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MOLECULAR BASIS OF OVARIAN DEVELOPMENT AND FUNCTION

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1. ABSTRACT

Molecular biology, and in particular the ability to modify genetic composition *in vivo* through transgenesis, has revolutionized the study of reproductive biology. Knowledge of the mechanisms of oocyte growth and hormone action have benefited from the identification and characterization of several novel genes involved in ovarian development and function. There are hundreds of genetically altered mice, appearing spontaneously or by design, that have impairments in female reproductive performance, and the cellular and molecular characterization of some of these deficiencies has lead to the discovery of similar abnormalities in infertile women. There is also growing appreciation for the ability of germ cells to play a significant role in ovarian function, and the recent identification of oocyte-specific factors with regulatory actions on the surrounding somatic cells will undoubtedly lead to critical advances in our understanding of the complex biochemical interactions that control ovarian folliculogenesis. This review will focus on the molecular mechanisms controlling the two primary functions of the ovary – oocyte development and steroid hormone production, with particular emphasis on the germ cell-somatic cell interactions that form the basis for these activities.

2. INTRODUCTION

The development of the ovary is a complex process that begins with its formation during embryogenesis, progresses by maturation at puberty to become the organ responsible for oocyte development, steroid hormone production and ovulation, and terminates at the end of the reproductive years by a gradual transition to quiescence. Decades of investigation have revealed the importance of cellular associations and hormone-dependent processes for the ovary to develop and function in a

coordinated and cyclical manner. More recent investigations have revealed the biochemical nature of some of the factors that mediate the cellular activities and the physiologic consequences of interfering with the expression of these factors.

Of the two primary functions of the ovary, oocyte development is more poorly understood in molecular terms than ovarian steroidogenesis. Investigations of the molecular mechanisms controlling this process have historically been very challenging because of the difficulty in obtaining oocytes in sufficient numbers to perform biochemical analyses. In addition, oocyte growth occurs within the context of a complex and continuously changing environment involving the proliferation, differentiation and apoptosis of the surrounding somatic or granulosa cells. While oocyte growth and acquisition of developmental competence rely most heavily on adequate communication between the oocyte and granulosa cells, the process is further complicated by the fact that these cell-cell interactions are strongly influenced by hormones and growth factors generated by the hypothalamic-pituitary-ovarian axis.

Spontaneously arising infertile animals offer unique opportunities to investigate the regulatory factors that govern ovary formation and folliculogenesis. For example, mutations causing loss of expression of Kit receptors by primordial germ cells or of its ligand by somatic cells result in ovaries that lack follicles due to the impaired development of the primordial germ cells during embryogenesis (1). The characterization of these mice has provided considerable knowledge about the molecular mechanisms controlling primordial germ cell proliferation and migration. Similarly, the recent application of transgenic technology to generate mice deficient in specific genes has yielded animal models whose disrupted ovarian function can provide valuable clues to our understanding of folliculogenesis. For example, follicle development is arrested at the primary stage in mice carrying a null mutation at the *Gdf9* locus (2), a gene which is expressed predominantly in oocytes. Similarly, mice deficient in connexin 37, which forms the gap junctions between oocytes and granulosa cells, show limited oocyte growth and premature luteinization of granulosa cells (3). Generation and analysis of genetically deficient mice with abnormal folliculogenesis provide novel opportunities to identify the contribution of specific genes to follicle development.

This article provides an overview of some of the molecular mechanisms that control ovary formation as well as several aspects of mature ovarian function. Given the depth of knowledge currently available in this field, some emphasis will be placed on the molecules associated with the germ cell-somatic cell communications that have such fundamental importance in the development and function of the ovary.

3. THE EMBRYONIC OVARY

The formation of the embryonic ovary is the result of three sequential processes: 1) specification of the primordial germ cells; 2) migration and proliferation of the primordial germ cells; and 3) colonization of the urogenital ridges by the primordial germ cells and formation of primordial follicles. These processes are best understood in the mouse, for which many of the molecular signals required for gonad development have been identified. During embryogenesis, and indeed through all phases of germ cell development, the germ cells are highly dependent on the surrounding somatic cells.

3.1. Specification of primordial germ cells

Mature germ cells develop from undifferentiated precursor cells that arise during embryogenesis. The molecular mechanisms underlying specification of the primordial germ cells vary significantly between species, involving maternally inherited molecules in *Drosophila* (*oskar)* and *Xenopus (Xdaz1)* and the asymmetric distribution of cytoplasmic factors ("germ plasm") in *C.Elegans* and zebrafish. In mammals, oocytes originate from the primordial germ cell (PGC) lineage that arises in extraembryonic epiblast cell precursors located outside the embryo. Specification of the PGCs involves at least two bone morphogenetic proteins, Bmp4 and Bmp8b, produced by the extraembryonic ectoderm (4-6). Bmp4 and Bmp8b homodimers bind to separate tetrameric receptors on the pluripotent epiblast cells to induce their specification. Once the PGCs are formed, Bmp4 and Bmp8b are no longer required for further development. In mice, the founding population of about 45 PGCs can be first visualized by their alkaline phosphatase staining by E7.2 (7), while in humans these cells are visible in the yolk sac at 4 weeks post-conception.

3.2. Migration and proliferation of primordial germ cells

In mammals, germ cells are specified at some distance from the gonads and later migrate to them, where they then differentiate into sex-specific germ cells. Following their formation, the PGCs in the yolk sac migrate during the next few days through the hindgut mesentary to the primordial gonad, or genital ridge, which develops from the mesoderm lining the abdominal cavity. During transit, the PGCs proliferate rapidly such that, in mice, when they reach the genital ridge by E11, the population has increased to more than 3,000 cells. Proliferation continues in the genital ridge until E13.5, reaching a maximum of about 25,000 (7). Although the mechanisms that guide the migration and proliferation of the PGCs are poorly understood, two components provided by the cells of the migratory pathway are clearly important: extracellular matrix components and cytokines. Studies using whole-mount immunocytochemistry have shown the changing distribution of three extracellular matrix glycoproteins, collagen IV, fibronectin, and laminin, during PGC migration (8). In addition, PGCs change the strength of their adhesion to each glycoprotein differentially during migration (8). As PGCs lacking integrin subunit beta1 fail to colonize the genital ridge efficiently, integrins are likely important mediators of PGC migration (9). Differential expression of cadherins in PGCs during migration suggests that these cell adhesion molecules also facilitate PGC

migration (10).

