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Control of oocyte maturation

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Abstract

Oocyte maturation is a complex process involving nuclear and cytoplasmic maturation. The nuclear maturation is a chromosomal segregation and the cytoplasmic maturation involves the reorganization of the cytoplasmic organelles, mRNA transcription and storage of proteins to be used during fertilization and early embryo development. The mechanism of oocyte maturation *in vivo* and *in vitro* still are not totally understood. However it is generally accepted that the second messenger cyclic adenosine monophosphate (cAMP) plays a critical role in the maintenance of meiotic blockage of mammalian oocytes. A relative increase in the level of cAMP within the oocyte is essential for maintaining meiosis block, while a decrease in cAMP oocyte concentration allows the resumption of meiosis. The oocyte cAMP concentration is regulated by a balance of two types of enzymes: adenylate cyclase (AC) and phosphodiesterases (PDEs), which are responsible for the synthesis and degradation of cAMP, respectively. After being synthesized by AC in cumulus cells, cAMP are transferred to the oocyte through gap junctions. Thus, specific subtypes PDEs are able to inhibit or attenuate the spontaneous meiotic maturation of oocytes with PDE4 primarily involved in the metabolism of cAMP in granulosa cells and PDE3 in the oocyte. Although the immature oocytes can resume meiosis *in vitro*, after being removed from antral follicles, cytoplasmic maturation seems to occur asynchronously with nuclear maturation. Therefore, knowledge of the oocyte maturation process is fundamental for the development of methodologies to increase the success of *in vitro* embryo production and to develop treatments for various forms of infertility. This review will present current knowledge about the maintenance of the oocyte in prophase arrest, and the resumption of meiosis during oocyte maturation, focusing mainly on the changes that take place in the oocyte.

Keywords: adenyl cyclase, cAMP, cumulus-oocyte-complex, meiosis, phosphodiesterase.

Introduction

The ovaries of neonate females contain oocytes in the diplotene stage of first meiotic prophase. These immature oocytes remain in a resting phase known as dyctiate or germinal vesicle (GV) stage (Downs, 1993; Sathananthan, 1994).

Within the follicle, granulosa cells (GC) that surround the oocyte undergo differentiation forming the cumulus-oocyte-complex (COC). A well-organized zona pellucida separates the oocyte from the cumulus cells (CC). The CC in close contact with the zona pellucida, which is known as cumulus oophorus (CO), communicates with the oocyte through gap junctions. The CC including the CO cells, due to their close contact with the oocyte, have a different function from that of GC from the follicular wall. Regulatory substances produced by the oocyte have important functions on the metabolism of CC and products of these somatic cells actively participate in the growth and maturation of oocytes.

At puberty some of the activated follicles continue to develop, eventually resulting in ovulation. Through activation and development of the follicle, the oocyte also starts to grow. During this time, it is very active with intense mRNA and protein synthesis and differentiation of organelles. These changes prepare the oocyte to be a competent gamete (Cran and Moor, 1990). The diameter of the oocyte also increases five times (attaining 100 to 120 μm in diameter), but it still remains at germinal vesicle stage of meiosis (Schultz *et al*., 1978; Downs, 1993).

The final process of oocyte maturation consists of the acquisition of the capacity to be fertilized and is characterized by several changes including the resumption of meiosis (Paynton and Bachvarova, 1990; Eppig, 1991). *In vivo* oocyte maturation coincides with differentiation of the pre-ovulatory follicle which includes changes in the oocyte and the CCs. The changes in the oocyte occur sequentially and synchronously under the stimulus of the LH, through its action on the GCs (Cran and Moor, 1990; Sathananthan, 1994). During oocyte maturation in preparation for fertilization and subsequent embryo development, changes in the nucleus and cytoplasm occur. As meiosis proceeds chromosomes segregate and organelles reorganize in the cytoplasm, transcription ceases, the stored mRNA is partially used, and the pattern of protein phosphorylation changes (Ponderato *et al*., 2001; Ferreira *et al*., 2009).

