplasm during meiotic maturation in porcine (Yoshida et al., 1993) and bovine (Hosoe and Shioya, 1997) models. At the end of maturation, when these oocytes reach the MII stage, the cortical granules arrest in the cortex (Hosoe and Shioya, 1997; Yoshida et al., 1993). Cortical granules undergo exocytosis to release their contents into the perivitelline space immediately after fertilization (Hosoe and Shioya, 1997; Yoshida et al., 1993). This secretory process is calcium dependent and is known as the cortical reaction (Schuel, 1978).

A study of human oocytes showed that cortical granules are also present over the spindle region, and they are usually absent in the mouse (**Figure 1**; Ducibella et al., 1988). Furthermore, cortical granules in human are more abundant compared with mouse (Sathananthan, 1994).

Several lines of evidence show that cortical granule migration is a cytoskeleton-dependent process, and microfilaments are required for cortical translocation in both non-mammalian and mammalian animal models, such as sea urchin, pig, human and mouse (Connors et al., 1998; Kim et al., 1996; Liu et al., 2010; Wessel et al., 2002). Once granules are positioned at the cortical area of the oocyte, they dissociate from the cytoskeleton structures and remain in the cortex (Ducibella, 1996; Wang et al., 1997).

Most vesicle-docking and membrane-fusion events are mediated by two classes of proteins, known as v- and t-SNAREs (soluble NSF-attachment protein receptors) (Fasshauer et al., 1998; Weber et al., 1998). Recent work in porcine oocytes revealed that the t-SNARE protein SNAP-23 and the v-SNARE protein VAMP1 are involved in docking cortical granules to the oolemma, and interactions between these SNARE proteins, as well as with the molecule complexin, are responsible for arresting cortical granules in the cortex of oocytes prior to exocytosis (Tsai et al., 2011). The trigger for the cortical reaction is the fusion of the spermatozoon plasma membrane with the oolemma. The fertilization-induced Ca^{2+} signal induces the fusion of cortical granules with the oolemma over the entire oocyte's surface (Miyazaki, 2007). The released cortical granule proteins are responsible for blocking polyspermy by modifying the oocyte's extracellular matrix, such as the zona pellucida in mammals, the vitelline envelope in echinoderms and the fertilization layers in amphibians (Cran and Esper, 1990; Schuel, 1978; Sun, 2003).

Reorganization of the cytoskeleton

Redistribution

Eukaryotic cells contain three main types of cytoskeletal filaments: microfilaments, intermediate filaments and microtubules. The cytoskeleton is a dynamic and adaptable structure that can remain unchanged or undergo modification according to the needs of the cell. The three filament types of the cytoskeleton are formed by specific subunits. Microtubules consist of globular and compacted tubulin subunits, whereas microfilaments consist of similarly globular and compacted actin subunits. Intermediate filaments consist of elongated and fibrous polypeptide subunits arranged in a tetramer analogous to the α- and β-tubulin subunits in the microtubule and the actin monomer. These three types of cytoskeletal ''polymers'' are maintained by weak, noncovalent interactions and can rapidly associate and dissociate without the need for new formations or breakage of covalent bonds (Theurkauf et al., 1992).

Microtubules display a relatively uniform distribution at the GV stage in mouse oocytes (**Figure 1**). Similarly, microfilaments are scattered throughout the ooplasm (Sun and Schatten, 2006). After GVBD, microtubules are condensed around the chromosomes and begin to migrate to the cortex, while microfilaments are densely accumulated in the subcortical region of oocytes, especially around the meiotic spindle of the oocyte. In MII-stage mature mouse oocytes,

microtubules and microfilaments mainly accumulate in the cortical cytoplasmic region (Gumus et al., 2010; Sun and Schatten, 2006; Verlhac et al., 2000). In addition, microtubules are observed as fully organized meiotic spindles that are symmetric, bipolar, barrel shaped and oriented parallel to the oocyte surface (Gumus et al., 2010; Sathananthan, 1994; Verlhac et al., 2000). Similar features of cytoskeletal distribution are observed in the GV human oocyte, while the MII oocyte differs in spindle orientation. The human MII spindle is often oriented perpendicular to the oocyte surface and it is barrel-shaped, anastral and lacks centrioles (Sathananthan, 1994).

