Oocyte maturation: gamete-somatic cells interactions, meiotic resumption, cytoskeletal dynamics and cytoplasmic reorganization

Giovanni Coticchio*, Mariabeatrice Dal Canto, Mario Mignini Renzini, Maria Cristina Guglielmo, Fausta Brambillasca, Diana Turchi, Paola Vittoria Novara, and Rubens Fadini

Biogenesi Reproductive Medicine Centre, Istituti Clinici Zucchi, Via Zucchi 24, 20900 Monza, Italy

*Correspondence address. Tel: +39-039-83833369; E-mail: coticchio.biogenesi@grupposandonato.it

Submitted on September 29, 2014; resubmitted on February 4, 2015; accepted on February 11, 2015

TABLE OF CONTENTS
- Introduction
- Methods
  - Literature search
- Results
  - Cellular bases of oocyte–CC interactions during maturation
  - Oocyte–CC signalling for meiotic arrest and resumption
  - Global chromatin remodelling, meiotic resumption and developmental competence
  - DNA damage and meiotic progression
  - GV positioning and oocyte polarity
  - Cytoskeletal forces governing spindle positioning
  - Cortical polarization and polar body extrusion
  - Changes in cortical mechanics important for oocyte maturation
  - Cytoplasm reorganization during maturation
  - Clinical correlates of oocyte maturation
- Conclusions

BACKGROUND: In a growth phase occurring during most of folliculogenesis, the oocyte produces and accumulates molecules and organelles that are fundamental for the development of the preimplantation embryo. At ovulation, growth is followed by a phase of maturation that, although confined within a short temporal window, encompasses modifications of the oocyte chromosome complement and rearrangements of cytoplasmic components that are crucial for the achievement of developmental competence. Cumulus cells (CCs) are central to the process of maturation, providing the oocyte with metabolic support and regulatory cues.

METHODS: PubMed was used to search the MEDLINE database for peer-reviewed original articles and reviews concerning oocyte maturation in mammals. Searches were performed adopting ‘oocyte’ and ‘maturation’ as main terms, in association with other keywords expressing concepts relevant to the subject. The most relevant publications, i.e. those concerning major phenomena occurring during oocyte maturation in established experimental models and the human species, were assessed and discussed critically to offer a comprehensive description of the process of oocyte maturation.

RESULTS: By applying the above described search criteria, 6165 publications were identified, of which 543 were review articles. The number of publications increased steadily from 1974 (n = 7) to 2013 (n = 293). In 2014, from January to the time of submission of this manuscript, 140
Introduction

The unparalleled size and unique ability to develop into a totipotent zygote place the fully grown mature oocyte at the very centre of the reproductive strategy of mammals and other metazoans. In fact, while, with the exception of mitochondrial DNA (mtDNA), oocytes and spermatogenesis contribute equally to the genetic make-up of the new individual, the cellular and molecular continuum across generations is ensured almost exclusively by the female gamete. This justifies the phenomenal investment in regulatory processes by which oocytes are generated, nurtured and matured for a period that can span as much as several decades in a woman.

The natural history of the oocyte begins during fetal life when its generation as a distinct cell type coincides approximately with the initiation of meiosis and assembly into the primordial follicle (Gougeon, 1996). Entry into meiosis is immediately followed by arrest at the diplotene stage and establishment of a condition of relative metabolic quiescence. In such a suspended state, the oocyte can pause for up to 40 years until when, under the impulse of a still not fully understood mechanism, it undertakes a phase of growth while remaining meiotically arrested. In the human, oocyte growth unfolds over 110–120 days, during which cell mass undergoes an astounding more than 100-fold increase and cell diameter shifts from <40 to ~120 μm. Meanwhile, macromolecules and organelles are produced and stored in very large amounts (Gosden and Lee, 2010). In such a way, the oocyte meets an essential precondition for preimplantation embryo development, stockpiling sufficient cytoplasmic mass required for the fertilized egg to achieve multicellularity in the absence of net growth. Notably, oocyte growth is much more than a quantitative increase in cell mass finalized to the housekeeping needs of the early embryo. Rather, growth is accompanied by precisely programmed qualitative changes in the endowment of organelles and informational molecules that will regulate crucial developmental functions. For example, maternal effect genes have been characterized as sequences whose transcriptional and translational products are generated during oocyte growth and whose regulatory function is required only after fertilization (Li et al., 2010). Typically, many maternal effect gene products are transcriptional regulators whose loss of function causes major developmental failures, such as arrest at cleavage or blastocyst stages, or inability to activate the zygote genome.

Oocyte growth occurs concomitantly and is mutually interdependent with the development and differentiation of the follicle (Hutt and Albertini, 2007). However, oocyte growth is already finalized at the early antral stage, i.e. significantly before follicle development is completed. In fact, while for several days the antral follicle experiences a further expansion in preparation for ovulation, no increase in oocyte size is observed (Griffin et al., 2006). Finally, stimulated by the mid-cycle LH surge, the fully grown diplotene stage-arrested oocyte resumes meiosis and progresses to the metaphase II stage (MII) in ~30 h in the human, thereby achieving meiotic maturity. Therefore, growth and maturation may be discerned as temporally and functionally distinct phases of oogenesis. By definition, the importance of oocyte growth for embryo development has appeared almost self-explanatory to scientists. On the contrary, a proper appreciation of maturation beyond the meiotic imperative has been, and still is, less obvious to appreciate. Oocyte meiosis, whose molecular regulation is not discussed here because it is thoroughly described elsewhere (Holt et al., 2013), is a complex process. In fact, not only does it require substantial modification of the mitotic cell cycle, but also it necessitates a machinery able to attain chromosome segregation with minimal loss of cytoplasm. To this end, the oocyte develops an asymmetric cell design and a peripheral position of the meiotic spindle. This dictates that the cleavage planes of meiotic divisions lie just beneath the oolemma. In this fashion, the extrusion of two small polar bodies is achieved. Attainment of asymmetry and polar body extrusion demand major rearrangements of the cytoskeleton, co-ordinated cytoplasmic dynamics and fine control of tension forces governing the cortex, elements that only recently have emerged in their elegant complexity (Brunet and Verlhac, 2010; Yi et al., 2011).

Oocyte maturation also involves many other intercellular, intracellular and molecular processes whose implications for embryo development are as important as the achievement of haploidy. Studies accomplished...
especially in the last decade have allowed us to better understand the complexity, choreography and significance of oocyte maturation. Examples of the intense cellular activity occurring during oocyte maturation are major changes in chromatin configuration that accompany meiotic resumption (Luciano et al., 2012), modifications in mitochondrial distribution, clustering and activity in response to changing energy demands of the cell machinery (Dalton and Carroll, 2013), and endoplasmic reticulum (ER) reorganization and maturation finalized to intracellular Ca$^{2+}$ signalling (Mann et al., 2010). These and other transformations, illustrated in this manuscript, are finely co-ordinated, and are often interdependent processes. Explicative in this respect is the accumulation of mitochondria and ER elements around the newly formed spindle, without which spindle positioning to the cortex in preparation for the first meiotic division does not occur (Dalton and Carroll, 2013).

In addition to the extreme asymmetry of the meiotic divisions and redistribution and regulation of organelles, another major theme of oocyte maturation, and indeed the larger processes of oogenesis and folliculogenesis, is the intense cross-talk and co-operativity between the oocyte and the surrounding follicle cells (Fig. 1). These cells, referred to as cumulus cells (CCs), originate as a subpopulation of granulosa cells as soon as the antrum is formed in the developing follicle (Matzuk, 2002). Oocyte–CC interactions are implemented through different signalling modalities and are recognized as a founding element of oocyte maturation. In fact, CCs support a wide variety of functions of the maturing oocyte, including metabolism, meiotic arrest and resumption, and cytoskeletal rearrangements.

In providing scope for this review, meiotic progression and attendant cytoplasmic changes are brought together in the same view to appreciate the importance of maturation as a process having direct implications for oocyte quality. The investigation of mammalian oocyte maturation became an increasingly attractive object of investigation in the 1930s, when it was first possible to culture fully grown oocytes and preimplantation embryos (Pincus and Enzmann, 1935). Most of the previous and current knowledge has been developed in experimentally amenable species, such as the mouse, a fact that is reflected in the contents of this review. Nevertheless, the implications of oocyte maturation studies in animal models for the understanding of human reproduction and the treatment of infertility are obvious. Indeed, the first human embryo generated extracorporally was derived from an in vitro-matured oocyte as a result of the pioneering efforts of John Rock and Miriam Menkin in the 1940s (Rock and Menkin, 1944). Therefore, in the context of this manuscript, oocyte in vitro maturation (IVM) is not illustrated as a clinical approach, but rather it is adopted as an experimental paradigm able to shed light on many aspects of the final act of oogenesis and inspire better criteria of oocyte quality and novel in vitro manipulation strategies.

**Methods**

**Literature search**

PubMed was systematically searched for peer-reviewed original articles and reviews identified by relevant keywords, such as ‘oocyte’, ‘oocyte maturation’, ‘in vitro maturation’, ‘meiosis’, ‘germinal vesicle’, ‘chromatin’, ‘DNA damage’, ‘actin’, ‘meiotic spindle’, ‘endoplasmic reticulum’, ‘mitochondria’, ‘Golgi apparatus’, ‘cortical granules’ and ‘cumulus cells’. Keywords were used in multiple and overlapping combinations in order to identify those publications strictly relevant to oocyte function. Additional studies were identified by thorough analysis of reference lists from relevant publications. The ‘English language’ limit was applied. The most relevant publications, i.e. those concerning major phenomena occurring during oocyte maturation, were assessed and discussed critically to offer a comprehensive description of the process of oocyte maturation. Concerning animal studies, priority was given to publications relevant to species more consistently used as experimental models for the human.

**Results**

By applying the above described search strategy, 6165 publications were identified, of which 543 were reviews. The number of publications increased steadily from 1974 (n = 7) to 2013 (n = 293). In 2014, from...
January to the time of submission of this manuscript, 140 original manuscripts and reviews were published. By focusing on studies concerning mainstream phenomena occurring during maturation and described in established experimental models and the human species, the number of publications of interest was narrowed down to a few hundreds, many of which are extensively described or cited in this review. The selected studies, grouped systematically in specific topics and illustrated in the below sections, extend previous knowledge and shed new and astounding knowledge on oocyte maturation.

**Cellular bases of oocyte–CC interactions during maturation**

The interaction between the oocyte and follicle cells is known to occur uninterruptedly from the formation of the primordial follicle until ovulation, but the modalities by which it is implemented can differ over time in response to the challenges imposed by the changing design of the developing follicle. For example, in the minimalist structure of the primordial follicle, direct cell-to-cell contacts prevail as a form of interaction (McLaren, 1988) while, as soon as the follicle becomes more complex and physical barriers are raised between cells, additional systems are deployed to ensure cell communication. Five types of interactions are recognized in the germinal-somatic regulatory loop: (i) direct contact-mediated signals in the absence of intercellular junctions, (ii) typical ligand-receptor interactions, (iii) paracrine signalling pathways, (iv) gap junctions and other junctional contacts via transzonal projections (TZPs) and (v) receptor tyrosine kinases (RTKs) (McGinnis et al., 2013).

Among these modalities, paracrine regulation is active throughout oogenesis and plays a substantial role also during maturation. Typical in such respect is the case of the transforming growth factor (TGF)-β family members growth differentiation factor 9 (GDF9) and bone morphogenetic factor 15 (BMP15). These agents are profoundly involved in oocyte–granulosa cell regulatory loops at early stages of oogenesis, but their action extends temporally also to the maturation phase (Edson et al., 2009). Produced by the oocyte, they diffuse through the cumulus mass and act in a paracrine fashion on follicle cells, by interaction with morphogenetic protein receptor type-II and activin receptor-like kinases to ultimately stimulate Sma- and Mad-related intracellular signaling (Kaiwo-oja et al., 2006). During maturation, GDF9 and BMP15 modulate a number of effects on surrounding CC, some of which are discussed elsewhere in this review, including growth, inhibition of apoptosis and luteinization, stimulation of energy and cholesterol metabolism, and cumulus expansion.

During a large part of oocyte growth and maturation, the presence of a thick zona pellucida clearly prevents extensive oocyte–CC contact. Instead, physical interaction takes the form of thin axon-like projections, the TZPs, that depart from the CC more closely arranged around the oocytes, extend through the entire depth of the zona pellucida and make contact with the oolemma (Fig. 2), where they form connexin-based gap junctions, desmosome-like structures and zonulae adherens (Albertini et al., 2001). Interestingly, abundance and characteristics of TZPs can vary under different phases and conditions. In fact in the human, at the pre-antral stage, TZPs are particularly numerous and form deep invaginations at the points of contact with the oolemma, while during the following antral stages their number and complexity of contact at the oocyte surface decrease (Combelles et al., 2004). Even more intriguingly, FSH appears to directly modulate TZP stability at the late pre-antral stage, as shown by the fact that in FSHβ knockout mice TZP development is enhanced, while retraction and a decrease in number occur upon treatment with FSH (Kumar et al., 1997). This has potentially significant implications for in vivo ovarian stimulation or IVM, in the context of which supra-physiological or inappropriate gonadotrophin dosage could affect TZP dynamics and ultimately oocyte–CC interaction (Luciano et al., 2011). Many or most of the TZP-mediated interactions occur via heterologous gap junctions formed from connexins 37 and 43, contributed by the oocyte and CC, respectively (Ackert et al., 2001; Mora et al., 2012). Through these intercellular contacts, oocyte, CC and indeed mural granulosa cells (MGCs) form a functional syncytium by which small molecules (up to 1 kDa in mass) are exchanged to meet the metabolic and regulatory demands of the oocyte, as described in following sections of this manuscript. Notably, gap junction communication can be promptly down-regulated not only by physiological stimuli, as in the case of meiotic resumption, but also by in vitro manipulation conditions (Lodde et al., 2007), with downstream effects that are not always compatible with the acquisition of developmental competence.

Live-cell fluorescence and time-lapse microscopy observations suggest that mitochondria, endosomes and lysosomes can be moved bi-directionally throughout the TZPs extension (Albertini et al., 2001). Consistent with this, microtubules, which normally mediate organelle mass) are exchanged to meet the metabolic and regulatory demands of the oocyte, extend through the entire depth of the zona pellucida and act in a paracrine fashion on follicle cells, by interaction with morphogenetic protein receptor type-II and activin receptor-like kinases to ultimately stimulate Sma- and Mad-related intracellular signaling (Kaiwo-oja et al., 2006). During maturation, GDF9 and BMP15 modulate a number of effects on surrounding CC, some of which are discussed elsewhere in this review, including growth, inhibition of apoptosis and luteinization, stimulation of energy and cholesterol metabolism, and cumulus expansion.

During a large part of oocyte growth and maturation, the presence of a thick zona pellucida clearly prevents extensive oocyte–CC contact. Instead, physical interaction takes the form of thin axon-like projections, the TZPs, that depart from the CC more closely arranged around the oocytes, extend through the entire depth of the zona pellucida and make contact with the oolemma (Fig. 2), where they form connexin-based gap junctions, desmosome-like structures and zonulae adherens (Albertini et al., 2001). Interestingly, abundance and characteristics of TZPs can vary under different phases and conditions. In fact in the human, at the pre-antral stage, TZPs are particularly numerous and form deep invaginations at the points of contact with the oolemma, while during the following antral stages their number and complexity of contact at the oocyte surface decrease (Combelles et al., 2004). Even more intriguingly, FSH appears to directly modulate TZP stability at the late pre-antral stage, as shown by the fact that in FSHβ knockout mice TZP development is enhanced, while retraction and a decrease in number occur upon treatment with FSH (Kumar et al., 1997). This has potentially significant implications for in vivo ovarian stimulation or IVM, in the context of which supra-physiological or inappropriate gonadotrophin dosage could affect TZP dynamics and ultimately oocyte–CC interaction (Luciano et al., 2011). Many or most of the TZP-mediated interactions occur via heterologous gap junctions formed from connexins 37 and 43, contributed by the oocyte and CC, respectively (Ackert et al., 2001; Mora et al., 2012). Through these intercellular contacts, oocyte, CC and indeed mural granulosa cells (MGCs) form a functional syncytium by which small molecules (up to 1 kDa in mass) are exchanged to meet the metabolic and regulatory demands of the oocyte, as described in following sections of this manuscript. Notably, gap junction communication can be promptly down-regulated not only by physiological stimuli, as in the case of meiotic resumption, but also by in vitro manipulation conditions (Lodde et al., 2007), with downstream effects that are not always compatible with the acquisition of developmental competence.

