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Cytoplasmic maturation of bovine oocytes: Structural and biochemical modifications and acquisition of developmental competence

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Abstract

Oocyte maturation is a long process during which oocytes acquire their intrinsic ability to support the subsequent stages of development in a stepwise manner, ultimately reaching activation of the embryonic genome. This process involves complex and distinct, although linked, events of nuclear and cytoplasmic maturation. Nuclear maturation mainly involves chromosomal segregation, whereas cytoplasmic maturation involves organelle reorganization and storage of mRNAs, proteins and transcription factors that act in the overall maturation process, fertilization and early embryogenesis. Thus, for didactic purposes, we subdivided cytoplasmic maturation into: (1) organelle redistribution, (2) cytoskeleton dynamics, and (3) molecular maturation. Ultrastructural analysis has shown that mitochondria, ribosomes, endoplasmic reticulum, cortical granules and the Golgi complex assume different positions during the transition from the germinal vesicle stage to metaphase II. The cytoskeletal microfilaments and microtubules present in the cytoplasm promote these movements and act on chromosome segregation. Molecular maturation consists of transcription, storage and processing of maternal mRNA, which is stored in a stable, inactive form until translational recruitment. Polyadenylation is the main mechanism that initiates protein translation and consists of the addition of adenosine residues to the 3′ terminal portion of mRNA. Cell cycle regulators, proteins, cytoplasmic maturation markers and components of the enzymatic antioxidant system are mainly transcribed during this stage. Thus, the objective of this review is to focus on the cytoplasmic maturation process by analyzing the modifications in this compartment during the acquisition of meiotic competence for development.

Keywords: Cytoplasmic maturation; Bovine oocytes; Organelles; Cytoskeleton; Molecular maturation

1. Introduction

The complex events that occur during oocyte maturation depend not only on the correct dynamics of chromosome separation during nuclear maturation, but also on the redistribution of cytoplasmic organelles and on the storage of mRNA, proteins, and transcription factors

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needed for this process to occur. The transcripts and proteins stored in the cytoplasm of the oocyte are of fundamental importance for the maturation process and for ensuring the progression of early embryo development to the eight-cell stage (in cattle), when the embryonic genome is activated and the synthesis of new proteins becomes necessary. This phase is denoted as embryonic genome activation (EGA) and the expression of certain genes during this period will determine the success of embryogenesis in the pre-implantation stage [1].

Although they are distinct processes, nuclear maturation and cytoplasmic maturation are interlinked events that occur simultaneously at determined times, even though the molecular programming of the cytoplasm may have already started during the phase of oocyte growth. The cytoplasmic maturation process can be divided into three main events: (1) redistribution of cytoplasmic organelles, (2) dynamics of the cytoskeletal filaments, and (3) molecular maturation.

Thus, the objective of the present review is to focus on the process of cytoplasmic maturation, in light of the changes that occur in this compartment during the acquisition of meiotic competence for development.

2. Redistribution of cytoplasmic organelles

It is well established that several ultrastructural changes regarding morphology and redistribution can be observed in cytoplasmic organelles during oocyte maturation. Trafficking of cytoplasmic organelles during maturation occurs through the actions of cytoskeletal microfilaments and microtubules and repositioning of the organelles depends on the needs of the cell during each stage of development.

2.1. Mitochondria

The activation of determined metabolic pathways involved in protein synthesis and phosphorylation is indispensable for cytoplasmic maturation. Within this context, mitochondria play an extremely important role since they are a key component of the metabolic machinery responsible for the supply of energy that is consumed during the maturation process [2,3]. The movement of mitochondria to areas of high energy consumption is crucial for the oocytes and the embryo blastomere during critical periods of the cell cycle. Previous studies showed that during the maturation period, mitochondria synthesizes the ATP necessary for the synthesis of proteins which, in turn, supports the completion of subsequent maturation processes and embryo development [2,3].

Ultrastructural analysis of bovine oocytes subjected to in vitro maturation (IVM) has shown that the mitochondria move from a more peripheral position to a more disperse distribution throughout the cytoplasm after 12–18 h of culture [4]. This event is similar to what occurs in vivo, which involves a more peripheral distribution before the luteinizing hormone (LH) surge, a clustered cortical formation in the final stages of nuclear maturation, and a dispersed distribution after the extrusion of the polar body, approximately 19 h after the LH surge [5,6]. Upon reaching metaphase II (MII), the mitochondria in bovine oocytes, together with lipid droplets, occupy a central position in the cell [6] (see Fig. 1A). In addition, recent studies have shown that the number of mitochondria present in the cytoplasm of mammalian oocytes varies according to the stage of development of the cell. During the pre-migratory stage of germ cells, the number of mitochondria is approximately 10 units, increasing to 200 units in the oogonium stage. Primary oocytes contain approximately 6000 mitochondria, and this number increases to more than 100,000 copies of mtDNA during oocyte maturation, with one or two copies of mtDNA per organelle [7–10].