Compared with microfilaments and microtubules, the third component of the cytoskeleton, the intermediate filaments, is still poorly understood. Plancha (1996) first described the organization and dynamics of cytoplasmic intermediate filaments during oocyte maturation in hamster. In prophase-I-arrested fully grown hamster oocytes, cytokeratins, the first cytoplasmic intermediate filament protein (Plancha et al., 1989), is confined to 4–10 large cortical aggregates, corresponding to extensive meshworks of intermediate filaments (**Figures. 1 and 6**). These large aggregates disperse into multiple small spots starting at MI until the end of the maturation period at MII, where cytokeratin exhibits a spotted pattern that is evenly distributed throughout the cytoplasm and large patches can no longer be identified (Plancha, 1996). However, meiotic progression to MII is not required for cytokeratin reconfiguration because precociously arrested MI oocytes also exhibit dispersed cytoplasmic foci at the end of the culture period (Plancha, 1996). Redistribution of cytokeratins is insensitive to nocodazole and cytochalasin D, which inhibit microtubules and microfilaments, respectively, suggesting that it occurs independently of microtubules and microfilaments (Plancha, 1996). However, the role of intermediate filaments during mammalian oocyte maturation remains an open question.

Control of the asymmetric divisions of the oocyte

Oocyte meiosis in higher organisms consists of two highly asymmetric divisions, and each division must ensure accurate segregation of the maternal genome and highly asymmetric partitioning of the cytoplasm. The main purpose of the asymmetric divisions is to retain most of the maternal stores in the oocyte for embryonic development. In mouse oocytes, the asymmetry relies on the migration and anchoring of the division spindle to the cortex in MI and by its anchoring to the cortex in MII (Brunet and Verlhac, 2011). Upon entry into the first meiotic division (MI), microtubules form bipolar spindles. During MI, the microtubule spindles are targeted to the oocyte periphery. During the second meiotic division (MII), the microtubule spindles are positioned at the periphery of the oocyte until fertilization triggers the emission of the second polar body (Brunet and Verlhac, 2011).

The reorganization of the spindles is tightly controlled by the microfilament network (Longo and Chen, 1985; Verlhac et al., 2000). However, it was only recently that the microfilament network involved in spindle motility could be visualized in live maturing oocytes using an F-actin-specific probe Utr-GFP, thereby enabling a better understanding of the processes (Schuh and Ellenberg, 2008). Prior to migration, microfilaments form a meshwork consisting of numerous thin filaments. This network is extremely dynamic and constantly remodelled. When spindle migration is initiated, the microfilaments are progressively organized along the spindle and migrate towards the cortex of mouse oocytes (Schuh and Ellenberg, 2008). Although the role of microfilaments in spindle migration is clearly established, the molecular mechanism of the actin network involved in this process is just now being elucidated.

Formin2 (Fmn2), a specific actin filament nucleator, is the earliest identified gene involved in the formation of a dynamic actin meshwork during migration of the MI spindle (Leader et al., 2002). Oocytes from Fmn2-deficient mice have chromosomes that remain centrally located, do not extrude the first polar body and, when fertilized, lead to aneuploid embryos that are not viable (Leader et al., 2002). Microinjection of mRNA encoding the formin homology1 (FH1) and FH2 domains of Fmn2 into $Fmn2^{-/-}$ mouse oocytes partially rescue the defect of polar body extrusion (Kwon et al., 2010). In addition, Fmn2 colocalizes with the spindle during MI (Kwon et al., 2010).

Spire1 and Spire2, two types of actin filament nucleators, are newly identified key factors in MI spindle migration in mouse oocytes (Pfender et al., 2011). Spire1 and Spire2 co-operate with Fmn2 to mediate asymmetric spindle positioning by assembling an actin network that serves as a substrate for spindle movement (Pfender et al., 2011). After the transition from MI to MII, the MII spindle forms rapidly subjacent to the cortex of the oocyte. However, little is known about the mechanisms responsible for anchoring the spindle to the cortex in MII.

