

## Oocyte Maturation: Events that support subsequent stages of development

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### SUMMARY

The competence of oocytes to develop to an advanced embryonic stage is dependent on the transformations that occur during oocyte maturation. In bovines, oocyte maturation begins in the phase that precedes ovulation and it is characterized by a series of morphological, molecular, and biochemical changes in the oocyte and somatic cells that surround it. These changes prepare the oocyte for fertilization and subsequent embryonic development. This review focuses on the basic principles in the nuclear and cytoplasmic maturation of oocytes through which they acquire meiotic competence and the ability for subsequent embryonic development.

### Maturação oocitária: eventos que suportam etapas subsequentes do desenvolvimento

### RESUMO

A competência de desenvolvimento do oócito há estágio embrionário avançado é dependente das transformações ocorridas durante a maturação oocitária. Em bovino a maturação oocitária in vivo inicia-se na fase que precede a ovulação e é caracterizada por uma série de mudanças morfológicas, moleculares e bioquímicas no oócito e nas células somáticas que o envolvem. Estas mudanças vão tornar o oócito apto a ser fecundado e ter um desenvolvimento embrionário subsequente. Esta revisão enfoca os princípios básicos da maturação nuclear e citoplasmática de oócitos, através dos quais eles adquirem competência meiótica e capacidade para o subsequente desenvolvimento embrionário.

### INTRODUCTION

Maturation is the process through which an oocyte acquires the intrinsic capacity to support subsequent stages of development until the activation of the embryonic genome (De La Fuente & Eppig 2001; Do et al., 2018). This process includes complex and distinct activities, such as the events of nuclear and cytoplasmic maturation (Adona et al., 2008; Coticchio et al., 2015). Nuclear maturation mainly involves chromosomal segregation, while cytoplasmic maturation includes biochemical-molecular modifications and reorganization of organelles (De La Fuente & Eppig, 2001; Coticchio et al., 2015; Do et al., 2018). Although the importance

of meiotic progression for oocyte maturation has been studied for a long time, many of the maturation events that are critical for oocyte development and enhancement of oocyte competence for subsequent embryonic development remain to be elucidated. For the purposes of this review, oocyte maturation will be divided into nuclear and cytoplasmic maturation, although these events occur concomitantly and intrinsically. This review is expected to inspire further studies into the advancement of assisted reproduction biotechnologies, such as the development of new in vitro maturation systems and the identification of mechanisms involved in oocyte competence.

## NUCLEAR MATURATION

At birth, bovines have an established number of primary oocytes, and the differentiation of primary oocytes into secondary oocytes occurs at sexual maturity. This process of oocyte differentiation is called maturation and occurs over a period of 24 hours via a series of changes that are stimulated initially by the gonadotrophic hormone (Zhang & Xia, 2012; García-Díaz et al., 2017; Sun et al., 2017). Under hormonal influence, the oocyte in the pre-ovulatory follicle progresses from the diplotenus phase of prophase I (PI) through the stages of metaphase I (MI), anaphase I (AI), and telophase I (TI, conclusion of meiosis I) to the stage of metaphase II (MII) of the second meiotic division, following which it is ovulated (Zhang & Xia, 2012). At the end of meiosis I, the cytoplasm is divided asymmetrically, generating two cells of different size (**Figure 1**): a small cell called the polar corpuscle and the large ovule (Adona et al., 2008).

Structural and molecular-biochemical reorganization is necessary for the occurrence of meiotic events in the oocyte. This reorganization is part of a complex interaction between the nucleus and cytoplasm, and it will be discussed in more detail throughout the text. However, the most striking events observed in nuclear maturation are germinal vesicle (GV) disruption, condensation, alignment, chromosomal segregation and the formation of the first polar corpuscle (Adona et al., 2008). Under appropriate conditions, these events are easily observed in the bovine species (Adona et al., 2008; Sugimura et al., 2018).