Although the immature oocytes can resume meiosis *in vitro*, after being removed from antral follicles (Edwards *et al*., 1965), cytoplasmic maturation

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seems to occur asynchronously with nuclear maturation (Janssenswillen *et al*., 1995; Huang *et al*., 1999). This is probably the main factor responsible for the lower rates of embryo production when oocytes are matured *in vitro*. Therefore, knowledge of the oocyte maturation process is fundamental for the development of methodologies to increase the success of *in vitro* embryo production and to develop treatments for various forms of infertility

This review will present current knowledge about the maintenance of the oocyte in prophase arrest, and the resumption of meiosis during oocyte maturation, focusing mainly on the changes that take place in the oocyte.

Control of meiotic arrest at the germinal vesicle stage

Although the exact mechanism of oocyte maturation *in vivo* or *in vitro* is not clearly understood, it is generally accepted that the second messenger cyclic adenosine monophosphate (cAMP) plays a critical role in the maintenance of meiotic arrest in mammalian oocytes (Conti *et al*., 2012). An increased level of cAMP in the oocyte is essential for the maintenance of the blockage of meiosis, while a decrease in concentration of cAMP allows the resumption of meiosis (Sela-Abramovich *et al*., 2006; Conti *et al*., 2012).

The concentration of cAMP in the oocyte is regulated by an equilibrium between two enzymes: adenyl cyclase (AC) and the phosphodiesterase (PDE), which are responsible for the synthesis and degradation, respectively, of cAMP (Conti *et al*., 2002). The production of cAMP in the oocyte is controlled by receptors linked to the G protein (GPR3 in mouse) which is essential for the activation of adenyl cyclase (Holt *et al*., 2013).

Since meiotic arrest at the germinal vesicle stage depends upon the interaction between the oocyte and surrounding CC, it has been hypothesized that cAMP passes from these cells to the oocyte through the gap junctions (Dekel *et al*., 1988). Besides, at least in rodents, GC play an important role in increasing cAMP in oocytes through the transfer of cyclic guanosine monophosphate (cGMP), an inhibitor of phosphodiesterase 3 (PDE3) through the gap junctions (Zhang *et al*., 2010). In rodents, cGMP is synthesized by CC under the influence of natriuretic peptide type C (NPPC) from the mural GC (Fig. 1; Holt *et al*., 2013). The effect of the cGMP in the CC is also mediated by the regulation of others phosphodiesterases (PDE2, PDE4 and PDE5; Zhang *et al*., 2010).

High levels of cAMP in the oocyte suppress the activity of the maturation promoting factor (MPF) through a mechanism which depends on protein kinase A (PKA; Maller, 1980; Bornslaeger *et al*., 1986). The MPF is a protein composed of a catalytic subunit, the cycline-dependent kinase 1 (CDK1) and a regulatory subunit, the cycline B (Downs, 1993; Taieb *et al*., 1997), and is regulated by the phosphorylation of the treonine 14 and tyrosine 15 residues of CDK1 (Bilodeau-Goeseels, 2012). This phosphorylation is catalyzed by the kinase Wee1B, while the dephosphorylation is dependent on the phosphatase Cdc25 (Lew and Kornbluth, 1996).