Evidence from heteroplasmic murine and human oocytes, showed that the segregation of mtDNA variants occurs as early as during the first mitotic divisions of germinal cell precursors and that these variants are then rapidly transmitted to future generations. Analysis of the distribution of pathogenic mtDNA mutations in the offspring of carrier mothers shows that chances of inheriting a pathogenic mutation increase with the proportion in the mother, but there is no bias toward transmitting more or less of the mutant mtDNAs. This suggests that there is no strong selection against this type of mutation. One of the possible factors responsible for the variations in the amount of inherited mutant mtDNA may be the massive reduction of these molecules that are transmitted by the mother. Observations of this type gave rise to the concept of mitochondrial genetic bottleneck or developmental bottleneck for the transmission of mtDNA [10,11]. Based on this concept, it was proposed that the large amount of mtDNA and, by inference, of the mitochondria present in the oocyte, may represent a genetic mechanism to guarantee the distribution of these organelles and molecules to the gametes and somatic cells of future generations. Thus, not only are the mitochondria important for oocyte metabolism, but also their numbers are a predictor of functional competence, since a reduced number of mitochondria may cause abnormal distribution of the organelle during early embryogenesis [10].

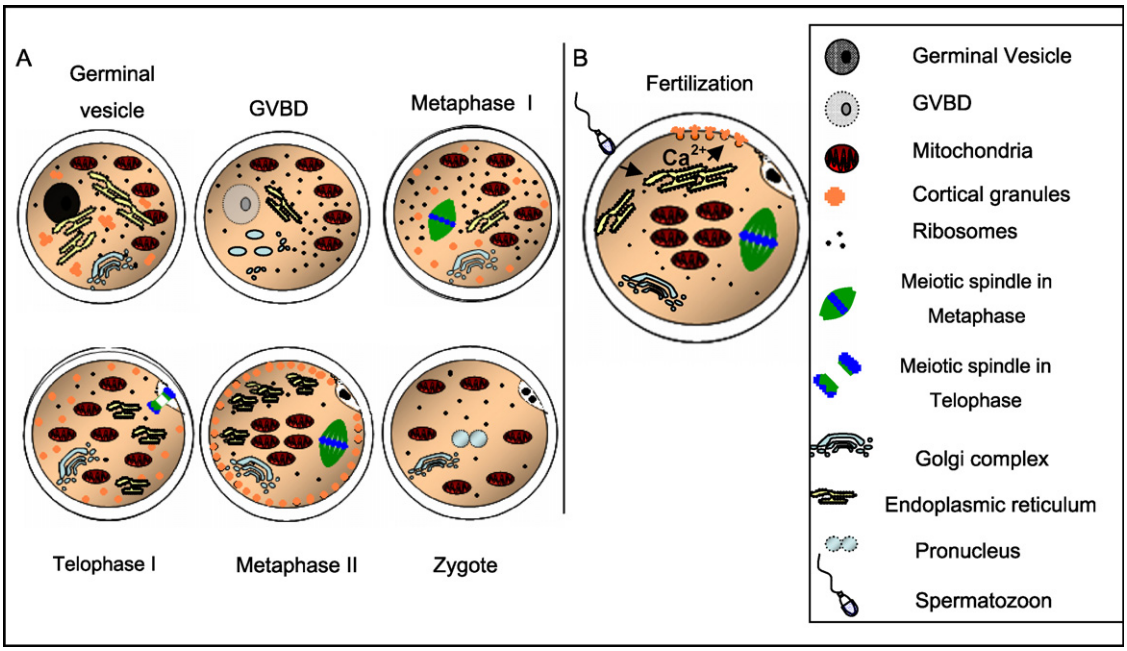

Fig. 1. Schematic overview of the distribution of cytoplasmic organelles during maturation, fertilization and bovine zygote formation. A. Nuclear maturation progression and cytoplasmic organelle movement from the immature stage of germinal vesicle to the mature stage of metaphase II and zygote formation. B. Organelle distribution and the mechanism of cortical granule content release, secondary to intracellular calcium (Ca^{2+}) release after the entry of the spermatozoon into the oocyte during fertilization.

Mouse oocytes derived from small antral follicles and matured in vitro had a reduced potential for development compared to oocytes of pre-ovulatory follicles and to oocytes matured in vivo. This statement is attributed to the observation of significant reductions in the number of mitochondrial DNA copies, the amount of ATP, and the proportion of oocytes with peripheral mitochondrial distribution. This reduced number of mitochondria may lead to a reduced amount of ATP, which is essential for subsequent stages of development [12]. Although these data demonstrated a correlation between the variables analyzed (number of copies of mitochondrial DNA, quantity of ATP and proportion of oocytes with peripheral mitochondrial distribution versus developmental potential), they are still insufficient to prove a causal relationship.

New data suggest that the mitochondria of human and murine oocytes and embryos may have heterogeneous intracellular domains from the viewpoint of the inner mitochondrial membrane potential (deltapsim). The magnitude of deltapsim would be involved in some mitochondrial activities, including ion flow regulation and ATP release. Highly polarized mitochondria, i.e., mitochondria with high deltapsim, have a specific and stable domain in the subcortical/sub-plasmalemmal region of the cytoplasm in these oocytes [13,14], and represent a small fraction of the total mitochondrial complement [15]. The presence of this subcortical region of highly polarized mitochondria in human and murine oocytes and embryos was proposed to be a microzone of differential activities related to the acquisition of developmental competence [13,14], which may affect the normal occurrence of the fertilization process [14].

