

Chapter 2

Control of Mammalian Oocyte Development by Interactions with the Maternal Follicular Environment

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Abstract Development of animal germ cells depends critically on continuous contact and communication with the somatic compartment of the gonad. In females, each oocyte is enclosed within a follicle, whose somatic cells supply nutrients that sustain basal metabolic activity of the oocyte and send signals that regulate its differentiation. This maternal microenvironment thus plays an indispensable role in ensuring the production of fully differentiated oocytes that can give rise to healthy embryos. The granulosa cells send signals, likely membrane-associated Kit ligand, which trigger oocytes within resting-stage primordial follicles to initiate growth. During growth, the granulosa cells feed amino acids, nucleotides, and glycolytic substrates to the oocyte. These factors are necessary for the oocyte to complete its growth and are delivered via gap junctions that couple the granulosa cells to the oocyte. In a complementary manner, growing oocytes also release growth factors, notably growth-differentiation factor 9 and bone morphogenetic protein 15, which are necessary for proper differentiation of the granulosa cells and for these cells to support oocyte growth. During the late stages of oocyte growth, cyclic GMP that is synthesized by the granulosa cells and diffuses into the oocyte is required to prevent its precocious entry into meiotic maturation. Finally, at the early stages of maturation, granulosa cell signals promote the synthesis of a subset of proteins within the oocyte that enhance their ability to develop as embryos. Thus, the maternal legacy of the follicular microenvironment is witnessed by the fertilization of the ovulated oocyte and subsequent birth of healthy offspring.

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2.1 Introduction

Maternal control of embryonic development typically describes the concept that factors which accumulate in the oocyte during its growth and development before fertilization influence the development of the embryo after fertilization. Many, though by no means all, of these factors are messenger mRNAs, reflecting that embryos are transcriptionally inactive during the early stages of postfertilization development, so new protein synthesis relies entirely on mRNAs that were synthesized by the oocyte. Thus, the maternal rather than embryonic genome determines the mRNA population of the early embryo. Here we describe maternal control of a different nature, focusing primarily on studies using the mouse as a model organism. Germ cells develop within a microenvironment that is created by the somatic cells of the gonad. In females, the cells of the ovarian follicle—principally the granulosa cells which surround and enclose the oocyte throughout its growth and development—create this environment. Because individual oocytes and their follicles undergo growth throughout reproductive life, and many aspects of growth can be faithfully recapitulated *in vitro*, much has been learned about the interactions between the female germ cell and its environment during this period. Such studies have revealed a continuous and multidimensional exchange of signals that not only drives oocyte development but also permits the oocyte to remodel the somatic microenvironment to meet its evolving needs.

2.2 Generating Primordial Follicles

The early life histories of oocytes and the granulosa cells are separate stories, reflecting their different embryological origin. Oocytes are descended from the primordial germ cells (PGCs), which arise from posterior epiblast under the influence of signals sent by adjacent tissues, notably bone morphogenetic protein (BMP) 4 from the extra-embryonic ectoderm (Gunesdogan and Surani 2016). Following migration to the genital ridge, the PGCs of female embryos differentiate into oogonia, which undergo multiple rounds of mitotic proliferation before entering meiosis (Pepling 2012; Jorgensen 2013; Grive and Freiman 2015). The granulosa cells are not born so far from home but instead thought to be derived from cells at the surface of the developing gonad (Mork et al. 2012; Hummitzsch et al. 2015). By near the time of birth, each oocyte has become enclosed by a small number of granulosa cells in a structure termed a primordial follicle. The signals that trigger formation of the primordial follicles are not fully understood. However, when oocytes lack the bHLH-type transcription factor, FIGLA, the follicles do not form (Soyal et al. 2000). Similarly, when *Sohlh2*, which also encodes a bHLH transcription factor, is deleted in oocytes, primordial follicles are able to form but disappear shortly after birth (Choi et al. 2008). These and other reports (Reddy et al. 2009; Saatcioglu et al. 2016) in which other genes have been deleted in the oocyte

highlight that, even at the primordial follicle stage, normal follicular structure and function is not sustained in the absence of a healthy oocyte. Conversely, oocytes that do not lie within a primordial follicle will undergo apoptosis (Pepling and Spradling 2001). These observations underscore the indispensable role of signaling between the germ line and soma in females, a mutual dependence that differs strikingly different from the male, where seminiferous tubules can remain organized when no germ cells are present.

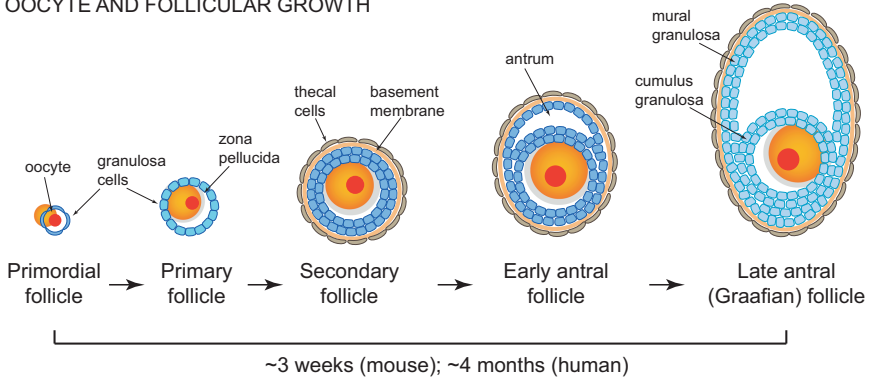
Within the primordial follicle, the oocyte and granulosa cells are closely apposed as revealed by electron micrographs, and cell adhesion complexes appear to physically couple the oocyte to the granulosa cells and the granulosa cells to each other (Jorgensen 2013). Oocytes and granulosa cells express a variety of junctional proteins, including cadherins and nectins that characterize adherens junctions (Mora et al. 2012). Even at this early stage, oocytes express mainly E-cadherin, whereas granulosa cells express mainly N-cadherin (Mora et al. 2012), meaning that the intercellular complexes are likely heterotypic. In addition, gap junctional structures have been detected using electron microscopy both between granulosa cells and between oocytes and granulosa cells (Mitchell and Burghardt 1986). In contrast, protein components of desmosomes have not been detected in the primordial follicles (Mora et al. 2012). Desmosomes generate very strong intercellular adhesion, and it was suggested that their absence facilitates the changes in the association between the oocyte and granulosa cells that will occur when the primordial follicle enters the growth phase, as described below (Mora et al. 2012).

2.3 Growth and Maturation of the Oocyte and Its Follicle

Prior to ovulation and fertilization, the oocyte undergoes a prolonged period of growth, followed by a briefer stage termed meiotic maturation (Fig. 2.1). Because available evidence indicates that no new functional oocytes are created after birth under physiological conditions (Zhang et al. 2012, 2015; Lei and Spradling 2013; Zarate-Garcia et al. 2016), the entry of primordial follicles into the growth phase must be regulated. Thus, some primordial follicles will remain in this arrested condition for up to decades before initiating growth. Oocytes from aged females, however, develop poorly as embryos (Nelson et al. 2013; Ben-Meir et al. 2015; Haverfield et al. 2016; Meldrum et al. 2016). Whether this reduced oocyte quality reflects damage that accumulates during its prolonged arrest within the primordial follicle or arises during the growth and maturation process is unclear; however, in both mice and humans, primordial follicles of aged females show increased DNA damage as reflected by immunological detection of γ H2A.X (Titus et al. 2013). This suggests that there may be an age-associated loss of oocyte quality within the primordial follicles.

The growth phase of oogenesis lasts about 3 weeks in the mouse and 3–4 months in human. The most obvious feature of the growth phase is the enormous increase—greater than 100-fold—in the volume of the oocyte. This increase

OOCYTE AND FOLLICULAR GROWTH



MEIOTIC MATURATION

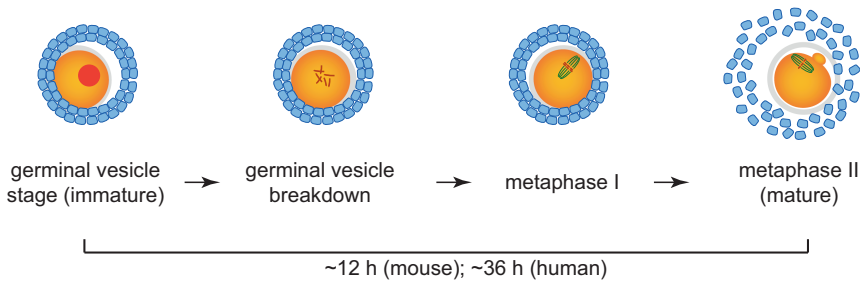


Fig. 2.1 Oocyte and follicular growth and meiotic maturation. *Upper* Near the time of birth, each oocyte becomes surrounded by a small number of squamous granulosa cells in a primordial follicle. During reproductive life, individual follicles enter the growth phase and are termed primary follicles. The granulosa cells assume a cuboidal morphology and begin to proliferate mitotically, continuing to fully cover the surface of the growing oocyte. At this stage, the oocyte elaborates the zona pellucida, which physically separates it from the granulosa cell bodies. A basement membrane appears outside the granulosa cell layer and thecal cells are recruited external to this membrane. As the granulosa cells continue to proliferate, they generate multiple layers. A fluid-filled cavity appears, which separates the granulosa into the cumulus granulosa, adjacent to the oocyte, and the mural granulosa, adjacent to the follicle wall. Preovulatory follicles contain a large antrum and may be termed Graafian. *Lower* Luteinizing hormone, which binds to receptors on the mural granulosa cells, triggers meiotic maturation of the oocyte. The nuclear (germinal vesicle) membrane breaks down and the chromosomes condense and become assembled on the first meiotic spindle. After translocation of the spindle to the oocyte cortex, the first meiotic division occurs and half of the chromosomes are discarded in the first polar body. The oocyte chromosomes become aligned on the second meiotic spindle (metaphase II). Fertilization will trigger the completion of the second meiotic division. Not shown are cytoplasmic events that occur during maturation. Adapted from Res Probl Cell Diff 58:191–224

reflects the accumulation of mitochondria and other organelles, mRNA, and proteins that will support early embryonic development after fertilization (Sánchez and Smits 2012). The growing oocyte also undergoes major ultrastructural rearrangements, including the accumulation of cortical granules that will play an

essential role in preventing polyspermic fertilization (Ducibella et al. 1994), the assembly of an internal lattice structure (Yurttas et al. 2008; Kim et al. 2014), establishment of oocyte-specific DNA methylation patterns (Lucifero et al. 2004; Smallwood et al. 2011; Tomizawa et al. 2012; Stewart et al. 2015), and the accumulation of proteins implicated in mRNA translational control into a subcortical complex (Li et al. 2008; Flemr et al. 2010).