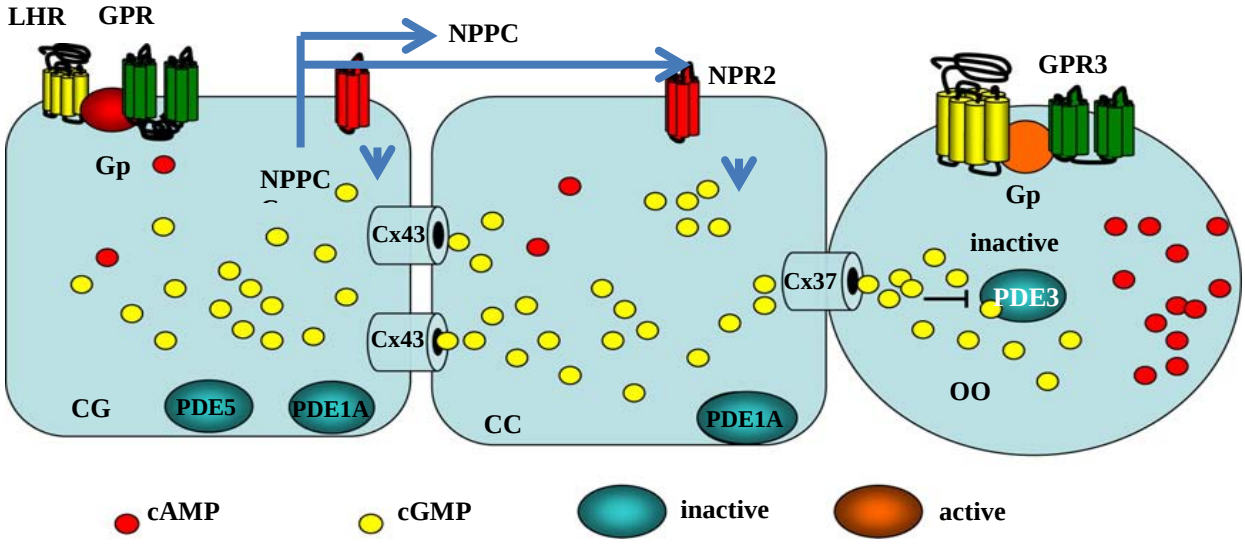

Figure 1. Schematic representation of meiotic arrest when the oocyte nucleus is at the germinal vesicle stage. GC= mural granulosa cells; CC= cumulus'cells; Oo= oocyte; PDE= phosphodiesterase; GPR= receptor linked to protein G; NPPC= natiuretic peptide; Cx= conexin. Adapted from Conti *et al*., 2012.

The inhibition of PDE3 increases the levels of cAMP activating protein kinase (PKA). The PKA regulates the activity of Wee1B and Cdc25 directly. In oocytes arrested in meiotic prophase, the PKA mediates the phosphorylation of Cdc25 down-regulating its function through sequestration to the cytoplasm (Zhang *et al*., 2008; Pirino *et al*., 2009). On the other hand, the phosphorylation of Wee1B increases the inhibition of MPF.

The prevention of premature resumption of meiosis is crucial for oocyte survival. In addition, the regulation of cyclin B1 levels is a second mechanism for the maintenance of meiotic arrest (Holt *et al*., 2013). In oocytes in the GV stage, a significant amount of cyclin B is already present. Therefore, the inactivation of CDK1 through phosphorylation is what maintains the oocyte in meiotic arrest (Holt *et al*., 2013). However, the cyclin B needs to be constantly degraded by the anaphase promoter complex/cyclossome (APC/C), in order to maintain blockage at prophase I. If the cyclin B accumulates during the GV stage, the increase in its concentration will activate the MPF leading to spontaneous resumption of meiosis (Reis *et al*., 2006).

Meiosis resumption

Re-initiation of meiosis depends upon several external factors. In mammals oocyte maturation is induced by the withdrawal of the inhibitory influence of GC *in vivo* the LH surge causes the breakdown of the gap junctions between the oocyte and the GC in the pre-ovulatory follicle (Eppig, 1991). Similarly, the removal of immature oocytes from the follicular environment can interrupt the transfer of regulators and metabolic support crucial for the maintenance of meiotic arrest, resulting in resumption of oocyte maturation.

The re-initiation of meiosis is regulated by changes in the pattern of phosphorylation of several proteins by specific kinases. Of importance is the activity of MPF (Masui and Markert, 1971), which is the universal cell cycle regulator of mitosis and meiosis (Nurse, 1990). The activation of MPF induces condensation of chromosomes, breakdown of the nuclear envelop (GVBD) and preparation of the cytoplasm for the M phase during both mitotic and meiotic cycles (Murray, 1989; Murray and Kirschner**,** 1989; Motilik and Kubelba, 1990).

In rodents and ruminants, the receptor for LH (LHR) is expressed mainly on cells of the theca and mural granulosa layers. A paracrine signal, as well as intercellular communication is crucial for the COC to respond to the LH surge (Peng *et al*., 1991). The effect of LH is to promote a decrease in the expression of NPPC receptors in CC and the consequent transfer of cGMP to the oocyte (Robinson *et al*., 2012). At the same time the gap junctions between the oocyte and CC are disrupted by factors from the EGF family such as the epiregulin, ampiregulin and beta-celulin (Norris *et al*., 2009; Vaccari *et al*., 2009). As a result of the drop in cGMP, there is an increase of PDE3 activity which promotes a rapid decline of cAMP levels in the oocyte and re-initiation of meiosis (Holt *et al*., 2013; Fig. 2).