The current discussion regarding the relation of mitochondria to the acquisition of developmental competence involves the definition of developmental potential itself, which is still controversial [9]. Zygotes that initiate the first mitotic cleavages faster than others have a better probability of reaching the blastocyst stage and therefore have a better developmental potential. On this basis, the timing between the insemination and first cleavage is emerging as a determinant factor in defining developmental competence [16]. Considering that during this process all events are controlled by the molecular machinery within the oocyte [1], we can establish a direct relationship between competent oocytes and zygotes with a high developmental potential. Among several other parameters, the relationship between mitochondrial activity and distribution (inherited from the mother, i.e., from the oocyte) and the amount of ATP during the early stages of development preceding EGA has also emerged as another important factor to be considered in determining bovine embryo

competence [9], which is in contrast to the data reported by Van Blerkom et al. [17] in a previous study on human embryos.

Recent studies on bovine and murine oocytes and embryos have also correlated with the reorganization of the mitochondria in the oocytes after IVM, the ATP levels and the total number of cells in the blastocysts. Embryos with less ATP in the cytoplasm had slower development and resulted in a smaller number of cells [3,18]. Before embryonic genome activation (approximately 72 h of culture), mitochondria have intermediate levels of activity, a fact that may be explained by adaptive protection against reactive oxygen species (ROS) as a result of mitochondrial metabolism [7,19]. This protection is provided by scavenger molecules such as glutathione and peroxidases, which are produced during molecular oocyte maturation or during the 2-cell embryo stage by permissive transcription [20]. Thus, if mitochondrial activity is high during the early stages of embryo development, the embryos probably would not survive, because they would be unable to eliminate the excessive production of ROS in these processes [9]. After embryonic genome transcription has begun, mitochondrial activity decreases as the embryo starts to utilize other metabolic pathways such as anaerobic glycolysis to produce energy [9,21].

Perhaps mitochondria have an important role in the acquisition of oocyte competence during development. However, none of the studies available in the literature was able to test this link directly, and a correlation does not mean that a causal relationship is present. That the real measure of competence, i.e., term development, was almost never the endpoint of these experiments, a causal relationship is difficult to justify. Regarding ATP content in the oocyte, several controversial data have been published, where no correlation was found between developmental competence and ATP content in the oocytes. Thus, this gap in understanding will need to be filled from future studies that analyze the presence or absence of a causal relationship between oocyte competence and mitochondrial distribution, as well as ATP content.

2.2. *Ribosomes, Golgi complex and endoplasmic reticulum (ER)*

Protein synthesis is indispensable not only for oocyte maturation *per se*, but also for zygote formation and early embryogenesis. To this end, an appropriate quantity of ribosomes must be present during maturation. Ribosomes are synthesized by the transcription of ribosomal RNA (rRNA) genes, by the processing of the transcripts, and by the addition of countless ribosomal proteins to their two subunits. The nucleolus is the site of formation of the ribosomal subunits and during the phases of oocyte growth and activation of the embryonic genome the nucleolus is present in the fibrillogranular form, reflecting the high activity of ribosome synthesis and therefore protein synthesis. The oocyte of a primordial follicle is transcriptionally quiescent and the nucleolus is exclusively composed of the granular portion, signaling an absence of ribosome synthesis activity [22,23]. During metaphase I of meiosis, protein synthesis in the oocyte is approximately three times greater than that during the germinal vesicle breakdown (GVBD) stage (Fig. 1A). When the cell reaches metaphase II, however, the oocyte exhibits basal levels of mRNA translation. Perhaps the absence of a functional nucleolus leads to an absence of rRNA transcription or ribosome production for mRNA translation [24]. Previous evidence suggested that the production of ribosomes in the GV stage, where there is a functional nucleolus and therefore presence of rRNA transcription or ribosome production for mRNA translation, may favor greater storage of these organelles in the oocytes during the MI stage [17]. Thus, the higher levels of protein synthesis observed in MI as opposed to MII in oocytes may be due to the larger ribosomal storage observed during MI. As a consequence of the high utilization of these organelles during the maturation process until MII, the protein stores are reduced and therefore the amount of ribosomes and the levels of protein synthesis may be comparatively lower. In any case, this evidence supports the idea that the presence of ribosomes is directly linked to protein synthesis during crucial periods of development.

The dynamics of the Golgi membranes during maturation and fertilization in mammals requires more study [25]. It is known that bovine oocytes in the GV stage present Golgi fragments that are transformed into vesicles during GVBD [4,25,26].