Growth of the oocyte is accompanied by growth of the follicle. Approximately coincident with the initiation of oocyte growth, the squamous granulosa cells that enclose it in the non-growing primordial follicle become cuboidal in shape and begin to proliferate mitotically (Hirshfield 1991; Da Silva-Buttkus et al. 2008). At this stage, the follicle is termed primary. A layer of theca cells is then assembled external to the granulosa cells, from which they are separated by a basement membrane (Hirshfield 1991; Liu et al. 2015). As the oocyte continues to grow, the granulosa cells proliferate so that they fully cover the expanding surface of the oocyte and also generate multiple layers around it. Studies using Ki67 as a marker of cell proliferation indicate that, in multilayered follicles, granulosa cells in the layer closer to the oocyte proliferate more rapidly than those in the outer layer adjacent to the basement membrane (Da Silva-Buttkus et al. 2008). This suggests that oocyte factors may promote granulosa cell proliferation (Gilchrist et al. 2006), as discussed further below. As the follicle continues to grow, a fluid-filled cavity termed the antrum develops. This separates the granulosa cells into two populations—the cumulus granulosa that surround the oocyte and the mural granulosa that line the follicular wall.

The final stage of oocyte development is termed meiotic maturation (Conti et al. 2012; Holt et al. 2013; Adhikari and Liu 2014; Coticchio et al. 2015). It is physiologically triggered by a release of luteinizing hormone, which also induces ovulation and thereby coordinates the completion of oocyte development with potential fertilization. During maturation, cyclin-dependent kinase (CDK) 1 becomes active, advancing the cell cycle from late G2 to M-phase. Thus, the nuclear membrane is disassembled, a process termed germinal vesicle breakdown, the chromosomes become fully condensed, and the first meiotic spindle is assembled. The spindle migrates from the center of the oocyte to its periphery and the first meiotic division occurs accompanied by cytokinesis to generate the first polar body (Bennabi et al. 2016). The chromosomes remaining in the oocyte then become organized on the second meiotic spindle at metaphase II. In addition to these nuclear events, cytoplasmic changes occur also as a subset of mRNAs become translationally activated, whereas others become silenced (Kang and Han 2011; Susor et al. 2015), the cortical granules complete their migration to the oocyte periphery (Cheeseman et al. 2016), and other ultrastructural events occur such as aggregation of mitochondria around the spindle (Van Blerkom 2010). During maturation of the oocyte, the surrounding cumulus granulosa cells secrete a matrix that separates individual cells from each other and also from the oocyte (Russell and Salustri 2006). This process, termed cumulus expansion, breaks physical contact between the oocyte and somatic cells of the follicle.

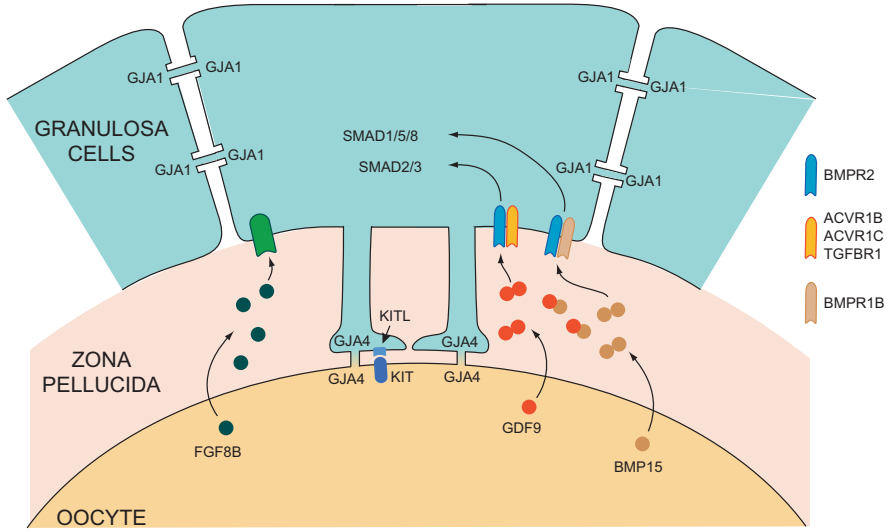


Fig. 2.2 Pathways of oocyte-granulosa cell communication. Gap junctions composed of GJA1 (connexin43) subunits link granulosa cells to each other, whereas those composed of GJA4 (connexin37) subunits likely link them to the oocyte, whose major gap junctional component is GJA4. KITL produced by the granulosa cells is also able to activate KIT on the oocyte membrane. GDF9 and BMP15 produced by the granulosa cells bind to receptors, either as homodimers or as heterodimers, on the oocyte membrane. FGF8B is also secreted by the granulosa cells and acts on the granulosa cells. Adapted from *Res Probl Cell Diff* 58:191–224

2.4 Mechanisms of Granulosa Cell-Oocyte Communication

Oocytes and granulosa cells communicate using both gap junctions, which allow direct transfer of molecules between coupled cells, and secreted factors, which by binding to membrane-associated receptors activate specific signaling pathways within the target cell (Fig. 2.2).

2.4.1 Gap Junctions

Gap junctions are intercellular channels that permit the exchange of molecules up to approximately 1 kDa between coupled cells (Evans 2015; Winterhager and Kidder 2015). The basic unit of the gap junction is the connexon, which is composed of six connexin proteins that become arranged in a ring in the plasma membrane surrounding a “hollow” core. Connexons of adjacent cells become associated to generate the gap junction. A gap junctional plaque is a group of connexons that are localized together in the plasma membrane. Gap junctions have long been known to couple the oocyte to the granulosa cells and the granulosa cells to each other, and gene-knockout studies confirmed their essential role in the development

of both compartments during follicular growth. These studies focused on two connexins: connexin-37 (*Gja4*), the main connexin expressed in mouse oocytes, and connexin-43 (*Gjal*), the main connexin expressed in granulosa cells. In mice lacking *Gja4*, there is no detectable gap junctional communication between the oocyte and granulosa cells (Simon et al. 1997; Veitch et al. 2004; Gittens and Kidder 2005). The oocytes are able to begin growth, but fail to reach full size, and do not acquire the ability (termed meiotic competence) to undergo meiotic maturation (Carabatsos et al. 2000). Although the absence of connexin-37 does not apparently disturb gap junctional communication between granulosa cells, the follicle does not progress beyond the late pre-antral stage (Gittens and Kidder 2005). Thus, impaired development of the oocyte also impairs follicular development.

Absence of connexin-43 leads to a complementary phenotype—oocyte-granulosa cell communication is retained, but communication between granulosa cells is severely impaired (Ackert et al. 2001; Veitch et al. 2004; Gittens and Kidder 2005). The granulosa cells of mice lacking *Gjal* are unable to generate a second layer of cells around the growing oocytes; hence the follicles are defined as remaining at the primary stage. As when connexin-37 is absent, the oocytes are unable to reach full-size, manifest multiple ultrastructural abnormalities, and do not acquire meiotic competence. Thus, reduced communication between granulosa cells impairs oocyte development.

It is not fully established whether connexin-37 or connexin-43 forms the granulosa cell component of the gap junctions with the oocyte. Although antibodies specific for connexin-43 stain punctae in the zona pellucida, it is not known whether these correspond to gap junctions between the oocyte and granulosa cells or between cytoplasmic processes, termed transzonal projections (discussed below) of granulosa cells that penetrate into the zona pellucida. Experiments using primary granulosa cells in culture indicated that granulosa cells lacking *Gja4* (connexin-37) could not form gap junctions with the oocyte (Veitch et al. 2004). When *Gja4*^{-/-} granulosa cells were aggregated with *Gja4*^{+/+} oocytes, however, gap junctional coupling was observed, and the oocytes grew apparently normally (Gittens and Kidder 2005). It may be that granulosa cells normally employ connexin-37 to couple with the oocyte and connexin-43 to couple with other granulosa cells (Gittens and Kidder 2005). This would require selective trafficking of each connexin to its appropriate location, a process that has been described for certain connexins (Evans 2015). Alternatively, heterotypic gap junctions have been identified in other cell types (Koval et al. 2014).

2.4.2 Secreted Factors

These experiments highlight the importance of signaling from the granulosa cells to the oocyte. It is not yet known whether the oocyte provides support to the granulosa cells via the gap junctions. The oocyte does, however, regulate granulosa

cell differentiation and activity via secreted growth factors (Gilchrist et al. 2008; Su et al. 2009). This role of the oocyte was hinted at many years ago by the observation that when the oocyte was removed from the follicle, the granulosa cells rapidly underwent a process termed luteinization, which normally occurs only after ovulation has expelled the oocyte from the follicle (el-Fouly et al. 1970). Its role was clearly revealed, however, in studies focused on a process termed cumulus expansion. At the time of ovulation, the cumulus cells secrete hyaluronic acid, a glycosylaminoglycan that expands to separate the cumulus cells from each other and embed them in a gelatinous matrix (Eppig 1991). When the oocyte was microsurgically removed from cumulus-oocyte complexes obtained from preovulatory follicles, however, the remaining shell of cumulus cells was unable to undergo expansion (Buccione et al. 1990; Vanderhyden et al. 1990). Co-culture of the shells with oocytes or with oocyte-conditioned medium, however, restored the ability to expand. Thus, oocytes secrete an expansion-enabling factor (Dragovic et al. 2007). Moreover, oocytes also maintain the cumulus granulosa cell lineage, as assessed by expression of marker mRNAs, preventing expression of mural granulosa cell markers (Diaz et al. 2007; Emori et al. 2013; Wigglesworth et al. 2015).