Recently, Yi et al. (2011) uncovered an Arp2/3 (actin-related protein 2/3 complex)-dependent mechanism for maintaining the cortical localization of the MII spindle in the mouse oocyte, the suppression of Arp2/3 activity apparently disrupted subcortical spindle positioning during MII arrest (Yi et al., 2011). The findings of Yi *et al*. show that MII chromosomes, through Ran signalling, localize and activate the Arp2/3 complex at the proximal cortex, and that the Arp2/3 complex in turn functions to keep the spindle close to the cortex. The role of the Arp2/3 complex in maintaining the spindle position is two-fold. First, active Arp2/3 creates a retrograde flow of actin filaments, resulting in cytoplasmic particle streaming that pushes the spindle towards the cortex. Second, the activity of the Arp2/3 complex seems to counteract myosin-II-induced cap contractility that could potentially reverse streaming to allow the spindle to move away from its cortical location (Yi et al., 2011). However, there are still a number of questions that need to be clarified (Verlhac, 2011). One of these is whether the Arp2/3 complex is the only actin nucleator required to maintain spindle anchoring during MII. More importantly, the model proposed by the authors is based on the existence of a flow of actin filaments, but it is not clear how this flow is produced. Another question is how actin filament flow induces cytoplasmic streaming.

Forces that drive spindle migration also rely on the activity of actin-associated motors called myosins. Myosin 10 is essential for spindle cortical anchoring in *Xenopus laevis* oocytes, where it functions as a microtubule–microfilament