Despite the information obtained thus far, data regarding the role of the Golgi complex during maturation and the subsequent events remain controversial. A recent study has shown that, in contrast to what is occurring in somatic cells, extracellular matrix proteins (such as GM130) found in the membranes of the Golgi complex in germinal cells during MII are phosphorylated and co-localized only with sites of ER export and not with the meiotic spindle. This suggests that the organizing mechanism of the Golgi complex is independent of the centrosome. This difference can be explained by the fact that during mitosis, there is a

uniform partition of proteins and Golgi membranes between the two daughter cells, an event that does not occur in oocytes due to the formation of the first and second polar bodies. In addition, the study showed that, in contrast to what was observed in mouse and *C. elegans* embryos, neither fertilization nor cell division up to the eight-cell stage required trafficking through the secretory pathway, since the bovine zygotes cultured in the presence of Brefeldin A (BFA), a fungal metabolite that inhibits protein secretion by interrupting the transport of ER-Golgi vesicles, was able to complete cytokinesis and divide up to the eight-cell stage. One explanation for this observation was that the BFA dose used was low and therefore insufficient to inhibit secretion [25]. Thus, further studies are needed in order to fill the gaps regarding the dynamics of proteins and of Golgi membrane and their direct importance for oocyte maturation, fertilization and early embryogenesis [26].

The membranes of the ER are physiologically active, interact with the cytoskeleton and contain different domains specialized in different functions. Among the known ER functions are protein folding and degradation, lipid metabolism, compartmentalization of the nucleus, regulation of the Ca^{2+} ion gradient, and membrane synthesis [27]. By storing and releasing calcium, this system plays an extremely important role in intracellular signaling. Complex mechanisms involved in remodeling and in the activity of calcium signaling pathways have been suggested, which points out the importance of calcium ion in various developmental events [28]. The Ca^{2+} signaling pathways depend on differences between intra- and extracellular calcium levels, which generate different concentration gradients between the two compartments. This gradient is regulated by the membrane potentials of the oolemma. In rodent and human oocytes, Ca^{2+} release from cytoplasmic stores is mediated by ligand-gated ion channels such as the inositol 1,4,5 triphosphate receptor (IP3R) and the ryanodine receptor, both located on the ER membrane. It was established that Ca^{2+} release via IP3 and its receptor, IP3R, is essential for oocyte activation during fertilization [29,30,28].

Biochemical and structural changes in the ER during maturation are critical for proper functioning of intracellular calcium regulation. In vivo analyses of mouse oocytes in the germinal vesicle (GV) stage showed that the ER is uniformly distributed in the ooplasm. As is the case for mouse oocytes as well as oocytes from other mammalian species, the ER is found in cortical regions and accumulates in small 1–2 μm wide clusters throughout the cytoplasm (except in the vicinity of the meiotic apparatus) as development progresses to the metaphase II stage [31,32] (Fig. 1A). The sensitivity of the system to calcium release is increased after maturation. During fertilization, the entry of the spermatozoon into the oocyte leads to a marked release of calcium from the reticulum, followed by the beginning of embryonic development [31] (Fig. 1B). At the time of second polar body formation, the ER clusters begin to disaggregate approximately 3–4 h after insemination and 2 h before calcium signaling ceases in the pronuclear stage of development [32,33].

2.3. Cortical granules

Cortical granules (CG) are derived from the Golgi complex [34]. Exocytosis of CGs involves cytoskeleton filaments [35] and proteins homologous to members of the SNARE hypothesis, a molecular model of trafficking, docking and exocytosis of vesicles in the secretory compartment of somatic cells, also observed in sea urchin eggs [36]. For oocytes in the GV stage, cortical granules are distributed in clusters throughout the cytoplasm [37]. At the end of the maturation period, when these oocytes reach the MII stage, the granules are distributed throughout the inner surface close to the plasma membrane [36,38], a pattern strategically arranged to await for spermatozoon entry and egg activation (Fig. 1A).

The cortical granules are organelles exclusively found in oocytes and their composition includes a diverse population of proteins, structural molecules, enzymes, and glycosaminoglycans. The exocytosis of cortical granules (cortical reaction) is one of the most common mechanisms used by the oocyte to prevent polyspermy [37]. If fertilization with more than one spermatozoon occurs, the resulting zygote will undergo abnormal cleavage and will become non-viable, eventually degenerating at the beginning of mitotic divisions. The mechanism of blockade is highly conserved among animal groups and is based on rapid modification of the oocyte extracellular matrix. This modification involves releasing CG contents to the outer surface after oocyte activation is triggered by calcium release from the ER in response to spermatozoon entry into the oocyte oolemma [39] (Fig. 1B). Evidence indicates that in mammalian oocytes the intracellular signals leading to cortical reaction involves the activation of inositol phosphate (PIP (2)) cascade. Furthermore, the spermatozoon-oocyte fusion as mediated by the GTP-binding protein (protein G) leads to the generation of two secondary messengers, i.e., inositol 1,4,5 triphosphate (IP(3)) and diacylglycerol (DAG). Activity of IP(3) is directly involved in calcium

release from intracellular stores. Concurrently, DAG activity activates protein kinase C (PKC) which in turn leads to exocytosis of cortical granules. Calmodulin-dependent kinase II is another key molecule involved in the translation of the calcium signal. The release of GC content promotes changes in the sperm receptors on the zona pellucida, consequently leading to hardening of the zona (zona reaction). Another factor acting on the blockade of polyspermy is the modification of the oolemma after the spermatozoon-oocyte fusion and the consequent formation of CG envelopes after the cortical reaction [40].