Following the maturation events, the oocyte in MII is capable of being fertilized (Adona et al., 2008). The mechanisms that trigger the activation of the oocyte in MII will promote the termination of the second meiotic division, characterized by the progression of MII to telophase II (TII) with the release of the second polar corpuscle (Adona et al., 2008; Souza-Fabjan et al., 2014). Thus, the genetic material of the oocyte is halved by two successive meiotic divisions (Alberts et al., 2010). The ploidy returns to its original value with the interaction (syngamy) of the spermatozoid with the oocyte, forming a zygote that will develop into embryo, which will proceed continue development via mitotic division (Khatir et al., 1998).

## CYTOPLASMIC MATURATION

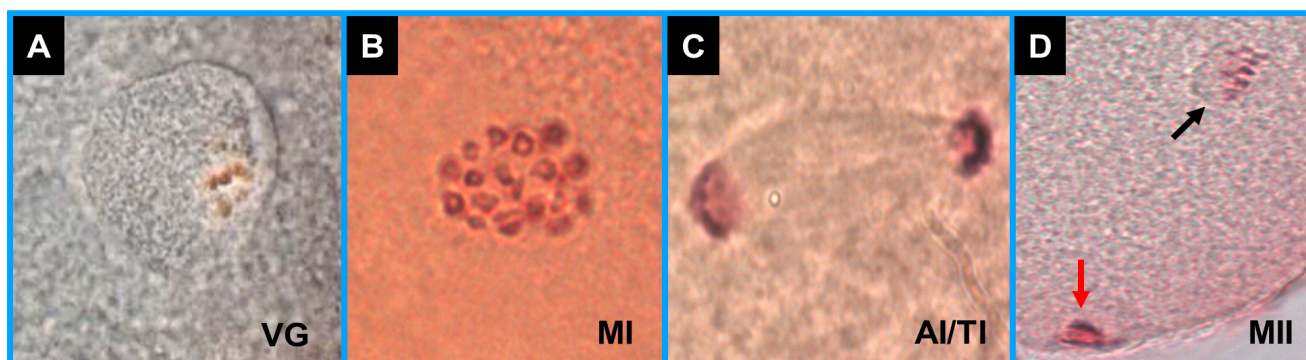
Competence development of oocytes is primarily dependent on the biochemical and structural transformations that occur in the cytoplasm (Ferreira et al., 2009; Khan et al., 2016). These transformations, involved in maturation, are highly intricate processes involving several simultaneous events, such as protein syntheses, molecular modifications, migration and reorganization of organelles in the cytoplasm (Chen et al., 2016; Khan et al., 2016). These changes in the oocyte cytoplasm will be discussed in more detail in the following sections.

Cytoplasmic maturation can be studied by different methodologies (embryo production, reorganization of the organelles, or molecular methods), but the most commonly used for the evaluation of *in vitro* cytoplasmic maturation is the proportion of blastocysts (Adona et al., 2008; Sugimura et al., 2018). Evaluations of cytoplasmic maturation (45% blastocysts) for study purposes are considered lower than nuclear maturation (85% MII). However, this disparity between maturation (cytoplasmic and nuclear) is expected, taking into account the type and the difficulty of the evaluations, mainly referring to cytoplasmic maturation (Adona et al., 2008; Ferré et al., 2018; Sugimura et al., 2018). The evaluation of cytoplasmic maturation rate in the embryo is quite complex, since it involves different steps in the process, such as the maturation itself, fertilization by semen, and embryo culture.