Cytokines play an important role in the proliferation and survival of the PGCs. Basic fibroblast growth factor (11), tumor necrosis factor-alpha (12) and neuregulin-beta (13), a ligand for ErbB2 and ErbB3, have been shown to increase the number of PGCs in culture. Leukemia inhibitory factor (LIF) promotes PGC survival by inhibiting apoptosis (14,15). Mice with spontaneously-arising deficiencies in expression of the tyrosine kinase receptor, Kit, or its ligand, Kit Ligand (KL), have revealed that survival of PGCs during migration depends on this ligand-receptor system. PGCs express the Kit receptor from E7.5 to E13.5, and KL is expressed along the migratory path of the PGCs (16,17). Addition of KL to PGCs in culture enhances survival by inhibiting apoptosis (14,18,19). Kit signaling is complex, activating multiple signaling cascades to effect diverse cellular responses. Ligand activation of Kit leads to autophosphorylation, but the downstream events required for PGC survival are not yet elucidated. Kit-mediated recruitment and activation of phosphatidylinositol 3'-kinase (PI3-K) plays a critical role in mediating cell adhesion, proliferation and survival in some Kit-bearing cell types, yet mutation of the binding site for the p85 subunit of PI3-kinase in the Kit gene using a knock-in strategy did not affect the proliferation and survival of the resulting PGCs (20). In contrast, others have demonstrated the requirement for PI3-K in cytokine-mediated germ cell survival during fetal oogenesis (21), suggesting that there are redundant pathways utilizing the PI3-K signaling pathway that are essential for PGC survival.

In many ways, PGCs are unique stem cells, as their development requires them to undergo extensive demethylation of the genome, erasure of allele-specific methylation associated with imprinted genes, and re-activation of the normally inactivated X chromosome (22-25). Germ line-specific epigenetic modifications are re-introduced during gametogenesis (26), and it is thought that the extensive genomic modifications may be necessary to bestow totipotency to the germ cells, and may also prevent inheritance of aberrant epigenetic modifications by subsequent generations.

3.3. Colonization of primordial germ cells and formation of primordial follicles

The colonization of the PGCs in the genital ridge and the subsequent formation of primordial follicles are essential for the continued survival of the germ cells. Upon arrival at the genital ridge, the PGCs, now called oogonia, intermingle with the somatic cells that will support their subsequent development. These pregranulosa cells arise from epithelial cells that invaginate into the genital ridge. By E12.5 in mice (22 weeks in the human), the oogonia become associated with cords of pregranulosa cells which develop further into primordial follicles, each consisting of a single oocyte wrapped in a single layer of flattened pregranulosa cells. Cellular aggregation likely involves cadherins (27), and KL-Kit interactions have also been shown to facilitate cell adhesion (28). Ovarian follicles do not develop in the absence of oocytes, indicating that oocytes participate in follicle formation from the earliest stages. The oocyte factor(s) that is required for the formation of primordial follicles is unknown, but the targeted deletion of the transcription factor FIG-alpha leads to the failure of primordial follicle formation and a massive depletion of oocytes that results in female sterility (29). This transcription factor, which coordinates the expression of the zona pellucida genes *Zp1*, *Zp2* and *Zp3* later in development, is apparently also required for the expression of one or more factors by the oocyte that are essential for the initial organization of primordial follicles.

Once colonization is complete, the oogonia enter meiosis (around E13.5). The germ cells, now called oocytes, enter prophase I but become arrested at the diplotene stage of the first meiotic prophase and remain in that state until fully grown and stimulated to ovulate. While the period of proliferation of PGCs is followed by the entry into meiosis for many oogonia, the timing is also coincident with a most significant period of attrition of oogonia from E13 to E16, resulting in the loss of 70% of the germ cells by apoptosis (30). TGF-beta1 and -beta2 and Fas ligand have been shown to induce the death of oogonia (31,32), and may do so by modulation of the activity of the Bcl-2 family of mitochondrial genes. Mice with a homozygous deletion of bcl-2 have significantly reduced numbers of primordial follicles (33). Studies using mice with altered expression of the anti-apoptotic Bcl-x and the pro-apoptotic Bax have demonstrated that a precise concentration of Bcl-x is required for PGC survival, and the absence of the Bax can suppress apoptosis (34). The shift from a gonadal environment that supports proliferation and survival to one that allows such significant germ cell loss temporally coincides with the formation of primordial follicles. It is reasonable to speculate that the balance of pro-apoptotic and anti-apoptotic cytokines, along with the association of the oogonia with pregranulosa cells may be determinants of oocyte survival.

Gonadal differentiation, or development of the somatic components of the gonads, is dependent upon a molecular cascade responsible for the development of the testes or ovaries from the bipotential genital ridge. Some of the genes required for this process have been identified. During embryogenesis, the Wilm's tumor suppressor gene, *wt1*, is expressed in the indifferent gonad and then becomes localized to the granulosa and epithelial cells of the ovary (35,36). Targeted disruption of *wt1* produces mice displaying arrest of gonadal development (37). WT1 can activate the Dax-1 promoter, suggesting that expression of DAX1, an orphan nuclear receptor, is an early event in the process of gonadal differentiation (38). Mutations in the *Dax-1* gene are associated with hypogonadotropic hypogonadism (39), and XY individuals with *Dax-1* deleted develop as males (40), suggesting that while this gene is required for ovary development, it does not appear to be an essential factor in testis formation. DAX-1 is first expressed in the somatic component of the urogenital ridge at E10.5 and peaks at E12 (41). It inhibits steroidogenesis by repressing expression of the steroidogenic acute regulatory protein (StAR; 42) and by binding to steroidogenic factor-1 (SF-1; 43) to suppress its normal