The decrease in cAMP levels results in a reduction of PKA activity and the Cdc25 is transferred to the nucleus (Oh *et al*., 2010). The accumulation of phosphatase Cdc25 in the nucleus promotes the activation of the MPF and the transportation of Wee1B to the cytoplasm. As meiosis is reinitiated, the Wee1B is inactivated and the Cdc25 is activated promoting an increase of CDK1 activity (Conti *et al*., 2012).

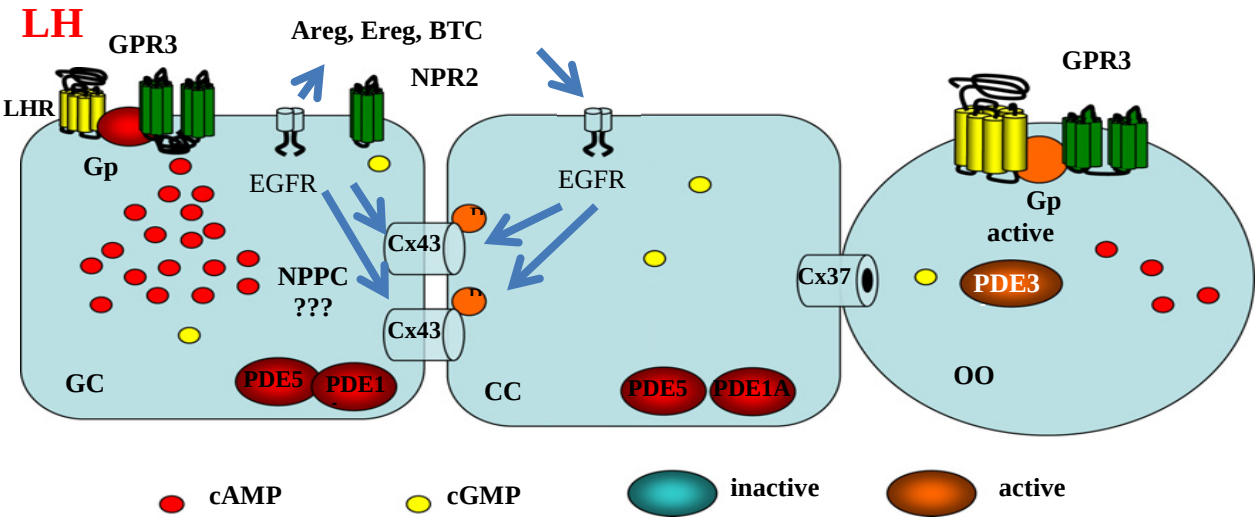

Figure 2. Schematic representation of the events taking place during the resumption of oocyte meiosis. There is a reduction in the levels of cAMP, activation of MPF, germinal vesicle breakdown (GVBD) and oocyte maturation. CG= mural granulosa cells; CC= cumulus cells; Oo= oocyte; PDE= phosphodiesterase, EGFR= EGF receptor; GPR= receptor linked to the protein G; NPPC= natriuretic peptide, NPR2= receptor of natriuretic peptide; P= phosphorylation, Cx= conexin. Modified from Conti *et al.*, 2012.

Mitogen-Activated Protein Kinase (MAPKs)

A group of proteins belonging to the serine/treonine family, the MAPKs, is involved in the progression of meiosis. These proteins are activated by extracellular signals and therefore are also known as ERK (extra cellular signal regulated kinase) with the variants ERK1 - p44 kDa and ERK2 - p42 kDa; Kubelka *et al*., 2000; Bilodeau-Goeseels, 2012).

The MAPKs have several substrates similar to phosphorylation, including phospholipases, transcription factors and cytoskeleton proteins. The MAPK path is universally activated during meiotic maturation of vertebrate oocytes. In cattle, MAPK increases after 8 h of *in vitro* culture, continuing gradually until 12-14 h, and then remaining stable until maturation is completed (Kubelka *et al*., 2000; Quetglas *et al*., 2010).