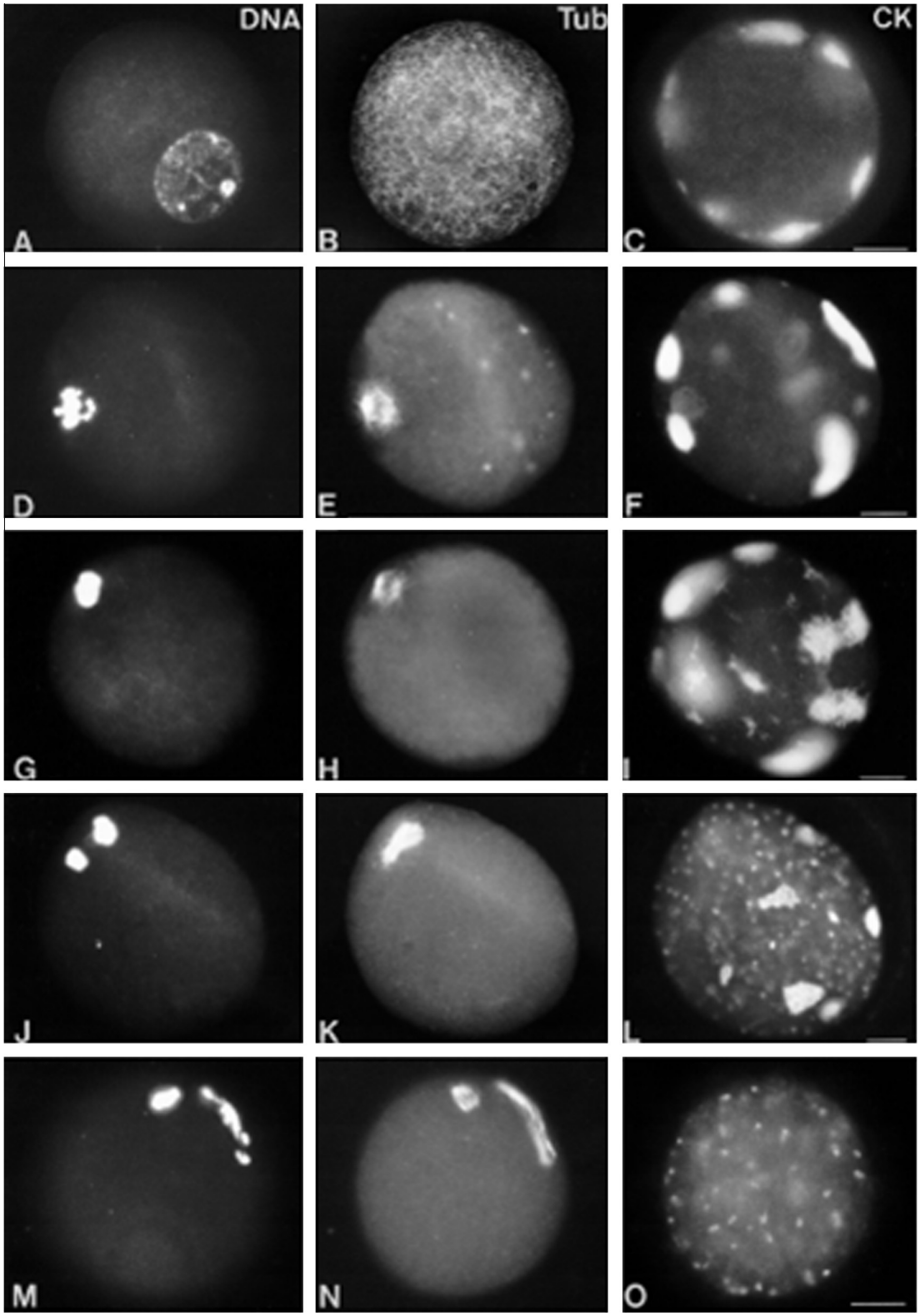

Figure 6 Redistribution of cytokeratins during in-vitro oocyte maturation in hamster. Oocytes are triple labelled for DNA (A, D, G, J, M), tubulin (B, E, H, K, N) and CK (C, F, I, L, O). (A—C) Prophase I. (D—F) Prometaphase I. (G—I) Metaphase I. (J—L) Late anaphase I or telophase I. (M—O) Metaphase II. Bars = 20 μm (reproduced from Plancha, 1996, with permission of the publisher).

linker (Weber et al., 2004). The myosin 10 transcript is relatively abundant in mouse oocytes (Evsikov et al., 2006), and this motor may link spindle microtubules to the actin network. Myosins are activated by myosin light chain kinase

(MLCK). MLCK inhibition disrupts spindle migration (Schuh and Ellenberg, 2008). MLCK is a substrate of mitogen-activated protein (MAP) kinase, whose activity depends, in turn, on the product of the c-mos proto-oncogene. The Moloney sarcoma oncogene (MOS)/MAP kinase pathway is essential for spindle positioning. The absence of spindle migration was initially observed in the oocytes of most knockout mice (Verlhac et al., 2000). Therefore, the MOS/MAP kinase pathway, via MLCK, most likely controls the activation of myosins involved in spindle movement.

Regulation of cellular organelles

Growth, maturation and fertilization of oocytes require the proper structure and relocalization of cellular organelles. This is achieved through rearrangement of microtubules and microfilaments (Sun and Schatten, 2006). As already mentioned, ER reorganization occurs at GVBD (Kline, 2000; Mehlmann et al., 1995).

To investigate the role of microfilaments and microtubules in ER reorganization, two different chemicals were applied. One of them was latrunculin A, a microfilament-depolymerizing agent, and the other was nocodazole. Reorganization of the ER at GVBD, although unaffected by latrunculin A, is strikingly absent in nocodazole-treated mouse oocytes (FitzHarris et al., 2007). This result indicates that microtubules, but not microfilaments, are essential for ER motility at GVBD. Further study revealed that it is microfilaments, not intact microtubules, which are necessary for formation of the cortical ER clusters at the MI–MII transition in mouse oocytes (FitzHarris et al., 2007).

However, in some species, the function of cytoskeletal components in the reorganization of ER is different. It has been reported that redistribution of ER from the cytoplasm to the nuclear area is dependent on microfilaments in starfish eggs (Terasaki, 1994). Translocation of mitochondria is also mediated by microtubules, not microfilaments, because disassembly of microtubules with nocodazole inhibites the movement of mitochondria whereas disruption of microfilaments by cytochalasin B has no effect in porcine oocyte (Sun et al., 2001). Moreover, microfilament depolymerization by cytochalasin does not affect central migration of mitochondria during mouse oocyte maturation (Calarco, 2005).

Effects on oocyte maturation

Among the three types of cytoskeletal filaments, microtubules are more directly involved in the processes of chromosome and organelle movement (Sun and Schatten, 2006), and microfilaments are more directly involved in processes of chromosome migration, cortical spindle anchorage, polarity establishment and first polar body emission during oocyte maturation (Tremoleda et al., 2001).