3. Dynamics of cytoskeletal filaments during maturation

The cytoskeletal filaments are dynamic and adaptable structures that can remain unchanged or undergo modifications according to the needs of the cell. In addition, this system is responsible for chromosome segregation during meiosis and mitosis, for cell division during cytokinesis, and for trafficking molecules and organelles inside the cells [41]. The three filament types of the cytoskeleton are formed by subunits that are characteristic for each one. The microtubules consist of globular and compacted tubulin subunits, whereas actin filaments consist of similarly globular and compacted actin subunits. The intermediate filaments consist of elongated and fibrous polypeptide subunits arranged in a tetramer analogous to both the αß-tubulin subunits in the microtubule and the actin monomer. Their function is mainly related to mechanical resistance in response to stress. These three types of cytoskeletal ''polymers'' are maintained by weak noncovalent interactions, and can rapidly associate and dissociate without the need for new formation or breakage of covalent bonds [41]. Among the three types of cytoskeletal filaments, microtubules are more directly involved in the processes of organelle movement [42] even though the participation of microfilaments was also observed during ER redistribution in starfish oocytes [43]. The microtubule subunits adhere to motor proteins such as dynein, dynactin and kinesin, which bind to molecules and to membranes of the organelles, and promote movement along the microtubules [44].

The microtubules have two distinct regions: a growth end facing the periphery of the oocyte (plus-ends) and another end facing the interior of the cell or the microtubule organizing center (minus-ends). The two major motor protein families are kinesin, which mediates the movement towards the plus-end region, and dynein which, in association with dynactin, mediates the movement towards the minus-end in *Xenopus laevis* oocytes [44]. These observations were confirmed by FitzHarris et al. [45] in a recent study in which the movement of the ER was analyzed during IVM of mouse oocytes. Their study demonstrated that the ER reorganization during oocyte maturation is a complex multi-step process involving distinct microtubule- and microfilament-dependent phases and indicated a role for dynein in the cytoplasmic changes which prepare the oocyte for fertilization. The cytoplasmic dynein is also directly involved in various cell functions, such as chromosome movement, organization and positioning of the meiotic spindle, and nuclear migration [46,47]. On the basis of this information, we will describe below the events associated with chromosome movement during oocyte maturation and the involvement of the cytoskeletal filaments in these events.

During the GV stage of oocyte growth, the spatial rearrangement of the organelles is related to the modified organization of the cytoskeleton that forms a network in which the organelles encased by a membrane move and occupy defined positions [48] (Fig. 2). When entering the M phase of the cell cycle (meiosis in the case of female gametes) microtubule asters appear close to the condensed chromatin in bovine oocytes after GVBD. Furthermore, during the transition from the GV stage to anaphase I, the microfilaments or actin filaments are distributed in the cortical area below the oolemma, without connecting to the microtubules [49] (Fig. 2). In metaphase I (MI), the microtubules are nucleated by tubulin polymerization in the oocyte cytoplasm with the centrosome, forming the meiotic spindle and the metaphase plate in which the chromosomes are arranged in an equatorial manner [3,48,50] (Fig. 2A). In MI, the metaphase plate is proportionally larger than that formed in MII and in this phase the actin filaments are abundantly distributed in the cortical region but are absent among the microtubules (Fig. 2). The spindle is barrel-shaped and its poles are flattened [49]. Although the microfilaments are absent among the microtubules, there seems to be an interaction between these polymers since the polarized movement of the chromosomes also depends on processes mediated by actin filaments [42,51].

As the cell approaches anaphase I, the chromosomes start to separate and therefore a large portion of microtubules can be seen located between the two segregating chromosome sets. The spindle elongates and a large quantity of actin filaments can be observed around the chromosomes (Fig. 2). In telophase I (TI), the microtubules located between the two chromosome sets form a cone-like triangular structure having a wider and a more tapered shape (Fig. 2B). The wider portion