In bovine oocytes, the two main isoforms (ERK1/2) of MAPK are activated near the time of GVBD (Kubelka *et al*., 2000; Quetglas *et al*., 2010). This suggests that MAPK is not required for the initiation of meiosis, but is crucial for the post- GVBD events (Kubelka *et al*., 2000; Ponderato *et al*., 2001).

The MAPK are found in the oocyte where they are activated by MOS kinase and in *CC* where they are activated by RAS/RAF. In both cell types the MAPK are activated by phosphorylation of tyrosine and treonine residues and by MEK, also named MAPKK (Mitogen-activated protein kinase kinase). Activation of MEK is also mediated by phosphorylation and the proteins *MOS* in the oocyte and RAS/RAF in the CC (Crocomo *et al*., 2013).

In the mouse, LH induces the phosphorylation of ERK1/2 in pre-ovulatory follicles 30 min after stimulation and phosphorylation levels increase after 2 h (Panigone *et al*., 2008). The activation of the ERK1/2 occurs first in the mural GC and later in CC.

The MAPK, when activated, promotes the MPF stabilization in oocytes through the inhibition of some negative regulators and activation of cdc25 phosphatase. In bovine oocytes, activation of MAPK occurs at the same time as or slightly before GVBD, with levels increasing gradually during oocyte maturation and remaining elevated until meiosis II.

The activation and inactivation of MAPK is also related to variation in cAMP and PKA in the oocyte and CC. According to Sun *et al*. (2002), the activation of CC depends on paracrine factors secreted by the oocyte, showing the ability of this cell to control its own meiotic maturation.

Nuclear maturation

The process of nuclear maturation begins when meiosis resumes from the diplotene stage, signaled by chromosome condensation and GVBD. It corresponds to the reversal of the first blockage of meiosis from the GV stage until the second blockage at metaphase II (MII). After GVBD, the oocyte goes through metaphase I (MI), anaphase I (AI) and telophase I (TI), ending with the first meiotic division and then rapidly passes through MII of second meiotic division where a second meiotic arrest occurs (second meiotic blockage; Kubelka *et al*., 2000).

By the end of the first meiotic division, homologues of oocyte chromosomes (2n) have separated into two nuclei with n chromosomes each (MI). The cytoplasm divides asymmetrically generating two cells, one keeping almost all the cytoplasm, the secondary oocyte and the other very small, the first polar body. Soon after, the second meiotic division begins and remains in this stage (MII) until the fertilization or parthenogenetic activation (Quetglas *et al*., 2010).

The stage of nuclear maturation might be evaluated directly by the configuration of the chromosomes (Fig. 3) and classified in different stages (Landim-Alvarenga, 1999):

- Germinal vesicle (GV): characterized by the presence of a spherical nucleus with intact envelope and filamentous chromatin;
- Germinal vesicle breakdown (GVBD): characterized by condensed chromatin and absence of a visible nuclear membrane;
- Metaphase I (MI): chromosomes arranged on the metaphase plate peripherally located in the ooplasm;
- Metaphase II (MII): characterized by the presence of metaphase plate with chromosomes arranged in the periphery of the ooplasm and by the extrusion of the first polar body (PB) represented by a dense group of chromosomes.

The integrity of the nuclear membrane including the GV is maintained by proteins called laminins. During GVBD, the CDK1 promotes disorganization of the nuclear envelop, phosphorylating the laminins (Adhikari and Liu, 2014). At same time as nuclear envelop disintegration, condensation of the chromosomes occurs and the metaphasic plate is organized.

The protein degradation which occurs during the transition from metaphase to anaphase is controlled by the anaphase promoter complex (APC) which is responsible for the ubiquitination of several protein substrates (Sullivan and Morgan, 2007). The APC is a multi-subunit of the ubiquitin ligase E3, whose substrates are degraded by the proteosome 26S. The action of APC requires the linking of a protein co-activator, the CDC20 or FZR1 which confers specificity to the enzyme. The link of APC to CDC20 allows the anaphase of mitosis or meiosis to continue by degradation of cyclin B1 (Jones, 2011).