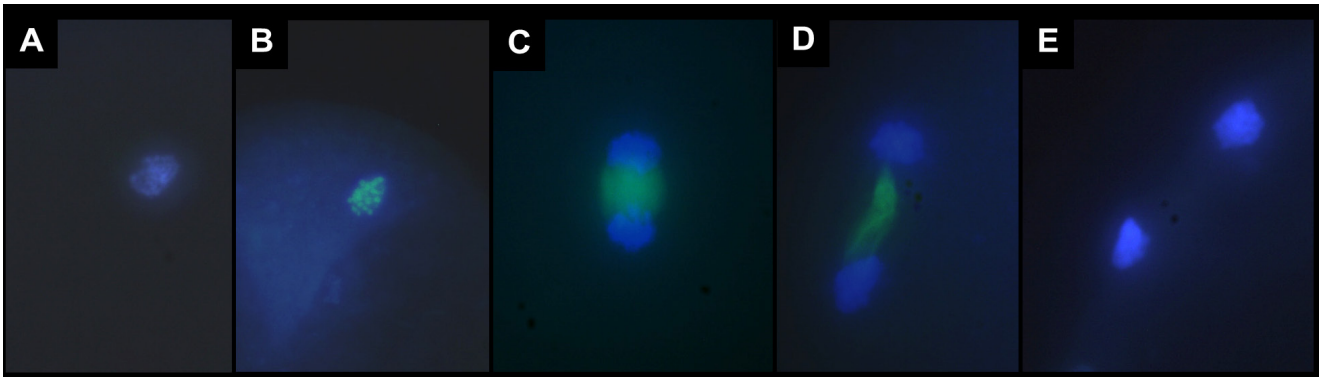
## STRUCTURAL CHANGES

### CYTOSKELETON

Microtubules (homologous polymers of alpha and beta tubulins) are the major components of the cellular cytoskeleton (**Figure 2**), providing the framework for cell division (Ferreira et al., 2009; Kalous et al., 2018; Xie et al., 2018). Actin microfilaments are also an integral part of the cytoskeleton and are located mainly in the cortex (layer just below the plasma membrane) of oocytes in the GV stage and in the meiotic axis of oocytes after GV rupture (Adona et al., 2008; Ferreira et al., 2009; Xie et al., 2018).



**Figure 1.** Classification of meiosis in bovine oocytes. Germinal vesicle (A); metaphase I (B); progression between anaphase I and telophase I (C) and metaphase II (D). Black arrow: metaphase plate and red arrow: 1st polar corpuscle (Classificação de meiosis em oócitos bovinos. Vesícula germinal (A); metafase I (B); progressão entre a fase I e metafase I (C) e metafase II (D). Seta preta: placa metafase e seta vermelha: 1º corpóriculo polar).



**Figure 2.** Dynamics of microtubules in bovine oocytes: Blue-stained chromatin (chromosomes) and green microtubules. Oocytes at the stage of Germinal vesicle (A); metaphase I (B); anaphase I (C); telophase I (D) and metaphase II (E) (Dinâmica de microtúbulos em oócitos bovinos: cromatina manchada de azul (cromossomos) e microtúbulos verdes). Oócitos na fase de vesícula germinal (A); metafase I (B); anafase I (C); telofase I (D) e metafase II (E).

During the maturation process (PI to MII), populations of centrosome (microtubule-organizing center) regulate microtubules coordinately, in nuclear and cytoplasmic events. These events include asymmetric-oriented meiotic axis formation, the extrusion of the first polar corpuscle, the formation of the second metaphase plate, and the positioning of the organelles inside the cell (Adona et al., 2008; Ferreira et al., 2009; Xie et al., 2018).

With the initiation of maturation and rupture of the GV in the oocyte, the microtubules gain access to the chromosomes, binding to the kinetochore (protein complex found in the centromere, region of constriction) and segregating the chromosomes to the cell poles (Ferreira et al., 2009; Alberts et al., 2010; Xie et al., 2018). The first meiotic division is reductional and involves the segregation of homologous chromosomes while the sister chromatids remain united. The second meiotic division is equational, and leads to the segregation of sister chromatids, similarly to what occurs during mitosis in somatic cells (Brunet et al., 2003). Errors of segregation during one or the other meiotic division may result in an aneuploid embryo after fertilization, which in turn may have serious developmental defects (Brunet et al., 2003; Forman et al., 2012). Specifically, in mammals, the loss or gain of autosomes results in physiological and developmental disorders (Jackson-Cook, 2011). Microtubules are also used by motor proteins (families of kinesins and dyneins) for the periodic migration of organelles within the cell (Barlan & Gelfand, 2017; Xie et al., 2018). Microtubule-dependent motor proteins play an important role in the positioning of organelles within the cell (Alberts et al., 2010; Barlan & Gelfand, 2017).