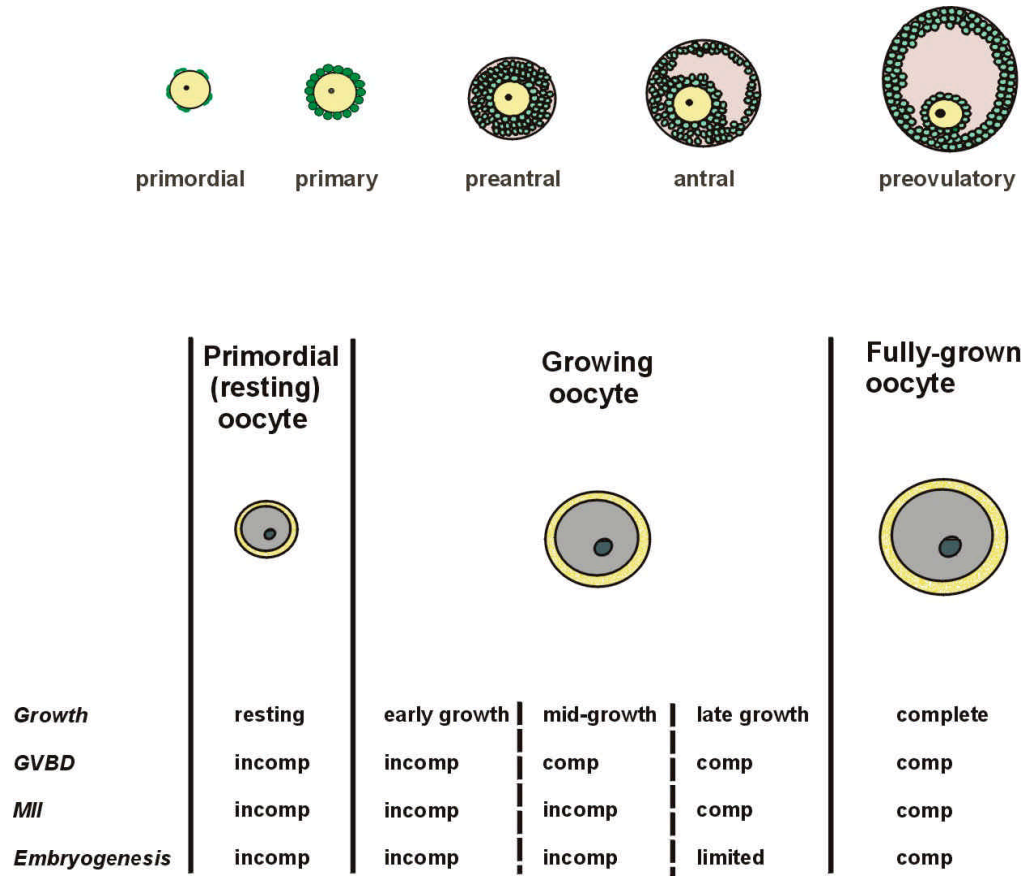

Figure 1. Diagram illustrating the stages of follicular development (upper) and the acquisition of developmental competence in the oocyte (lower). Competence (comp) to undergo germinal vesicle breakdown (GVBD), progression to metaphase II (MII), and to complete preimplantation embryogenesis is acquired gradually through oocyte growth. incomp = incompetent

activation of steroid hormone production. SF-1, another orphan nuclear receptor, is expressed in the urogenital ridges of both sexes beginning at E9.5 and its expression is essential for normal testis differentiation (44). However, targeted disruption of the Ftz-F1 gene, which encodes SF-1, results in complete gonadal agenesis in both sexes (44), suggesting a role for SF-1 in gonad formation in both sexes, although its specific involvement in female gonad development remains unclear.

4. MOLECULAR CONTROL OF FOLLICLE DEVELOPMENT

4.1. Overview of follicle development

At the time of birth in most mammals, the ovary is populated predominantly by primordial follicles, each composed of a meiotically-arrested primary oocyte surrounded by a single layer of flattened pregranulosa cells (Figure 1). In mammals, follicles develop continuously from the pool of primordial follicles throughout the reproductive lifespan of the animal. Follicle development is morphologically characterized by an increase in the diameter of the oocytes, and a synchronous proliferation of the granulosa cells, resulting in multiple layers of cells that surround each oocyte (Figure 1). The development of follicles involves the recruitment of primordial follicles from the resting pool, the continued growth of the follicles, selection of a dominant follicle, ovulation and luteinization. These growing follicles support oocyte growth and the acquisition of meiotic and developmental competence, and the granulosa cells have clearly established roles in supporting the growth of the oocyte and maintenance of meiotic arrest (45). The last decade has yielded an increasing amount of evidence that the communication is bidirectional, i.e. that the oocyte can influence several aspects of granulosa cell development, including proliferation, differentiation, and extracellular matrix and steroid hormone production (46,47). While evidence for this cellular interdependence continues to accumulate, much less is known about the molecules that mediate these interactions. Some responses rely on cell-cell contact, while other responses can be elicited by co-culturing the two cell types or by culturing one cell type in the presence of conditioned medium from the other, indicating an important role for paracrine factors. Consequently, it is now very clear that the process of follicle development is a carefully orchestrated event that involves bidirectional communication between germ cells and somatic cells using at least two modes of intercellular communication: gap junctions and paracrine factors.

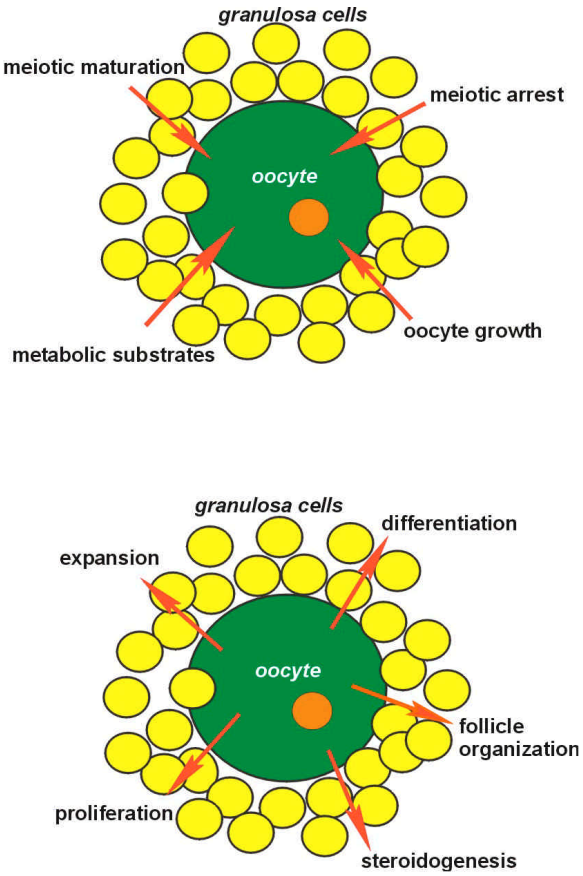

Figure 2. Diagram summarizing the physiologic activities dependent upon the bi-directional communication between oocytes and granulosa cells. Granulosa cells influence oocyte growth and meiosis (upper) and oocytes regulate several aspects of granulosa cell function (lower).