In vertebrate oocytes, the metaphase' spindle organizes to lead to an unequal division of cytoplasm, which results in the expulsion of first PB. Parallel to the expulsion of the PB, there is a decrease in MPF activity,

which shortly increases again inducing the organization of the metaphase' spindle for the second meiotic division, without formation of a nuclear envelop, chromosome de-condensation or DNA replication.

High levels of MAPK and MPF are needed for the maintenance of oocytes in MII since fertilization or parthenogenetic activation causes an abrupt intra-oocyte drop of both kinases and the completion of meiosis (Hashimoto and Kishimoto, 1988; Choi *et al*., 1991; Naito and Toyoda, 1991; Jelinkova *et al*., 1994; Dedieu *et al*., 1996; Taieb *et al*., 1997; Wu *et al*., 1997; Oh *et al*., 1998). When a spermatozoon enters the oocyte, the chromosomes separate and the organization of the nuclear envelop terminates meiosis with the extrusion of the second PB (2nd PB). After extrusion of the 2nd PB fusion of female and male pro-nuclei occurs, which is the beginning of the embryonic development. In order to ensure normal development of the embryo, nuclear changes during the oocyte maturation and fertilization are needed, which are coordinated by movements of the genetic material and organelles, and by biochemical changes in the cytoplasm (Van Blerkom, 1991).

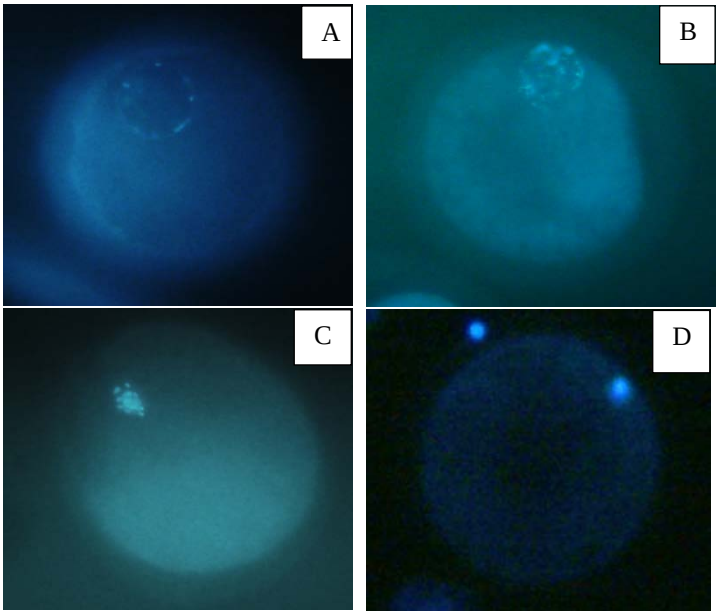

Figure 3. Meiotic stages of oocytes stained with Hoechst 33342 evaluated with an inverted microscope with ultra-violet light. The oocytes are classified as: A) Germinal vesicle (GV); B) Germinal vesicle breakdown (GVBD); C) Metaphase I (MI) and D) Metaphase II (MII). Amplification 400X.

Cytoplasmic maturation

The progression of meiosis that characterizes nuclear oocyte maturation, does not by itself guarantee further embryonic development; additional cytoplasm modifications or cytoplasm maturation is also necessary (Ferreira *et al*., 2009).

Cytoplasmic maturation includes structural and molecular changes occurring in the oocyte from the GV stage to the end of MII. Evaluation of maturation might be done indirectly through the ability of the mature oocyte to cleave and develop into a blastocyst after fertilization or parthenogenetic activation.

The ultrastructural changes in the cytoplasm include migration of several of the organelles. The mitochondria and Golgi complex, which are located in the periphery of the oocyte in immature oocytes, move to a perinuclear distribution. The cortical granules, originating from the Golgi complex, and originally situated in the center of the oocyte migrate to the periphery and become anchored to the oocyte membrane (Cran and Moor, 1990).

Redistribution of organelles in the cytoplasm

The location of the organelles in the cytoplasm depends on the cell's needs at each stage of development and their relocation is made possible by the action of cytoskeleton microfilaments and microtubules.