In bovine oocytes, inhibition of microtubule polymerization by nocodazole does not prevent GVBD, but progression to metaphase fails to occur. However, in cytochalasin B-treated oocytes, inhibiting microfilament polymerization does not affect either GVBD or metaphase formation, but movement of chromatin to the proper position is inhibited (Tremoleda et al., 2001). Both latrunculin A and nocodazole are capable of preventing second polar body extrusion after mouse oocyte activation (FitzHarris et al., 2007), suggesting that both microfilaments and microtubules are essential for mouse oocyte maturation. When the cytoskeleton is disrupted by heat shock, bovine oocytes are blocked at the first metaphase, first anaphase or first telophase stages, and the majority of arrested oocytes have abnormal spindles (Roth and Hansen, 2005). In some oocytes, the spindles have a rounded shape with a few unaligned chromosomes, whereas in other oocytes, spindles have an amorphous shape with substantial disruption to the chromosomal alignment (Roth and Hansen, 2005). One of the explanations for the arrest is that incorrect chromosome positioning or improper attachment to the spindle microtubule activates the spindle checkpoint and delays the onset of anaphase by inhibiting the anaphase-promoting complex/cyclosome (Cleveland et al., 2003; Zhou et al., 2002).

Influence of assisted reproduction technology on oocyte organelles

Assisted reproduction technology has been regarded as an attractive option for infertility treatment with great promise since 1978, when the first assisted reproduction baby was born. There is great clinical incentive to improve the efficiency of assisted reproduction technology, and further studies are needed to improve pregnancy rates. Reduced oocyte developmental competence is one of the main reasons for the decreased potential of in-vitro-produced embryos. It is known that both nuclear and cytoplasm maturation can affect the developmental competence of the oocyte. The clinical protocol is one of the main factors influencing cytoplasmic maturation (Ge et al., 2012a). Two clinical protocols that are routinely used to obtain fully mature, fertilization-competent oocytes are ovarian stimulation and IVM. Ovarian stimulation involves injection of high doses of exogenous gonadotrophins and can recruit multiple follicles simultaneously, avoiding follicle dominance and atresia (the natural physiological mechanism for selecting high-quality oocytes). This enables the generation of several embryos that can be selected for transfer following IVF.

It has been observed that the amount of mtDNA decreases significantly in mouse oocytes retrieved from ovarian stimulation compared with oocytes matured normally *in vivo* (Ge et al., 2012a). Additionally, lower ATP concentrations are detected in mature mouse oocytes from the ovarian stimulation group (Ge et al., 2012a). High-dosage injection or elevated exposure to gonadotrophins also has an effect on the mtDNA structure and copy number, metabolic function and distribution of mitochondria in monkey, hamster and bovine oocytes (Gibson et al., 2005; Lee et al., 2006b; Liu et al., 2011). Repeated ovarian stimulation decreases the number of oocytes that have normally distributed mitochondria, with a concomitant decrease in mtDNA copy number in mice (Miyamoto et al., 2010). Meanwhile, repeated ovarian stimulation alters ATP content (Combelles and Albertini, 2003; Lee et al., 2006b) and induces mtDNA mutations (Chao et al., 2005; Gibson et al., 2005) and oxidative damage (Chao et al., 2005; Miyamoto et al., 2010; Yin et al., 2006).

However, the effect of ovarian stimulation on mitochondrial distribution remains controversial. Some studies have reported that ovarian stimulation does not significantly alter the distribution of mitochondria in mouse, monkey or human oocytes (Ge et al., 2012a; Liu et al., 2011; Yin et al., 2006). Other studies have found significant differences in distribution patterns of mitochondria between the ovarian stimulation and control groups in human and porcine oocytes (Dell'Aquila et al., 2009; Sha et al., 2010). Overall, it is well accepted that impaired mitochondrial function may contribute to the low developmental potential of oocytes produced via ovarian stimulation.

Ovarian stimulation is also associated with several reproductive pathologies, including an increased risk of ovarian hyperstimulation syndrome (Jurema and Nogueira, 2006), an increased rate of aneuploidy (Allen et al., 2006) and an increased incidence of genomic imprinting disorders (Liu et al., 2011; Sato et al., 2007). IVM of oocytes from small antral follicles may reduce the need for exogenous gonadotrophin treatment and may offer an alternative to hyperstimulation of ovulation during IVF, but pregnancy and live birth rates are generally lower than those achieved using standard IVF (Ge et al., 2008). Zeng et al. (2007, 2009) has demonstrated that matured oocytes of both rats and humans produced via IVM have significantly lower concentrations of mtDNA compared with oocytes matured *in vivo*. However, ATP content of oocytes from the IVM group is not significantly reduced in either mouse or rats (Ge et al., 2012a; Zeng et al., 2009).