#### MITOCHONDRIA

Mitochondria are specialized cytoplasmic organelles that occupy a substantial portion of the cellular volume, and are the catalysts of ATP (adenosine triphosphate) synthesis through the metabolism of carbohydrates and fatty acids contained in the cytoplasm, as well as from the external medium of the cells (Ferreira et al., 2009; Woods et al., 2018). The efficiency of the mitochondrial matrix in the conversion of pyruvate to ATP is essential for oocyte maturation, fertilization, and subsequent embryonic development (Ferreira et

al., 2009; Sanchez-Lazo et al., 2014). During the maturation process, the mitochondria rearranges within the cellular cytoplasm. In oocytes at the GV stage, mitochondria are found in greater quantities in the periphery of the cytoplasm, with small groups scattered in the center of the oocyte. In oocytes at the MII stage the mitochondria are more centralized in the cytoplasm (Adona et al., 2008; Ferreira et al., 2009; Reader et al., 2015).

#### ENDOPLASMIC RETICULUM (ER)

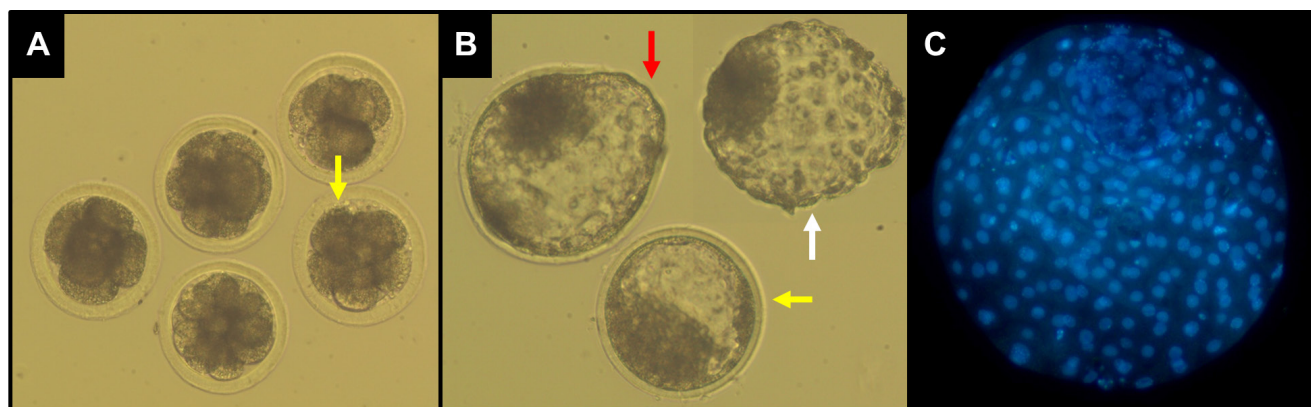
The ER is a multifunctional organelle composed of a three-dimensional network of interconnected tubules and cisterns, which runs from the nuclear membrane (forming the same) to the plasma membrane. This organelle plays a central role in protein and lipid biosynthesis, and stores glycogen (Alberts et al., 2010; Westrate et al., 2015). Its membrane is the site of production of all transmembrane proteins and lipids for most of the organelles, including the ER itself, the Golgi complex, lysosomes, endosomes, mitochondria, secretory vesicles, and the plasma membrane (Westrate et al., 2015). In addition, it has an important role in the control of intracellular  $Ca^{2+}$ , allowing rapid movement of  $Ca^{2+}$  outwards and into its cisterns (Stricker & Smythe, 2003; Westrate et al., 2015).

The structural organization of ER is largely modified during the period comprising oocyte maturation (GV - MII stage) in different species (Payne & Schatten, 2003; Ferreira et al., 2009; Mann et al., 2010). In humans, immature oocytes (GV stage) are largely devoid of cortical ER, while in mature oocytes (MII stage) there is a large accumulation, showing cortical and cytoplasmic ER accumulations (Mann et al., 2010).