The resumption of meiosis is accompanied by extensive reorganization of the cytoplasmic microtubules in the oocyte. During interphase, long and relatively stable microtubules are distributed in the cytoplasm, while during metaphase, the microtubule organizer centers (MTOCs) are phosphorylated and the activity of microtubule organization increases (Fan and Sun, 2004). Thus, mitochondria, endoplasmic reticulum, Golgi complex and cortical granules assume different positions from those observed at GV stage (Ferreira *et al*., 2009).

Mitochondria

The activation of metabolic pathways through protein synthesis and phosphorylation is indispensable for cytoplasmic maturation. The mitochondria have a very important role in this process since they are a key component of energy metabolism (Krisher and Bavister, 1998; Meirelles *et al*., 2004). In this sense, the dislocation of the mitochondria to areas of high energy consumption is crucial for oocytes and embryos in critical periods of the cellular cycle.

During maturation, mitochondria synthesize ATP needed for the production of proteins used during late embryo development (Meirelles *et al*., 2004). A structural analysis of bovine oocytes submitted to *in vitro* maturation (IVM) show that, after 12 to 18 h in culture, the mitochondria change from a position more peripheral to a dispersed location throughout the cytoplasm (Hyttel *et al*., 1986).

This behavior is similar to what occurs *in vivo*, which means that the distribution is more peripheral before the LH peak, beginning to diffuse during the final phases of nuclear maturation and then dispersing after the polar body extrusion, approximately 19 h post-LH peak (Kruip *et al*., 1983; Hyttel *et al*., 1997).

Studies of bovine and murine oocytes indicate that the reorganization of mitochondria in cytoplasm post-IVM is correlated to the ATP levels in the embryos. Therefore, embryos with less ATP in the cytoplasm develop more slowly and have lower number of cells (Liu *et al*., 2000).

Before the embryonic genome is activated (after 72 h of culture) the mitochondria has an intermediate level of activity, which might be explained by the adaptive protection against the reactive types of oxygen (ROs) This protection occurs as a result of catalyzer molecules such as glutathione and peroxidases, which are produced during oocyte maturation and early embryonic development (Krisher and Bavister, 1998).

Besides the activities described above, the mitochondria regulate the process of cellular apoptosis, acting as reservoirs of activator proteins of programmed cell death (PCD) process, as for example cytocrome c. Permeabilization of the mitochondrial membrane allows the liberation of cytocrome and consequent activation of the apoptosis cascade resulting in cell death (Van Blerkom, 2004).

Endoplasmic reticulum

The endoplasmic reticulum (ER) membranes are physiologically active, containing specialized domains interacting with the cytoskeleton in the accomplishment of different functions. Among the known functions of the ER are the folding and degradation of proteins, lipid metabolism, nucleus compartmentalization, establishment of calcium (Ca^2) gradients, and its own synthesis (Ferreira *et al*., 2009). Throughout calcium storage, the system plays an important role in intracellular signaling. The mechanisms and paths of calcium ion (Ca^2) mobilization point to its importance in several cellular events.

The paths of Ca^2 signaling are dependent on differences in its extra and intra cellular contents, which are responsible for concentration gradients between both compartments. The established gradient is regulated by the ooplasm membrane. In rodent and human oocytes the content of Ca^2 in the cytoplasmic reticulum is mediated by proteins present in the reticulum canals; the receptors for inositol 1,4,5 triphosphate (IP3R) and ryanodine are both located in the ER membrane and are responsible for the control of Ca^2 movement into the cytosol. The Ca^2 liberation via IP3 and its receptor IP3R is crucial for oocyte activation during fertilization (Kline and Kline, 1994).

Biochemical and structural modifications of the ER during maturation are essential for the satisfactory functioning of intracellular calcium regulation. Examination of mouse oocytes in the GV stage shows that the ER are uniformly distributed in the ooplasm. During the progression of development until the MII stage, the ER are found in the cortical region accumulating in small stacks of 1-2 um over all cytoplasm (Stricker, 2006).