Live-cell fluorescence and time-lapse microscopy observations suggest that mitochondria, endosomes and lysosomes can be moved bi-directionally throughout the TZPs extension (Albertini et al., 2001). Consistent with this, microtubules, which normally mediate organelle movement, contribute to the formation of the TZPs cytoskeleton in bovine CC (Allworth and Albertini, 1993). It therefore appears possible that paracrine factors could be released or taken up at the TZP–oolemma interface. Therefore, in different ways, TZPs represent a fundamental dimension of oocyte–CC interaction.
RTKs and their respective ligands are well-known elements of intercellular regulatory networks of several phases of oogenesis, but only recently they have been described as a distinct route by which CC and the oocyte communicate during maturation. In particular, it has been observed in the mouse that tyrosine phosphorylation of cortical proteins is modest in fully grown germinal vesicle (GV) oocytes obtained from prevulatory follicles, but undergoes a significant increase after maturation in vitro (McGinnis and Albertini, 2010). This change is thought to be supported by CC because growth factors that act through CC, such as epidermal growth factor (EGF), enhance cortical phosphorylation (McGinnis and Albertini, 2010). Fyn kinase, a member of the Src-Family kinases, has been identified as another element able to mediate phosphorylation of cortical proteins (Luo et al., 2009), but full elucidation of this phenomenon, although biochemically prominent, remains elusive.

Collectively, it appears that the maturation process is supported by diverse manifestations of the interaction between the oocyte and CC. Among these, TZPs are of particular importance, not only for their function and regulation, but also because the preservation of their delicate structure, involving extremely thin cellular projections extending several microns in length, may be compromised by in vitro manipulation.

**Oocyte–CC signalling for meiotic arrest and resumption**

Throughout the long period of quiescence at the primordial stage and during follicle development, the oocyte remains arrested at the prophase stage of meiosis. The process of chromosome segregation is resumed only at ovulation, when the GV breaks down, the first meiotic division is accomplished and the meiotic cycle progresses, before pausing again at MI in preparation for fertilization. It has long been known that as soon as a well-defined antrum is formed oocytes become progressively competent to undergo GV breakdown (GVBD) and progress to MI. However, during antral expansion meiotic resumption is prevented by an inhibitory influence of the follicular environment, as shown by the fact that in many mammalian species GVBD and extrusion of the polar body I (PBI) occur spontaneously when oocytes are released from their follicles (Pincus and Enzmann, 1935; Edwards, 1965). For many years, different lines of evidence generated mainly in the mouse model have indicated cyclic adenosine monophosphate (cAMP) as the fundamental factor by which meiotic arrest is ensured before ovulation. First, the mid-cycle LH surge is preceded by a reduction in intraoocyte levels of cAMP (Schultz et al., 1983). Secondly, oocyte release from the follicle and meiotic resumption in vitro also coincide with a decrease in intraoocyte cAMP (Vivarrelli et al., 1983). Thirdly, membrane-permeable cAMP analogues (Schultz et al., 1983) or inhibitors of the oocyte-specific cAMP phosphodiesterase type 3 (PDE3) (Nogueira et al., 2003), the enzyme by which cAMP is hydrolysed, prevent spontaneous meiosis resumption in vitro. cAMP levels dictate the arrest or reinitiation of meiosis by regulating the protein complex referred to as M-phase promoting factor (MPF), formed from the kinase CDK1 and cyclin B (Jones, 2004). High intraoocyte concentrations of cAMP activate protein kinase type A (PKA, types I and II) (Viste et al., 2005). In turn, PKA phosphorylates the phosphatase CDC25 and the kinases WEE1B and MYT1, causing inactivation of the phosphatase and activation of the two kinases, respectively (Jones, 2004). In this fashion, tyrosine and threonine residues of CDK1 are phosphorylated, MPF activity is repressed and GVBD is inhibited. A decrease in intraoocyte cAMP levels overturns this state, ultimately allowing activation of MPF and, as a direct consequence of this, meiotic resumption (Jones, 2004).

As a result of evidence mainly generated in murine oocytes, it has long been thought that, to ensure meiotic arrest, cAMP was generated in the granulosa cells and transferred to the oocytes through gap junctions (Dekel, 2005). Initial evidence showed that forskolin, an adenylyl cyclase stimulant, caused meiotic arrest in cumulus-enclosed, but not denuded, oocytes (Dekel et al., 1984; Racowsky, 1984). However, the hypothesis that intraoocyte cAMP has a somatic origin has been questioned by more recent studies. In fact, while it is true that gap junction communication between oocyte and somatic cells is crucial to maintain meiotic arrest in mouse and rat oocytes (Sela-Abramovich et al., 2006; Norris et al., 2008), gap junction permeability to cAMP is unknown. Also, in the somatic compartment of the rabbit antral follicle cAMP levels are rather low before LH surge (Hunzicker-Dunn, 1981), making unlikely the possibility that granulosa cells can produce sufficient cAMP and deliver it to the oocyte. On the contrary, the evidence has become progressively stronger that the mouse oocyte has the potential to generate autonomously high intracellular concentrations of cAMP (Vaccari et al., 2008). This is suggested by the fact that mouse and human oocytes are equipped with a complete system of cAMP production, including a G-protein (GαS) (Mehlmann et al., 2002, 2004; DiLuigi et al., 2008) that is known to activate the cAMP signalling pathway, GPR3, a G-protein coupled receptor (Vaccari et al., 2009), and adenylyl cyclase type 3, that converts ATP in cAMP (Tsafriri et al., 1996; Freudzon et al., 2005; Mehlmann, 2005; Conti, 2011). Importantly, inactivation of any of such elements in vivo or in vitro leads to spontaneous meiotic resumption (Vaccari et al., 2008). Regardless of the compelling evidence that argues against an extracellular origin of cAMP, it is possible however that in non-murine species cAMP can be generated both endogenously by the oocytes as well as exogenously by somatic cells. This can be inferred by experiments in the bovine in which forskolin and a PDE3 inhibitor together, but not separately, were able to maintain denuded oocytes in meiotic arrest, while forskolin alone caused an increase in cAMP in cumulus-enclosed oocytes (Thomas et al., 2002). Therefore, caution should be exercised in extrapolating concepts from one species to another, considering realistically that alternative pathways involved in intracellular cAMP regulation may coexist and have different significance depending on specific conditions.

Previous and more recent findings have clarified how the follicular environment controls meiotic arrest and resumption in those species where cAMP is endogenously generated by the oocyte. Early experiments carried out in the rat established that shortly before GVBD, in addition to cAMP, the intraoocyte levels of cyclic guanosine monophosphate (cGMP) also fall dramatically following stimulation with LH or release from the follicle (Törnell et al., 1990). Furthermore, it was found that high concentrations of cGMP are inhibitory to the activity of PDE3 in mouse oocytes (Bornslaeger et al., 1984). Further suggestion of a role of cGMP in meiotic arrest derived from studies showing that experimental closure of gap junctions causes a drop in mouse intraoocyte cGMP levels that shortly precedes GVBD (Norris et al., 2009). Consequently, cGMP has become the object of many studies aimed at identifying the substance that is produced by follicle cells and conveyed to the oocyte to prevent meiotic resumption. Indeed, cGMP has emerged as the key regulator of a finely orchestrated inhibitory system that involves different factors and follicular compartments. It appears that MGCs and CCs play complementary roles to ensure the generation of cGMP, which can then
be transferred to the oocyte. In particular in the mouse model, CCs preferentially express the mRNA of the membrane bound guanylyl cyclase natriuretic peptide receptor 2 (NPR2), while MGCs express much more intensely the mRNA of the cognate ligand, natriuretic peptide precursor type C (NPPC) (Zhang et al., 2010, 2011b). Such differential expression suggests a scenario in which NPPC is primarily produced by MGC, diffuses throughout the follicle and, by binding to its receptor NPR2, stimulates CC to produce cGMP. Finally, the cyclic nucleotide is transferred via gap junctions from CC to the intraoocyte environment, where it represses the activity of PDE3 maintaining high cAMP levels. Several in vivo and in vitro observations confirm the above hypothesis: (i) NPPC prevents GVBD in cumulus–oocyte complexes (COCs), but not denuded oocytes; (ii) following exposure of COC to NPPC, cGMP increases in both CCs and oocytes; (iii) treatment of COC with NPPC induces a rise in cAMP in oocytes but not CCs and (iv) in Nppc or Npr2 mutant mice oocyte meiosis is resumed precociously in antral follicles (Zhang et al., 2010, 2011b). Similar to the mouse, in the pig (Hiradate et al., 2014) and cow (Franciosi et al., 2014) models, the Nppc and Npr2 genes were found to be preferentially expressed in MGC and CC, respectively. In such species, NPPC was also shown to inhibit spontaneous meiotic resumption in COC, suggesting that the NPPC/NPR2 system is shared among several mammalian species.

Recent observations have indicated that expression of the NPPC/NPR2 system is subject to regulation by different factors. In vivo in the mouse, gonadotrophin stimulation of follicular development induces expression of Nppc by MGC and Npr2 mRNA by CC (Zhang et al., 2010, 2011b). In addition, the ability of NPPC to prevent meiotic resumption in vitro and the competence of CC to produce cGMP are transient unless estradiol is present in the culture environment (Zhang et al., 2011b). In fact, this steroid is essential for the expression of Npr2 in CC. It also co-operates with FSH to enhance the expression of Nppc by MGC. Importantly, oocytes take an active and important part in this regulatory network, as indicated by the synergistic ability of the oocyte-derived factors GDF9 and BMP15, as homo- or heterodimers, to stimulate in CC the expression of Npr2 and the inosine monophosphate dehydrogenase gene, whose product is the limiting element for the generation of cGMP from guanylic metabolites (Wigglesworth et al., 2013).

Further experiments in the mouse have also shed light on the events by which the midcycle LH surge neutralizes the inhibitory mechanisms based on cGMP. Suppression of gap junction communication, which actively participate in the mechanism of meiotic arrest, is achieved by activation of a mitogen-activated protein kinase (MAPK)-dependent pathway that causes phosphorylation of connexin-43 on several regulatory serines (Norris et al., 2008). However, other elements concur to the regulation of meiotic resumption. In fact, acute LH stimulation is also followed by a decrease in cGMP levels in the entire follicle (Norris et al., 2009; Vaccari et al., 2009) and a suppression of NPPC synthesis (Kawamura et al., 2011). Recent evidence obtained in the rat model indicates that a fall in cGMP concentration in the follicle may derive from positive regulation of the cGMP PDE5, but it is also caused by inhibition of the guanylyl cyclase activity of NPR2 in the CC compartment (Egbert et al., 2014). Thus, reduced production of the ligand NPPC, inhibition of the guanylyl cyclase activity of the cognate NPR2 receptor and suppression of gap junction-mediated communication act in parallel to reduce the intraoocyte levels of cGMP. In this fashion, within 1 h of LH stimulation the intraoocyte cGMP concentration falls dramatically. In species other than the mouse and the rat, similar mechanisms may mediate meiotic resumption, as suggested by the finding that in the human exposure to ovulatory doses of hCG causes a reduction in NPPC present in the follicular fluid (Kawamura et al., 2011).

Different lines of evidence indicate that members of the EGF family amphiregulin (AREG) and epiregulin (EREG) play an important role as mediators of the pathway linking LH stimulation and cGMP regulation. It has long been known that EGF can induce meiotic resumption in intact rat follicles (Dekel and Sherizly, 1985) or mouse COC cultured in the presence of hypoxanthine (Downs et al., 1988), conditions that otherwise prevent GVBD. More recent investigations have established that, rather than EGF, AREG and to a lesser extent EREG are involved in signalling meiotic reinitiation at ovulation. Stimulation with LH of mouse MGC in vitro or rat follicles in vivo causes prompt expression of AREG and EREG mRNA before GVBD (Park, 2004). Production of such transcripts is likely to depend on the typical increase in cAMP by which follicle cells respond to gonadotrophins. This is suggested by the fact that forskolin, an activator of adenylate cyclase, is as efficient as LH in promoting the synthesis of AREG and EREG in cultured equine and bovine MGC (Sayasith et al., 2013). The two EGF-like factors are synthesized as precursors integrated in the oolemma and released as active forms following cleavage of their extracellular domains by the transmembrane metalloprotease ADAM17/TACE (Bielob et al., 2009). The involvement of AREG and EREG in the regulation of meiotic resumption is indicated by genetic, pharmacological and in vitro studies: (i) mice carrying inactivating mutations of the EGF receptor (EGFR), activated by AREG and EREG, fail to support oocyte meiotic resumption following acute LH stimulation (Hsieh et al., 2007), consistent with a delayed or reduced oocyte maturation observed in animals deficient in AREG or EREG synthesis; (ii) suppression of the phosphorylation-mediated activation of the EGFR, which occurs in vivo in MGC following LH treatment (Park, 2004), by the kinase inhibitor AG1478 prevents the stimulatory effect of LH on meiotic resumption in the mouse (Norris et al., 2010) and (iii) in vitro, exposure to AREG or EREG induces GVBD in intact follicles or COC cultured in the presence of hypoxanthine (Park, 2004). Other experiments have clarified further details of the involvement of EGF-like factors in the mechanism of meiotic resumption. Adopting the whole follicle culture as a model, it was observed in the mouse that the ability of AREG or EREG to promote GVBD is accompanied by a large decrease in cGMP in follicle cells (Vaccari et al., 2009; Norris et al., 2010). However, suppression of the EGFR kinase activity with the inhibitor AG1478 only partially prevents the large decrease in cGMP induced by LH (Norris et al., 2010), a sign that the effect of this hormone on cGMP levels derives from two or more redundant pathways. Also, treatment with EREG alone or in association with AREG determines only a partial down-regulation of gap junction communication, while exposure to the inhibitor AG1478 prevents LH-induced gap junction closure (Norris et al., 2010). Therefore, activation of the EGFR by EGF-like factors appears to play an important role in gap junction closure and decrease in cGMP that are instrumental for GVBD, although other as yet unidentified pathway(s) co-operate to achieve the same effect.