Two factors secreted by growing oocytes are growth-differentiation factor (GDF) 9 and BMP15, both members of the transforming growth factor β superfamily of growth factors. GDF9 binds to a receptor dimer composed of BMPR2-TGFBR1 and signals through SMAD2/3, whereas BMP15 binds to a BMPR2-BMPR1B dimer and signals through SMAD1/5/8. GDF9:GDF9 homodimers are thought to be the major form that signals in rodents, whereas GDF9:BMP15 heterodimers are the major active form in other species including humans (Dragovic et al. 2007; Peng et al. 2013; Mottershead et al. 2015). The granulosa cells are believed to be the principal or sole target of oocyte-derived GDF9 and BMP15; notably, oocyte-specific deletion of *Smad4*, which is required for all SMAD-dependent signaling, has no significant phenotypic effect (Li et al. 2011).

Mice lacking *Gdf9* are unable to ovulate and are sterile (Dong et al. 1996). Strikingly, during folliculogenesis, no more than a single layer of granulosa cells is generated around the growing oocyte. This defect appears to be due to an increase in the intrafollicular level of inhibin A, because multiple layers of granulosa cells are restored in *Gdf9*^{-/-}; *Inha*^{-/-} mice (Wu et al. 2004). Although mice lacking *Bmp15* are fertile, *Gdf9*^{+/-}:*Bmp15*^{-/-} individuals manifest severe follicular defects (Su et al. 2004), highlighting the shared role of these oocyte-derived growth factors in folliculogenesis.

In addition to these defects in the granulosa cells, however, oocyte development is also compromised in the absence of GDF9 (Dong et al. 1996; Carabatsos et al. 1998). Although the oocytes grow slightly larger than wild-type oocytes, they show ultrastructural abnormalities as well as an impaired ability to undergo meiotic maturation. As GDF9 likely does not act directly on the oocyte, these results suggest that the GDF9-deprived granulosa cells are unable to interact normally with the oocyte. Consistent with this, oocyte-derived factors promote the expression in granulosa cells of mRNAs encoding glycolytic enzymes (BMP15 and

fibroblastic growth factor 15) (Sugiura et al. 2007) and those required for synthesis of cholesterol (GDF9, BMP15) (Su et al. 2008), and for amino acid transport (Eppig et al. 2005), which enable the synthesis of products transferred from the granulosa cells to the oocyte. Recent studies have revealed that the oocyte promotes these activities in part by suppressing expression of *Ddit4l*, a negative regulator of MTOR. Thus, oocyte-derived factors likely increase protein synthesis in the neighboring granulosa cells (Guo et al. 2016).

2.5 Transzonal Projections: Bridges for Granulosa Cell-Oocyte Communication

Metazoan oocytes are surrounded by a protective extracellular matrix composed of glycoproteins. In mammals, this coat is termed the zona pellucida and is made of three (rodents) or four (primates) glycosylated proteins that are secreted by the oocyte (Wassarman and Litscher 2012, 2013). The zona pellucida is not present at the primordial follicle stage, so the oocyte and adjacent granulosa cells are in direct contact. Upon initiation of oocyte growth, the encoding genes become transcriptionally activated within the oocyte. The zona pellucida is not elaborated as a continuous structure enveloping the oocyte. Rather, as revealed through electron microscopy, it is assembled as aggregates or clumps that are subsequently knit together to form the continuous structure (Wassarman and Litscher 2012). As the oocyte continues to grow, the zona pellucida thickens to reach a final diameter of about 7 μm in mouse and 20 μm in humans (Litscher and Wassarman 2014). As a result, the granulosa cell bodies become physically separated from the oocyte, and it may be asked how the cell types are able to maintain the physical contact that is necessary for oocyte growth and development.

In all metazoan species that have been studied, including a wide range of mammals, thin cytoplasmic projections extend from the granulosa cells through the zona pellucida and contact the oocyte plasma membrane (Fig. 2.3) (Anderson and Albertini 1976; Albertini and Rider 1994; Motta et al. 1994; Makabe et al. 2006; Li and Albertini 2013). These projections mainly originate from the granulosa cells in the layer immediately adjacent to the oocyte but have also been observed to arise from granulosa cells in more distal layers (Jaffe and Egbert 2016). Both these structures, termed transzonal projections (TZPs), and their granulosa cell origin were identified over a century ago, and it was proposed at the time that they might allow the granulosa cells to feed nutrients to the oocyte (Hadek 1965). TZPs frequently form a bulbous foot-like structure at their site of attachment to the oocyte surface, but have also been described to penetrate deeply into invaginations of the oocyte plasma membrane (Motta et al. 1994; Makabe et al. 2006).

The gap junctions connecting the granulosa cells to the oocyte are located at the tips of the TZPs (Fig. 2.2), and gap junctions likely also couple TZPs to each other (Hadek 1965). Although gap junctions may be able to serve an adhesive function

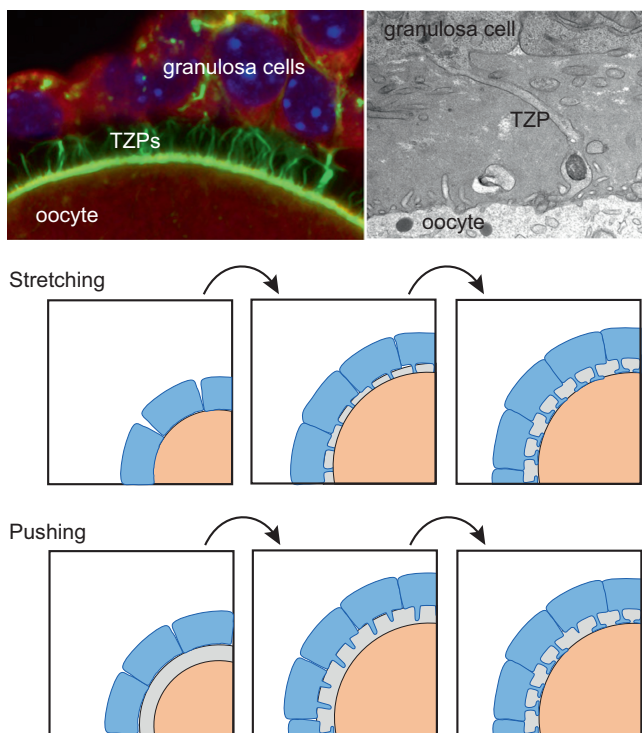


Fig. 2.3 Transzonal projections (TZPs) physically link the granulosa cells to the oocyte. *Upper* A confocal image of a portion of a granulosa cell-oocyte complex stained using fluorescent phalloidin, which binds to filamentous actin, reveals the TZPs and oocyte cortex. Granulosa cell nuclei are stained *blue*. The electron micrograph on the *right* shows a TZP extending from the granulosa cell through the zona pellucida to the oocyte plasma membrane. *Lower* Two models of TZP formation. The stretching model proposes that sites of cell adhesion that are established between the oocyte and granulosa cells before the deposition of the zona pellucida remain during and after its deposition. This generates a stretched cytoplasmic filament that becomes the TZP. The pushing model proposes that TZPs are elaborated from the granulosa cells after deposition of the zona pellucida and grow toward the oocyte where they establish contact with its plasma membrane. The models are not mutually exclusive

(Evans 2015), it seems probable that other proteins maintain the intercellular contact at the TZP tips; notably, TZPs are present in the ovarian follicles of mice lacking *Gja4* (Simon et al. 1997). Intriguingly, the number of TZPs is reduced by about 30% when *Ptk2* (protein tyrosine kinase) is deleted from oocytes (McGinnis and Kinsey 2015). This is accompanied by reduced gap junctional coupling as assayed by connexin-37 immunoreactivity in the zona pellucida and dye-transfer from the oocyte to the adjacent granulosa cells. As PTK2 promotes the formation of intercellular junctions by stabilizing complexes containing nectins or cadherins, it may help to stabilize the connection of the TZPs to the oocyte.

How TZPs arise remains unknown, and (at least) two models may be envisioned through which they could be generated (Fig. 2.3). The first (Chiquoine 1960;

Hadek 1965) is rooted in the physical contact between the oocyte and granulosa cells that exists in the primordial follicle. It is proposed that the sites of intercellular adhesion remain as the zona pellucida is assembled. As the granulosa cell bodies are pushed away from the oocyte, cytoplasmic filaments are generated where the granulosa cells remain tethered to the oocyte surface. As these filaments elongate, they become the TZPs. The second model posits that the TZPs are elaborated from the granulosa cells in a manner similar to the growth of filopodia. Although TZPs are within the size range observed for filopodia (diameter approximately 100 nm), little direct evidence yet supports either model. However, several observations are worth noting. First, as the oocyte grows, the number of TZPs that project to it increases substantially (Makabe et al. 2006). This is consistent with the consideration that the number of granulosa cells adjacent to the oocyte must increase in order for them to continue to fully cover the expanding oocyte surface. Second, because the zona pellucida becomes fully formed at an early stage of oocyte growth, a substantial fraction of the granulosa cell population in the oocyte-adjacent layer will be born after the zona has been elaborated. These granulosa cells likely project TZPs, which appear to be uniformly distributed around the surface of oocyte. It is not immediately obvious how the first model can accommodate these observations. Intriguingly, at the time of primordial follicle formation, the pre-granulosa cells extend filopodia that enclose the oocyte (Lechowska et al. 2011; Pepling 2012). Moreover, oocytes can occasionally be observed in the adrenal gland where, although they produce an extracellular coat resembling a zona pellucida, no TZPs are observed to project from the adjacent adrenal cells (Zamboni and Upadhyay 1983). These results suggest that the granulosa cells may have an inherent and distinct predisposition to generate filopodia.