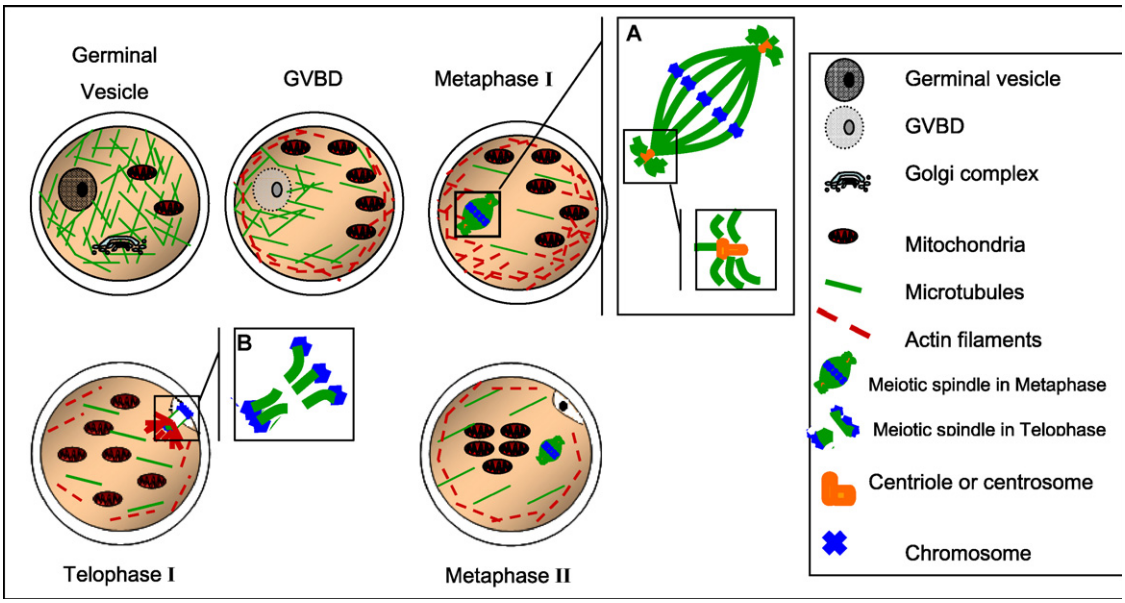

Fig. 2. Dynamics of cytoskeleton filaments during cytoplasmic and nuclear maturation of bovine oocytes. (A) Detail of the meiotic spindle apparatus at metaphase I and the centriole/centrosome structure. (B) Detail of the meiotic spindle at telophase I, in which we can see the microtubules among the chromosome sets.

of the microtubules is linked to the chromosome set destined for extrusion out of the cell, thus forming the first polar body. The tapered portion is associated with the set that will remain in the oocyte and enter meiosis II, again forming the metaphase plate. Thus, during oocyte meiosis, this cytoskeletal structural system still plays a fundamental role by ensuring that almost the entire cytoplasm of the dividing cell will remain in the secondary oocyte, which has accumulated all the information needed for the progression to subsequent steps. Only a small cytoplasmic portion will then be eliminated at the time of polar bodies extrusion. Based on this, the actin filaments associated with the chromosomes will also be eliminated at the time of extrusion [49].

During the transition from TI to MII there is no additional interphase period, but only a rapid chromatin condensation and the disappearance of the microfilaments and microtubules of the meiotic spindle in a process referred to as interkinesis [50]. The metaphase plate reappears soon after this event. As stated earlier, the meiotic apparatus is smaller in MII than in MI, a fact possibly explained by the reduction of chromosome number to half the initial amount [49] (Fig. 2).

Thus, in addition to what was described earlier regarding the movement of cytoplasmic organelles, the filaments of the cytoskeleton still actively participate in the capacitation of the oocyte as a whole for the nuclear acquisition of competence to progress through development. The dynamics of the cytoskeletal filaments discussed thus far has been observed and related to the acquisition of nuclear developmental competence in studies on bovine [52] and swine [53] oocytes. In murine and bovine oocytes, the events involving the cytoskeleton during oocyte maturation are regulated by molecules such as the serine/threonine kinase Aurora-A [54,55] and the cytoplasmic polyadenylation element-binding protein (CPEB) [55], which is synthesized and accumulated in the oocyte cytoplasm during its molecular maturation [55]. The protein kinase Aurora-A and other Auroras kinases co-localize with the centrosomes, chromosomes and midbody and their activity can be observed in the regulation of chromosome segregation, in the maintenance of MII and in the formation of the first polar body. On the other hand, the massive destruction of the CPEB protein is related to the transition from MI to MII [55]. These activities may be a consequence of these molecules acting on the other crucial molecular mechanisms that are described below.

4. Molecular maturation

Molecular maturation corresponds to the phases of oocyte growth and maturation and it involves the transcription, storage and processing of the mRNAs expressed by the chromosomes, which will be further translated into proteins by the ribosomes. The proteins derived from these mRNAs are involved both in

maturation and in subsequent cellular events such as fertilization, pronucleus formation and early embryogenesis. Thus these proteins are being stored until the appropriate time for their utilization [56].

Since many transcripts will be consumed, until the activation of embryonic genome, their correct storage in the oocyte cytoplasm is of great importance. After meiosis is resumed there is no longer any gene expression [56], and therefore what was produced during the growth phase will be metabolized at the appropriate time.

The mRNA transcribed during the molecular maturation of the oocyte is accumulated in a stable, but transiently inactive manner [24,56]. The biosynthetic machinery of the cytoplasm processes this mRNA into ribonucleoprotein particles [57]. In this ''packed'' form, the mRNA is protected from nucleolytic degradation and remains stored until the signals for translation are generated during maturation and early embryo development [58].

4.1. Polyadenylation

Several mechanisms are involved in the activation of translationally inactive mRNA [24,59]. These mechanisms involve the phosphorylation of many factors that initiate translation (such as eIF-4F [60]), the phosphorylation of S6 protein on the 40S ribosomal subunit [50,61], and the dephosphorylation of poly(A)-polymerase [62]. According to this model, polyadenylation (the addition of adenine) of the 3′ terminal portion of the cytoplasmic mRNA would stimulate the release of repressor molecules linked to the 5′ portion [24,63], thereby initiating translation. This polyadenylation process starts in the nucleus, and 250 to 300 adenine (A) residues are added to mRNA for the formation of the poly-(A)-3′ tail. The transport of this mRNA to the cytoplasm occurs through a characteristic splicing of the poly-(A) tail which, after reaching the cytoplasmic compartment, becomes smaller and heterogeneous in size [24,64]. When mRNA molecules have short poly(A) tails they are not effectively translated [65] and deletion of this sequence is an early step in the process of degradation [24,66]. The cytoplasmic prolongation of the poly-(A) tail, however, is related to the activation of translation [67] and this means that the addition of adenine to mRNA in the oocyte cytoplasm during maturation leads to the translation of proteins and deadenylation. This in turn leads to the degradation of the particular mRNA.