In the last few years, research on a possible involvement of EGF-like factors in oocyte meiotic resumption has been extended to the human and non-human primates. By using material donated by IVF patients, AREG was found at detectable concentrations in the follicular fluid only after a spontaneous LH surge or exogenous administration of hCG (Zamah et al., 2010), suggesting an accumulation of the factor as
Table I Factors involved in CC–oocyte interactions that are believed to control meiotic arrest and resumption.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Function</th>
<th>Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAMP</td>
<td>High intraoocyte concentrations of cAMP activate PKA (types I and II) leading ultimately to inhibition of MPF activity and maintenance of meiotic prophase arrest. cAMP is generated endogenously by the oocyte, but some studies suggest it is also produced by the somatic compartment and subsequently transferred into the oocyte compartment.</td>
<td>Mouse, rat, cow, human</td>
<td>Schultz et al. (1983), Dekel et al. (1984), Racowsky (1984), Mehlmann et al. (2002), Thomas et al. (2002), Thomas (2004), Vaccari et al. (2008), DiLuigi et al. (2008)</td>
</tr>
<tr>
<td>cGMP</td>
<td>cGMP is produced by CC and transferred through gap junctions into the oocyte, where it represses the activity of PDE3 preventing cAMP degradation.</td>
<td>Mouse, rat</td>
<td>Bornslaeger et al. (1984), Törnell et al. (1990), Norris et al. (2009), Vaccari et al. (2009)</td>
</tr>
<tr>
<td>NPR2</td>
<td>NPR2 is a membrane bound guanylyl cyclase NPR2 receptor preferentially expressed by CC. It is positively regulated to produce cGMP by binding with NPPC.</td>
<td>Mouse, rat, cow, pig</td>
<td>Zhang et al. (2010, 2011a, b), Kawamura et al. (2011), Hiradate et al. (2014), Franciosi et al. (2014)</td>
</tr>
<tr>
<td>GDF9, BMP15</td>
<td>Secreted as homo- or heterodimers, GDF9 and BMP15 act synergistically and induce in CC the expression of Npr2 and the inosine monophosphate dehydrogenase gene. The protein product of the latter gene is the limiting element for the generation of cGMP from guanylyl metabolites.</td>
<td>Mouse</td>
<td>Wigglesworth et al. (2013)</td>
</tr>
<tr>
<td>AREG, EREG</td>
<td>Produced by granulosa cells in response to acute LH stimulation, AREG and EREG activate the EGF receptor(s) expressed by CCs. This causes a decrease in intraoocyte cGMP and meiotic resumption as an effect of down-regulation of gap junction communication and inhibition of cGMP production by follicles cells.</td>
<td>Mouse, monkey, human</td>
<td>Park (2004), Vaccari et al. (2009), Norris et al. (2010), Peluffo et al. (2012), Zamah et al. (2010), Ben-Ami et al. (2011), Humaidan et al. (2011)</td>
</tr>
</tbody>
</table>

The table includes a schematic description, divided by species, of current understanding of the function of such factors in relation to oocyte meiosis.
cAMP, cyclic adenosine monophosphate; PKA, protein kinase type A; MPF, M-phase promoting factor; cGMP, cyclic guanosine monophosphate; CCs, cumulus cells; PDE3, phosphodiesterase type 3; NPPC, natriuretic peptide precursor type C; MGCs, mural granulosa cells; NPR2, natriuretic peptide receptor 2; GDF9, growth differentiation factor 9; BMP15, bone morphogenetic protein 15; AREG, amphiregulin; EREG, epiregulin.

an effect of acute gonadotrophin stimulation. In addition, in follicle cells of the same patients, AREG mRNA was highly expressed in both MGC and CC, while the gene of the cognate receptor hErbb1 was preferentially expressed by CC (Zamah et al., 2010), a sign that the CC compartment is the target of AREG. Finally, it was observed that in individual follicles low levels of AREG were associated with the recovery of immature (GV-stage) oocytes (Zamah et al., 2010). Another investigation explored the possibility that different modalities of triggering ovulation, i.e. HCG administration or induction of endogenous LH surge with GnRH agonist (GnRH-a), influence intrafollicular AREG levels and oocyte quality (Humaidan et al., 2011). It was found that concentrations of the EGF-like factor were significantly lower in the GnRH-a groups. This was associated with a higher rate of mature oocytes and, following fertilization, embryos suitable for transfer, suggesting that in ovulation induction LH and hCG may induce non-equivalent follicular responses and that the effect of AREG on oocyte maturation and quality may be dose-dependent. In the rhesus monkey, experiments carried out in ovarian cycles involving naturally selected follicles confirmed that AREG is not detectable until several hours after the administration of an ovulatory bolus of hCG (Peluffo et al., 2012). In the same study, immature COC retrieved from small antral follicles were cultured for 48 h to achieve maturation in vitro. In the presence of FSH and LH, AREG used at concentrations of 10 or 100 ng/ml had little impact on the rate of GVBD, but there was a trend towards a higher rate of MII oocytes in the group treated with the lower concentration of the growth factor. AREG and EREG were also tested in IVM experiments involving a human experimental model (Ben-Ami et al., 2011). Left-over immature GV-stage oocytes recovered from stimulated cycles and left partially enclosed in their vestment of corona radiata cells were exposed to AREG and EREG together. Similar to the case of rhesus monkey oocytes, the growth factors did not appear to improve the rate of GVBD but generated an increase in the overall availability of MII oocytes.

In conclusion, much information has been gained on the mechanism of meiotic arrest and resumption in the mouse and rat models, especially with reference to the mode by which cGMP produced in the somatic compartment generates intraoocyte conditions that are not permissive for GVBD. Also, in the same context EGF-like factors have emerged as important, although perhaps not exclusive, mediators of LH in the process of meiotic resumption (Table I). Less clear is whether the same paradigm is applicable to primates and in particular the human, where details of the meiotic arrest mechanism and the role of EGF-like factors have been much less investigated. This is reflected in the fact that the use of such factors is far from reaching clinical application, remaining entirely experimental.
Global chromatin remodelling, meiotic resumption and developmental competence

During the protracted phase of quiescence in the primordial follicle, the oocyte chromosomes are only moderately dispersed and transcriptionally active to assure a sufficient level of housekeeping functions. Subsequent follicle development is accompanied by a parallel phase of oocyte growth, by which the female gamete reaches full size in preparation for fertilization and preimplantation development. Growth can only be achieved through intense and sustained RNA transcription. To this end, the oocyte chromatin acquires a condition of maximal dispersion to allow accessibility to multiple transcription sites by the transcription machinery. Once growth is achieved and the oocyte develops the ability to resume meiosis and progress to the mature stage in the later stages of folliculogenesis, the chromatin is subject to an extensive process of condensation. In several species, chromatin condensation is accompanied by intense transcription down-regulation. Such concomitance has generated the hypothesis that chromatin condensation is the modality by which transcription silencing is achieved in preparation for meiotic resumption. This seems to emerge from experiments showing that in mouse oocytes knock-down of the gene encoding the developmental pluripotency-associated protein 3 causes failure of chromatin condensation and incomplete transcriptional repression (Liu et al., 2012). However, this matter remains controversial. In fact, in mouse oocytes obtained from nucleoplasmin 2 (Npm2−/−) mutants, chromatin condensation is not observed, while transcription silencing occurs apparently undisturbed (De La Fuente et al., 2004). Progressive heterochromatinization occurs with modalities that are believed to be variations on the same theme in different species and appear to obey precise spatio-temporal cues. In the mouse model, chromatin condensation reveals itself as a sequential transition between different conformational patterns (Mattson and Albertini, 1990). In small grown oocytes, chromatin is dispersed and decondensed throughout the GV, with only a few domains showing a higher degree of condensation. As cell growth proceeds, the foci of heterochromatin increase in size and number, tending subsequently to coalesce in larger clusters that eventually envelop the nucleolus. Following staining with fluorescent dyes, such aggregation of chromatin appears as a rim surrounding the nucleolus and is the origin of the classification of oocytes into two broad categories, ‘surrounded nucleolus’ (SN) and non-surrounded nucleolus (NSN) (Zuccotti et al., 1995). It has been established that acquisition of the SN configuration normally occurs in oocytes that have almost reached the apex of their growth curve, at the time when follicles have already formed a small antrum and massive transcription repression has started (Chouinard, 1975; Parfenov et al., 1989; Mattson and Albertini, 1990). Development of the SN type appears to be a highly regulated process because in mouse and human oocytes it occurs in close coordination with the oocyte ability to support the G2 to M phase transition (Mirre et al., 1980; Bouniol-Baly et al., 1999). However, sometimes temporal co-ordination of chromatin condensation fails, with the consequence that both the SN and NSN types are represented among oocytes collected from mouse antral follicles (Zuccotti et al., 1998). Several studies indicate that timely evolution of the chromatin from an NSN to an SN status supports the acquisition of meiotic and developmental competences. In fact, in comparison to the NSN population, SN oocytes released from antral follicles and exposed to in vitro conditions display an increased ability to undergo GVBD and progression to MII. Also, while after IVM and fertilization NSN oocytes are unable to cleave beyond the 2-cell stage, SN oocytes have the potential to develop to the blastocyst stage (Zuccotti et al., 1998). How chromatin condensation is achieved in oocytes approaching the stage of meiotic resumption is not fully understood. However, studies in the mouse are suggestive of a role for histone deacetylation. In this species, in fact, pharmacological inhibition in vitro of histone deacetylase interferes with chromatin transition from NSN to SN and meiotic progression (De La Fuente et al., 2004). Granulosa cells are believed to be involved in the process of chromatin condensation in the GV, as indicated by the finding that in vitro the transition from NSN to SN is observed in mouse oocytes cultured in association with companion granulosa cells, but not in denuded oocytes (De La Fuente and Eppig, 2001).

Evidence derived from species other than the mouse suggests that the association between progressive chromatin condensation and acquisition of oocyte meiotic and developmental competence is a general phenomenon, although specific details may vary. For example in the cow, oocytes recovered from early and mid-antral follicles are characterized by four alternative and sequential stages of chromatin condensation (Lodde et al., 2007). In the G0 stage, chromatin presents a fibrillar constitution throughout the GV. In the G1 and G2 stages, chromatin is found in a progressively more condensed state, displaying few foci of heterochromatin in the former and well-defined larger clumps of condensed material in the latter. The G3 stage represents the highest condition of condensation, with a single large clump of heterochromatin. Such stages are indicative of oocyte meiotic and developmental potential. G0 oocytes are in most cases unable to resume meiosis and mature in vitro, while G1, G2 and G3 oocytes are competent to undergo GVBD and progress to MII. However, G1, G2 and G3 oocytes are not equally developmentally competent, because the G3 type displays a significantly higher potential to develop to the blastocyst stage (Lodde et al., 2007). The somatic compartment of the follicle is known to regulate in the oocyte the process of progressive chromatin condensation. In the same cow IVM model described above, the role of CC has been thoroughly investigated. In particular, it has been shown that conditions that support maintenance of oocyte–CC gap junction communication (i.e. exposure to low FSH levels), not only delay meiotic resumption and prevent abrupt non-physiological transcriptional silencing of the oocyte chromatin, but also induce a progressive orderly conversion of the oocyte chromatin from a dispersed (G0) to a more condensed form (Luciano et al., 2011). Importantly, the same conditions that preserve oocyte–CC gap junction communications also increase the proportion of GV oocytes that is able mature in vitro and, following fertilization, support development to the blastocyst stage (Luciano et al., 2011).

Data on human oocytes are limited but are nevertheless reminiscent of those generated in animal models. An accumulation of heterochromatin around the oocyte nucleolus, described as a ‘karyosphere’, was initially observed in electron microscopy studies on the ultrastructure of the antral follicle (Parfenov et al., 1989), but more recently chromatin conformation was studied in left-over GV-stage oocytes derived from stimulated assisted reproductive technology (ART) cycles. In such oocytes, chromatin is found arranged in four alternative configurations: (i) chromatin partially surrounding the nucleolus and dispersed in fibrillar formations in the rest of the nucleus; (ii) all the chromatin surrounding the nucleolus; (iii) chromatin completely surrounding the nucleolus and partially organized in condensed masses throughout the nucleus and
Oocyte maturation

DNA damage and meiotic progression

DNA damage is a very common insult to which all cells are exposed during their lifetime (Jackson and Bartek, 2009). Cells interpret DNA single strand breaks (SSBs) or double strand breaks (DSBs) as a serious threat to their integrity and react to that with a DNA damage response (DDR) involving an arrest of the cell cycle as a downstream effect of the activation of DNA damage checkpoints (DDCs), in order to give time for repair mechanisms to intervene (Bartek and Lukas, 2007). If the scale of damage is overwhelmingly superior to the capacity of the repair mechanism, the response takes the form of programmed cell death by which affected cells are removed by apoptosis (Ciccia and Elledge, 2010). Mammalian oocytes are particularly exposed to DNA damage. In fact, before undergoing growth and maturation they remain dormant in the ovary for long periods of time (up to more than 40 years in the human). As a consequence, DNA damage can accumulate, jeopardizing oocyte function and the genetic integrity of the ensuing embryo. DDR may be activated principally at both the G1/S- and G2/M-phase transitions of the cell cycle. Clearly, only the latter option can be adopted by oocytes, being the arrest at the meiotic prophase and meiotic resumption at ovulation reminiscent of the somatic G2/M-phase transition. At both cell phase transitions, SSBs and DSBs trigger a DDC response by activating two major kinases, i.e. ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (Reinhardt and Yaffe, 2009; Smith et al., 2010).

At G2, in the presence of DNA injury these regulators inhibit entry into the M-phase by activation of the checkpoint kinases Chk1 and Chk2, that, by destroying CDC25A or phosphorylating CD25B or CDC25C, prevent the activation of CDK1 and, with it, the cyclin B-CDK1 complex that acts as the major regulator of the cell cycle (see also ‘Oocyte–CC signalling for meiotic arrest and resumption’ section for the involvement of these regulators in meiotic resumption) (Bartek and Lukas, 2007; Smith et al., 2010; Carroll and Marangos, 2013). Taking advantage of the DDC arrest, the cell can repair various types of DNA damage. At the DNA lesion site, ATM gives rise to the phosphorylated form of the H2AX histone (γH2AX), which acts as a catalyst for the recruitment of the necessary checkpoint and repair factors (Burgoyne et al., 2007). Base excision repair, nucleotide excision repair and mismatch repair can intervene in case of single strand damage, while DSBs can be resolved by either homologous recombination or non-homologous end joining (Jackson and Bartek, 2009). In oocytes, DSBs have physiological significance in the context of meiotic recombination, by allowing the exchange of portions of DNA between homologues non-sister chromatids (McDougall et al., 2005; Burgoyne et al., 2009). However even in such a case, if not occurring in a properly regulated fashion, DSBs trigger a cell response that culminates in an apoptotic event at the time of formation of the primordial follicle (Pittman et al., 1998; Baudat et al., 2000).

At the antral and pre-ovulatory stages of follicle development, DNA damage is anticipated to induce some form of response in fully grown oocytes, but in such respect the DDR has remained a poorly described phenomenon until recently. Fully grown oocytes are naturally arrested at the meiotic prophase and a meiotic block in response to DNA damage may not appear as a strict necessity. However, ability to resume meiosis in response to an ovulatory stimulus is already developed at the antral stage. Therefore, in addition to DNA repair mechanisms, it

![Figure 3](https://example.com/image-url)  
**Figure 3** Optical sections of GVs of human fully grown immature oocytes analysed by confocal laser scanning microscopy. Micrographs represent specific areas (A and B) or an entire section of the GV. Chromatin, stained with Hoechst 33258, appears (A) exclusively surrounding the nucleolus; (B) surrounding the nucleolus, but also partially organized in condensed masses in adjacent domains of the nucleus; (C) only partially condensed, but mostly dispersed in fibrillar formations in the rest of the nucleus. Scale bars represent 10 μm.
is plausible that antral oocytes deploy cell cycle arrest strategies in response to DNA damage. The similarity of the mechanisms regulating the G2/M transition in somatic cells and meiotic resumption in oocytes would predict that fully grown oocytes can activate a DDR cascade. If necessary, this would prevent the resumption of the meiotic cycle for a time required for the intervention of DNA repair mechanisms. Initial studies involving the mouse model were indicative of a limited DDR in fully grown oocytes (Bradshaw et al., 1995), consistent with the finding that injection of females with etoposide, a topoisomerase II inhibitor and DSBs inducer, does not cause significant delay of meiotic progression to metaphase I (MI). More recent experiments have expanded the knowledge of oocyte response to DSBs. Mouse fully grown oocytes released from antral follicles were initially exposed to increasing concentrations of etoposide and their ability to resume meiosis and progress into M phase of meiosis I was subsequently assessed (Marangos and Carroll, 2012). At the highest concentrations of etoposide, oocytes were unable to undergo GVBD and progress to MII. However, at lower concentrations of the topoisomerase inhibitor, the oocytes were capable of undergoing GVBD and entering M phase with unaltered kinetics. Importantly, the intensity of γH2AX staining shown by the DNA of the oocytes that underwent GVBD following etoposide treatment was much higher to that of control oocytes. This suggests that the absence of a cell cycle arrest in response to DSBs is not the result of the intervention of an efficient DNA repair mechanism, but rather the consequence of an inadequate DDR response. Further experiments have demonstrated that prophase arrest after exposure to high concentrations of etoposide, which causes extensive of DNA damage, is ensured by sequential activation of ATM and Chk1 (Marangos and Carroll, 2012). On the contrary, Chk1 remains inactive at low concentrations of etoposide (which produce only limited DNA damage), explaining why GVBD occurs under such conditions. ATM also remains inactive at low concentrations of etoposide. Therefore, mouse oocytes are equipped with an ATM/Chk1 mechanism to respond to DNA DSBs. However, this system is insensitive to low levels of injury, becoming activated only above a certain threshold. It is interesting to note how γH2AX, which is usually generated by the active form of ATM, is detectable also at low levels of DNA damage, an indication that phosphorylation of H2AX can also occur via an ATM-independent regulation. Finally, phosphorylation of CDC25B rather than degradation of CDC25A phosphatase seems to be the downstream step promoted by the active form of Chk1 that ultimately negatively regulates CDK1, inhibiting meiotic resumption (Marangos and Carroll, 2012).