Follicle growth is initially characterized by the transition of granulosa cells from flattened to cuboidal cells. Continued growth features both an increase in oocyte diameter and proliferation of granulosa cells. This phase of preantral follicle growth is relatively slow, comprising ~85% of the total duration of follicle growth in some species. During preantral follicle growth, theca cells become associated with the growing follicle, although they remain separated by a basement membrane. Granulosa cells of preantral follicles are a relatively homogeneous population of proliferating cells that acquire receptors for follicle-stimulating hormone (FSH) and steroid hormones (48,49). Under the influence of FSH, cyclin D2 expression is induced in granulosa cells (50) and the follicle continues to grow (Figure 1). Transition to an antral follicle is associated with the formation of a fluid-filled cavity, and the granulosa cells differentiate into two sub-populations: cumulus granulosa cells, which are those most closely associated with the oocyte and are ovulated with it; and mural granulosa cells, which form a multi-layered wall against the basement membrane of the follicle and acquire differentiated functions, including steroidogenesis (51) and the expression of luteinizing hormone (LH) receptors (48,52). During oocyte growth, usually at the early stages of antrum formation, oocytes acquire meiotic competence (i.e. the ability to resume meiosis). The final stages of oocyte development are characterized by the gradual acquisition of full developmental competence, including ability to undergo embryonic development.

In the absence of sufficient FSH, or by the natural process of follicle selection, some follicles that have initiated growth will fail to reach ovulation and will undergo apoptosis or atresia. Indeed, more than 99.9% of ovarian follicles present at birth never reach ovulation due to follicular atresia. This process is regulated by endocrine factors, notably FSH and LH, and these signals are mediated within the ovary by a variety of paracrine factors (see 53 for review).

4.2. Granulosa cell control of oocyte development

Oocytes cannot grow in isolation from their granulosa cells, although limited development is possible when in co-culture with soluble factors from granulosa cells (54). This dependence of the oocyte upon the granulosa cells includes not only oocyte growth, but regulation of meiosis as well (Figure 2). In addition to the essential role of FSH in stimulating follicle growth, numerous intra-follicular factors have been identified to support oocyte development; however, like FSH, their actions on the oocytes are mediated by the granulosa cells, and the molecular mechanisms by which granulosa cells support oocyte development are not well-defined. One study has demonstrated that the factors involved in oocyte-somatic cell interactions necessary to support oocyte development are conserved between rats and mice (55). By forming chimeric reaggregated ovaries with interspecific exchange of somatic and germ cell components, the authors showed that mouse oocytes grown in rat follicles underwent fertilization and subsequent embryonic and fetal development. Interestingly, species-specific characteristics of the oocytes were retained, despite the xenogeneic follicular environment. The mechanisms by which granulosa cells influence oocyte activity are not defined, although it is clear that granulosa cells modulate both protein expression (56) and transcriptional activity in oocytes (57).

Gap junctions play a most important role in allowing granulosa cells to provide essential components to the oocyte during growth (58). In addition, the presence of this type of intercellular communication among the granulosa cells permits coordinated cellular activity. The structural proteins comprising these channels, called connexins, are members of a multigene family that consists of at least 17 members (59). The presence of these functional units have been demonstrated using metabolic cooperativity studies in which uptake of radiolabelled compounds (eg. uridine, choline or 2-deoxyglucose) by granulosa cells is monitored for the transfer of radioactivity to unlabelled oocytes (58). In addition to the provision of metabolic substrates, gap junctions also mediate signals involved in both the maintenance of oocyte meiotic arrest, and the resumption of meiosis induced by the LH surge (60). Mouse models with connexin deficiency have provided *in vivo* confirmation of the importance of gap junctions in oocyte and follicle development. Gap

junctions between granulosa cells contain predominantly connexin 43, and follicles lacking this connexin failed to develop past early preantral stages (61). Deletion of connexin 37, which is present in the gap junctions between oocytes and granulosa cells, interfered with the development of antral follicles and caused inappropriate luteinization and failure to ovulate (3). These results suggest that gap junctional communication between granulosa cells and between oocytes and granulosa cells are essential for normal folliculogenesis.

The first ligand-receptor system to be characterized for its role in mediating granulosa-oocyte interactions was KL and the Kit tyrosine kinase receptor. In the postnatal ovary, both Kit receptors and KL are expressed in developing and preovulatory follicles. Kit is found in oocytes at all stages of follicular development in mouse (62-64) and human (65). KL is expressed in rat (66), mouse (67,68) and human granulosa cells (69) and in the ovarian surface epithelium (70). Kit may play a role in oocyte growth as determined by an increase in oocyte diameter when follicles are cultured in the presence of KL (71). Furthermore, in *Kitl/Kitl*$^{Sl\text{-}t}$ and *Kitl*$^{Sl\text{-}pan}$ mutant mice which have reduced levels of KL expression, oocytes are present, although fewer in numbers, and follicular development is arrested. The impairment of follicle growth suggests the necessity of functional KL for appropriate follicular development (72,73). This was further demonstrated by the *in vivo* administration of antibodies blocking Kit activation at various times after birth. The blockade of Kit function disturbed the onset of primordial follicle development, primary follicle growth, follicular fluid formation in preantral follicles, and the penultimate-stage of ovarian follicle maturation before ovulation. These results suggested that ovarian follicle growth is dependent on Kit at a time when functional FSH receptors are not yet expressed in mouse ovary (74). *In vitro* studies support the possibility that KL alone is sufficient for the initiation of follicle growth. When placed in culture, primordial follicles in ovaries from neonatal rats will spontaneously develop, but initiation of growth is completely inhibited in the presence of Kit blocking antibodies (75). Klinger and De Felici (76) used a multistep culture system for mouse oocytes obtained from E15.5-E16.5 embryos, and found that KL alone can induce the onset of growth, but was not sufficient to fully activate the mechanisms governing the acquisition of meiotic competence (76). Thus, evidence to date suggests that KL is necessary and sufficient to induce primordial follicle development.

KL is expressed as either membrane-bound or soluble proteins that arise from alternatively spliced mRNAs (77). Both transcripts, when translated, yield membrane-associated products; however, one form, KL-1, is efficiently cleaved due to the presence of an 84 base pair exon which encodes a proteolytic cleavage site, and thus is released as a soluble product. The other form, KL-2, lacks this exon and therefore remains more stably on the membrane (77). In the testis, membrane-bound KL is the more potent of the two forms in its ability to induce the proliferation of Kit-bearing germ cells (78,79). Although both membrane-bound and soluble forms of KL are present in the ovary (67), the relative activity of these proteins in regulating oocyte growth is not yet determined.