2.6 Granulosa Cell-Oocyte Communication at the Initiation of Oocyte and Follicular Growth

An early morphological indicator that a primordial follicle and its enclosed oocyte have entered the growth phase is the transition of the granulosa cells from a squamous to cuboidal morphology. This suggests that oocyte growth might be initiated by signals sent by the granulosa cells, and several lines of evidence point to Kit ligand (KITL) as a strong candidate (Fig. 2.4). KITL is constitutively expressed by the granulosa cells (Hutt et al. 2006; Thomas and Vanderhyden 2006) and interacts with the Kit receptor (KIT) expressed by oocytes (Manova et al. 1990; Kidder and Vanderhyden 2010). Oocyte-specific expression of a constitutively active form of KIT (*Kit^{D818V}*) causes most oocytes within primordial follicles to begin growing shortly after birth (Saatcioglu et al. 2016). Conversely, deletion of its kinase prevents oocytes from beginning to grow, even when the adjacent granulosa cells become cuboidal and began to proliferate. Within the granulosa cells, deletion of *Tsc1*, which encodes a

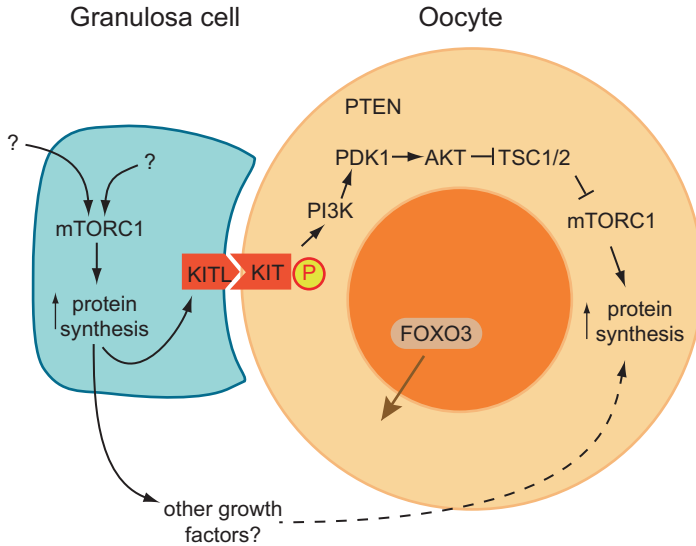


Fig. 2.4 Granulosa cell-oocyte communication at the initiation of oocyte growth. In response to an intra- or extracellular signal of unknown origin, the granulosa cells of a primordial follicle may increase their rate of protein synthesis. This leads to an increase in the production of KITL, possibly in particular the membrane-associated variant. The granulosa cell-derived KITL activates its receptor, KIT, on the oocyte plasma membrane. This in turn increases protein synthesis on the oocyte via PI3-kinase signaling. Reduced nuclear activity of the transcription factor, FOXO3, is a key downstream event driving oocyte growth. A role for other granulosa cell-derived factors is not excluded. Adapted from Res Probl Cell Diff 58:191–224

repressor of MTORC1 and whose deletion would be expected to increase protein synthesis, leads to an increase in KITL and widespread initiation of oocyte growth (Zhang et al. 2014). Conversely, deletion of *Rptor* in the granulosa cells, which would decrease protein synthesis, prevents the squamous-cuboidal transition in these cells as well as the initiation of oocyte growth (Zhang et al. 2014). Taken together, these results suggest that an increase in KIT activity in the oocyte triggers its growth.

The mechanism by which KIT activation triggers oocyte growth remains to be fully established. KIT typically signals through phosphatidylinositol kinase-3, leading to activation of AKT and an increase in global protein synthesis. Consistent with this, deletion in oocytes of *Pten*, which antagonizes this pathway, promotes oocyte growth (John et al. 2008; Reddy et al. 2008). Growth also depends on other molecular signals, however. For example, deletion of *Nobox* in the mouse prevents normal oocyte growth, strongly implicating this transcription factor (Rajkovic et al. 2004). Additionally, the transcription factor, FOXO3, translocates from the nucleus to the cytoplasm at an early stage of growth (albeit clearly after growth has begun), and genetic deletion of *Foxo3* causes most oocytes in primordial follicles to begin to grow (Castrillon et al. 2003; John et al. 2008). Taking these results together, a plausible proposal for the transition of primordial follicles into the growth phase

may be that an increase in protein synthesis within the granulosa cells, which is associated with cuboidalization in some manner, leads to increase in the production and availability of KITL. This activates, or increases the activation of, KIT on the oocyte membrane, leading to an increase in MTOR-regulated protein synthesis and consequent oocyte growth.

Although the initiation of oocyte growth normally is accompanied by cuboidalization of the granulosa cells, it is worth noting that this morphological change is apparently not indispensable. Expression of a constitutively active *Kit* mutant (Saatcioglu et al. 2016) or deletion of *Tsc1* or *Tsc2* in the oocyte (Adhikari et al. 2009, 2010) triggers its growth, even though the granulosa cells retain their squamous morphology. Similarly, deletion of the oocyte-specific transcription factor *Sohlh2* or oocyte-specific deletion of transcription factor *Lhx8* also triggers initiation of oocyte growth, while the adjacent granulosa cells remain squamous (Pangas et al. 2006; Choi et al. 2008; Ren et al. 2015). Nevertheless, the squamous-cuboidal transition may be required for oocytes to grow efficiently or beyond a certain size and in any case remains a reliable marker of the physiological activation of oocyte growth.

Although an increase in the rate of protein synthesis in the adjacent granulosa cells likely plays a central role in triggering oocyte growth, the source and the nature of the signal that increases protein synthesis in the granulosa cells of one primordial follicle but not those of its neighbor remains unknown. Indeed, it could be hypothesized that the oocyte is the source of the signal. A clue may lie in observations that, when ovary-like structures have been generated by aggregating oocytes and granulosa cells, many of the reconstituted follicles rapidly initiate growth (Eppig et al. 2000; Hayashi et al. 2012). This strongly suggests that the regulated entry of primordial follicles into the growth pool depends on cellular interactions in the intact ovary that are not recapitulated in the de novo-generated structures.

2.7 Granulosa Cell-Oocyte Communication During Oocyte and Follicular Growth

The granulosa cells play an essential role not only in initiating oocyte growth but also in sustaining it. This was dramatically illustrated many years ago when granulosa-oocyte complexes (GOCs) were isolated from ovarian follicles and cultured intact or following physical separation of the two cell types (Eppig 1979). Oocytes within intact GOCs continued to grow, whereas as oocytes not in direct contact with the granulosa cells grew little or not at all, even when the granulosa cells were provided in co-culture. Subsequent studies showed that the rate of oocyte growth was proportional to the number of granulosa cells that were associated with it (Brower and Schultz 1982). These results highlight the indispensable role played by the granulosa cells as well as the crucial importance of

physical contact between the two cell types. As discussed above, gap junctions couple the granulosa cells to the oocyte and to each other. Gap junctions permit the transfer of molecules up to about 1 kDa between cells, and these include factors essential for normal oocyte growth and development (Eppig 1991). For example, the oocyte expresses only low levels of mRNAs encoding factors required to convert glucose to pyruvate and thus relies on pyruvate transferred from the granulosa cells. Via the gap junctions, the granulosa cells also provide the oocyte with ribonucleotides and some amino acids that it cannot efficiently take up from extracellular sources. More broadly, it has been suggested that, because the surface/volume ratio of the oocyte steadily decreases as it grows, the coupling with the granulosa cells provides oocyte access to the cell surface of the granulosa cells, thereby mitigating the effect of its growth (Eppig 1991).

Although oocytes cannot reach full size when gap junctional coupling with the granulosa cells is lost, they nonetheless undergo substantial growth, reaching about half the volume of wild-type oocytes (Carabatsos et al. 2000; Gittens and Kidder 2005). Since oocytes grow only very little or not at all when the granulosa are physically removed, this suggests that contact-dependent but gap junction-independent signaling from the granulosa cells also drives oocyte growth. The nature of these signals remains to be established. However, KITL promotes oocyte growth *in vitro* (Packer et al. 1994; Thomas et al. 2008). Importantly, the membrane-associated form of the ligand, which is generated by alternative splicing of the mRNA, is considerably more effective than the soluble form (Thomas et al. 2008). *In vivo*, both isoforms of the mRNA are present, but decline at the end of follicular growth, when oocyte growth has ceased, via an oocyte-regulated process (Joyce et al. 1999, 2000). These results suggest that the granulosa cells promote oocyte growth by extracellular, albeit membrane-associated, signaling as well as through gap junctional coupling.

In addition to providing factors that sustain the metabolic activity of the growing oocyte and enable its continual increase in volume, the granulosa cells also transmit signals that regulate its developmental progression. The best-known of these regulate the concentration of cyclic AMP (cAMP) in the oocyte. Activation of cAMP-dependent protein kinase A inhibits entry of the oocyte into M-phase, by promoting phosphorylation of sites on CDK1 that inhibit its activity; thus, a high level of cAMP prevents the initiation of meiotic maturation, whereas a sufficient decrease in cAMP concentration within the oocyte permits maturation (Holt et al. 2013; Adhikari and Liu 2014). Based on evidence including that blocking gap junctional communication between the oocyte and cumulus granulosa within the follicle can trigger maturation in the absence of LH (the physiological trigger) (Sela-Abramovich et al. 2006; Richard and Baltz 2014), it was long thought that these permitted the granulosa cells to deliver cAMP to the oocyte. Later studies demonstrated, however, that meiotic arrest depends on cAMP generated by the oocyte itself (Bornslaeger and Schultz 1985; Mehlmann et al. 2004).