Further experiments have clarified some other aspects of oocyte sensitivity and response to DNA damage. Of particular interest is the fact that, while mouse oocytes are to some extent responsive to the presence of DSBs in their DNA as discussed above, they appear rather insensitive to interstrand cross-links (ICLs) caused by alkylating agents, such as mitomycin C (Yuen et al., 2012). Immature mouse oocytes treated with mitomycin C are able to resume meiosis in vitro and progress to MI but, following parthenogenetic activation, develop into severely compromised embryos. Unlike the case of DSBs in which the lack of sensitivity to relatively low levels of damage is not caused by the absence of γH2AX at the injury sites, failure to respond to ICLs probably resides in the inability to activate a specific signalling system at the damaged sites. In fact, in mouse oocytes treated with mitomycin C, the Fanconi anaemia protein FANCD2, which is part of the Fanconi anaemia pathway and as such participates in mechanism of detection and repair of ICLs, does not appear to form associations with lesion sites, while in cleaving embryos this protein is found where ICLs occur (Yuen et al., 2012). Additional data indicate that DNA damage not detected or not repaired during oocyte maturation can be passed on to the embryo. DSBs can be experimentally induced also by bleomycin or laser microbeam dissection. Immature mouse oocytes exposed to these agents show some delay in the GVBD kinetics, but nevertheless they are able to extrude the PBI, progress to MI and support parthenogenetic development (Ma et al., 2013). Notably, however, also in this case the resulting embryos display different anomalies, including multiple pronuclei and multiple micronuclei.

In conclusion, mouse oocytes appear to have limited ability to detect and repair DNA lesions. Such limitation may have different origins. In fact, in the case of ICLs a suitable mechanism of detection of the injury sites seems to be missing. On the contrary, low or moderate levels of DSBs appear to escape the DNA repair mechanism because oocytes are apparently unable to activate ATM, the master regulator of DDR, despite the sites of lesions are signaled by the presence of γH2AX. The fact that oocytes do not always arrest the meiotic process in the presence of significant levels of DNA damage has potentially important implications, because genetic instability caused by DNA lesions could be passed on from oocytes to embryos, possibly affecting fetal and postnatal life. In the human, nothing is known of the possibility that DNA damage can be transmitted from oocytes to embryos, although recent observations indicate that human GV stage oocytes (Fig. 4) are affected by DSBs (Guglielmo et al., 2012).

**GV positioning and oocyte polarity**

At the MI and MII stages the meiotic spindle is located cortically, separated just a few microns from the cell surface. This assures that the two meiotic divisions occur with minimal loss of cytoplasm through the emission of two small polar bodies. In this fashion, the endowment of organelles and molecules stockpiled during oogenesis is maintained almost intact, assuring maximum support in terms of cell mass to embryo development, in the absence of net growth during cleavage and blastocyst formation. The eccentric position of the MI spindle in the mature oocyte and the lack of overt asymmetry that characterizes the primordial oocyte pose the question as to when and how asymmetry is created during oogenesis. In this scenario, the GV has attracted much attention for its involvement in the establishment of polarity in different systems and for the fact that its breakdown is preliminary to the formation of an asymmetrically positioned MI spindle. Examples of the involvement of the cell nucleus in the establishment of cell polarity are numerous. For example, in somatic cells, cortical localization of the nucleus is associated with precise cellular and functional attributes, such as axon projection in neurons and release of secretory organelles in epithelial cells (Drubin and Nelson, 1996). In Drosophila, repositioning of the GV from a central position to the dorsal cortex contributes to the design of oocyte polarity and, with it, the body plan of the future organism (Doerflinger et al., 2003). In mammals, there are clues that at some point in time during the growth phase the GV, initially localized centrally in the primordial oocyte, acquires an asymmetric position. In fact, when observed in situ, fully grown oocytes of most mammalian species display an eccentric GV (Hertig and Adams, 1967; Allworth and Albertini, 1993; Albertini and Barrett, 2004). On the contrary, mouse and...
rat oocytes released from pre-ovulatory follicles and cultured under conditions that prevent meiotic resumption exhibit a central GV (Messinger and Albertini, 1991; Chaigne et al., 2012). This inconsistency is probably more apparent than effective, because observation of fully grown mouse oocytes while still enclosed in their follicles indicates that the GV is positioned eccentrically (Albertini and Barrett, 2004). Therefore, in one or more species a pre-existing asymmetry is lost when oocytes are placed in extracorporeal conditions. This is not a mere academic matter because it bears implications for the morphodynamics of oocyte maturation and above all the fate of the ensuing embryo, as discussed below. Studies of the last few years lend credit to the hypothesis that oocyte eccentricity is not entirely dictated by intrinsic regulatory mechanisms, but depends at least to some extent on external influences (Barrett and Albertini, 2010). Mouse oocytes were recovered and fixed for confocal microscopy analysis to assess GV/spindle position and oocyte–CC contacts at different hours after the administration of hCG to promote meiotic resumption in vivo. Over a period of 6 h after hCG injection, TZPs were initially uniformly distributed around the oocyte, except the portion of the oocyte cortex coincident with an eccentric position of the GV, where TZPs density (number) was higher. At 4–6 h post-hCG, the newly formed MI spindle was localized cortically in a position where TZPs were highly enriched. Therefore in vivo, while surrounding the entire oocyte, TZPs become progressively more abundant in the region of the cortex where the GV and MI spindle are eccentrically localized. Analysis of COC cultured in vitro in the absence of FSH revealed a different dynamics. In fact, after 2 h of culture the GV was repositioned centrally and the MI spindle appeared in the same central position shortly afterwards, before being repositioned cortically at 6 h. The TZPs, initially present throughout the cortex, progressively disappeared during the same time frame. Live cell differential interference contrast time-lapse microscopy confirmed that in vitro the GV, initially localized cortically, is repositioned in the centre, while the MI spindle forms centrally and only subsequently migrates to the cortex. Interestingly, culture of immature COC in the presence of FSH, whose receptors are expressed by CC but not the oocyte, prevents the disappearance of TZPs and the disanchoring of the GV from the cortex. Therefore, also in vitro the MI spindle forms eccentrically and does not require repositioning, provided that COCs are exposed to FSH. A direct TZP involvement in the maintenance of GV eccentricity and oocyte asymmetry is suggested by the fact that maturation in vitro in the presence of latrunculin, an agent that depolymerizes actin filaments that form the TZP cytoskeleton, determines TZPs disappearance and migration of the GV from the cortex to the centre, irrespective of the exposure of COC to FSH (Barrett and Albertini, 2010). GV disanchoring from the cortex and repositioning to the centre involves significant developmental consequences. In fact, centrally positioned GVs become larger
before undergoing GVBD. In addition, they give rise to MI spindles that are larger in comparison to in vivo matured oocytes and display dispersed chromosomes, conditions that are associated with an increase in the rate of oocyte aneuploidy (Barrett and Albertini, 2010). Generation of a large MI spindle involves also another important developmental phenomenon consisting of the emission of a large polar body (Plancha et al., 2005), a condition that reduces the cytoplasmic mass of the MII oocyte to levels that may not be sufficient to ensure successful development of the ensuing embryo (Fig. 5). Collectively, the above evidence leads to the conclusion that in mammals oocyte asymmetry, signified by cortical localization of the GV, occurs before the oocyte achieves full size. In some species like the mouse, such asymmetry may be lost as an effect of removal of regulatory cues generated by the follicular environment. Loss of asymmetry at the GV stage can be re-established after GVBD by migration of the MI spindle to the cortex, whose formation occurs centrally under non-physiological conditions. Therefore in the mouse oocyte, although the forces that govern GV eccentricity display limited stringency, polarity can be regained—if lost—by back up mechanisms acting downstream of GVBD. However, reacquisition of polarity comes with a cost, represented by abnormal spindle morphogenesis and increased developmental risks. Crucial for the maintenance of the original asymmetry is the presence of the TZPs that connect the oocyte with surrounding CC. The pattern of distribution of such intercellular contacts mirrors the positioning of the GV and the MI, suggesting the concept, novel for mammals but not for other model species such as Drosophila, that elements of oocyte design are dictated by polarized signals of somatic origin.

In the human, oocyte asymmetry has been poorly explored, although preliminary observations seem to suggest that polarity is established before GVBD. In left-over immature oocytes recovered from stimulated ART cycles, in most cases the GV is found cortically although it can also be localized centrally. Time-lapse microscopy has revealed that initial positioning may be subject to changes, with subsequent effects on meiotic resumption. In particular, cortically positioned GVs may undergo significant and continued displacement for several hours, in the vast majority of cases displaying the ability to undergo GVBD as long as contact with the cortex is not lost (Coticchio et al., 2011). Centrally localized GVs can also be subject to intense translational movements, but GVBD is unlikely to occur unless they are repositioned to the cortex. Overall, such cellular behaviours are consistent with the hypothesis that, although oocyte polarity may or may not be established in fully grown oocytes, the GV has an affinity for the cortex as an elective domain where to undergo breakdown. If confirmed, this would indicate a previously unrecognized role for the oocyte cortex. Also, while the reason why immature oocytes from stimulated cycles can exhibit the GV at different positions remains obscure, it is tempting to speculate that the forces that control spindle relocation in mouse oocytes may be also involved in GV repositioning in human oocytes. Regardless, these observations have significant clinical implications, in consideration of the use of immature oocytes for various fertility treatment approaches.

**Cytoskeletal forces governing spindle positioning**

The above discussion illustrates the importance of repositioning the MI spindle to the cortex when its assembly occurs in the vicinity of the oocyte centre. Irrespective of whether spindle migration may be a...
safeguard mechanism to recover a previously lost asymmetry, it represents a major change in the design of the oocyte that exemplifies the fundamental role of the cytoskeleton in the choreography of oocyte maturation.

In somatic cells undergoing symmetric or asymmetric division, spindle positioning relies on the action of astral microtubules and microtubule-associated motor proteins (Verlhac et al., 2000). A similar solution does not seem to be adopted by oocytes, as indicated by the fact that the meiotic spindle is devoid of astral microtubules and chromosome migration still occurs after treatment of MI oocytes with microtubule depolymerizing agents (Verlhac et al., 2000). Instead, recent findings highlighted a fundamental role for actin in the repositioning of the MI spindle to the cortex of the mouse oocyte, confirming and detailing classical studies performed in the 1980s (Maro et al., 1986). In fact, mice mutant for Fmn2, a gene encoding the actin-nucleating protein formin-2 (FMN2), are defective for spindle migration at MI (Leader et al., 2002). In GV-stage oocytes, FMN2 is localized at the cortex, while following GVBD sophisticated staining techniques allow the visualization of actin filaments bridging the spindle and the cortex (Schuh and Ellenberg, 2008). Consistent with the observation that myosin II is found at the spindle poles and myosin II inhibitors prevent spindle migration, this has induced some authors to hypothesize that the spindle is pulled towards the cortex by an actomyosin-based contractile mechanism (Chaigne et al., 2012). An alternative model has been developed from observations that have (i) indicated additional regions where FMN2 localizes and (ii) precisely described speed and direction of spindle movement during the process of symmetry breaking and repositioning in the mouse oocyte. Shortly after GVBD, FMN2 clusters around the forming spindle where it generates a cloud of actin filaments (Yi et al., 2013). FMN2 and elements of the ER colocalize in the immediate surroundings of the spindle, suggesting also that precise positioning of FMN2 depends on this organelle. Such colocalization was crucial in experiments in which FMN2 distribution was experimentally disrupted to test the hypothesis that actin polymerization is essential for spindle migration. Indeed, agents that cause dispersion of the ER also disperse FMN2 distribution around the spindle, but not at the cortex, and inhibit spindle migration (Yi et al., 2013). However, how an initially symmetric distribution of FMN2 and actin filaments around the spindle could assist polarized spindle displacement towards the cortex remained an unsolved problem until chromosome movement, as a measure of spindle displacement, was tracked and precisely analysed by time-lapse live cell imaging techniques. During spindle migration, chromosome trajectory indicates two types of movement. Initially, chromosomes move with low instantaneous speed and straightness within a range of 5–10 μm from the oocyte centre. Such kinetic behaviour has been described as ‘confined random walk’. In a second phase that occurs abruptly, chromosomes move towards the cortex with much higher speed and much straighter trajectory (Yi et al., 2013). This is suggestive of the existence of two distinct mechanisms by which chromosomes and the spindle are relocated to the cortex. Interestingly, the same pattern of movement is observed if the spindle is experimentally depolymerized shortly after GVBD.

The involvement of FMN2 in the first phase of random walk emerges not only from the inability of Fmn2<sup>−/−</sup> mutants to support spindle movement, but also from the observation that, if the spindle is disrupted, FMN2 accumulates opposite to the direction taken by chromosomes as they move closer to the cortex (Yi et al., 2013). It seems plausible, therefore, that the initial phase of slow movement of the spindle is generated by clustering of FMN2 around that spindle that, by inducing local nucleation of F-actin, promotes an undirected pushing force that moves the spindle randomly within a short range from the oocyte centre. However, it is possible that as some point FMN2 accumulation and F-actin polymerization in the vicinity of the spindle can occur stochastically in a polarized fashion, making the spindle drift more decisively to the cortex and creating the premises for a symmetry breaking event (Li, 2013).

If FMN2 is responsible for the initial slow and random displacement of the spindle, what causes the subsequent much faster and polarized movement towards the cortex? Time-lapse video microscopy has revealed that in the mouse oocyte the second phase of chromosome and spindle movement is accompanied by an intense activity of cytoplasmic streaming (Yi et al., 2013). An uninterrupted flow of cytoplasmic particles may be observed departing from the area of the cortex approached by the chromosomes and the spindle. Drawing two symmetrical paths, these currents of cytoplasmic particles drift away cortically, reach the opposite oocyte pole and return centrally towards the spindle (Fig. 6). This pattern is rotationally symmetric around the axis connecting the spindle and the oocyte centre, while the speed of cytoplasmic particles mirrors that of the spindle, increasing gradually as spindle movement progresses.