4.3. Oocyte control of granulosa cell function

Discoveries in the past decade have brought considerable excitement to the study of folliculogenesis, most particularly because of the identification and early characterization of oocyte proteins involved in this process. The physiological importance of the interactions between the oocyte and granulosa cells was originally suggested by an *in vivo* study in which ovectomy lead to spontaneous transformation of the Graafian follicle into a corpus luteum (80), which indicated that the oocyte or oocyte-secreted factors contribute to the suppression of luteinization. Additional evidence for such a factor was provided by *in vivo* and *in vitro* experiments in rats and rabbits, showing that the removal, absence, or destruction of oocytes leads to granulosa cell luteinization (81,82). Much of our understanding of the specific roles of the oocyte in follicle development were subsequently derived from studies which examined granulosa cell function following removal of the oocyte from preantral follicles or oocyte-cumulus cell complexes (oocytectomy) (83). Using the procedure of oocytectomy, the hypothesis that oocytes suppress luteinization was supported by the observation that mouse oocytes secrete a factor(s) that inhibits progesterone and stimulates estradiol production by cumulus granulosa cells (84-86). Oocyte-secreted factors also inhibit expression of LH receptors in granulosa cells which may contribute to their actions as luteinization inhibitors (87). In terms of follicle growth, oocytes have been shown to promote granulosa cell proliferation and follicular integrity (88). During follicle development, some of the granulosa cells differentiate into cumulus cells, and the use of oocytectomy has determined that granulosa cells seem to be dependent on gap junctional communication with the oocyte for this aspect of their development, since granulosa cells from oocytectomized preantral follicles fail to differentiate into cumulus cells (Figure 2) (89).

The identification of the oocyte-secreted factors that influence granulosa cell function has been difficult and none are yet confirmed, but members of the transforming growth factor-beta (TGF-beta) superfamily have aroused considerable interest by their ability to mimic the actions of the oocyte in regulating granulosa cell activities. Investigation of these factors has lead to significant advances in our understanding of the actions of oocytes on granulosa cells and follicle development. Some members of this family, growth differentiation factor 9 (GDF-9), bone morphogenetic protein 15 (BMP-15) and BMP-6, are expressed by oocytes and are therefore logical candidates to consider as possible mediators of the effects attributed to the oocyte. The expression of both GDF-9 and BMP-15 begins in oocytes of small primary follicles and continues through ovulation (90,91), and both of these growth factors are mitogens for granulosa cells (92,93). The essential role of these factors in folliculogenesis has been demonstrated by their targeted deletion in mice. Mice deficient in GDF-9 have a block in folliculogenesis at the primary follicle stage, and are characterized by sweeping changes in gene

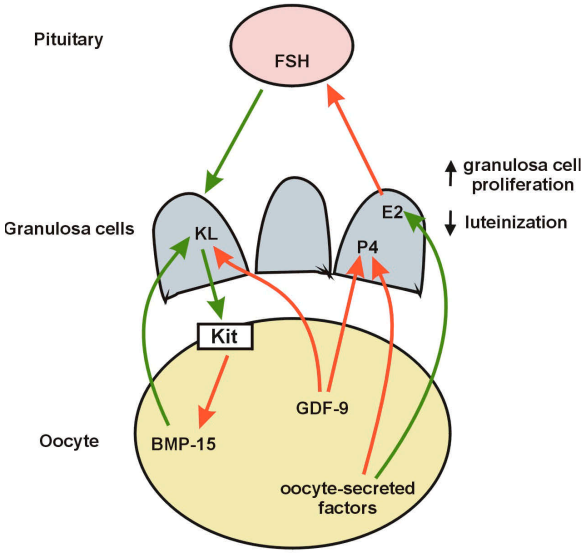

Figure 3. Model showing paracrine and hormonal factors controlling oocyte growth and follicle development. Oocyte-secreted factors, including BMP-15, GDF-9 and others yet to be identified, act on the granulosa cells to regulate KL expression and steroid hormone production to enhance follicle development and the inhibition of luteinization. In turn, granulosa cells produce KL that acts through Kit receptors to promote oocyte growth. Green arrows indicate stimulatory actions; red arrows indicate actions that are inhibitory.

expression associated with failed follicle development (2,94,95). Bmp15 null females are subfertile with decreased ovulation and fertilization rates in mice (96), but a natural X-linked mutation in sheep identified BMP-15 as an essential factor for female fertility with homozygous mutants having follicular development arrest at the primary stage (97). In contrast, BMP-6 null female mice are fertile, indicating that BMP-6 is not essential for follicle development in this species (98).

The aberrant folliculogenesis found in GDF-9 and BMP-15 deficient mice and sheep (2,96,97) has lead to the investigation of these factors in human ovaries. Both factors are expressed in human oocytes (99), and there are no mutations associated with these genes in women with premature ovarian failure or polycystic ovary syndrome (100); however, the level of expression of GDF-9 has been reported to be reduced in polycystic ovaries (99). Treatment of human cortical tissue slices with GDF-9 *in vitro* enhanced both follicle survival and progression of follicular development to the secondary stage, suggesting that this growth factor may have clinical utility in designing culture conditions for human follicles (101).

It is already evident that the molecular basis of oocyte-granulosa cell interactions is quite complex, as the actions of many of the oocyte-secreted TGF-beta family members on granulosa cell activity seem to be dependent on the context, particularly as it relates to stage of follicle development, the presence of gonadotropins, and the species. In addition, the interplay between the various TGF-beta family members needs to be elucidated. For example, fully grown oocytes suppress KL expression in granulosa cells (102), and this action may be mediated by GDF-9, as recombinant GDF-9 can also inhibit KL expression (102) and the ovaries of GDF-9 deficient mice show elevated levels of KL transcripts (95). Recent evidence indicates that another oocyte-secreted factor, BMP-15, can stimulate KL expression in rat granulosa cells (103). Furthermore, both KL and BMP-15 stimulated the mitotic activity of granulosa cells cultured in the presence of oocytes, and both required oocyte Kit activity to elicit their stimulatory effect, suggesting that yet another oocyte factor, downstream of Kit, can modulate the response of granulosa cells to BMP-15. Although these interactions are likely to be quite complex, the consequence of this plethora of newly identified oocyte-secreted factors has been to give potential identities to oocyte factors previously identified only by their actions, and to turn the investigation of follicle development into a dynamic area of study. The molecular definition of the oocyte-granulosa cell interactions has generated a complex model of the regulatory mechanisms controlling follicle growth (Figure 3).