Studies over the last decade have established that cGMP, rather than cAMP, is the maturation-inhibiting molecule transferred by the granulosa via the gap

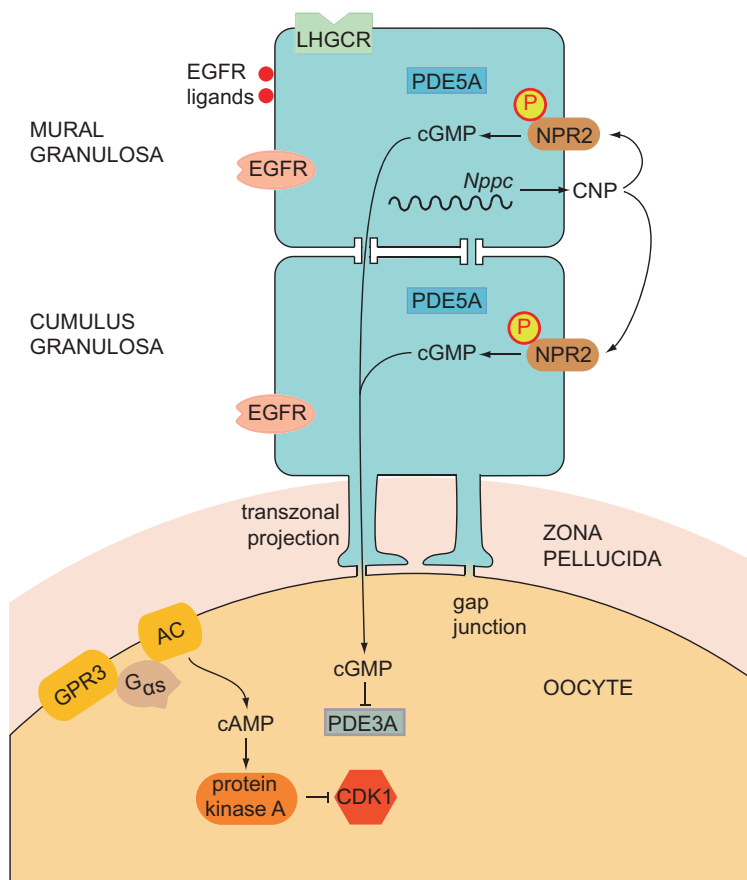


Fig. 2.5 Granulosa cell-oocyte communication regulates meiotic maturation. C-type natriuretic peptide (CNP) is manufactured and secreted by mural granulosa cells where it associates with the guanylyl cyclase natriuretic peptide receptor (NPR) 2 on the mural and cumulus granulosa cell membranes, triggering the synthesis of cyclic GMP (cGMP). Via the gap junctions that couple the granulosa cells to the oocyte, cGMP diffuses to the oocyte, where it inhibits the activity of phosphodiesterase (PDE) 3A. Owing to PDE inhibition, the concentration of cAMP, which is synthesized by the oocyte, remains high enough to activate cAMP-dependent protein kinase A. This inhibits cyclin-dependent kinase (CDK) 1. When luteinizing hormone (LH) binds to its receptor on the mural granulosa cells, production of cGMP decreases, leading to a drop in its concentration within the oocyte. PDE3A becomes active, degrades cAMP, thereby leading to an increase in CDK1 activity. This triggers meiotic maturation. Adapted from Res Probl Cell Diff 58:191–224

junctions (Fig. 2.5) (Norris et al. 2009; Conti et al. 2012; Jaffe and Egbert 2016). cGMP acts by inhibiting the activity of the major cAMP-directed phosphodiesterase, PDE3A, that is present in the oocyte. cGMP is manufactured by the granulosa cells through the activity of the guanylyl cyclase natriuretic peptide receptor (NPR) 2, located on the plasma membrane of both mural and cumulus granulosa cells

(Zhang et al. 2010). NPR2 is activated by C-type natriuretic peptide (CNP, encoded by the *Nppc* gene) that is released by the mural granulosa cells. Thus, cGMP constitutively synthesized by the granulosa cells diffuse into the oocytes via gap junctions and, by inhibiting PDE3A, maintains a high intra-oocyte concentration of cAMP and prevents precocious initiation of meiotic maturation.

2.8 Granulosa Cell-Oocyte Communication During Meiotic Maturation

Meiotic maturation is triggered physiologically by luteinizing hormone (LH), which binds to receptors located on the mural granulosa cells. The consequence of activation of the LH receptors is a decrease in cGMP concentration throughout the follicle. This can be attributed to a decrease in the production of CNP by the mural granulosa cells and dephosphorylation and inactivation of NPR2 (Egbert et al. 2014; Shuhaibar et al. 2015, 2016). Other mechanisms may also contribute to the decrease in cGMP. How LH triggers these changes, however, remains to be fully elucidated. A key early event following LH receptor activation is the release from the mural granulosa cells of, members of the epidermal growth factor (EGF) family, notably epiregulin and amphiregulin (Conti et al. 2012). These bind to EGF receptor (EGFR) located on both the mural and cumulus granulosa cells and activate EGFR-dependent signaling. The relative contribution of EGFR-dependent and EGFR-independent signaling to the drop in intrafollicular cGMP is not fully established, and it is possible that multiple pathways participate (Conti et al. 2012; Wang et al. 2013; Jaffe and Egbert 2016). Regardless of the mechanism, however, the consequence is that the direction of cGMP flow reverses—it now leaves the oocyte and returns to the granulosa cells to maintain a uniform concentration across the coupled cells. The resulting decrease in oocyte cGMP derepresses PDE3A, leading to a decrease in intra-oocyte cAMP and activation of CDK1. Thus, the gap junctions linking the oocyte to the granulosa cells play a crucial role, not only in preventing precocious maturation but also in enabling oocyte maturation in response to LH.

Recent studies have uncovered an additional role for the cumulus granulosa cells during maturation. Oocytes of mice lacking amphiregulin (*Areg*^{-/-}) show an increased incidence of spindle abnormalities at metaphase II and a reduced ability to develop as embryos (Chen et al. 2013). Moreover, providing amphiregulin to cumulus cell-enclosed oocytes during maturation *in vitro* increases the fraction that are able to develop as embryos. This important observation confirms that amphiregulin exerts this beneficial effect during oocyte maturation. Further studies revealed that amphiregulin increases AKT phosphorylation in the oocyte and that this effect depends on the presence of the cumulus cells. Finally, amphiregulin increases the synthesis of proteins encoded by mRNAs that are translationally activated during maturation. Taken together these results indicate that

amphiregulin-stimulated cumulus cells send signals to the oocyte that increase the translation of a subset of mRNAs during maturation and increase the developmental competence of the oocytes. The nature of the signals and the mechanism by which they are transmitted to the oocyte are important questions that remain to be answered.

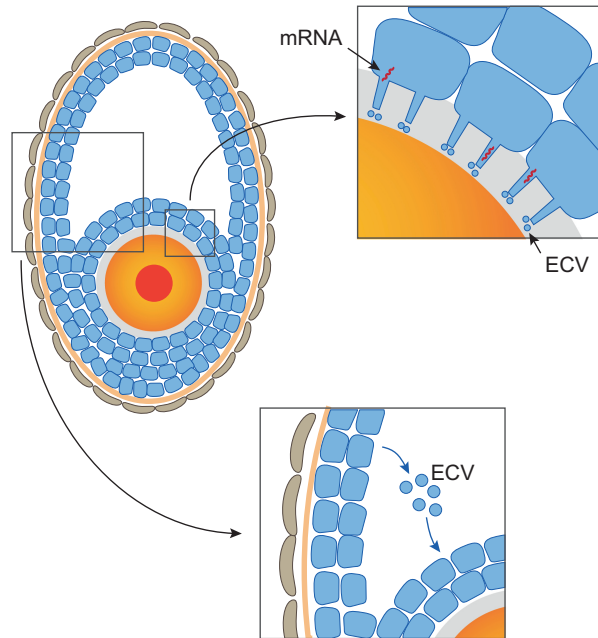
2.9 Newly Identified Pathways of Granulosa Cell-Oocyte Communication

Recent studies described below have identified new and unanticipated mechanisms of intercellular communication within the follicle (Fig. 2.6). Membrane-bound structures termed extracellular vesicles (ECV) have been identified in a variety of normal and pathological contexts (Machtinger et al. 2016; Navakanitworakul et al. 2016). Two classes are currently recognized, distinguished by their origin. Exosomes range from 10 to 100 nm in diameter and arise from the fusion of multivesicular bodies with the plasma membrane. Microvesicles range from 100 to 1000 nm and are budded off from the plasma membrane. Because the origin of vesicles cannot be reliably determined after they have been released from the cell, they have been collectively termed ECV. ECV can contain a diverse array of macromolecules including mRNAs, proteins, and microRNAs and thus hold the potential to alter the physiology of the cells with which they fuse. Several groups have independently identified and characterized ECV in follicular fluid of mammals including humans (da Silveira et al. 2012; Soheli et al. 2013; Santonocito et al. 2014; Hung et al. 2015; Navakanitworakul et al. 2016). Similar to ECVs in other contexts, the follicular ECVs contain abundant small RNAs whose size is consistent with miRNAs. The composition of the ECVs changes during follicular growth, and, importantly, the follicular ECV can be taken up by cumulus cells and are able to promote expansion of the cumulus cell matrix, identifying a potential biological function for them (Hung et al. 2015).

These observations raised the intriguing possibility that ECVs might also be a vehicle for information transfer between the oocyte and granulosa cells. Indeed, recent work has identified small vesicles at the tips of TZPs (Macaulay et al. 2014, 2016). Moreover, live-cell imaging has demonstrated that cargo including RNAs can be transported along the TZPs toward the tips from where the vesicles presumably bud. These observations suggest that such vesicles could provide a mechanism to deliver macromolecules from the cumulus granulosa cells to the oocyte. In support of this possibility, when oocytes were incubated on a monolayer of granulosa cells expressing EGFP, the encoding *Egfp* mRNA was subsequently detected in the oocytes (Macaulay et al. 2014). Future studies should uncover the extent of the role played by ECV in delivering cargo between and within the somatic and germ-line compartments of the follicle.

Fig. 2.6 New pathways of granulosa cell-oocyte communication.