Changes in the mitochondrial distribution pattern have been observed during oocyte maturation *in vitro*. De los Reyes et al. (2011) reported an increased rate of canine in-vivo MII ovulated oocytes, with larger mitochondrial clusters spread throughout the cytoplasm compared with IVM MIIs cumulus–oocyte complexes (COC), which have smaller mitochondrial clusters. Human cumulus-removed oocytes matured *in vitro* with abnormal morphologies demonstrate an abundance of swollen mitochondria with blurred cristae and membranes in a single or clustered distribution, showing apoptotic characteristics (Yang et al., 2009).

The organization of the cytoskeleton is also significantly different between in-vivo-matured and IVM mouse oocytes (Sanfins et al., 2003). Metaphase spindles at both MI and MII in in-vivo-matured oocytes are compact with focused spindle poles, whereas IVM oocytes without cumulus have barrel-shaped spindles. MII IVM oocytes exhibite a significant increase in spindle size when compared with MII in-vivo-matured oocytes.

With respect to meiotic progression, in-vivo-matured oocytes are more synchronous in the rate and extent of anaphase to telophase of MI and first polar body emission compared with their IVM counterparts (Sanfins et al., 2003). The majority of IVM COC that fail to cleave are arrested at a metaphase stage (MI and mature, unfertilized MII oocytes) in primates (Delimitreva et al., 2012). In COC of primates arrested at MI, the largest groups are those with disorganized spindles and broad poles. A relatively small portion of MI-arrested COC have normal spindles. This contrasts with MII spindles where more than half appear normal. However, it has been observed that most of these ''normal'' spindles have misaligned chromosomes, and some chromosomes are incompletely condensed and COC arrested both at MI and at MII have a high proportion of unaligned chromosomes (Delimitreva et al., 2012).

Immature (germinal vesicle and MI) and mature (MII) oocytes have been collected from polycystic ovary syndrome patients (Li et al., 2006). The level of abnormality in spindle and chromosome configurations in oocytes matured *in vitro* is significantly higher than in oocytes matured *in vivo* (Li et al., 2006). However, previous studies demonstrated that the quality of cytoskeleton structures in mammalian oocytes can be improved by adjusting the maturation conditions (Ibanez et al., 2005; Tkachenko et al., 2010).

Discussion

Oocyte maturation is a complex process including both nuclear and cytoplasmic maturation. Nuclear maturation primarily involves chromosomal segregation, whereas cytoplasmic maturation involves a series of complicated processes. Due to developments in modern experimental techniques, including electron microscopy and immunofluorescence, the redistribution and morphological changes of organelles have been extensively studied. Recently, more studies have focused on the molecular mechanism involved in cytoplasmic maturation, but further studies are needed to better understand this process. To date, it is accepted that the correct changes in the localization, morphology and biochemical properties of organelles and cytoskeleton must occur for the oocyte to acquire high developmental potency.

Figure 1 describes the organelle organization in mouse (A) and human (B) oocytes. The general organization of the human oocyte is similar to that of mouse but has some unique features. The human oocyte contains basic cellular organelles, such as mitochondria, SER, Golgi apparatus, cortical granules and cytoskeleton, while it lacks lipids, vacuoles and cytoplasmic lattices, which are very common in other mammalian species (Sathananthan, 1994; Sathananthan et al., 2006). Mitochondria form voluminous aggregates with SER tubules and vesicles at the end of the maturation process in human oocytes (Familiari et al., 2006; Motta et al., 2000; Sathananthan and Trounson, 2000). In human MII oocytes, ER clusters are present not only in the cortex but also throughout the entire oocyte, with no apparent polarity in relation to the meiotic spindle (Mann et al., 2010). Cortical granules are also present over the spindle region, which is usually not observed in the mouse (Ducibella et al., 1988). Additionally, cortical granules are more abundant in human compared with mouse (Sathananthan, 1994). The MII spindle, often oriented perpendicular to the oocyte surface, is barrel-shaped, anastral and lacks centrioles (Sathananthan, 1994).