4.4. Theca support of follicle development

During follicle growth, theca cells are recruited to develop from the stromal cell population. These cells are responsive to LH and produce androgens that are utilized by granulosa cells, through the action of aromatase, to produce estradiol (reviewed in 104). In addition to the hormonal regulation of theca cell function, several intra-ovarian factors have been identified that may contribute to the recruitment and function of the theca cells, and to the coordinated activity of theca cells and the developing follicle. KL was the first granulosa cell-derived growth factor that was shown to directly stimulate theca cell proliferation and androstenedione production in the absence of gonadotropins (105). Theca cells express Kit (63) and it has been suggested that KL promotes early follicle development by inducing proliferation and organization of stromal stem cells around small follicles (106). Coordination of activity between theca and granulosa cells to drive the rapid transition of follicles from late preantral to antral may be achieved by a positive feedback loop of theca cell-derived keratinocyte growth factor (KGF) and hepatocyte growth factor (HGF) and granulosa cell-derived KL. In bovine (107) and human ovaries (108), KGF and HGF have been shown to stimulate granulosa cell KL expression and KL increases the expression of KGF and HGF in theca cells. A possible role for the oocyte in regulating theca cell activity has also been proposed, as GDF-9 stimulates both basal and LH-stimulated rat theca-interstitial cell androgen biosynthesis (109); however, recent studies have shown that granulosa cells in rhesus monkeys also express GDF-9, and treatment of human theca cells with GDF-9 increased their proliferation and blocked forskolin-stimulated progesterone and androgen synthesis, suggesting a broader role for this growth factor in ovarian function in primates (110).

5. OOCYTE MATURATION

In mammalian ovaries, follicles committed to

ovulate undergo two major events following the preovulatory surge of gonadotropins. The oocytes within the preovulatory follicle resume meiosis and progress to metaphase II, a process called oocyte maturation, and the cumulus cells surrounding the oocytes undergo a process of expansion and mucification.

5.1. Meiotic (nuclear) maturation

In most mammals, meiosis is initiated in female germ cells during fetal life; however, the process is arrested at prophase I at or around the time of birth. Even after oocyte growth has been initiated, oocytes continue to be maintained in meiotic arrest, despite a significant increase in cell size. This arrest results from either an absence of essential cell cycle regulatory proteins, or the presence of meiosis-arresting substances, or both. As oocytes enter the final stages of growth, the mechanisms controlling meiosis undergo a dramatic change, such that the oocytes acquire the ability to spontaneously resume meiosis when liberated from the follicles and placed in culture. Oocytes that can achieve spontaneous oocyte maturation (i.e. have meiotic competence) clearly have acquired the molecules required for the resumption and completion of meiosis, yet they do not resume meiosis if they are retained within the follicle. Under normal conditions, the surge of LH triggers the resumption of meiosis, which then proceeds through completion of the first meiotic division which is morphologically seen as germinal vesicle breakdown and first polar body formation, with subsequent arrest at metaphase II. The final phases of meiosis are completed only after sperm penetration. Acquisition of the competence to mature is a step-wise event that occurs during the final stages of oocyte growth, while the oocytes are normally arrested at the germinal vesicle stage (Figure 1). While oocytes are increasing in size, they sequentially acquire the ability to undergo germinal vesicle breakdown, produce a polar body, cleave to the 2-cell stage after insemination, and develop to the blastocyst stage (111).

A number of molecules found within follicles have been shown to inhibit spontaneous oocyte maturation including cyclic adenosine monophosphate (112,113), purines (114), müllerian inhibiting substance (115) and KL (66). Of these, the role of cAMP is the best characterized. Oocytes cultured in the presence of membrane-permeable analogues of cAMP or phosphodiesterase inhibitors, which maintain high levels of intraoocyte cAMP levels, inhibit oocyte meiosis in a dose-dependent and reversible manner (112,113). More recent studies have determined that PDE3A is the major phosphodiesterase form expressed in mammalian oocytes, and that PDE3A activity increases prior to resumption of meiosis in both spontaneous and gonadotropin-stimulated maturation (116-118). Therefore, oocyte PDE3A activity appears to be one of the intraoocyte mechanisms for controlling resumption of meiosis in oocytes.

The molecular mechanisms by which the oocyte undergoes meiotic maturation involve changes in the presence or activity of meiosis inhibitors, as well as the activation of cell cycle kinases. Maturation-promoting factor (MPF), which is composed of a catalytic subunit, p34cdc2, and a regulatory subunit, cyclin B, has been found to be essential for this process (reviewed in 119). MPF activity increases during oocyte maturation, then decreases at anaphase of the first meiotic division. The increase in MPF activity is associated with the increased synthesis of cyclin B (120,121), and the activation of p34cdc2 (122). The importance of the cdc25b phosphatase in regulating activation of MPF has recently been demonstrated in cdc25b deficient mice, which are infertile because oocytes remain arrested at prophase with low MPF activity (123). A historical perspective on the discovery of MPF has been provided recently by the individual who first reported it (124).

We have previously shown that KL acts as a negative regulator of rat oocyte meiosis through activation of Kit receptors (66). Kit receptors are present on the surface of oocytes at all stages of follicular development (63); its expression is highest in mice in meiotically-arrested oocytes and this expression decreases after maturation (64). In contrast, the presence of Kit receptor on fully-grown rat oocytes isolated from preovulatory antral follicles persists after hCG treatment (125). The continued presence of Kit receptor during oocyte maturation indicates that the LH surge probably does not inhibit the expression of Kit as a means of inducing meiosis. In both rats (66) and mice (126), KL mRNA levels in granulosa cells increase in response to hCG stimulation, indicating that meiotic resumption is probably not mediated by an overall decrease in follicular KL levels. However, there is a localized decrease in the expression of both forms of KL by the cumulus cells that precedes, by at least 2 hours, the occurrence of germinal vesicle breakdown in oocytes of preovulatory follicles, which supports at least a reasonable temporal association between the loss of KL with the resumption of meiosis. As well, in response to hCG, there is a shift in steady-state transcript levels from membrane-bound KL to soluble KL in mural granulosa cells. The significance of this shift is currently unknown, however, it is clear that the two isoforms have differential activity, such that the presence of the soluble form cannot compensate for loss of the transmembrane form *in vitro* (79) or *in vivo* (127). Therefore, Kit activation contributes to the maintenance of meiotic arrest and it appears that LH stimulation of oocyte meiosis may be mediated, at least in part, by a decrease in Kit activity that is achieved by a reduction in Kit expression in mouse oocytes. In rats, Kit activity may be diminished by a loss of KL expression in the cumulus cells and a reduction in the relative amounts of membrane-bound KL compared to soluble forms in the mural granulosa cells.