Extracellular vesicles derived from the granulosa cells are present in follicular fluid and can fuse with cumulus granulosa cells, providing a means to transfer macromolecules between granulosa cells, including between the mural and cumulus subpopulations. Vesicles that may be related to ECV have also been observed at the base of TZPs where they meet the oocyte surface and may similarly transfer macromolecules from the granulosa cells to the oocyte



2.10 Conclusion

The developing oocyte relies on its somatic environment—specifically, the granulosa cells that enclose it—throughout its development from a resting state in the primordial follicle until it is ovulated in preparation for fertilization. The granulosa cells provide nutrients that sustain the metabolic activity of the oocyte and send signals that regulate its developmental progression. In turn, the oocyte sends signals to the granulosa cells that regulate their differentiation and function. This bidirectional communication between the germ cell and its somatic support cells relies on both intra- and extracellular pathways and dynamically remodels the microenvironment of the follicle corresponding to the needs of the oocyte, thus ensuring that ovulation releases a fully formed oocyte that is competent to develop as a healthy embryo.

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References

- Ackert CL, Gittens JE, O'Brien MJ, Eppig JJ, Kidder GM (2001) Intercellular communication via connexin43 gap junctions is required for ovarian folliculogenesis in the mouse. *Dev Biol* 233:258–270
- Adhikari D, Liu K (2014) The regulation of maturation promoting factor during prophase I arrest and meiotic entry in mammalian oocytes. *Mol Cell Endocrinol* 382:480–487
- Adhikari D, Flohr G, Gorre N, Shen Y, Yang H, Lundin E, Lan Z, Gambello MJ, Liu K (2009) Disruption of Tsc2 in oocytes leads to overactivation of the entire pool of primordial follicles. *Mol Hum Reprod* 15:765–770
- Adhikari D, Zheng W, Shen Y, Gorre N, Hamalainen T, Cooney AJ, Huhtaniemi I, Lan Z-J, Liu K (2010) Tsc/mTORC1 signaling in oocytes governs the quiescence and activation of primordial follicles. *Hum Mol Genet* 19:397–410
- Albertini DF, Rider V (1994) Patterns of intercellular connectivity in the mammalian cumulus-oocyte complex. *Microsc Res Tech* 27:125–133
- Anderson E, Albertini DF (1976) Gap junctions between the oocyte and companion follicle cells in the mammalian ovary. *J Cell Biol* 71:680–686
- Ben-Meir A, Burstein E, Borrego-Alvarez A, Chong J, Wong E, Yavorska T, Naranian T, Chi M, Wang Y, Bentov Y, Alexis J, Meriano J, Sung HK, Gasser DL, Moley KH, Hekimi S, Casper RF, Jurisicova A (2015) Coenzyme Q10 restores oocyte mitochondrial function and fertility during reproductive aging. *Aging Cell* 14:887–895
- Bennabi I, Terret ME, Verlhac MH (2016) Meiotic spindle assembly and chromosome segregation in oocytes. *J Cell Biol* 215:611–619
- Bornslaeger EA, Schultz RM (1985) Adenylate cyclase activity in zona-free mouse oocytes. *Exp Cell Res* 156:277–281
- Brower PT, Schultz RM (1982) Intercellular communication between granulosa cells and mouse oocytes: existence and possible nutritional role during oocyte growth. *Dev Biol* 90:144–153
- Buccione R, Vanderhyden BC, Caron PJ, Eppig JJ (1990) FSH-induced expansion of the mouse cumulus oophorus in vitro is dependent upon a specific factor(s) secreted by the oocyte. *Dev Biol* 138:16–25
- Carabatsos MJ, Elvin J, Matzuk MM, Albertini DF (1998) Characterization of oocyte and follicle development in growth differentiation factor-9-deficient mice. *Dev Biol* 204:373–384
- Carabatsos MJ, Sellitto C, Goodenough DA, Albertini DF (2000) Oocyte-granulosa cell heterologous gap junctions are required for the coordination of nuclear and cytoplasmic meiotic competence. *Dev Biol* 226:167–179
- Castrillon DH, Miao L, Kollipara R, Horner JW, DePinho RA (2003) Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. *Science* 301:215–218
- Cheeseman LP, Boulanger J, Bond LM, Schuh M (2016) Two pathways regulate cortical granule translocation to prevent polyspermy in mouse oocytes. *Nat Commun* 7:13726
- Chen J, Torcia S, Xie F, Lin CJ, Cakmak H, Franciosi F, Horner K, Onodera C, Song JS, Cedars MI, Ramalho-Santos M, Conti M (2013) Somatic cells regulate maternal mRNA translation and developmental competence of mouse oocytes. *Nat Cell Biol* 15:1415–1423
- Chiquoine AD (1960) The development of the zona pellucida of the mammalian ovum. *Am J Anat* 106:149–169
- Choi Y, Yuan D, Rajkovic A (2008) Germ cell-specific transcriptional regulator sohlh2 is essential for early mouse folliculogenesis and oocyte-specific gene expression. *Biol Reprod* 79:1176–1182
- Conti M, Hsieh M, Musa Zamah A, Oh JS (2012) Novel signaling mechanisms in the ovary during oocyte maturation and ovulation. *Mol Cell Endocrinol* 356:65–73
- Coticchio G, Dal Canto M, Mignini Renzini M, Guglielmo MC, Brambillasca F, Turchi D, Novara PV, Fadin R (2015) Oocyte maturation: gamete-somatic cells interactions, meiotic resumption, cytoskeletal dynamics and cytoplasmic reorganization. *Hum Reprod Update* 21:427–454

- Da Silva-Buttkus P, Jayasooriya GS, Mora JM, Mobberley M, Ryder TA, Baithun M, Stark J, Franks S, Hardy K (2008) Effect of cell shape and packing density on granulosa cell proliferation and formation of multiple layers during early follicle development in the ovary. *J Cell Sci* 121:3890–3900
- da Silveira JC, Veeramachaneni DN, Winger QA, Carnevale EM, Bouma GJ (2012) Cell-secreted vesicles in equine ovarian follicular fluid contain miRNAs and proteins: a possible new form of cell communication within the ovarian follicle. *Biol Reprod* 86:71
- Diaz FJ, Wigglesworth K, Eppig JJ (2007) Oocytes determine cumulus cell lineage in mouse ovarian follicles. *J Cell Sci* 120:1330–1340
- Dong J, Albertini DF, Nishimori K, Kumar RM, Lu N, Matzuk M (1996) Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature* 383:531–535
- Dragovic RA, Ritter LJ, Schulz SJ, Amato F, Thompson JG, Armstrong DT, Gilchrist RB (2007) Oocyte-secreted factor activation of SMAD 2/3 signaling enables initiation of mouse cumulus cell expansion. *Biol Reprod* 76:848–857
- Ducibella T, Duffy P, Buetow J (1994) Quantification and localization of cortical granules during oogenesis in the mouse. *Biol Reprod* 50:467–473
- Egbert JR, Shuhaibar LC, Edmund AB, Van Helden DA, Robinson JW, Uliasz TF, Baena V, Geertsi A, Wunder F, Potter LR, Jaffe LA (2014) Dephosphorylation and inactivation of NPR2 guanylyl cyclase in granulosa cells contributes to the LH-induced decrease in cGMP that causes resumption of meiosis in rat oocytes. *Development* 141:3594–3604
- el-Fouly MA, Cook B, Nekola M, Nalbandov AV (1970) Role of the ovum in follicular luteinization. *Endocrinology* 87:286–293
- Emori C, Wigglesworth K, Fujii W, Naito K, Eppig JJ, Sugiura K (2013) Cooperative effects of 17 β -estradiol and oocyte-derived paracrine factors on the transcriptome of mouse cumulus cells. *Endocrinology* 154:4859–4872
- Eppig JJ (1979) A comparison between oocyte growth in coculture with granulosa cells and oocytes with granulosa cell-oocyte junctional contact maintained in vitro. *J Exp Zool* 209:345–353
- Eppig JJ (1991) Intercommunication between mammalian oocytes and companion somatic cells. *Bioessays* 13:569–574
- Eppig JJ, Wigglesworth K, Hirao Y (2000) Metaphase I arrest and spontaneous parthenogenetic activation of strain LTXBO oocytes: chimeric reaggregated ovaries establish primary lesion in oocytes. *Dev Biol* 224:60–68
- Eppig JJ, Pendola FL, Wigglesworth K, Pendola JK (2005) Mouse oocytes regulate metabolic cooperativity between granulosa cells and oocytes: amino acid transport. *Biol Reprod* 73:351–357
- Evans WH (2015) Cell communication across gap junctions: a historical perspective and current developments. *Biochem Soc Trans* 43:450–459
- Flemr M, Ma J, Schultz RM, Svoboda P (2010) P-body loss is concomitant with formation of a messenger RNA storage domain in mouse oocytes. *Biol Reprod* 82:1008–1017
- Gilchrist RB, Ritter LJ, Myllymaa S, Kaivo-Oja N, Dragovic RA, Hickey TE, Ritvos O, Mottershead DG (2006) Molecular basis of oocyte-paracrine signalling that promotes granulosa cell proliferation. *J Cell Sci* 119:3811–3821
- Gilchrist RB, Lane M, Thompson JG (2008) Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. *Hum Reprod Update* 14:159–177
- Gittens JE, Kidder GM (2005) Differential contributions of connexin37 and connexin43 to oogenesis revealed in chimeric reaggregated mouse ovaries. *J Cell Sci* 118:5071–5078
- Grive KJ, Freiman RN (2015) The developmental origins of the mammalian ovarian reserve. *Development* 142:2554–2563
- Gunesdogan U, Surani MA (2016) Developmental competence for primordial germ cell fate. *Curr Top Dev Biol* 117:471–496