The oocyte plays a crucial role in determining embryo competence and therefore, the results of assisted reproduction technology. However, all oocytes retrieved by ovarian stimulation or IVM are exposed to spermatozoa with little ability to evaluate oocyte quality. Oocytes are currently evaluated for developmental potential based on morphology, a criterion that lacks quantification and accuracy. Oocyte nuclear maturity, as assessed by light microscopy, is assumed to represent the MII stage, when the first polar

body is visible in the proper perivitelline space (Rienzi et al., 2005). The MII stage is characterized by the alignment of homologous chromosomes in the centre of the meiotic spindle (Rienzi et al., 2005). Conventional methods to image the meiotic spindle, such as immunostaining or transmission electron microscopy, require prior fixation, causing the oocyte's death, limiting or prohibiting its clinical use (Pickering et al., 1990).

However, recently a digital, orientation-independent polarized light microscope, the polscope, has been applied to human and mouse oocytes (Keefe et al., 2003). The polscope has demonstrated the exquisite sensitivity needed to image the low levels of birefringence exhibited by mammalian spindles, thus providing a method to quantify spindle architecture in living oocytes (Cooke et al., 2003; Wang et al., 2001). At the moment, many studies have reported that polarized microscopy might be used as a noninvasive technique to assess the meiotic spindle in human oocytes (Braga et al., 2008; Cooke et al., 2003; De Santis et al., 2005; Wang et al., 2001).

Furthermore, one of the most severe cytoplasmic abnormalities of MII oocytes is the appearance of SER clusters (SER discs) within the cytoplasm (Rienzi et al., 2012). SER discs can be identified as translucent vacuole-like structures in the cytoplasm by phase-contrast microscopy (Rienzi et al., 2012). It has been demonstrated that embryos derived from oocytes with SER discs are associated with a risk of serious, significantly abnormal outcomes (Balaban and Urman, 2006; Ebner et al., 2006). However, a recent study (Mateizel et al., 2013) has shown that embryos derived from MII oocyte with visible SER discs (SER + MII) have the capacity to develop normally and may lead to newborns with no major malformations; however, the authors also stressed that embryo transfer with embryos originated from SER+ cycles should be approached with caution due to lower cycle efficiency, and follow up of children born after embryo transfer with embryos originating from SER+ cycles is encouraged.

On the other hand, ATP content and mtDNA quantity have been associated with oocyte quality and further embryo development, so they have been proposed as indicators for the developmental potential of mouse (Combelles and Albertini, 2003), cattle (Tamassia et al., 2004) and human oocytes (Van Blerkom et al., 1995; Zeng et al., 2007). However, these characteristics are not currently used in the clinical setting due to a lack of critical metrics.

The first polar body is extruded from the oocyte before fertilization and can be biopsied without damaging the oocyte. Reich et al. (2011) demonstrated that human polar bodies reflect the oocyte transcript profile and suggest that mRNA detection and quantification through high-throughput quantitative PCR may provide insight into oocyte quality, a critical metric needed before fertilization and transfer of the resulting embryo back into the woman.

For patients who continually experience poor embryo development and implantation failure during IVF procedures, the cytoplasmic transfer technique, which allows for the introduction of potentially beneficial ooplasmic components from donor oocytes, may restore normal growth and viability to their own developmentally compromised embryos. Cytoplasmic transfer has been performed in a very limited number of cases and, although the success rate exceeds expectations (Cohen et al., 1997, 1998; Brenner et al., 2000; Barritt et al., 2000, 2001), the technique remains controversial. Among the limited cases, the incidence of chromosomal anomalies is higher than the rate of major congenital abnormalities observed in the natural population (Cohen et al., 1997, 1998; Brenner et al., 2000; Barritt et al., 2000, 2001). Although ooplasmic transplantation does not involve transfer of any nuclear DNA, mtDNA may be transmitted via the donor ooplasm to any resulting offspring. It has been proposed that the resulting offspring are the recipients of two nuclear DNA genomes from the mother and the father and two mtDNA genomes from the mother and the donor. Therefore, the technique of cytoplasmic transplantation is still considered experimental and it is still years away from clinical practice.

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