**Figure 6** Schematic representation of forces governing spindle repositioning to the cortex prior to PBI emission. Initially, the spindle moves with low instantaneous speed and straightness in the vicinity of the oocyte centre as an effect of symmetric distribution of FMN2 (pink circles) and actin filaments (not shown). Afterwards, FMN2 accumulation and F-actin polymerization in the vicinity of the spindle acquires a polarized distribution, causing the spindle to drift towards the cortex. As the spindle moves towards the cell periphery, Arp2/3 (red circles) becomes progressively localized in the adjacent cortical domain. Dynamic actin polymerization generated by Arp2/3 promotes an uninterrupted flow of cytoplasmic material (blue broken arrows) that drifts away cortically, reach the opposite oocyte pole and return centrally towards the spindle. This is believed to produce a pushing force that ultimately determines spindle positioning at the cortex.
Overall, this is strongly indicative that streaming of cytoplasmic material generates a pushing force that accounts for the fast phase of spindle movement. Interestingly, cytoplasmic streaming does not stop with the repositioning of the MI spindle to the cortex. It continues even after PBII extrusion, maintaining the MI spindle at the cortex. Therefore, cytoplasmic streaming appears to emerge as a major phenomenon by which oocyte polarity is achieved during maturation (Yi et al., 2011).

Experiments involving mouse MI and MII oocytes indicate that cytoplasmic streaming is a downstream effect of an interplay between the chromatin and the cortex. Central to this interaction is the actin-related protein 2/3 (Arp2/3) complex, a factor that promotes actin nucleation in cell motility and membrane trafficking (Goley and Welch, 2006). Following GVBD when chromosomes are still positioned centrally, Arp2/3 is not detectable by immunofluorescence. However, as chromosomes move towards the cell periphery, Arp2/3 becomes progressively localized in the cortical domain where the MI spindle will finally become repositioned (Yi et al., 2013). This suggests the concept that at some point of the journey away from the oocyte centre, the chromosomes (but not the spindle, as explained below) trigger a cortical polarized localization and activation of Arp2/3. In its turn, by inducing actin nucleation, Arp2/3 is believed to orchestrate flows of cytoplasmic material that push decisively the MI spindle towards the cortex. The role of Arp2/3 clearly emerges from experiments in which the specific inhibitor CK-666 was found to prevent the generation of cytoplasmic flows and spindle repositioning to the cortex (Yi et al., 2013). The spindle microtubular apparatus is not essential for this mechanism, because its depolymerization does not affect cytoplasmic streaming and the second phase of fast chromosome movement towards the cortex (Yi et al., 2013).

Little is known on the signal that induces Arp2/3 localization in the cortical domain adjacent to the MI spindle. However, fast spindle movement does not occur in Fmn2−/− oocytes in which chromosomes are positioned naturally towards the cortex, indicating that FMN2 may play a role in assisting the communication between the chromosomes and the adjacent cortex to achieve Arp2/3 localization or activation (Yi et al., 2013).

Information has also been gained on the involvement of cytoplasmic streaming in maintaining the meiotic spindle in a cortical position in MII oocytes. As mentioned above, in such oocytes cytoplasmic streaming occurs according to trajectories entirely comparable to those observed in MI oocytes (Yi et al., 2011). Similarities in cytoplasmic streaming between MI and MII oocytes also involve Arp2/3, which at the mature stage is detectable by immunofluorescence microscopy in the cortex overlying the spindle (Yi et al., 2011) and whose inhibition by CK-666 disrupts cytoplasmic streaming and causes spindle detachment from the cortex (Yi et al., 2011). Neural Wiskott-Aldrich syndrome protein (N-WASP), an upstream activator of Arp2/3 (Campellone and Welch, 2010), can also be detected in the cortex near the spindle. Its knockdown in mouse oocytes by morpholino injection determines disruption of Arp2/3 localization and spindle detachment (Yi et al., 2011), indicating an active role for this regulator in spindle positioning. Therefore, it is believed that also at the MI stage cortical localization of Arp2/3 and activation of its actin-nucleating ability generates a flow of cytoplasmic material that pushes and maintains the spindle at the cortex. Spatio-temporal image correlation spectroscopy analysis confirmed the importance of the actin-nucleating activity of Arp2/3, showing that actin filaments move along trajectories equivalent to those of cytoplasmic streaming and that Arp2/3 inhibition with CK-666 drastically attenuates actin flow in addition to disrupting cytoplasmic streaming (Yi et al., 2011).

In mouse MII oocytes, further details are known on how chromosomes induce Arp2/3 localization in the adjacent cortex. Previous studies had shown that MII chromatin is source of a Ras-related nuclear protein (Ran) GTPase signal that organizes an actin-rich cortical cap required for polar body extrusion, as illustrated in the following section (Deng et al., 2007). To test the hypothesis that the same signal was responsible for Arp2/3 localization and consequent spindle positioning, mouse MII oocytes were injected with a dominant-negative Ran protein. The treatment disrupted localization of N-WASP and Arp2/3 and caused spindle detachment from the cortex (Yi et al., 2011). Overall, the above evidence demonstrate that, through Ran signalling, MII chromosomes activate a cascade of events that sequentially involve N-WASP and Arp2/3 localization, activation of Arp2/3, generation of actin flows and finally cytoplasmic streaming, by which the cortical position of the MI spindle is maintained.

Cortical polarization and polar body extrusion
At both meiotic divisions, the peripheral positioning of the spindle not only orientates the cleavage plane in a fashion that results in the extrusion of a very small polar body, but also induces structural changes in the overlying cortex. In mouse MI and MII oocytes, it has been known for long time that chromosomes cause significant modifications of the adjacent cortex, including disappearance of microvilli from the oocyte surface and above all a thickening of the cortical cytoskeleton involving a prominent actin cap surrounded by a ring of myosin II (Longo and Chen, 1984, 1985; Maro et al., 1986). Such a specialized contractile actomyosin structure, referred to as cap, is crucial for the mechanics of PBII extrusion (Brunet and Verlhac, 2010). In mouse MII oocytes, more recent findings have shown that chromosomes (not the spindle microtubules) are the source of a cue that, in addition to triggering cytoplasmic streaming (see above), is responsible for the assembly of the actomyosin cap. Chromosome action is believed to be a function of mass and distance from the cortex. This can be inferred from experiments in which oocytes were injected with beads coated with plasmid DNA (Deng et al., 2007). The beads were able to induce formation of cortical actomyosin structures as long as their distance from the cortex was shorter than 20 μm. In addition, the size and strength of the actomyosin cap was inversely proportional to the distance of the beads from the cortex and directly proportional to the quantity of beads. Therefore, MII chromosomes generate a signal progressively decreasing with distance that is used as a molecular ruler to trigger changes in the nearest region of the cortex. Such a solution ensures that the actomyosin cap forms only when and where it will be needed to assist PBII extrusion. The biochemical nature of this signal is believed to be similar to the system that promotes microtubule polymerization and spindle organization in the vicinity of the chromosomes, involving a Ran-GTPase gradient. The signal is centred at the chromosomes and depends on two chromatin-bound factors—chromatin-associated RCC1 (regulator of chromosome condensation 1) and RAN GEF (guanine nucleotide exchange factor)—while another factor (cytoplasmic GTPase activating protein for RAN) is dispersed throughout the cytoplasm (Dumont et al., 2007; Yi et al., 2011; Azoury et al., 2012). An important clue of the involvement of a Ran gradient in the chromosome ability to influence the cortex derives from experiments showing that in mouse dominant-
negative Ran mutants the formation of the actomyosin cap is inhibited. In MII oocytes, chromosomes are also a source of a Ran gradient. However, in dominant-negative Ran mutants disruption of the actomyosin cap is not observed at the MI stage and PBI extrusion occurs undisturbed, raising the question of whether cortical polarization is regulated by two different mechanisms at MI and MII (Deng et al., 2007).

How the chromatin-borne Ran gradient is translated into a regulatory signal that induces the formation of an actomyosin structure is only partially understood. It is known that the actin nucleator Arp2/3 is localized in the cortex overlaying the spindle and that inhibition of Arp2/3, or its upstream activator N-WASP, disrupts formation of the actin cap (Halet and Carroll, 2007; Yi et al., 2011). However, evidence for other possible upstream regulators is sketchy. CDC42 and RAC (two Rho-type GTPases that regulate actin polymerization in other cell systems) are suspected to control N-WASP activity and therefore actin nucleation in the vicinity of the spindle (Na and Zernicka-Goetz, 2006). Recent work has also revealed that several other factors intervene in the process of actin polarization, such as myosin light chain kinase (Deng et al., 2005), FYN Tyr kinase (Luo et al., 2009) and Ser/Thr kinase MOS (Deng et al., 2005), a major cell cycle regulator, making it more difficult to decipher the overall scenario by which the actomyosin cap and the mechanics of PBII extrusion are regulated.

Human mature oocytes also display a network of sub-oolemmal actin, but the presence of an actin-rich domain in the vicinity of the spindle has remained elusive until recently. In fact, conventional fluorescence microscopy does not show the existence of an actin cap. However, high-resolution confocal microscopy has recently demonstrated that in MII, but not GV-stage, oocytes cortical actin is indeed measurably more abundant in the vicinity of the spindle (Fig. 7) (Coticchio et al., 2014). In the context of human assisted reproduction, further research on the polarity of the cortical cytoskeleton and the mechanism of PBII extrusion is warranted. Interest in this matter is exemplified by the fact that in some cases, often in a cycle- or patient-specific fashion, oocytes fertilized by a single sperm develop abnormally into triplo-nucleate eggs as a consequence of PBII retention (Porter et al., 2003). Interestingly, the degree of actin polarization and abundance was found to diminish with increasing maternal age (Coticchio et al., 2014), an observation that sheds new light on the phenomenon of oocyte ageing. In fact, while it is widely recognized that a progressive increase in oocyte aneuploidy is the major cause of a decrease in female fertility with age (Hassold and Hunt, 2009), it cannot be rule out a priori that other factors, such as a weakening of the actin cytoskeleton, contribute to oocyte ageing.

Changes in cortical mechanics important for oocyte maturation

In mitotic cells that give rise to daughter cells of equivalent size, it is known that cytokinesis requires a timed stiffening of the cortex (Théry and Bornens, 2006). This prompts the question of whether and how cortical tension changes during oocyte maturation, especially considering the highly asymmetric divisions that characterize the meiotic process and the polarization of the cortex described in the previous section. Two recent studies carried out in mouse oocytes have shed light on the mechanics of the oocyte cortex, highlighting fundamental and previously unrecognized aspects of oocyte cytokinesis. An initial investigation revealed the rather surprising notion that, unlike mitotic cells, the overall stiffness of the oocyte cortex progressively decreases 6-fold as meiosis progresses from the GV to the MII stage (Larson et al., 2010). However, this decrease does not occur homogeneously but reflects the polarity that is established in the cortex during maturation. In fact, in MII oocytes the cortical tension of the domain overlying the cortex is almost 3-fold less than that of the remaining cortex, despite sphericity of the cell being preserved. Therefore, the very basic mechanics of the oocyte cortex differ from what is normally observed in somatic cells, most likely in order to assist the extreme asymmetry of polar body extrusion. Further studies have identified some of the factors that regulate cortical tension. In the first place, it has been important to rule out that an overall decrease in stiffness is not merely caused by a progressive reduction in actin content, whose polymeric and soluble fractions do not vary during maturation. Paradoxically, a thickening of cortical actin is progressively observed during maturation, reaching its maximum at the sites where polar bodies are emitted (Chaigne et al., 2013). By exposing mouse oocytes to specific inhibitors, thickening of cortical actin has been shown to depend on at least two regulatory elements, i.e. the actin nucleator Arp2/3 and the Mos-MAPK pathway. It is hypothesized...
that the function of these elements is organized sequentially, according to a model in which Mos-MAPK acts as an upstream signal that promotes Arp2/3 activation by phosphorylation of the Arp2/3 regulator Wave2. On another hand, softening of the cortex that takes place during maturation seems to depend on another factor, myosin II, whose role is well established in contractile activities that shape the cortex of mitotic cells. Indeed, at the GV stage myosin II is predominantly localized throughout the cortex, but as soon as maturation begins and cortical tension decreases it is progressively displaced in the cytoplasmic domain overlying the MII spindle. Why the oocyte regulates the mechanical properties of its cortex by reducing the overall tension is only partially understood, but it is significant that treatment with concanavalin A that softens the cortex also prevents MI spindle migration from the oocyte centre to the periphery (Chaigne et al., 2013).

Schematic description, divided by species, of changes occurring in organelles and the cortical domain in oocytes during the transition from the prophase (GV) stage to the MII stage.

<table>
<thead>
<tr>
<th>Cytoplasmic component or domain</th>
<th>Prophase</th>
<th>Metaphase II</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endoplasmic reticulum Mouse</td>
<td>poorly defined organelle clusters are visible throughout the ooplasm, while larger less well-defined accumulations are localized only in the cell interior and without obvious polarization. Cow and hamster: elements of the organelle are found in a cortical position. Human: organelles elements are organized in a fine network extending throughout the cortex and the cell interior.</td>
<td>Mouse: organelle elements form a reticular network extending over the entire cell, with large accumulations in the oocyte cortex. Such clusters are polarized, being absent from the microvilli-free cortical domain overlaying the MII spindle. Cow and hamster: small clusters are more homogeneously dispersed. Human: clusters are found throughout the oocyte, also in the vicinity of the MII spindle.</td>
<td>Mehlmann et al. (1995), Shiraishi et al. (1995), Payne and Schatten (2003), Mann et al. (2010)</td>
</tr>
<tr>
<td>Mitochondria Mouse</td>
<td>aggregates are finely dispersed throughout the cell, showing only moderate concentration around the GV</td>
<td>Mouse: aggregates are found throughout the oocyte, with partial accumulation in the vicinity of the meiotic spindle. Human: mitochondria with higher potential difference across the inner mitochondrial membrane, and therefore more functionally competent, are found beneath the oolemma.</td>
<td>Dalton and Carroll (2013), Van Blerkom (2003, 2011)</td>
</tr>
<tr>
<td>Golgi apparatus Mouse, cow, rhesus monkey: the organelle is not organized in a typical single ribbon-shaped array, but is formed from many ‘mini-Golgis’ dispersed in the cytoplasm. These aggregates appear moderately more concentrated in the interior than in the cortex.</td>
<td>Mouse, cow, rhesus monkey: Mini-Golgis fragment in even smaller clusters and diffuse evenly throughout the ooplasm.</td>
<td>Hyttel et al. (1989), Moreno et al. (2002)</td>
<td></td>
</tr>
<tr>
<td>Cortical granules Mouse, cow, human: CGs are centrally localized a finely dispersed in the oocyte</td>
<td>Mouse, cow, human: CGs are found beneath the oolemma but in the mouse are excluded by the cortex adjacent the MII spindle.</td>
<td>Szollosi (1967), Nicosa et al. (1977), Liu et al. (2003)</td>
<td></td>
</tr>
<tr>
<td>Cortex Mouse: cortex characterized by high stiffness</td>
<td>Mouse: 6-fold reduction in cortex stiffness in comparison to the GV prophase stage. Stiffness of domain overlaying the spindle almost 3-fold less than that of the remaining cortex.</td>
<td>Larson et al. (2010)</td>
<td></td>
</tr>
</tbody>
</table>

GV, germinal vesicle; MII, metaphase II; CGs, cortical granules.

Table II

Cytoplasm reorganization during maturation

During the transition from the prophase (GV) stage to the MII stage virtually all major organelles undergo important changes in structure, function and/or distribution. Such modifications are described in the following sections and schematically summarized in Table II, together with those concerning the oocyte cortex, as discussed in the previous section.