#### GOLGI COMPLEX (GC)

The GC is an organelle formed by several cisternae and vesicles surrounded by membranes, through which molecules, synthesized initially in the ER, transit and may undergo additional changes, such as glycosylation, sulfation, phosphorylation, activation of proteins, packaging, and specific distribution to various cellular or extracellular regions (Alberts et al., 2010; Wilson et al., 2011). In addition to the innumerable functions that GC exerts on cells, it is also responsible for the formation of cortical granules (Burkart et al., 2012).





**Figure 3.** Embryos at different stages of in vitro development. Embryos in early stage, White arrow: zona pellucida and yellow arrow: blastomeres (A); Embryos in advanced stage, yellow arrow: blastocyst, red arrow: blastocyst in hatching and white arrow: hatched blastocyst (B) and Blastocyst stained for visualization of nuclei for counting the number of cells (C) (Embriões em diferentes estágios de desenvolvimento in vitro. Embriões em estágio inicial, flecha branca: zona pellucida e flecha amarela: blastomeres (A); Embriões em estágio avançado, seta amarela: blastocisto, flecha vermelha: blastocisto em eclosão e flecha branca: blastocisto eclodido (B) e Blastocyst manchados para visualização de núcleos para contar o número de células (C)).

The role of GC during meiosis in mammalian oocytes is not fully elucidated. However, the dynamics of the GC structural distribution in oocytes during meiosis progression was shown to be dispersed, with vesicles present in almost the entire cytoplasm (Payne & Schatten, 2003; Ferreira et al., 2009). This reorganization of GC in the oocyte in meiosis seems to follow a pattern similar to that found in somatic cells in mitosis (Alberts et al., 2010).

#### CORTICAL GRANULES (CG)

The CG are organelles produced from the Golgi complex and are present only in female gametes of all mammals, most vertebrates and many invertebrates (Wessel et al., 2001; Burkart et al., 2012). They possess a population of molecules, including proteases, glucosidases, enzymes, and structural proteins that contribute to the physical and biochemical barrier blocking polyspermy (Burkart et al., 2012; Xie et al., 2018) (Burkart, 2012 #41; Liu, 2011 #51; Adona, 2011 #14). They are non-renewable secretory vesicles, whose contents stop being synthesized after fertilization, and only return to be codified and transcribed in the new oocytes of the reproductive cycle (Wessel et al., 2001; Burkart et al., 2012).

The main functions of GC contents are to construct (echinoderms) or modify (mammalian) the extracellular matrix (zona pellucida) in the oocytes, which becomes a biochemical and mechanical barrier that prevents the entry of more than one sperm into the oocyte (Wessel et al., 2001; Xie et al., 2018). The CG are formed in growing oocytes, but they are reorganized in the cell during maturation (Burkart et al., 2012; Xie et al., 2018). CG can be identified in small groups in the cytoplasm of the oocytes at the GV stage. Its migration to the periphery of the oocyte occurs with the progression of maturation. In oocytes in MII the CG are distributed in the cortex near the plasma membrane (Adona et al., 2008; Ahmed et al., 2017; Xie et al., 2018).

#### MOLECULAR CHANGES

During oocyte growth and maturation, molecules are synthesized and stored. These molecules will support maturation and development after fertilization,

until the genome of the embryo becomes transcriptionally active, and embryo-derived signals begin to regulate embryogenesis (De La Fuente & Eppig, 2001; Meirelles et al., 2004). Most of the oocyte mRNA is synthesized and accumulated during the period of oocyte growth (Meirelles et al., 2004; Do et al., 2018). Metabolism in the oocyte is characterized by transcription and active translation during the pre-ovulatory period. However, gene transcription ceases before ovulation, so the oocyte, zygote, and embryo (Figure 3), with less than 16 blastomeres (bovine), are dependent on the pool of mRNAs and proteins accumulated during the period of growth and maturation (Meirelles et al., 2004; Salilew-Wondim et al., 2018). Oocytes depend on the synthesis and activation of a variety of proteins so they can enter and continue the process of oocyte maturation (Soeda et al., 2013; Chen et al., 2016).