Recently, supporting evidence on the patterns of polyadenylation in bovine oocytes and embryos was published. In a study on the polyadenylation of various mRNAs involved in the beginning of oocyte meiosis resumption to the first embryo cleavages, Brevini et al. [68] observed that, depending on the transcript investigated, mRNA polyadenylation may vary according to four distinct patterns: no changes, gradual reduction, gradual elongation, and reduction followed by elongation. When evaluating oocytes from the GV stage to the MII stage, the authors observed that the poly-(A) tail of the mRNAs was significantly shorter at MII than during GV, although an elongation or no change in size in the poly-(A) tail was observed in some transcripts. In subsequent stages (i.e., during fecundation and the formation of zygote as well as the two-cell embryo), the polyadenylation pattern was, with few exceptions, the same. When comparing better quality embryos (i.e., embryos that cleaved up to 27 h after insemination) to embryos with low developmental potential (cleavage after 27 h post-insemination), the authors observed several differences in the polyadenylation patterns and concluded that these specific changes in mRNA polyadenylation are associated with modulation of gene expression in the embryos during this phase. In addition, they also concluded that abnormal levels of polyadenylation in some maternal mRNAs are accompanied by a lower potential for embryo development [68].

In addition to those directly related to the acquisition of developmental competence, the main transcripts produced during molecular maturation of the cumulus-oocyte complexes (COCs) encode regulators of the cell cycle: maturation promoting factor (MPF) and its forming subunits, cyclin B and $p34^{cdc2}$; the protein of the *c-mos* pro-oncogene (MOS), and mitogen-activated protein kinase (MAPK) in addition to countless other mRNAs to be described later on [69]; the proteins and molecules that are markers of cytoplasm maturation such as glutathione [70] and ATP molecules [3]; and the components of the antioxidant enzyme system catalase, superoxide dismutase, and glutathione peroxidase [71].

4.2. Regulators of the cell cycle

We know that an event preceding the resumption of meiosis in bovine oocytes is the synthesis of new proteins [72,73]. Most of the proteins and factors involved in this process are synthesized during the first hours of in vitro culture, with MPF being responsible for the resumption of meiosis [73]. MPF is a heterodimer consisting of the regulatory subunit cyclin B1 and the catalytic subunit $p34^{cdc2}$ [74]. Maternal stores of cyclin B1 mRNA are detected in bovine oocytes in the GV

stage from ovaries recovered at abattoirs, but the protein is not yet present. The mRNA of cyclin B1 is stored along with a short poly-(A) tail in a translationally inactive manner and the accumulation of this transcript leads to extensive cytoplasmic polyadenylation and subsequent translation and synthesis of the protein cyclin B, which is accumulated and detected in the oocyte cytoplasm 3 h after the beginning of IVM [72,73]. Studies on *Xenopus* oocytes and on human somatic cells cultured in vitro have revealed that the mRNA of the protein kinase $p34^{cdc2}$ or Cdk1 also undergoes lengthening of the poly-(A) tail by polyadenylation. This process involves the phosphorylation of CPEB through the action of the mitotic kinase Aurora-A. In bovine oocytes, it has been observed that both CPEB and Aurora-A are synthesized and accumulated in the cytoplasm during oocyte maturation [55]. CPEB is bound in a repressive manner to a specific RNA sequence (UUUUUAU). The cytoplasmic polyadenylation element (CPE), located in the non-translated 3′ region of the mRNAs, consists of an mRNA recognition site for the beginning of polyadenylation. Thus the Aurora-A-CPEB phosphorylation pathway leads to the beginning of polyadenylation and consequently to the translation of $p34^{cdc2}$ [75–77]. After translation, the $p34^{cdc2}$ protein joins cyclin B1 and this complex is then phosphorylated by the Wee1/Myt1 kinases. This leads to the formation of the so-called preMPF which, after undergoing dephosphorylation by phosphatase cdc25, is activated and starts to have the functional activity of MPF proper [74].

The MOS protein, a product of the c-MOS proto-oncogene, is also coded by maternal mRNA stored during oocyte growth. During the maturation period, this mRNA is translated and degraded together with other mRNAs in later phases of development according to the species studied. The MOS protein activates the MAPK cascade, which modulates MPF activity and leads to entry into the cell cycle (transition from the G2 phase to the M phase) [78]. In bovine COCs, the polyadenylation of mRNAs that code for the proteins necessary for MPF and MAPK activation occurs between 9 and 12 h of in vitro culture [79,80]. Thus, appropriate synthesis and storage of these transcripts in the oocyte cytoplasm will also be crucial for the continuity of nuclear resumption and progression of meiosis.