- Guo J, Shi L, Gong X, Jiang M, Yin Y, Zhang X, Yin H, Li H, Emori C, Sugiura K, Eppig JJ, Su YQ (2016) Oocyte-dependent activation of MTOR in cumulus cells controls the development and survival of cumulus-oocyte complexes. *J Cell Sci* 129:3091–3103
- Hadek R (1965) The structure of the mammalian egg. *Int Rev Cytol* 18:29–71
- Haverfield J, Nakagawa S, Love D, Tschlaki E, Nomikos M, Lai FA, Swann K, FitzHarris G (2016) Ca(2+) dynamics in oocytes from naturally-aged mice. *Sci Rep* 6:19357
- Hayashi K, Ogushi S, Kurimoto K, Shimamoto S, Ohta H, Saitou M (2012) Offspring from oocytes derived from in vitro primordial germ cell-like cells in mice. *Science* 338:971–975
- Hirshfield AN (1991) Development of follicles in the mammalian ovary. *Int Rev Cytol* 124:43–101
- Holt JE, Lane SI, Jones KT (2013) The control of meiotic maturation in mammalian oocytes. *Curr Top Dev Biol* 102:207–226
- Hummitzsch K, Anderson RA, Wilhelm D, Wu J, Telfer EE, Russell DL, Robertson SA, Rodgers RJ (2015) Stem cells, progenitor cells, and lineage decisions in the ovary. *Endocr Rev* 36:65–91
- Hung WT, Hong X, Christenson LK, McGinnis LK (2015) Extracellular vesicles from bovine follicular fluid support cumulus expansion. *Biol Reprod* 93:117
- Hutt KJ, McLaughlin EA, Holland MK (2006) Kit ligand and c-Kit have diverse roles during mammalian oogenesis and folliculogenesis. *Mol Hum Reprod* 12:61–69
- Jaffe LA, Egbert JR (2016) Regulation of mammalian oocyte meiosis by intercellular communication within the ovarian follicle. *Annu Rev Physiol*. doi:10.1146/annurev-physiol-022516-034102
- John GB, Gallardo TD, Shirley LJ, Castrillon DH (2008) Foxo3 is a PI3K-dependent molecular switch controlling the initiation of oocyte growth. *Dev Biol* 321:197–204
- Jorgensen JS (2013) Defining the neighborhoods that escort the oocyte through its early life events and into a functional follicle. *Mol Reprod Dev* 80:960–976
- Joyce IM, Pendola FL, Wigglesworth K, Eppig JJ (1999) Oocyte regulation of kit ligand expression in mouse ovarian follicles. *Dev Biol* 214:342–353
- Joyce IM, Clark AT, Pendola FL, Eppig JJ (2000) Comparison of recombinant growth differentiation factor-9 and oocyte regulation of KIT ligand messenger ribonucleic acid expression in mouse ovarian follicles. *Biol Reprod* 63:1669–1675
- Kang MK, Han SJ (2011) Post-transcriptional and post-translational regulation during mouse oocyte maturation. *BMB Rep* 44:147–157
- Kidder GM, Vanderhyden BC (2010) Bidirectional communication between oocytes and follicle cells: ensuring oocyte developmental competence. *Can J Physiol Pharmacol* 88:399–413
- Kim B, Zhang X, Kan R, Cohen R, Mukai C, Travis AJ, Coonrod SA (2014) The role of MATER in endoplasmic reticulum distribution and calcium homeostasis in mouse oocytes. *Dev Biol* 386:331–339
- Koval M, Molina SA, Burt JM (2014) Mix and match: investigating heteromeric and heterotypic gap junction channels in model systems and native tissues. *FEBS Lett* 588:1193–1204
- Lechowska A, Bilinski S, Choi Y, Shin Y, Kloc M, Rajkovic A (2011) Premature ovarian failure in nobox-deficient mice is caused by defects in somatic cell invasion and germ cell cyst breakdown. *J Assist Reprod Genet* 28:583–589
- Lei L, Spradling AC (2013) Female mice lack adult germ-line stem cells but sustain oogenesis using stable primordial follicles. *Proc Natl Acad Sci USA* 110:8585–8590
- Li R, Albertini DF (2013) The road to maturation: somatic cell interaction and self-organization of the mammalian oocyte. *Nat Rev Mol Cell Biol* 14:141–152
- Li L, Baibakov B, Dean J (2008) A subcortical maternal complex essential for preimplantation mouse embryogenesis. *Dev Cell* 15:416–425
- Li X, Tripurani SK, James R, Pangas SA (2011) Minimal fertility defects in mice deficient in oocyte-expressed Smad4. *Biol Reprod* 86:1–6
- Litscher ES, Wassarman PM (2014) Evolution, structure, and synthesis of vertebrate egg-coat proteins. *Trends Dev Biol* 8:65–76
- Liu C, Peng J, Matzuk MM, Yao HH (2015) Lineage specification of ovarian theca cells requires multicellular interactions via oocyte and granulosa cells. *Nat Commun* 6:6934

- Lucifero D, Mann MR, Bartolomei MS, Trasler JM (2004) Gene-specific timing and epigenetic memory in oocyte imprinting. *Hum Mol Genet* 13:839–849
- Macaulay AD, Gilbert I, Caballero J, Barreto R, Fournier E, Tossou P, Sirard MA, Clarke HJ, Khandjian EW, Richard FJ, Hyttel P, Robert C (2014) The gametic synapse: RNA transfer to the bovine oocyte. *Biol Reprod* 91:90
- Macaulay AD, Gilbert I, Scantland S, Fournier E, Ashkar F, Bastien A, Saadi HA, Gagne D, Sirard MA, Khandjian EW, Richard FJ, Hyttel P, Robert C (2016) Cumulus cell transcripts transit to the bovine oocyte in preparation for maturation. *Biol Reprod* 94:16
- Machtinger R, Laurent LC, Baccarelli AA (2016) Extracellular vesicles: roles in gamete maturation, fertilization and embryo implantation. *Hum Reprod Update* 22:182–193
- Makabe S, Naguro T, Stallone T (2006) Oocyte-follicle cell interactions during ovarian follicle development, as seen by high resolution scanning and transmission electron microscopy in humans. *Microsc Res Tech* 69:436–449
- Manova K, Nocka K, Besmer P, Bachvarova R (1990) Gonadal expression of c-kit encoded at the W locus of the mouse. *Development* 110:1057–1069
- McGinnis LK, Kinsey WH (2015) Role of focal adhesion kinase in oocyte-follicle communication. *Mol Reprod Dev* 82:90–102
- Mehlmann LM, Saeki Y, Tanaka S, Brennan TJ, Evsikov AV, Pendola FL, Knowles BB, Eppig JJ, Jaffe LA (2004) The Gs-linked receptor GPR3 maintains meiotic arrest in mammalian oocytes. *Science* 306:1947–1950
- Meldrum DR, Casper RF, Diez-Juan A, Simon C, Domar AD, Frydman R (2016) Aging and the environment affect gamete and embryo potential: can we intervene? *Fertil Steril* 105:548–559
- Mitchell PA, Burghardt RC (1986) The ontogeny of nexuses (gap junctions) in the ovary of the fetal mouse. *Anat Rec* 214:283–288
- Mora JM, Fenwick MA, Castle L, Baithun M, Ryder TA, Mobberley M, Carzaniga R, Franks S, Hardy K (2012) Characterization and significance of adhesion and junction-related proteins in mouse ovarian follicles. *Biol Reprod* 86:153
- Mork L, Maatouk DM, McMahon JA, Guo JJ, Zhang P, McMahon AP, Capel B (2012) Temporal differences in granulosa cell specification in the ovary reflect distinct follicle fates in mice. *Biol Reprod* 86:37
- Motta P, Makabe S, Naguro T, Correr S (1994) Oocyte follicle cells association during development of human ovarian follicle. A study by high resolution scanning and transmission electron microscopy. *Arch Histol Cytol* 57:369–394
- Mottershead DG, Sugimura S, Al-Musawi SL, Li JJ, Richani D, White MA, Martin GA, Trotta AP, Ritter LJ, Shi J, Mueller TD, Harrison CA, Gilchrist RB (2015) Cumulin, an oocyte-secreted heterodimer of the transforming growth factor-beta family, is a potent activator of granulosa cells and improves oocyte quality. *J Biol Chem* 290:24007–24020
- Navakanitworakul R, Hung WT, Gunewardena S, Davis JS, Chotigeat W, Christenson LK (2016) Characterization and small RNA content of extracellular vesicles in follicular fluid of developing bovine antral follicles. *Sci Rep* 6:25486
- Nelson SM, Telfer EE, Anderson RA (2013) The ageing ovary and uterus: new biological insights. *Hum Reprod Update* 19:67–83
- Norris RP, Ratzan WJ, Freudzon M, Mehlmann LM, Krall J, Movsesian MA, Wang H, Ke H, Nikolaev VO, Jaffe LA (2009) Cyclic GMP from the surrounding somatic cells regulates cyclic AMP and meiosis in the mouse oocyte. *Development* 136:1869–1878
- Packer AI, Hsu YC, Besmer P, Bachvarova RF (1994) The ligand of the c-kit receptor promotes oocyte growth. *Dev Biol* 161:194–205
- Pangas SA, Choi Y, Ballow DJ, Zhao Y, Westphal H, Matzuk MM, Rajkovic A (2006) Oogenesis requires germ cell-specific transcriptional regulators *Sohlh1* and *Lhx8*. *Proc Natl Acad Sci USA* 103:8090–8095
- Peng J, Li Q, Wigglesworth K, Rangarajan A, Kattamuri C, Peterson RT, Eppig JJ, Thompson TB, Matzuk MM (2013) Growth differentiation factor 9:bone morphogenetic protein 15 heterodimers are potent regulators of ovarian functions. *Proc Natl Acad Sci USA* 110:E776–E785
- Pepling ME (2012) Follicular assembly: mechanisms of action. *Reproduction* 143:139–149