Endoplasmic reticulum

The ER is an organelle formed from membranous tubules and vesicles organized in multiple continuous sub-components, namely rough ER, smooth ER and nuclear envelope. Protein synthesis and assembly and
lipid synthesis are major functions of the ER in all cells. In oocytes, the ability of this organelle to store and release free Ca\(^{2+}\) in the cytoplasm acquires a specific and fundamental importance. In fact, at fertilization, the ER is central to a mechanism that generates transient rises in the concentration of intracellular free Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\), referred to as oscillations, by which MII oocytes respond to gamete fusion (Swann and Yu, 2008). Amplitude, frequency and duration of these oscillations are then decoded by downstream regulators into signals that trigger the events of fertilization, i.e. cortical granules (CGs) release, exit from MII, PBII extrusion and formation of pronuclei (Ducibella and Fissore, 2008). Studies carried out over decades in different species have finally identified in the sperm-borne phospholipase C zeta (PLC\(_{\zeta}\)) the origin of the stimulus that ultimately induces the Ca\(^{2+}\)-releasing activity of the ER (Swann, 1990; Swann et al., 2006). Introduced into the ooplasm as a result of oocyte-sperm fusion, PLC\(_{\zeta}\) is in fact believed to activate the phosphoinositide signalling pathway by breaking down phosphatidylinositol 4,5-bisphosphate into diacylglycerol and inositol 1,4,5-trisphosphate (IP\(_3\)). Downstream from this signalling pathway, IP\(_3\) finally binds its receptor (IP\(_3\)R) located in the membranes of the ER that, as a consequence of a conformational change caused by the binding, acts as an IP\(_3\)-gated channel allowing release of Ca\(^{2+}\) from the lumen of the organelle (Swann and Lai, 2013). The molecular and biochemical aspects of the regulation of the Ca\(^{2+}\)-releasing mechanism in mature oocytes have been extensively described in recent publications and will not be discussed in this context (Wakai et al., 2013; Wakai et al., 2013). On the contrary, here attention will be focused on the changes to which the ER is subject during oocyte maturation that are part of a larger cytoplasmic reorganization and are relevant to the acquisition of developmental competence.

In mouse oocytes, changes in the arrangement of the ER during maturation become immediately apparent from the comparison of GV and MII oocytes. In the GV, no well-defined organelle clusters are visible throughout the ooplasm, while larger less well-defined accumulations are localized only in the cell interior and without obvious polarization. A different picture emerges from the analysis of MII oocytes, in which the ER takes the form of a reticular network extending over the entire cell, with large (1–2 \(\mu\)m in diameter) accumulations of elements in the oocyte cortex. Such clusters display a polarized distribution because they are not found in the microvilli-free cortical domain overlaying the MII spindle (Mehlmann et al., 1995). In other mammalian species, the pattern of ER rearrangement during maturation may be different, as observed in the cow (Payne and Schatten, 2003) and hamster (Shiraishi et al., 1995) where elements of the ER are found in a cortical position in GV-stage oocytes and more homogeneously dispersed in small clusters in mature oocytes. Differences in the distribution of the ER in mouse GV and MII oocytes are associated with changes in the ability to respond to Ca\(^{2+}\)-releasing stimuli. In fact, MII oocytes respond to sperm penetration or treatment with IP\(_3\) with Ca\(^{2+}\) transients that are much larger and more frequent in comparison to those generated by GV oocytes under the same conditions (Jones et al., 1995). Therefore, it is possible that the transition of the ER from a structure organized in non-polarized, poorly defined accumulations localized deep in the cytoplasm into a network of more well-defined asymmetrically distributed cortical clusters represents a cellular manifestation of an increased responsiveness to the activating stimulus triggered by the sperm. Other studies in mouse oocytes suggest that regulation of the IP\(_3\)R may account for the increased sensitivity of the Ca\(^{2+}\)-releasing mechanism that accompanies maturation. In fact, Western blot analysis indicates that the amount of the IP\(_3\)R almost doubles during the transition from the GV to the MII stage (Mehlmann et al., 1996). As shown by immunofluorescence microscopy, this increase is associated with a redistribution of the IP\(_3\)R, which in GV oocytes is diffusely localized in the cortex without apparent polarization, while in MII oocytes the same molecule appears distributed in clusters of 1–2 \(\mu\)m occupying the cortex, except the region around the MII spindle. In addition, during maturation the IP\(_3\)R appears to increase its sensitivity, i.e. response to IP\(_3\), as an effect of phosphorylation by M-phase kinases, namely cdk1 (VWakai et al., 2011). Overall, the above evidence is consistent with the hypothesis that during maturation the oocyte enhances the sensitivity of its Ca\(^{2+}\)-releasing mechanism by redistributing to the cortex the elements of the ER that act as intracellular stores of this ion, and by increasing the number and reactivity of IP\(_3\)R molecules, through which the ER responds to the generation of IP\(_3\) that follows the action of the sperm PLC\(_{\zeta}\). In this fashion, the oocyte is thought to set the stage for the initial phases of fertilization. The apparent importance of ER redistribution during maturation has inspired studies investigating the role of the cytoskeleton, by which organelles are usually moved within cells. Immediately following GVBD and before becoming organized in clusters of 1–2 \(\mu\)m occupying the cortex, in mouse oocytes the ER develops into a dense array of membranes that surrounds the newly formed MII spindle. As discussed in the previous section, this is instrumental for the localization of FMN2 and the initial phase of spindle movement towards the cortex (Yi et al., 2013). Such a distribution in the vicinity of the spindle appears to depend on the breakdown of the GV because it does not occur if GVBD is experimentally prevented (FitzHarris et al., 2007). Nocodazol, a microtubule depolymerizing agent, or inhibition of cytoplasmic dynein, a motor protein associated with microtubules, also prevent formation of the ER network around the spindle. At later stages of maturation, the cloud of ER accompanies the spindle during its journey to the cortex and, in coincidence with the MII–MII transition, acquires the typical organization in clusters of 1–2 \(\mu\)m localized at the cortex. This final phase of reorganization is not affected by depolymerization of microtubules, but is prevented by disruption of microfilaments. Thus, reorganization of the ER during maturation appears as a finely regulated phenomenon whose initial phase following GVBD depends on microtubules and the motor protein dynein, while the subsequent and final rearrangement is supported by microfilament action. Notably, in oocytes of diabetic mice the ER network does not surround the forming MII spindle and does not become organized into typical cortical clusters of 1–2 \(\mu\)m diameter following maturation (Zhang et al., 2013), suggesting that this organelle may be involved in developmental anomalies, reported also in the human, dictated by maternal pathologies.

During maturation, human oocytes display modifications in the pattern of ER distribution and a variation in the abundance of the IP\(_3\)R that are reminiscent of the changes observed in the mouse. In fact, human GV-stage oocytes are characterized by elements of the ER organized in a fine network extending throughout the cortex and the cell interior, while MII oocytes have large distinct clusters 2–3 \(\mu\)m in diameter throughout the cortex and the cell interior (Mann et al., 2010). Interestingly, in MII oocytes ER clusters are not absent from the cortex in the vicinity of the MII spindle, consistent with the fact that in the human the oocyte surface overlying the spindle is not devoid of microvilli, unlike the mouse. Also, during the GV–MII transition, Western blot analysis indicates that oocytes increase their endowment of IP\(_3\)R by 50%,
while the ability to release Ca\(^{2+}\) in response to IP\(_3\) almost doubles (Mann et al., 2010). Therefore, in a fashion similar to the mouse model, during maturation human oocytes undergo changes in the distribution and constitution of the elements of the ER that account for an increased ability to release Ca\(^{2+}\). Interestingly, human MII oocytes matured \textit{in vitro} from GV-stage oocytes fail to support such increases in IP\(_3\)R abundance and Ca\(^{2+}\)-release ability despite an apparently normal ER distribution in clusters of 2–3 μm, suggesting that a reduced response to IP\(_3\) may explain the reportedly lower developmental competence of \textit{in vitro} matured oocytes (Mann et al., 2010). However, these results should be interpreted with caution, because they were obtained using left-over GV-stage oocytes derived from stimulated IVF cycles, i.e. material that is believed to be intrinsically developmentally incompetent. In addition, in such experiments GV-stage oocytes were cultured without CC, a condition known to profoundly affect many cellular and biochemical aspects of the process of maturation.

In human IVF, the ER has drawn the attention of clinical embryologists. In fact, a fraction of mature oocytes recovered from treatment cycles involving ovarian stimulation with gonadotrophins show large aggregates (many microns in diameter) of smooth ER (Otsuki et al., 2004), whose network is not usually detectable by conventional transmitted light microscopy. Interestingly, accumulations of ER are not visible in GV-stage oocytes, a sign that they form during maturation \textit{in vivo}, irrespective of the underlying mechanism. In several reports, an association between such aggregates and birth anomalies was described (Ebner et al., 2008; Sá et al., 2011), although a recent review showed that healthy babies can be born from oocytes displaying ER anomalies (Shaw-Jackson et al., 2014). However, data on this matter remain limited and clearly more systematic research is needed.

Mitochondria

The massive organelle and cytoskeletal reorganization occurring during oocyte maturation is energetically costly, requiring adequate levels of ATP at precise times and positions. Oxidative phosphorylation supported by mitochondrial function is the primary source of ATP because fully grown oocytes have a limited ability to metabolize glucose into pyruvate (Biggers et al., 1967; Leese, 1995). Therefore, conditions that perturb mitochondrial function are expected to cause important developmental disturbances, such as meiotic chromosome segregation and maturation and fertilization failure. In addition, being that the mitochondrial legacy is entirely maternal, compromised mitochondrial function in oocytes can have direct effects on the early embryo, including cleavage arrest, abnormal cytokinesis and blastomere fragmentation, as a consequence of mitochondrial-driven apoptosis (Van Blerkom, 2011). Intriguingly, during maturation mitochondria efficiently support oocyte function despite their apparently underdeveloped, primitive aspect characterized by a small spherical shape, few truncated cristae and highly electron-dense matrix (Motta et al., 2000).

During maturation, mitochondria are subject to changes in abundance, function and localization that are crucial for their ability to meet the oocyte energy demands. Estimates of mitochondrial number in maturing oocytes are difficult, considering the extremely large cell size and the challenge posed by ultrastructural morphometric analysis. However, mitochondrial number is thought to be around 92,000 and 740,000 in mouse and sheep oocytes, respectively (Picó and Matsumoto, 1976; Cotterill et al., 2013). In the human, indirect assessments based on the assumption that each organelle contains one copy of mtDNA have suggested figures ranging from 30,000 to more than 1,000,000 (Brenner et al., 2000; Van Blerkom, 2004; May-Panloup et al., 2007). Studies carried out in pig oocytes are consistent with the hypothesis that during the transition from the GV to the MII stage, mitochondrial number can decrease by two-thirds (Cran, 1985). However, possible numerical differences at the different maturation stages do not necessarily reflect changes in the mitochondrial mass because individual organelles can interact with each other and undergo phenomena of fusion and fission, as recently shown in mouse oocytes (Wakai et al., 2014). Another factor that may reconcile the apparent paradox of a decrease in mitochondrial number during maturation is mtDNA copy number. Throughout oogenesis from the primordial to the pre-ovulatory stage, mtDNA content per oocyte expands dramatically, growing from thousands to many hundred thousand copies. In the pig, a peak in mtDNA synthesis has been reported to occur during maturation (Spikings et al., 2007), a phenomenon that could give rise to the presence of more than one copy of DNA in each individual organelle and therefore counterbalance the concomitant reduction in mitochondrial number. The prevalent importance of mtDNA copy number over mitochondrial number also emerges from studies in which mtDNA synthesis was inhibited at various stages of maturation, from which it is possible to conclude that a precise mtDNA threshold is essential to ensure the normal unfolding of fertilization and early development (Spikings et al., 2007). Intriguingly, mtDNA can be subject to degradation during the early cleavage stages (Spikings et al., 2007; Cree et al., 2008), suggesting that a balance between synthesis and degradation may represent a homeostatic mechanism by which mitochondrial function is regulated during maturation and early preimplantation development in response to a changing involvement of oxidative phosphorylation in energy-generating processes. Whether a burst of mtDNA synthesis occurs in human oocytes during maturation is currently unknown. Preliminary data are consistent with this possibility, although it remains uncertain whether this phenomenon can have a clinical significance (Van Blerkom, 2011).

During transition from the GV to the MII stage, in addition to possible changes in overall relative organelle mass and mtDNA copy number, mitochondrial function can be regulated through other modalities. Specific organelle distributions at the different maturation stages can profoundly influence the quality of the maturing oocyte and the destiny of the ensuing embryo. In fact, compromised oocyte quality is associated with aberrant mitochondrial rearrangement and low ATP levels (Van Blerkom et al., 1995; Van Blerkom, 2004). Changes in mitochondrial distribution during maturation have been precisely mapped in mouse oocytes. At the GV stage, mitochondria aggregates are finely dispersed throughout the cell, showing only moderate concentration around the GV. Following GVBD, mitochondria accumulate in large amounts (over 40% of the overall population) in the immediate surroundings of the forming MI spindle (Dalton and Carroll, 2013). Importantly, as in other maturation phases, many mitochondria appear juxtaposed to elements of the ER, a vicinity that at this stage is motivated by the energy demands of the mechanics of formation and translocation of the MI spindle (see ‘Cytoskeletal forces governing spindle positioning’ section). This is consistent with the fact that a dense cloud of mitochondria accompanies the MI spindle during its relocation from the oocyte centre to the periphery and, following PBI extrusion, reconstitutes around the forming MII spindle (Dalton and Carroll, 2013). However, by the time the oocyte arrest at the MII stage, the spindle is no longer enclosed in a shell of...
mitochondria, which nevertheless remain more densely localized in this area.

Recent findings have described mitochondrial rearrangement in even greater detail. In most eukaryotic systems, mitochondrial are known to be transported along microtubules. In mouse oocytes this seems to be also the case, because inhibition of microtubule polymerization with nocodazole prevents accumulation of mitochondria around the MI spindle while the same effect is not observed by disrupting actin filaments with latrunculin. Predictably, motor proteins have been found to assist mitochondrial accumulation. In fact, oocytes react to treatment with function-blocking antibodies specific for dynein or kinesin-1, which move cargoes towards the minus and plus ends of microtubules, respectively (Hirokawa et al., 1998). In particular, inhibition of dynein function reduces the clustering of mitochondria around the MI spindle, while suppression of kinesin-1 activity enhances mitochondrial accumulation (Dalton and Carroll, 2013). Therefore, microtubules provide the major infrastructure for mitochondrial displacement and a regulated balance of the activity of different motor proteins accounts for the appropriate vectorial movement towards the spindle. Live cell time-lapse microscopy and semi-quantitative data generated by confocal microscopy have revealed an intriguing aspect of mitochondrial distribution during PBI extrusion. As described above, a massive array of mitochondria accompany the spindle during its relocalization from the centre to the cortex. At MI this arrangement is still present, but at around the time of anaphase I mitochondria acquire a polarized arrangement and appear predominantly clustered at the spindle pole oriented inward and virtually absent from the area near the pole facing the cortex.

With the progression of the first meiotic division, this has the effect of retaining almost all mitochondria within the oocyte, while a much smaller fraction of organelles is segregated in the PBI (Dalton and Carroll, 2013). Although such a mechanism has not been yet elucidated in fine detail, it appears obvious that it represents a solution to the need of preserving for the oocyte, and therefore the ensuing embryo, the original endowment of organelles, especially considering that mitochondrial replication does not occur during preimplantation development. Interestingly, in mouse oocytes during PBI extrusion the ER follows a similar, although leakier, pattern of segregation (Dalton and Carroll, 2013), suggesting that the two types of organelle may be regulated by the same mechanism. Further experiments have established that the distance of the spindle from the cortex is crucial for mitochondrial polarization at anaphase I. In fact, a direct relationship exists between distance of the spindle from the cortex and degree of mitochondrial polarization (Dalton and Carroll, 2013). Therefore, it is plausible that asymmetric mitochondrial segregation occurs as an effect of not yet known spindle–cortex interactions, while it would not take place in conditions that prevent spindle migration. Indeed, following GVBD, when spindle repositioning to the cortex is prevented by inhibiting the actin-nucleating factor Arp2/3, the first meiotic division generates two daughter cells that inherit comparable proportions of mitochondria (Dalton and Carroll, 2013). Mitochondrial segregation appears to be differentially regulated at the second meiotic division. As previously discussed, as soon as the PBI is extruded, mitochondria form a dense cloud around the forming MI spindle, but tend to disperse shortly afterwards by the time the oocyte pauses at MI. In mouse eggs treated with ethanol to achieve parthenogenetic activation, during anaphase II mitochondrial accumulation near the pole oriented towards the oocyte centre and rarefaction around the pole facing the cortex are not observed, with the consequence that mitochondrial segregation is not asymmetric (Dalton and Carroll, 2013). However, the contingent of mitochondria removed from the oocytes and eliminated with the PBI is small, because mitochondrial accumulation around the MI spindle becomes much less pronounced once the oocyte progresses through meiosis and pauses at MI. This probably makes unnecessary the mechanism of polarization that vice versa acts in concomitance with PBI extrusion. Overall, the changing arrangements of mitochondria during maturation represent a notable example of the oocyte’s ability to regulate mitochondrial function. The mitochondrial population is redistributed in time and space in a very specific fashion and concentrated when and where it is most needed, i.e. around the meiotic spindle, from its formation to the time when its migration from the cell centre to the cortex is completed. Afterwards, mitochondrial polarization at the innermost spindle pole at anaphase I adds another level of regulation, by which mitochondrial loss with the extrusion of the PBI is minimized.