#### CYCLIC ADENOSINE MONOPHOSPHATE (cAMP)

cAMP plays a key role in the control of oocyte maturation, especially in mammals and amphibians (Hinckley et al., 2005; Sun et al., 2017). Maintenance of arrested meiosis at the stage of PI, or its resumption, is controlled by different levels of cAMP, and the reduction of cAMP in the oocyte seems to be involved with the rupture of the GV, at least in vitro (Sun et al., 2017). Thus, elevated cAMP levels maintain meiotic arrest and its decrease is a necessary signal for the rupture of the meiotic block (Hinckley et al., 2005; Sun et al., 2017).

The loss of intercellular communication resulting from peaking luteinizing hormone (LH) may be a trigger for the oocyte to resume meiosis. This loss of intercellular communication will cease the supply of cAMP from the cumulus cells, thus reducing intra-oocyte concentrations, which in turn leads to the inactivation of the protein kinase A pathway (Perniss et al., 2017; Sun et al., 2017). However, GV disruption occurs prior to metabolic decoupling between the oocyte and cumulus cells, and the level of intra-oocyte cAMP does not necessarily decline during the resumption of meiosis (van den Hurk & Zhao, 2005; Sun et al., 2017).

The LH peak promotes a rapid increase of cAMP and intracellular calcium ( $\text{Ca}^{2+}$ ) in cumulus cells and the direct transport of inositol triphosphate (IP3) to the oocyte, followed by the release of calcium into the oocyte (Whitaker, 2006; Ferrer-Buitrago et al., 2018). Calcium is responsible for protein modifications by stimulating the pathway of phosphorylation or dephosphorylation, or by activating proteases (Ferrer-Buitrago et al., 2018). The LH peak induces a high-frequency calcium signal in mammalian oocytes, which in domestic animals is accompanied by the synthesis of cyclins and possibly other cell cycle proteins (van den Hurk & Zhao, 2005). Modification of cyclins by the calcium signal is required for the activation of the maturation promoting factor, which results in GV disruption and oocyte activation (Ferrer-Buitrago et al., 2018).

#### FACTOR PROMOTING MATURATION (MPF)

The MPF is the inducer of the cell division process and its activity can be detected in the oocytes at the MI and MII stages, declining its activity during the transition between MI and MII (Kubelka et al., 2000; Han et al., 2017). MPF activity is reactivated for oocyte entry into the MII stage, and its inactivation in oocytes arrested at MII is induced by fertilization (Han et al., 2017). Inactivation of MPF is the trigger to resume meiosis arrested in MII (Kubelka et al., 2000; Han et al., 2017). The regulation of MPF activity depends on the association of its subunits: catalytic subunits such as serine-threonine kinase ( $\text{p34}^{\text{cdc}2}$ ) and regulatory subunits such as cyclin B (Jones 2004; Han et al., 2017) RNA binding protein immunoprecipitation, and luciferase reporter assay, we investigated how rates of mRNA translation, protein synthesis and degradation contribute to the steady state level of Cyclin B1 and B2 in mouse oocytes. Ribosome loading onto *Ccnb1* and *Mos* mRNAs increases during cell cycle reentry, well after germinal vesicle breakdown (GVBD). However, activity of the MPF complex is dependent on subsequent phosphorylation on tyrosine 15 (Y15), threonine 14 (T14), and threonine 161 (T161) residues (Kikuchi et al., 2000; Gaffré et al., 2011). In general terms,  $\text{p34}^{\text{cdc}2}$  associated with cyclin B, is phosphorylated at Y15 and at T14 by the *Myt1* and *Wee1* protein kinases; at this stage, MPF (pre-MPF) is still inactive. Therefore, activation of MPF still depends on the dephosphorylation of Y15

and T14 by protein phosphatase *cdc25* (Kikuchi et al., 2000). The process of inactivation of the MPF complex is independent of its catalytic activation, which is generally caused by the proteolysis of cyclin B (Kikuchi et al., 2000; Han et al., 2017). In fertilized oocytes, degradation of cyclin B was related to the inactivation of the MPF complex (Kikuchi et al., 2000).