Nuclear or meiotic maturation of oocytes can be achieved in chemically-defined media, which helped to identify the minimal nutritional requirements for the resumption of meiosis. However, those conditions proved to be insufficient to promote full fertilization and developmental capabilities. Initial failures in efforts to fertilize oocytes matured *in vitro* were attributed to incomplete or aberrant cytoplasmic maturation, despite the apparent completion of nuclear maturation (128,129). Although the most striking abnormality in these oocytes

was the failure to form the male pronucleus, full cytoplasmic maturation encompasses a number of relatively ill-defined processes that prepare the oocyte for fertilization, activation and early embryo development. What is clear is that cumulus cells play an essential role in promoting normal cytoplasmic maturation of oocytes necessary for pronuclear formation and subsequent developmental capability (129,130). The importance of the presence of cumulus cells during maturation is evident from observations that the rate of male pronucleus formation after fertilization was higher in oocytes that had been matured cumulus-enclosed *vs.* those that were matured cumulus-free (131). The specific cumulus-derived factors that promote cytoplasmic maturation of oocytes remain undefined; however, mechanisms as simple as nutrient support cannot be eliminated since male pronucleus formation following sperm penetration of porcine oocytes was reported to be much greater when the oocytes were matured in the presence of amino acids (131).

5.2. Cumulus Expansion

In response to the LH surge, the morphology of cumulus cells changes dramatically, including an extensive rearrangement of cytoskeleton through the assembly of actin microfilaments and the induction of the synthesis of hyaluronic acid which transform the tightly packed cumulus cells into a much larger mass of sticky (mucified) cells. In addition to facilitating fertilization, this expansion is required for optimal extrusion of the oocyte-cumulus cell masses from the follicle at ovulation (132,133). This was confirmed in mice with targeted deletion of cyclooxygenase-2 (COX-2; 134), an enzyme involved in prostaglandin synthesis. COX-2 deficient mice fail to ovulate and the defect in ovulation has been attributed to abnormal expansion of the cumulus cells (135). A decrease in ovulation number was also reported for mice lacking the prostaglandin E2 receptor, in which cumulus expansion is also impaired (136), suggesting that COX-2 is required for the gonadotropin induction of ovarian prostaglandin levels and that COX-2-related prostanoids are required for stabilization of the cumulus oophorus during ovulation.

Rather surprisingly, experiments using oocytectomy revealed the absolute requirement for oocyte-secreted factors for successful cumulus expansion in mice. Oocytectomized complexes failed to expand in response to FSH, but could undergo maximal expansion if cultured in the presence of oocytes or in media conditioned by oocytes. Oocyte-secreted factors enabled the expansion of oocytectomized cumulus complexes by promoting the ability of the cells to produce hyaluronic acid complexes (83,89). The effects are also seen with dispersed cumulus cells (137,138). The ability of the oocytes to secrete the cumulus expansion enabling factor (CEEF) and the capacity of cumulus cells to expand in response to FSH are temporally correlated with the acquisition of meiotic competence (89). Oocyte-secreted factors also suppress expression of urokinase plasminogen activator in granulosa cells (139), which indicates that mouse oocytes promote preovulatory matrix accumulation by modulating the gonadotropin action on both the synthesis and the degradation of specific matrix components.

Although TGF-beta1 mimics the oocyte-secreted factor(s) in enabling FSH-stimulated hyaluronic acid synthesis by mouse dispersed cumulus cells (137,138) and oocytectomized complexes (140), TGF-beta1 is less effective than the oocyte factor, and TGF-beta1 neutralizing antibodies do not inhibit the response to the oocyte factor (138,140), suggesting that TGF-beta1 is not the CEEF. GDF-9 has also been shown to enable cumulus expansion in the absence of the oocyte (94) and GDF-9 null oocytes do not have the ability to secrete the expansion enabling activity (140), rendering GDF-9 an excellent candidate for the CEEF. Recombinant GDF-9 also upregulates the expression of COX-2 and HAS-2, the major hyaluronic acid synthase involved in cumulus expansion, and inhibits the protease urokinase plasminogen activator, an enzyme suppressed during production of the hyaluronic acid extracellular matrix (94). Despite the overwhelming evidence, there remains a discrepancy in the timing of expression of GDF-9 vs CEEF in oocytes that needs to be explained. GDF-9 is expressed in oocytes starting very early in follicle development (90), whereas CEEF is not secreted by oocytes until around the time of acquisition of meiotic competence (89).

6. MOLECULAR BASIS OF OVARIAN STEROIDOGENESIS

6.1. Hormonal control of granulosa cell differentiation

The principal steroid hormones in the female, estradiol and progesterone, play key roles in ovarian physiology. The rate of production of steroid hormones is determined primarily by the availability of cholesterol to the first enzyme in the steroidogenic pathway, cytochrome P450 side-chain cleavage, as well as by the level and activities of this and other enzymes that catalyze the subsequent biosynthetic steps. Trophic hormones, primarily FSH and LH, acutely increase steroidogenesis by enhancing both of these mechanisms. In particular, the availability of cholesterol is increased by upregulation of the expression of StAR, a protein that facilitates the uptake of cholesterol by mitochondria (141,142).