Compartmentalization of mitochondria subpopulations with different functional attributes may be another option for the regulation of ATP supply in maturing oocytes. In fact, although morphometrically and ultrastructurally similar, mitochondria may belong to different functional categories in oocytes and embryos. Potentiometric dyes, such as JC-1, have revealed that mitochondria can differ in $\Delta \Psi m$, i.e. the potential difference across the inner mitochondrial membrane (Van Blerkom, 2003). Because the magnitude of $\Delta \Psi m$ is directly correlated to important mitochondrial functions, including ATP production, there is the awareness that mitochondria are not a homogeneous population and that specific localization of subpopulations characterized by different $\Delta \Psi m$ may correspond to precise oocyte regulatory needs. An example of this scenario may be represented by a small contingent (~5%) of sub-oolemmal mitochondria that shift to a higher level of $\Delta \Psi m$ when the mouse oocyte approaches the MI stage, while the vast majority of mitochondria remain in a state of low $\Delta \Psi m$ (Van Blerkom, 2011). Current findings suggest that this change is actively prevented by nitric oxide produced by corona cells until the ovulatory stimulus is triggered (Van Blerkom et al., 2008). Irrespective of the mechanism of regulation, however, it is notable that the localization of highly polarized, and therefore more functional, mitochondria occurs in a timely fashion in a specific oocyte domain where they are believed to be crucially needed. Several lines of evidence in fact suggest the notion that hyperpolarized mitochondria are essential for supporting the very first steps of fertilization at the cortical level (Van Blerkom et al., 2002).

The relationship between ATP production/demand and mitochondrial distribution during maturation was assessed further in recent investigations. In mouse oocytes matured in vitro, mitochondrial activity is characterized by three waves of increase in ATP production, separated by two shorter phases of down-regulation to basal levels (Yu et al., 2010). These waves of enhanced activity of ATP generation are temporally coincident with GVBD, migration of the spindle to the cortex and the transition between MI and MII. Above described studies illustrate how an accumulation of mitochondria is found around the nucleus at the time of GVBD, surrounds the spindle during its migration from the centre to the cortex, and become polarized at the innermost spindle pole shortly before body emission (Dalton and Carroll, 2013). However, these patterns do not appear to be essential to, or associated with, the above-mentioned peaks in ATP production because when mitochondrial rearrangement is disrupted by inhibitors of microtubule
polymerization, changes in ATP production remain detectable (Yu et al., 2010). Conversely, formation of small mitochondrial aggregates appears to be instrumental for the occurrence of the bursts in ATP production. In fact, constitution of these small clusters is temporally coincident with the ATP peaks observed at GVBD and the successive maturation steps. Formation of such small mitochondrial aggregates seems to be directed by actin microfilaments. Assembly and disarrangement of this cytoskeletal component, in fact, parallels the formation and disappearance of small mitochondrial clusters. Furthermore, disruption of actin microfilaments with cytochalasin B, which causes actin depolymerization, is incompatible with the formation of mitochondrial clusters and the increases in ATP production (Yu et al., 2010). Therefore, in mouse oocytes the actin microfilament network appears responsible for mitochondrial redistribution on a small scale and the increase in ATP production to support GVBD, spindle translocation and first polar body emission. However, the reasons why mitochondrial clustering in small aggregates are a requisite for an increase in ATP generation remains unknown. Finally, a role of CC for establishing adequate levels of ATP during maturation should not be overlooked. In this respect, it is indicative that mouse denuded oocytes and COC display similar patterns of ATP changes at precise maturation events, with the crucial difference that in the absence of CC the absolute levels of such patterns are lower in denuded oocytes (Dalton et al., 2013).

In conclusion, during maturation mitochondria undergo major changes involving redistribution on a large scale, formation of small aggregates on a smaller scale, compartmentalization of subpopulations characterized by different ATP distribution and activity, fusion/fission phenomena and mtDNA synthesis. By finely orchestrating these events, the maturing oocyte deploys a strategy by which appropriate levels of ATP, mainly derived by oxidative phosphorylation, are made available at precise times and spatial domains, in concomitance with crucial maturation steps.

The Golgi apparatus

The Golgi apparatus (GA) is the organelle by which macromolecules are post-translationally modified, sorted, packaged and destined for release in the extracellular environment or delivery to other intracellular compartments. In fully grown oocytes, the overall function of this organelle is not completely understood considering that protein secretion activity is negligible, although its role in the generation of CG is well known. In somatic interphase cells, the GA takes the form of a single ribbon-shaped mass of membranous structures known as ‘cistermae’ associated with the single centrosome positioned near the nucleus (Thyberg and Moskalewski, 1999). Indeed, GA organization depends on the microtubules that radiate from the centrosome (Thyberg and Moskalewski, 1999). During the S phase the centrosome duplicates and in the following mitosis the couple of centrosomes are crucial for the organization of the mitotic spindle (Green et al., 2012). Meanwhile, the structure of the GA fragments and disperses throughout the cytoplasm to ensure that the two daughter cells inherit comparable fractions of the organelle. This dynamic cannot be reproduced in fully grown oocytes, as the centriole—the centrosome’s core—is lost during early prophase (Szollosi et al., 1972). Investigations concerning mouse (Moreno et al., 2002), cow (Hyttel et al., 1989) and rhesus monkey (Moreno et al., 2002) oocytes converge towards the notion that at the GV stage the GA is not organized in a single ribbon-shaped array, but is formed from a multitude of ‘mini-Golgis’ dispersed in the cytoplasm. In the mouse, these small Golgi aggregates appear moderately more concentrated in the interior than in the cortex. Following GVBD, the mini-Golgis fragment into even smaller clusters and diffuse evenly throughout the ooplasm. This condition of fine fragmentation and homogeneous distribution persists until the MII stage. Fragmentation and dispersion of mini-Golgs occurs also in cow oocytes during GVBD, with the difference that in this species a second phase of redistribution occurring at MI concentrates a larger contingent of these organelles in the oocyte interior (Racedo et al., 2012). Preliminary observations are consistent with the hypothesis that redistribution and fragmentation of the GA in cow oocytes is regulated by microtubules and cytoplasmic dynein (Racedo et al., 2012).

During oocyte maturation, a specific role for the GA remains elusive but different lines of evidence suggest that this organelle is not simply subject to passive redistribution, but may actively participate in important regulatory mechanisms. This can be inferred by the observation that treatments, such as brefeldin A that block membrane trafficking from the ER to the GA, have the ability to inhibit reversibly the maturation in vitro of mouse oocytes (Moreno et al., 2002). It is presumed, therefore, that membrane trafficking is required for the meiotic progression of murine oocytes, to assure post-translational modification of proteins at the Golgi level, or assignment of these proteins to appropriate (post-Golgi) sites. Recent evidence suggests new, previously unsuspected possibilities for a role of the GA during oocyte maturation. GM130 is a protein of the cis-Golgi matrix involved in the trafficking between the ER and the GA, glycosylation and maintenance of Golgi organization (Nakamura, 2010). Novel information indicates that GM130 intervenes in completely different functions, such as cell polarization and division. This is consistent with the fact that GM130 is able to recruit microtubule nucleating factors, namely AKAP450 and γ-tubulin (Rivero et al., 2009), allowing the GA to act as a microtubule nucleating apparatus. In immature mouse oocytes, immunofluorescence analysis reveals that GM130-positive granules are present throughout the cytoplasm, although more abundant around the GV. At GVBD, GM130 begins to concentrate in the central part of the oocytes and, as assembly of the spindle progresses, localizes preferentially at the spindle poles. This distribution persists until anaphase I when the protein is found in the midbody bridging the oocyte and the PBI. Finally at MII, GM130 concentrates again at the spindle poles (Zhang et al., 2011a). Consequently, it is tempting to speculate that at least a subpopulation of GA elements has the ability to promote microtubule nucleation through the activity of GM130, thereby assisting meiotic spindle formation. Such a hypothesis seems to find confirmation in the fact that GM130 morpholino injection causes multiple spindle defects, i.e. elongation and disruption of pole focusing or formation of multipolar structures, and disturbances of polar body extrusion (Zhang et al., 2011a). In mouse oocytes, an involvement of the GA in spindle formation is also apparent from the observation that overexpression of a negative-dominant form of the regulator CDC42, which is involved in spindle formation in mitotic cells, causes phenotypes very similar to those associated with GM130 inactivation (Kodani et al., 2009). These findings open new hypotheses on the role of the GA in oocyte maturation, beyond the well-established function of CG generation. Nevertheless, extrapolation of these conjectures to other species should be made with caution. For example, in mouse oocytes numerous microtubule organizing centres (MTOCs) act as nucleators for microtubule assembly and could recruit elements of the GA carrying GM130. On the contrary, MTOCs are not present in oocytes of other...
species, including the human, a condition that must necessarily requires different mechanisms of microtubule nucleation. Thus, the notion that the GA is actively involved in the formation and regulation of the meiotic spindle in species other than the mouse will have to be tested in future studies.

**Cortical granules**

CGs are small individual membranous organelles distributed just beneath the oolemma in the mature oocyte that intervene in a very early and crucial step during the fertilization process. At the mature stage, CGs appear as secretory vesicles measuring 0.2–0.6 μm in diameter (Szollosi, 1967). They are produced exclusively in oocytes and are not replaced after release. Functionally, they are associated with the modification occurring to the zona pellucida at fertilization. Following gamete fusion and the start of intracellular Ca\(^{2+}\) signalling that drives the activation events, CGs fuse their membrane with the oolemma and shed their content of enzymes in the perivitelline space. Such GC-derived proteolytic proteins modify by cleavage one of the key zona pellucida proteins (ZP2) ad convert it in a version (ZP2f) that is unable to bind other spermatozoa, thereby preventing further penetration through the zona pellucida (Moller and Wassarman, 1989). In this fashion, polyspermic fertilization is prevented. Distribution of CG beneath the sub-oolemmal domain characterizes the mature MI oocyte, but at earlier stages of oogenesis CG formation and localization involves other cellular domains. Formation of CG starts at different stages of folliculogenesis in various mammalian species. In murine oocytes, CGs are present already at the primary follicle stage (Szollosi, 1967). Conversely, in the monkey (Hope, 1965) and human (Baca and Zamboni, 1967), these organelles do not appear until the secondary follicle stage. CGs have a sub-cellular origin associated with the activity of the GA. As discussed above, during the GV–MI transition, the GA undergoes significant re-arrangement (Liu, 2011) assisted by the microtubules cytoskeleton. However, the peripheral relocation of CG, which represents final products of the GA, is thought to be guided by microfilaments (Sun et al., 2001). In mouse mature oocytes, the sub-oolemmal distribution of GC that is typically observed in almost all mammalian species acquires a particular pattern. In fact, while being localized at the extreme periphery of the oocyte, GCs are virtually absent from cortical regions overlying MI and MII spindles (Nicosia et al., 1977; Okada et al., 1986). It is not clear how GCs are excluded from such areas, but evidence generated in the mouse and the hamster indicates that a peri-spindle GC-free area (CGFA) may derive from displacement and redistribution at MI of pre-existing GC (as suggested by an increase in CG density in the area surrounding the CGFA) or precocious and confined GC exocytosis occurring during the MI–MII transition (Ducibella et al., 1988, 1990; Liu et al., 2003). Doubts also remain on the reasons that lead to the establishment of a CGFA in these species. It cannot be ruled out that targeted release of CG in the cortex in the vicinity of the MI/MII spindles modifies the zona pellucida or the oolemma in a way that locally prevents sperm–oocyte fusion. This would assure that decondensation of the sperm head and formation of the male pronucleus occurs at a safe distance from the MI spindle and that the integrity of maternal chromatins is not affected as it undergoes remodelling. This seems to be confirmed by the observation that in mouse oocytes sperm penetration is less likely to occur in regions of the cortex where the MII spindle is located (Nicosia et al., 1977).

**Clinical correlates of oocyte maturation**

From the studies illustrated above, oocyte maturation emerges as an extremely complex process, although it unfolds during a minute fraction of the time taken by the follicle to convert a primordial oocyte into a mature egg. Understanding how oocyte maturation occurs in vivo is challenging in animal models and almost impossible in the human. Nevertheless, IVM experiments have allowed us to observe more closely how meiosis is resumed and, to some extent, how the cytoplasm is reshaped in preparation for fertilization. By observing oocyte maturation in vitro, we have also tested some hypotheses on the regulatory networks that govern, and the conditions that make possible, oocyte maturation. However, by its very nature, IVM remains an oversimplified system. In addition, caution should be exercised in translating evidence gathered in animal models, which in any case are essential to generate novel proofs of principles, into axioms adopted for developing new treatment strategies in a human ART scenario.

Regardless of the years our perception of oocyte maturation has dramatically improved thanks to studies that have examined the CC–oocyte interactions, the signalling system that guides the meiotic process, and the internal forces by which the cytoskeleton and organelles are reorganized. For example, in the previous section we have already discussed how studies carried out in the mouse have prompted questions on the role of AREG in oocyte maturation in vivo following controlled ovarian stimulation. Also, increasing knowledge has inspired approaches by which maturation can be achieved in vitro to serve clinical needs. Even more in the future, novel findings in this field will provide the bases for the development of more efficient IVM systems and the definition of more reliable criteria of oocyte quality.