#### MITOGEN-ACTIVATED PROTEIN KINASE (MAPK)

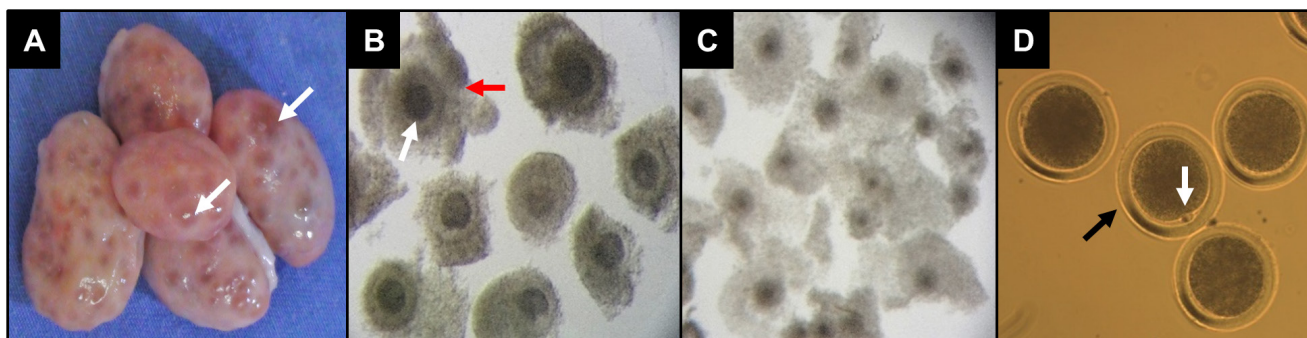
The MAPK belongs to the serine/threonine kinase family and is activated within specific signal transduction pathways by extracellular signals (Kubelka et al., 2000; Sha et al., 2017). For this reason, MAPK is also called extracellular signal-regulated kinase (ERK). Its activation in maturation in vertebrate oocytes is universal. However, the time required for its activation is different in different species (Kubelka et al., 2000; Sha et al., 2017). In bovine oocytes, it occurs after 8 hours of *in vitro* culture, and presents a gradual increase until 12-14 hours, then remains stable until the end of maturation (Kubelka et al., 2000). The two major isoforms (ERK1 and 2) of MAPK are activated with the proximity of GV breach at oocyte maturation. This suggests that MAPK is not required to restart meiosis, but is essential in post-GV events (Sha et al., 2017).

#### PROTO-ONCOGENE PROTEIN (C-MOS)

*c-mos* is a mitogen-activated kinase protein belonging to the same family of serine/threonine kinase found in sperm and oocytes (Sheng et al., 2002; Zhang et al., 2015). It is usually the first to be expressed during regulation of oocyte maturation, and is identified as a key regulator of meiosis that acts at multiple stages throughout the meiotic process (Phillips et al., 2002; Kong et al., 2012). This protein has an important regulatory role in the maintenance of oocytes at the MII stage in different species (Wu et al., 1997; Nishimura et al., 2009). In cattle it was detected around 4 hours after the resumption of meiosis, being actively synthesized in mature oocytes, and disappears after fertilization (Wu et al., 1997).