- Pepling ME, Spradling AC (2001) Mouse ovarian germ cell cysts undergo programmed breakdown to form primordial follicles. *Dev Biol* 234:339–351
- Rajkovic A, Pangas SA, Ballow D, Suzumori N, Matzuk MM (2004) NOBOX deficiency disrupts early folliculogenesis and oocyte-specific gene expression. *Science* 305:1157–1159
- Reddy P, Liu L, Adhikari D, Jagarlamudi K, Rajareddy S, Shen Y, Du C, Tang W, Hamalainen T, Peng SL, Lan Z-J, Cooney AJ, Huhtaniemi I, Liu K (2008) Oocyte-specific deletion of *Pten* causes premature activation of the primordial follicle pool. *Science* 319:611–613
- Reddy P, Adhikari D, Zheng W, Liang S, Hamalainen T, Tohonen V, Ogawa W, Noda T, Volarevic S, Huhtaniemi I, Liu K (2009) PDK1 signaling in oocytes controls reproductive aging and lifespan by manipulating the survival of primordial follicles. *Hum Mol Genet* 18:2813–2824
- Ren Y, Suzuki H, Jagarlamudi K, Golnoski K, McGuire M, Lopes R, Pachnis V, Rajkovic A (2015) *Lhx8* regulates primordial follicle activation and postnatal folliculogenesis. *BMC Biol* 13:39
- Richard S, Baltz JM (2014) Prophase I arrest of mouse oocytes mediated by natriuretic peptide precursor C requires GJA1 (connexin-43) and GJA4 (connexin-37) gap junctions in the antral follicle and cumulus-oocyte complex. *Biol Reprod* 90:137
- Russell DL, Salustri A (2006) Extracellular matrix of the cumulus-oocyte complex. *Semin Reprod Med* 24:217–227
- Saatcioglu HD, Cuevas I, Castrillon DH (2016) Control of oocyte reawakening by *Kit*. *PLoS Genet* 12:e1006215
- Sánchez F, Smitz J (2012) Molecular control of oogenesis. *Biochim Biophys Acta* 1822:1896–1912
- Santonocito M, Vento M, Guglielmino MR, Battaglia R, Wahlgren J, Ragusa M, Barbagallo D, Borzi P, Rizzari S, Maugeri M, Scollo P, Tatone C, Valadi H, Purrello M, Di Pietro C (2014) Molecular characterization of exosomes and their microRNA cargo in human follicular fluid: bioinformatic analysis reveals that exosomal microRNAs control pathways involved in follicular maturation. *Fertil Steril* 102:1751–1761. (e1751)
- Sela-Abramovich S, Edry I, Galiani D, Nevo N, Dekel N (2006) Disruption of gap junctional communication within the ovarian follicle induces oocyte maturation. *Endocrinology* 147:2280–2286
- Shuhaibar LC, Egbert JR, Norris RP, Lampe PD, Nikolaev VO, Thunemann M, Wen L, Feil R, Jaffe LA (2015) Intercellular signaling via cyclic GMP diffusion through gap junctions restarts meiosis in mouse ovarian follicles. *Proc Natl Acad Sci USA* 112:5527–5532
- Shuhaibar LC, Egbert JR, Edmund AB, Uliasz TF, Dickey DM, Yee SP, Potter LR, Jaffe LA (2016) Dephosphorylation of juxtamembrane serines and threonines of the NPR2 guanylyl cyclase is required for rapid resumption of oocyte meiosis in response to luteinizing hormone. *Dev Biol* 409:194–201
- Simon A, Goodenough D, Li E, Paul D (1997) Female infertility in mice lacking connexin 37. *Nature* 385:525–529
- Smallwood SA, Tomizawa S, Krueger F, Ruf N, Carli N, Segonds-Pichon A, Sato S, Hata K, Andrews SR, Kelsey G (2011) Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. *Nat Genet* 43:811–814
- Sohel MM, Hoelker M, Noforesti SS, Salilew-Wondim D, Tholen E, Looft C, Rings F, Uddin MJ, Spencer TE, Schellander K, Tesfaye D (2013) Exosomal and non-exosomal transport of extracellular microRNAs in follicular fluid: implications for bovine oocyte developmental competence. *PLoS One* 8:e78505
- Soyal SM, Amleh A, Dean J (2000) FIG alpha, a germ cell-specific transcription factor required for ovarian follicle formation. *Development* 127:4645–4654
- Stewart KR, Veselovska L, Kim J, Huang J, Saadeh H, S-i T, Smallwood SA, Chen T, Kelsey G (2015) Dynamic changes in histone modifications precede de novo DNA methylation in oocytes. *Genes Dev* 29:2449–2462

- Su Y-Q, Wu X, O'Brien MJ, Pendola FL, Denegre JN, Matzuk MM, Eppig JJ (2004) Synergistic roles of BMP15 and GDF9 in the development and function of the oocyte-cumulus cell complex in mice: genetic evidence for an oocyte-granulosa cell regulatory loop. *Dev Biol* 276:64–73
- Su Y-Q, Sugiura K, Wigglesworth K, O'Brien MJ, Affourtit JP, Pangas SA, Matzuk MM, Eppig JJ (2008) Oocyte regulation of metabolic cooperativity between mouse cumulus cells and oocytes: BMP15 and GDF9 control cholesterol biosynthesis in cumulus cells. *Development* 135:111–121
- Su YQ, Sugiura K, Eppig JJ (2009) Mouse oocyte control of granulosa cell development and function: paracrine regulation of cumulus cell metabolism. *Semin Reprod Med* 27:32–42
- Sugiura K, Su Y-Q, Diaz FJ, Pangas SA, Sharma S, Wigglesworth K, O'Brien MJ, Matzuk MM, Shimasaki S, Eppig JJ (2007) Oocyte-derived BMP15 and FGFs cooperate to promote glycolysis in cumulus cells. *Development* 134:2593–2603
- Susor A, Jansova D, Cerna R, Danylevska A, Anger M, Toralova T, Malik R, Suplikova J, Cook MS, Oh JS, Kubelka M (2015) Temporal and spatial regulation of translation in the mammalian oocyte via the mTOR-eIF4F pathway. *Nat Commun* 6:6078
- Thomas F, Vanderhyden B (2006) Oocyte-granulosa cell interactions during mouse follicular development: regulation of Kit ligand expression and its role in oocyte growth. *Reprod Biol Endocrinol* 4:19
- Thomas FH, Ismail RS, Jiang J-Y, Vanderhyden BC (2008) Kit ligand 2 promotes murine oocyte growth in vitro. *Biol Reprod* 78:167–175
- Titus S, Li F, Stobezki R, Akula K, Unsal E, Jeong K, Dickler M, Robson M, Moy F, Goswami S, Oktay K (2013) Impairment of BRCA1-related DNA double-strand break repair leads to ovarian aging in mice and humans. *Sci Transl Med* 5:172ra121
- Tomizawa S, Nowacka-Woszek J, Kelsey G (2012) DNA methylation establishment during oocyte growth: mechanisms and significance. *Int J Dev Biol* 56:867–875
- Van Blerkom J (2010) Mitochondrial function in the human oocyte and embryo and their role in developmental competence. *Mitochondrion* 11:797–813
- Vanderhyden B, Caron P, Buccione R, Eppig J (1990) Developmental pattern of the secretion of cumulus expansion-enabling factor by mouse oocytes and the role of oocytes in promoting granulosa cell differentiation. *Dev Biol* 140:307–317
- Veitch GI, Gittens JE, Shao Q, Laird DW, Kidder GM (2004) Selective assembly of connexin37 into heterocellular gap junctions at the oocyte/granulosa cell interface. *J Cell Sci* 117:2699–2707
- Wang Y, Kong N, Li N, Hao X, Wei K, Xiang X, Xia G, Zhang M (2013) Epidermal growth factor receptor signaling-dependent calcium elevation in cumulus cells is required for NPR2 inhibition and meiotic resumption in mouse oocytes. *Endocrinology* 154:3401–3409
- Wassarman PM, Litscher ES (2012) Influence of the zona pellucida of the mouse egg on folliculogenesis and fertility. *Int J Dev Biol* 56:833–839
- Wassarman PM, Litscher ES (2013) Biogenesis of the mouse egg's extracellular coat, the zona pellucida. *Curr Top Dev Biol* 102:243–266
- Wigglesworth K, Lee KB, Emori C, Sugiura K, Eppig JJ (2015) Transcriptomic diversification of developing cumulus and mural granulosa cells in mouse ovarian follicles. *Biol Reprod* 92:23
- Winterhager E, Kidder GM (2015) Gap junction connexins in female reproductive organs: implications for women's reproductive health. *Hum Reprod Update* 21:340–352
- Wu X, Chen L, Brown CA, Yan C, Matzuk MM (2004) Interrelationship of growth differentiation factor 9 and inhibin in early folliculogenesis and ovarian tumorigenesis in mice. *Mol Endocrinol* 18:1509–1519
- Yurttas P, Vitale AM, Fitzhenry RJ, Cohen-Gould L, Wu W, Gossen JA, Coonrod SA (2008) Role for PADI6 and the cytoplasmic lattices in ribosomal storage in oocytes and translational control in the early mouse embryo. *Development* 135:2627–2636
- Zamboni L, Upadhyay S (1983) Germ cell differentiation in mouse adrenal glands. *J Exp Zool* 228:173–193

- Zarate-Garcia L, Lane SI, Merriman JA, Jones KT (2016) FACS-sorted putative oogonial stem cells from the ovary are neither DDX4-positive nor germ cells. *Sci Rep* 6:27991
- Zhang M, Su YQ, Sugiura K, Xia G, Eppig JJ (2010) Granulosa cell ligand NPPC and its receptor NPR2 maintain meiotic arrest in mouse oocytes. *Science* 330:366–369
- Zhang H, Zheng W, Shen Y, Adhikari D, Ueno H, Liu K (2012) Experimental evidence showing that no mitotically active female germline progenitors exist in postnatal mouse ovaries. *Proc Natl Acad Sci USA* 109:12580–12585
- Zhang H, Risal S, Gorre N, Busayavalasa K, Li X, Shen Y, Bosbach B, Brännström M, Liu K (2014) Somatic cells initiate primordial follicle activation and govern the development of dormant oocytes in mice. *Curr Biol* 24:2501–2508
- Zhang H, Panula S, Petropoulos S, Edsgard D, Busayavalasa K, Liu L, Li X, Risal S, Shen Y, Shao J, Liu M, Li S, Zhang D, Zhang X, Gerner RR, Sheikhi M, Damdimopoulou P, Sandberg R, Douagi I, Gustafsson JA, Liu L, Lanner F, Hovatta O, Liu K (2015) Adult human and mouse ovaries lack DDX4-expressing functional oogonial stem cells. *Nat Med* 21:1116–1118

Oocytes

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