The system sensibility to the needs of Ca^2 liberation increases after maturation. During fertilization, the entrance of the spermatozoon into the oocyte provokes the exit of Ca^2 from the ER which is followed by the beginning of embryonic development (Ferreira *et al*., 2009).

Golgi Complex (GC)

The dynamics of Golgi membranes during maturation and fertilization of mammalian oocytes requires additional research. In the GV oocyte, the Golgi complex appears in the periphery of the ooplasm surrounded by small vesicles. Two kinds of vesicles are observed: coated vesicles resembling pinocytosis granules are seen next to the cis face of the Golgi complex while, next to the trans face smooth vesicles with irregular size and electron density are observed (Landim-Alvarenga and Alvarenga, 2006). Electron dense membranous granules appeared in association with the Golgi complex or distributed through the cytoplasm which were classified as cortical granules. On the other hand, in matured oocytes, Golgi complexes were still present but less developed (Landim-Alvarenga and Alvarenga, 2006).

Cortical granules

The cortical granules (CG) originate in the Golgi complex. The exocytosis of the CGs involves cytoskeleton filaments and homologue proteins. In GV

oocytes, the cortical granules are in clusters through the cytoplasm. By the end of the maturation period, when MII is attained, the granules are near the inner surface of oocyte cell membrane. This pattern is a strategic distribution in preparation for arrival of the spermatozoon and oocyte activation (Hosoe and Shioya, 1997).

The cortical granules are organelles exclusive of oocytes and their composition consists of a variable population of proteins, structural molecules, enzymes and glycosaminoglycans. The exocytosis of the cortical granules (cortical reaction) is one of the mechanisms most frequently used by the oocytes to avoid polyspermy. If the oocyte is fertilized by more than one spermatozoon, the resulting zygote will undergo abnormal cleavage, becoming nonviable and will degenerate as soon as the mitotic divisions begin (Hosoe and Shioya, 1997).

Molecular maturation

Molecular maturation consists of several stages such as transcription, storage and processing of the mRNA that will be used in ribosome synthesis of the proteins that will directly influence the subsequent cellular events, such as fertilization, pro-nucleus formation, and the beginning of the embryogenesis (Crocomo *et al*., 2013).

Transcription and mRNA storage occur during the folliculogenesis, while the nucleus is quiescent, and ends when meiosis is reinitiated, soon after the chromosomes condense and became inactive. However, the capacity of mRNA translation and protein synthesis is maintained throughout oocyte development and further embryogenesis (Sirard, 2001).

Most of the ooplasm mRNA is stable, but inactive due to its short poly-A tail. Under the action of signals generated during maturation, fertilization and the beginning of embryo development, there is poly-adenylation, which is the addition of adenines to the 3'end under the action of the poly-a polymerase. Poly-adenylation promotes the release of repressor molecules coupled to the 5'segment, allowing for the beginning of translation (Gottardi and Mingoti, 2009).

The transportation of mRNA to the cytoplasm occurs as a result of a characteristic shortening of the poly-A tail, which upon reaching this compartment becomes shorter and heterogeneous (Tomek *et al*., 2002). The mRNA molecules are not translated when they have short poly-A tails. Therefore, deletion of that sequence is the initial step in their degradation process (Tomek *et al*., 2002). The cytoplasm elongation of the poly-A tail means the activation of translation, which is, the addition of adenine to mRNA in the oocyte cytoplasm during maturation, leading to protein synthesis, and deadenylation for degradation of those mRNA (Ferreira *et al*., 2009).

Therefore, the efficiency of storage and reactivation of mRNAs, is regulated by polyadenylation and determines oocyte competence to support later developmental stages. The pronounced increase of kinase activity initiates a complex and specific cascade of protein phospho-dephosphorylation (Gottardi and Mingoti, 2009).

Conclusion

The information reviewed above shows that the process of maintenance of meiotic arrest involves a complex system of cellular signals that is modified by the pre-ovulatory stimulus of LH, resulting in re-initiation of meiosis and oocyte maturation. Most of the events reported here are intracellular, while others involve paracrine controls depending on intimate relations between follicular cells and the oocyte. All events are necessary to the production of a functional gamete with the capacity to develop to a healthy embryo after fertilization.

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