#### CYTOSTATIC FACTOR (CSF)

The CSF, by definition, is not a single molecule or protein, but an activity found in the oocyte (Wu & Kornbluth, 2008; Kalous et al., 2018). It is responsible for



**Figure IV.** Ovaries of cattle, white arrows: ovarian follicles (A); cumulus-oocyte complex, white arrow: oocyte and red arrow: cumulus cells (B); cumulus-oocyte complex expanded in vitro (C) and oocytes free from cumulus cells for visualization of the polar corpuscle, black arrow: zona pellucida and white arrow: 1st polar corpuscle (D) (Ovários de gado, flechas brancas: folículos ovarianos (A); complexo cumulus-oócito, flecha branca: oócito e seta vermelha: células cumulus (B); cumulus-oócito complexo expandiu in vitro (C) e óocitos livres de células cumulus para visualização do corpo corpuscle polar, seta preta: zona pellucida e seta branca: 1st corpuscle polar (D))



arresting the oocyte in MII, while maintaining MPF stability, preventing spontaneous parthenogenetic activation of the oocyte that is ready to be fertilized (Kalous et al., 2018). However,  $\text{Ca}^{2+}$  addition, or  $\text{Mg}^{2+}$  removal, causes rapid loss of CSF activity in oocyte extract. Conversely, removal of  $\text{Ca}^{2+}$ , or addition of  $\text{Mg}^{2+}$  stabilizes the activity of CSF (Meyerhof & Masui, 1977). These results indicate that they are activated proteins, with  $\text{Mg}^{2+}$ -dependent phosphorylation, and their activity is inhibited by  $\text{Ca}^{2+}$ /calmodulin ( $\text{Ca}^{2+}$  ions) (Masui, 2000).

Stabilization of MPF is maintained through CSF and c-mos protein, and mediated via MAPK (Perry & Verlhac, 2008; Kalous et al., 2018). In *Xenopus* oocytes, the c-mos protein and the ribosomal kinase S6 (p90rsk) have been reported as candidates for CSF (Sagata et al., 1989; Gross et al., 1999). These proteins appear to be involved directly or indirectly in inhibition of the anaphase promoter complex (APC), which targets cyclin B protein, maintaining MPF stabilization and arrest of the oocyte in MII (Tunquist & Maller, 2003; Cui et al., 2012).

#### CUMULUS-OOCYTE COMPLEX (COC)

Cumulus cells are highly specialized and have a cytoplasmic trans-zonal structure that penetrates the zona pellucida and comes in contact with the plasma membrane of the oocyte, giving shape to the COC (Sun et al., 2015). The structures of the communicating gap junctions allow the transfer of molecules of small molecular weight throughout the COC, and larger molecules are transported by endocytosis mediated by receptors (Gilchrist et al., 2004; Sun et al., 2015; Zhou et al., 2016). Molecules that pass through gap junctions include ions, metabolites, and amino acids that are required for development of the oocyte (Sun et al., 2015; Zhou et al., 2016). This model of ovary communication plays a key role for local dissemination of endocrine signals from the oocyte via cumulus cells, or vice versa (Gilchrist et al., 2004; Smith et al., 2012).

Among follicular cells, only cumulus cells have the capacity to undergo modifications, such as cell expansion, and this attribute is regulated by factors secreted by the oocyte (Gilchrist et al., 2004). COC expansion in vivo is initially induced by the LH peak in COC of pre-ovulatory follicles, which leads to extracellular production of a matrix protein produced by cumulus cells, creating a large spherical mass of expanded cumulus cells (Su et al., 2003). These cells also prevent changes in the oocyte that are unfavorable to normal fertilization (Van Soom et al., 2002; Tanghe et al., 2003). In cattle, (Figure 4) cumulus cell expansion and gap junction loss occur along meiosis progression, regulating maturation and developmental capacity of the oocyte. Mistakes in COC expansion may cause a reduction in oocyte fertilization (Van Soom et al., 2002; Luciano et al., 2004).

#### CONCLUSIONS

Comprehensive understanding of new mechanisms involved in oocyte maturation is of fundamental importance for the enhancement of assisted reproduction biotechniques, which are aimed at improving repro-

ductive efficiency in cattle or other species. The full development of in vitro oocyte competence is sought to realize the optimization of oocytes/embryos during the in vitro production of embryos or for opening new avenues for studies on reproductive diagnoses in different species.

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