4.3. Other mRNAs involved in the acquisition of competence for development

Other transcripts coded during COC maturation are added to those listed here as a function of the equilibrium and coordination of the cellular events involved in the acquisition of competence as a whole. Gonadotrophins play an early role in physiological coordination and therefore mRNAs that code for FSH, LH and connexin 43 receptors (FSHr, LHr and Cx 43r) must also be transcribed by the cumulus-oocyte complex so that the hormones will act during oocyte maturation when added in vitro, as it occurs in vivo. Connexins are transmembrane proteins that form gap junctions, as well as being responsible for communication between the oocyte and the surrounding cumulus cells [69]. Connexin 43 (Cx43), in particular, is involved in follicular growth and its mRNA and protein are present during the maturation of ovine COCs [81]. The mRNAs of FSHr, LHr and Cx43 may be considered to be predictors of oocyte competence in vitro by performing such important functions in cell signaling and folliculogenesis. Recent studies have shown that levels of mRNA coding these proteins depend on maturation time and oocyte quality [70,82].

4.4. Markers of cytoplasmic maturation and the antioxidant system

In addition to cell cycle regulators, other critical molecules for the development and progression of maturation and early embryogenesis are synthesized and accumulated inside the oocyte, with their presence considered to be a marker of having acquired cytoplasmic competence to support the subsequent phases. We consider good biological markers of cytoplasmic maturation to be those that can be analyzed by reproducible, precise and minimally invasive techniques [83]. Within this context, glutathione is one of the markers of cytoplasm maturation that has been extensively investigated. Several studies have shown that this enzyme plays a fundamental role in cell protection against oxidative damage [84] by eliminating the ROS produced during mitochondrial metabolism. Its intracellular concentration increases as the oocytes develop from the GV stage to MII [70,85]. After fertilization, its activity is related to the decondensation of sperm chromatin, with consequent oocyte activation and also the transformation of the sperm head into a male pronucleus [84]. If the medium used for IVM is deficient in cysteine, a precursor of glutathione [86], the glutathione synthesis will be affected by substrate insufficiency and then the oocytes will be cultured under suboptimal conditions resulting in unsatisfactory embryo development [70,84]. In addition to glutathione, ATP molecules have also been used as markers of cytoplasmic maturation in bovine [3] and

gilt oocytes [87]. In a recent study, it was reported that the number of ATP molecules in morphologically competent oocytes (category 1, homogeneous oocyte cytoplasm, compact multilayered cumulus oophorus; category 2, cytoplasm with small inhomogeneous areas, more than five layers of compact cumulus; category 3, heterogeneous/vacuolated cytoplasm, three to five layers of cumulus including small areas of denuded zona pellucida) increases from 1.4–1.8 pmol before IVM to 2.3–2.5 pmol after maturation, with a positive reflex also on early embryo development [3].

Together with glutathione, other molecules in the antioxidant enzymatic system also play an important role in attenuating the deleterious effects of ROS-induced oxidative stress [71]. It was demonstrated that catalase, superoxide dismutase and glutathione are present in bovine oocytes and in cumulus cells after IVM. In addition, their levels in denuded oocytes are lower than those observed in the cumulus-oocyte complex, although no difference has been detected between the two groups regarding ROS production [71].

5. Conclusions

Oocyte maturation is a long process during which oocytes acquire the intrinsic ability to progress through subsequent events of development and involves complex and distinct, although linked, mechanisms of nuclear and cytoplasmic maturation. The structural and biochemical changes described here regarding organelle redistribution, cytoskeletal filament dynamics and molecular maturation - which start during the growth phase and are completed with the progression of development as a direct consequence of oocyte cytoplasmic maturation - are linked mechanisms depending on the functional integrity and the coordinated participation of all the elements involved in the process. On this basis, ultrastructural analysis shows that mitochondria, ribosomes, endoplasmic reticulum, cortical granules and Golgi complex assume different positions from those observed in germinal vesicle oocytes during metaphase II. The microfilaments and microtubules of the cytoskeleton present in the cytoplasm promote these movements and act on chromosome segregation. Molecular maturation, characterized by the transcription, storage and processing of maternal mRNA, is also involved in regulating the cytoskeleton. Thus, the process of cytoplasmic maturation should be interpreted as a gradual capacitation that, together with nuclear meiotic competence, corresponds to the acquisition of critical cellular functions by the oocyte.

Considering that the real measure of competence (i.e., term development) was almost never the end point of experiments that evaluated the correlation between organelle quantity and distribution, it is difficult to justify a causal relationship between cytoskeleton function and molecular events, and oocyte competence. Further studies with an adequate methodology are needed to better understand this association.

Despite the great interest this topic has attracted over the last decades with the advent of new technologies, many gaps remain. As an example, controversies continue regarding the function of several cytoplasmic organelles and molecules in the gradual process of oocyte capacitation. There is lack of consistent information on ribosome, ER, and Golgi apparatus involvement during bovine oocyte maturation. Much of what is known about the participation of these organelles in the process of maturation comes from studies conducted on species other than cattle. There is some evidence indicating an important role for mitochondria in the acquisition of oocyte competence for development. However, with regards to the ATP content of the oocyte, several reports found no correlation between developmental competence and ATP content of the oocytes. In addition, understanding the mechanisms related to intra- and extracellular signaling that coordinate all the events involved in the acquisition of competence also represents an interesting approach that requires further elucidation.

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