In the context of human IVM, AREG has initially attracted considerable interest as a potential biochemical tool by which to increase yield and quality of in vitro matured oocytes. Progress in IVM is much needed, especially considering that current maturation rates after 30 h of culture usually do not exceed 50–55% (De Vos et al., 2011; Coticchio et al., 2012; Guzman et al., 2012), unlike the mouse and bovine models where 80–90% of GV-stage fully grown oocytes progress to the MI stage in vitro (Albuz et al., 2010). In the human, higher maturation rates can be achieved by extending the time of culture to 48–52 h after removal of CC and assessment of meiotic status at 30 h (Son et al., 2008), although culture in the absence of CC is known to be highly detrimental to oocyte quality. Data supporting the notion that AREG can have a decisive role in improving human IVM are lacking, stressing the perception that progress in IVM can be hardly achieved by the adoption of a single factor or condition. Rather, it seems more reasonable to focus on general processes that regulate oocyte maturation to obtain multiple clues suitable for designing innovative systems. Progress in this direction has been made, leading to a new approach by which oocyte maturation can be pursued in vitro. These attempts derive from an appreciation of the differences occurring between conditions in vivo and in IVM, especially during the initial phases of the process. In vivo, resumption of meiosis and associated cytoplasmic changes result from a delicate balance between forces that prevent precocious initiation of the maturation process and signals that induce the oocyte to start the meiotic process, as described in the previous section. On the contrary, IVM is believed to occur in a rather deregulated fashion, simply as an effect of the absence of the inhibitory influence of the follicular environment. Therefore, it was hypothesized that maturation in vitro could be attained
more successfully by reproducing in an IVM system at least part of the conditions that regulate maturation in vivo. In this vision, the simulated physiological oocyte maturation (SPOM) system was developed by using the mouse and the cow models (Albuz et al., 2010). This approach involves a 'pre-IVM' phase in which, immediately after collection, COCs are exposed for a few hours in vitro to potent stimulators of cAMP production, such as forskolin. This allows reproducing the 100-fold transient increase in cAMP that is observed in whole COC during maturation in vivo (Mattioli et al., 1994). As a second step, and for the remaining period of maturation in vitro, the SPOM system implies the use of a specific inhibitor of the oocyte PDE3, typically cilostamide, to prevent the almost instantaneous fall in cAMP that occurs in oocytes in vitro in the absence of stimulants of cAMP synthesis. In addition, the PDE3 inhibitor is used in association with FSH to actively promote meiotic resumption. Overall, these conditions have been suggested to recapitulate the process of maturation in vivo by (i) mimicking the spike in cAMP levels that occurs in COC at the time of ovulation that is thought to be somehow involved in the meiotic resumption regulatory cascade and (ii) ensuring that meiotic resumption is specifically induced while the oocyte is still under the influence of an inhibitory signal. It is a fact that this treatment extends the time in which gap junctional communication remains active during IVM, with clear implications concerning the degree and quality of oocyte–CC co-operativity. More importantly, following IVF of in vitro matured oocytes, the SPOM system appears to improve significantly oocyte developmental potential, as shown by an increase in blastocyst yield and quality in the bovine system and improvements in blastocyst rate, implantation rate and fetal yield in the mouse (Albuz et al., 2010). It is anticipated that the SPOM system is currently being tested with human material and future developments will clarify whether it will bring about major progress in human IVM. Indeed, other interesting results in the human have been obtained adopting a pre-IVM approach. In a preliminary study, prior to IVM left-over cumulus-enclosed GV-stage oocytes retrieved in stimulated IVF/ICSI cycles were maintained in meiotic arrest for 24–48 h by treatment with the PDE3 inhibitor Org9935. In oocytes surrounded by compact layers of CC, treatment with the inhibitor increased the rate of maturation, although it did not have effects on the rate of fertilization or proportion of good quality embryos (Nogueira et al., 2006). In a similar study, left-over denuded GV-stage oocytes obtained from stimulated ICSI cycles were exposed to cilostamide, another specific PDE3 inhibitor, for 24 h before culture under standard IVM conditions. Transient cilostamide treatment maintained the large majority of oocytes in meiotic arrest and increased the proportion of in vitro-matured oocytes having a bipolar spindle and equatorially aligned chromosomes (Vanhoutte et al., 2007). Although such studies are affected by the major flaw of the use of poor quality oocytes, i.e. supernumerary GV oocytes (with or without surrounding CC) retrieved in stimulated cycles, nevertheless they offer the proof-of-principle that control of meiotic resumption could represent a valuable approach for the improvement of oocyte IVM systems in the human. Another downstream implication of the studies that have attempted to shed light on oocyte maturation is the use of oocyte-secreted factors (OSFs) as part of a more complex environment in which mature oocytes can be achieved in vitro. OSFs, in particular the TGF-β family members GDF9 and BMP15, are paracrine factors produced and released by the oocyte primarily for activating or enhancing in the surrounding CC functions that meet specific oocyte demands. In this way, the oocyte creates an intrafollicular microenvironment where its growth and maturation can be maximally supported. For example, in the mouse both in vivo and in vitro BMP15 and GDF9 appear to increase in CC the rate of transcription of genes encoding key enzymes of the cholesterol biosynthetic pathway (Su et al., 2007), while the same pathway is barely used by the oocyte. Therefore, it is clear that the oocyte influences the metabolism of adjacent CC to compensate for its own need of cholesterol supply in the absence of autonomous production. A similar situation occurs in the case of glycolysis, a function poorly expressed by the oocyte that however is important for its development. This problem is resolved by the ability of CC to provide the oocyte with the products of glycolysis through gap junction-mediated transfer. However, in CC sustained glycolytic activity does not occur spontaneously but is supported by two OSFs, BMP15 and fibroblast growth factor-8 (Sugiura et al., 2008). Similar results have been observed in cow COCs, where BMP15 contributes to maintain active gap junction communication by which the products of glycolysis, a pathway positively regulated by AREG, are transferred from CC to the oocyte (Sugimura et al., 2014). These and other studies exemplify the importance of OSFs for the oocyte–CC interplay and oocyte function, a fact that could have major implications for improving the efficiency of oocyte maturation systems. This has already been confirmed by studies carried out in the mouse and the cow. In the mouse, oocyte exposure to GDF9 during IVM improves the rates of blastocyst formation and hatching, the blastocyst cell number and the fetal yield of the resulting embryos (Gilchrist et al., 2008). In the cow, GDF9 and BMP15 used alone or in combination during IVM also increase blastocyst rate (Hussein et al., 2006), probably by enhancing oocyte–CC gap junction communication. Unfortunately, however, the proof that these factors can decisively improve the outcome of human IVM is still lacking.

Collective knowledge gained from oocyte maturation studies has implications also for practices that are attempted with the aim of optimizing the outcome of ART procedures. The potential use of left-over GV-stage oocytes recovered after standard ovarian stimulation is exemplifying in this respect. Such oocytes occur with a frequency of 5–10% but, in specific cases, at retrieval much larger fractions of oocytes may be found meiotically arrested. GV arrest can be ascertained without ambiguity only after CC removal immediately after oocyte retrieval or a few hours afterwards. Although GV-arrested oocytes are in most cases discarded, their use after IVM for clinical purposes may be found in many reports (Vlaisavljević et al., 2007), especially in cases in which there is little or no availability of mature oocytes. While the effort of IVF specialists to rescue such oocytes and therefore offer the largest chances of success to IVF patients should be recognized, the use of left-over GV-stage oocytes should be discouraged. In fact, despite meiotic resumption and progression to MII being observed, at the current stage of knowledge the culture of immature oocytes to achieve maturation in vitro in the absence of CC raises more concerns than hopes. Much of the evidence discussed in this manuscript leaves little doubt on the central role of CC in oocyte maturation and the disturbances that can occur to oocyte function in their absence. In addition, it should be considered that GV-arrested oocytes recovered in stimulated IVF cycles are likely to be inherently developmentally incompetent (Nogueira et al., 2000). This can also be inferred from different chromatin and cytoskeletal patterns shown by such oocytes, most of which are incompatible with the ability to resume meiosis (Combelles, 2003). Preliminary, but informative, observations also suggest that a large fraction of
GV-arrested oocytes are affected by significant DNA damage as indicated by positivity to γ-H2AX staining (Guglielmo et al., 2012). It should not surprise, therefore, that clinical data show very poor, if any, benefit from the use of such qualitatively compromised oocytes, especially if used after removal of companion CC (Reichman et al., 2010). However, although negligible from a clinical standpoint, GV-arrested oocytes can have a role in research. In fact they represent a model, although imperfect, to study human oocyte maturation, thereby alleviating the perennial shortage of oocytes in basic research. Such opportunities should not be overlooked because significant knowledge can be gained, even recognizing the limits of the model. This concept is explained by a recent study that not only laid the foundations of MI spindle morphometry and mechanics but also, by comparing oocytes matured in vivo and GV oocytes matured in vitro, dispelled the myth that IVM affects spindle morphology in human oocytes (Coticchio et al., 2013).

The importance for oocyte maturation of functional CC–oocyte contacts is relevant to the cryopreservation of immature COCs obtained from unstimulated cycles. These oocytes may be cryopreserved by slow cooling or vitrification before or after IVM. Several reports suggests that while cryopreservation of in vitro matured COCs does not significantly affect oocyte developmental competence (Huang et al., 2010), cryostorage of immature COCs poses a major threat to CC–oocyte communication. This may be inferred by confocal microscopy studies showing massive disruption of TZPs as an effect of cryopreservation (VandeVoort et al., 2008; Brambillasca et al., 2013). Therefore, immature COCs recovered from liquid nitrogen are found in a condition of functional, if not physical, dissociation from surrounding CC, with predictably negative implications for the acquisition of oocyte developmental competence following IVM.

Oocyte maturation research can also assist in the interpretation of phenomena involving mature oocytes. Typical in this respect is the case of PBI morphology. Different manifestations of this trait have been suggested to be associated with oocyte quality and therefore clinical outcome. In particular, it has been reported that oocytes displaying a PBI characterized by large size, irregular shape, roughness of its surface or fragmentation are developmentally less competent, cleaving more irregularly in vitro after fertilization and giving rise to lower pregnancy rates after embryo transfer (Ebner et al., 1999, 2002). Nevertheless, there is no consensus around this matter, except the case of oocytes showing large polar bodies, which indeed appear to be developmentally compromised. Animal studies can assist in interpreting such cases. In fact, in the mouse the generation of a large polar body is a well-documented effect of intrinsic or extrinsic factors that can perturb oocyte maturation and ultimately affect developmental competence. Indeed, oocytes from c-mos<sup>−/−</sup> females generate a large PBI and are developmentally compromised (Choi et al., 1996). Similar consequences are observed when wild-type mouse oocytes are matured in vitro under inappropriate culture conditions (Sanfins et al., 2003; Plancha et al., 2005). On the contrary, there is no firm evidence that in non-human species PBI shape, roughness or fragmentation may predict oocyte quality, consistent with a lack of consensus on the hypothesis that these characteristics can be adopted for oocyte selection in human IVF.

From the above discussion, it emerges how basic studies on oocyte maturation not only enlighten us on a crucial phase of oogenesis, but also have practical implications inspiring novel applications and approaches to human ART.

**Conclusions**

In mammals, oocyte growth is already completed several days before ovulation. However, at ovulation the fully grown oocyte must undergo an intense and laborious period of final maturation to become developmentally competent. The significance of oocyte maturation for the attainment of haploidy has been a well-established concept for several generations of scientists. In contrast, changes occurring in the cytoplasmic compartment and the role of CC during the maturation process have been much more challenging to observe and understand. CCs develop as a distinct cell lineage concomitantly with the formation of the antrum as an effect of regulatory signals, such as GDF9 and BMP15, emitted by the oocyte (Diaz et al., 2007). By modifying the fate of the more adjacent granulosa cells, the germ cell creates a specialized intrafollicular microenvironment more suitably equipped to meet its own metabolic and cellular demands. Before and during maturation, CCs are instructed to activate specific metabolic pathways and make available molecules poorly synthesized by the oocyte, such as pyruvate, cholesterol and various amino acids (Su et al., 2009), whose shortage would otherwise compromise the maturation process. Notably, CC support to the oocyte is not limited to the mere supply of nutrients and metabolites. Perhaps even more important, the CC control vital functions. Meiotic arrest and resumption is exemplar in this respect. Fully grown oocytes are competent to undergo GVBD and progress to MI. Nevertheless, they pause at the GV stage as a consequence of a concerted inhibitory action of both CC and MGC. At ovulation, meiotic resumption is also under the control of follicle cells, which interrupt their inhibitory influence and at the same time emit a stimulatory signal (Conti et al., 2012; Liu et al., 2014). Some elements of this regulatory network (e.g. NPPC and EGF family members) have been identified in the mouse model and are under close scrutiny as candidates for the development of more advanced IVM systems (Albuz et al., 2010; Peluffo et al., 2012; Franciosi et al., 2014). In fact, current IVM approaches are highly imperfect and in need of major improvements (Smitz et al., 2011). In vitro, meiotic resumption is obtained as a result of simple removal of the inhibitory influence of the follicular environment in the absence of specific and genuine positive stimulation, a scenario very different from physiological conditions (Albuz et al., 2010). This has major detrimental influences on oocyte developmental competence. For example in the cow model, under in vitro conditions oocyte–CC communication is interrupted prematurely or severely affected. As a consequence, the process of chromatin condensation occurring in preparation for meiotic resumption is deregulated and oocyte ability to mature and undergo preimplantation development is compromised (Luciano et al., 2011). Recent studies have offered a glimpse into another dimension of the role of CC in the maturation process. Development of cell polarity and the ability to undergo highly asymmetric divisions are characteristics that largely rely on self-organizing processes (Li and Albertini, 2013). However, oocyte polarity appears to depend also on CC. In vivo, pre-ovulatory oocytes are connected to CC through a dense array of TZPs, which are particularly abundant and polarized in the portion of cortex arching the asymmetrically positioned GV. In vitro, inadequate culture conditions, i.e. the absence of FSH supplementation or exposure to the actin-depolymerizing agent latrunculin, cause disruption of TZPs, GV disanchoring from the cortex, loss of asymmetry and ultimately a series of downstream cellular and developmental consequences, suggesting that CC, through their TZPs, are instrumental...
for the design and maintenance of oocyte asymmetry (Barrett and Albertini, 2010).

While these findings underpin the concept that acquisition of oocyte developmental competence during maturation is crucially influenced by the surrounding environment, of which CCs are integral part, other lines of evidence indicate that specific oocyte traits are entirely intrinsic. Typical in this respect is the oocyte’s ability to respond to DNA damage. Oocytes are particularly exposed to DNA damage as a consequence of physiological mechanisms associated with chromosome recombination in fetal life or physical and chemical insults accumulating over long periods of time during follicular quiescence at the primordial stage. Fully grown GV stage oocytes appear to detect DSBs over long periods of time during follicular quiescence at the primordial stage. Meiotic chromosomes are in fact sources of a Ran-GTPase gradient that promote cytoplasmic streaming through actin polymerization activated by activation of the Arp 2/3 complex (Yi et al., 2013). During spindle migration to the cortex, as chromosomes reach sufficient proximity to the cortex, the Ran-GTPase gradient triggers an intense activity of cytoplasmic streaming by which the meiotic spindle is pushed to the extreme cell periphery. This action completes the cell symmetry breaking process, creating the geometrical premises for the extrusion of a small PB. Importantly, the same Ran-GTPase gradient is responsible for the formation of a specialized actomyosin domain in the more adjacent cortex, whose function supports the mechanics of PB extrusion (Deng et al., 2007).

Organelle rearrangement is a further major theme of oocyte maturation. Not only are elements of the ER and the GA and other organelles regulated in their function during maturation, but they are also redistributed in response to precise spatio-temporal cues. For example, a cloud of mitochondria and ER elements surrounds the spindle during its migration to the cortex (Dalton and Carroll, 2013; Yi et al., 2013), providing energy and spatial information for the localization of the actin-nucleating factor FMN2, which promotes spindle displacement. These and other numerous findings described in this review offer us a picture of oocyte maturation that extends far beyond the achievement of meiotic maturity. Despite interspecies differences which may delay the translation of research into practice, our increasing understanding of oocyte–CC interactions will be key to the development of more efficient IVM systems, highly demanded by animal biotechnology and human ART.

Acknowledgements

We would like to kindly thank Prof. David F. Albertini for inspiring many of the concepts discussed in the manuscript.

Authors’ roles


Funding

No external funding was either sought or obtained for this study.

Conflict of interest

None declared.

References


Doerflinger H, Benton R, Shulman JM, St Johnston D. The role of PAR-1 in regulating the polarized microtubule cytoskeleton in the Drosophila follicular epithelium. Development 2003;130:3965–3975.


FitzGarris G, Marangos P, Carroll J. Changes in endoplasmic reticulum structure during mouse oocyte maturation are controlled by the cytoskeleton and cytoplasmic dynein. Dev Biol 2007;305:133–144.


Freudzon L, Norris RP, Hand AR, Tanaka S, Saeki Y, Jones TLZ, Rasenick MM, Berlot CH, Mehlmann LM, Jaffe LA. Regulation of meiotic prophase arrest in


Oocyte maturation


Payne C, Schatten G. Golgi dynamics during meiosis are distinct from mitosis and are coupled to endoplasmic reticulum dynamics until fertilization. Dev Biol 2003;264:50–63.