Although its primary role is stimulation of the reproductive tract via estrogen receptor (ER)-alpha, estradiol produced by the granulosa cells also acts by an autocrine mechanism on ER-beta to stimulate granulosa cell proliferation. Mice lacking ER-alpha exhibit impaired follicular growth and infertility, and follicular development is arrested at the antral stage, suggesting a role for ER-alpha only during the later stages of follicle development (143). The combined actions of FSH and estradiol promote both proliferation and differentiation of granulosa cells. The components of the cell cycle kinase cascades that are important for hormone-induced granulosa cell proliferation have been reviewed previously (144). One of the most dramatic changes during follicular development is the transformation of the highly proliferative, preovulatory, estradiol-producing granulosa cells into the nonproliferative, terminally differentiated, progesterone-producing cells of the corpus luteum. Although this process of differentiation or luteinization normally occurs at preovulatory stage of follicle development in response to

the LH surge, granulosa cells removed from the follicular environment prior to the LH surge spontaneously acquire many of the characteristics of LH-differentiated cells. These characteristics include the increased expression of LH receptors and an enhanced ability to produce progesterone (145), suggesting that elements of the follicular environment contribute to the inhibition of luteinization.

6.2. Role of the oocyte in granulosa cell differentiation

As noted earlier, various models of germ cell deficiency indicate that the presence of the oocyte is critical for preventing premature progesterone production by rodent follicles (80-92). Oocyte-secreted factors also inhibit expression of LH receptors in granulosa cells which helps to maintain them in a less differentiated state (87). Using the procedure of oocytectomy, oocyte-secreted factors have been shown to regulate mouse and human granulosa cell steroidogenesis (84,85,146). Similar observations have been reported for pigs (147) and chickens (148) and has also been reported for Xenopus laevis oocytes, where the presence of early-stage oocytes caused the follicle cells to make estradiol rather than progesterone, an influence not dependent upon physical association with the oocyte (149). Thus, it appears that the default steroidogenic pathway for granulosa cells in many species is the synthesis of progesterone, and the oocyte is key to the inhibition of this pathway.

To date, the molecules that mediate the influence of the oocyte on granulosa cell luteinization have not been identified, but GDF-9 is a reasonable candidate, as recombinant GDF-9 suppresses FSH-induced progesterone production by rat granulosa cells (93), and GDF-9 deficient oocytes do not have the abilities of normal oocytes to promote estradiol and suppress progesterone production (140). It should be noted, however, that there are discrepancies in the action of GDF-9 that distinguish it from the actions of oocytes. While GDF-9 treatment alone enhances the expression of StAR and progesterone production in cultured granulosa cells (94), oocytes do not share this effect. Also, the expression of GDF-9 in oocytes continues until ovulation, past the time when granulosa cell differentiation is initiated, although the process of luteinization has been previously demonstrated to reduce the responsiveness of granulosa cells to oocyte-secreted factors (86). The actions of GDF-9 on granulosa cell steroidogenesis may be species-dependent and strongly affected by the presence of gonadotropins, as recombinant GDF-9 stimulates StAR expression and progesterone secretion by murine granulosa cells (94), whereas GDF-9 suppresses FSH-stimulated progesterone and estradiol production by rat granulosa cells (93).

At least two other members of the TGF-beta superfamily have been shown to have actions that inhibit luteinization: BMP-6 and BMP-15. BMP-6 has no effect on granulosa cell proliferation, but has been shown to produce a marked decrease in FSH-induced progesterone production, and thus shares a specific activity with oocytes (150). Similarly, the ability of BMP-15 to suppress FSH-stimulated progesterone production (92) warrants further investigation of both of these growth factors as luteinization inhibitors. Although they have actions in common with oocytes in the promotion of follicle development, it should be noted that the mechanism of action of the oocyte activity and the BMPs may be markedly different. The site of action of some of the oocyte-secreted factors appears to be downstream of the generation of cAMP (83), whereas BMP-6 appears to down-regulate FSH-stimulated adenylate cyclase activity (150). Likewise, BMP-15 seems to act by reducing the expression of FSH receptors (151) and therefore neither of these factors are likely to be the oocyte-secreted factor that regulates cumulus expansion or steroid hormone production.

7. PERSPECTIVE

The development of mature female germ cells and the production of steroid hormones are the two primary functions of the mammalian ovary. Both of these activities are strongly regulated by gonadotropic hormones produced by the pituitary and a multitude of intra-ovarian factors that mediate the actions of these hormones. Molecular biology, in combination with the ability to modify genetic composition *in vivo* through transgenesis, has helped to identify and characterize the importance of many of these paracrine factors. From the formation of the ovary during gestation to the time of ovulation, the organization and normal functioning of this organ is heavily dependent upon close interactions between the germ cells and the surrounding somatic cells. The Kit receptor expressed by germs cells appears to play a crucial role at all stages of oocyte development, and is essential for oocyte growth, yet we know little about the downstream events associated with Kit activation in these cells.

There is growing appreciation for the ability of germ cells to play a significant role in many aspects of ovarian function, including steroidogenesis. The recent identification of oocyte-specific factors with regulatory actions on the surrounding granulosa cells will undoubtedly lead to major advances in our understanding of the complex biochemical interactions that control ovarian folliculogenesis. Members of the TGF-beta superfamily, and in particular the growing number of these factors that show often unique expression in the ovary, will undoubtedly play an important role in follicle growth and hormone production. The investigation of the biochemical interactions and physiologic responses of the various intra-ovarian molecules that serve as paracrine factors, and the mechanisms by which they contribute to the complex, finely-controlled processes of oocyte development and steroidogenesis, promise to be dynamic areas of study for some time.

8. REFERENCES

1. Russell, E.S.: Hereditary anemias of the mouse: A review for geneticists. *Adv. Genet.* 20, 357-459 (1979)

2. Dong, J., D.F. Albertini, K. Nishimori, T.R. Kumar, N. Lu & M.M. Matzuk: Growth differentiation factor-9 is

required during early ovarian folliculogenesis. *Nature* 383, 531-535 (1996)

3. Simon, A.M., D.A. Goodenough, E. Li & D.L. Paul: Female infertility in mice lacking connexin 37. *Nature* 385, 525-529 (1997)

4. Ying Y., X.M. Liu, A. Marble, K.A. Lawson & G.Q. Zhao: Requirement of Bmp8b for the generation of primordial germ cells in the mouse. *Mol Endocrinol* 14, 1053-1063 (2000)

5. Ying Y. & G.Q. Zhao: Cooperation of endoderm-derived BMP2 and extraembryonic ectoderm-derived BMP4 in primordial germ cell generation in the mouse. *Dev Biol* 232, 484-492 (2001)

6. Ying Y., X. Qi & G.Q. Zhao: Induction of primordial germ cells from murine epiblasts by synergistic action of BMP4 and BMP8B signaling pathways. *Proc Natl Acad Sci USA* 98,7858-7862 (2001)

7. Tam P.P. & M.H. Snow: Proliferation and migration of primordial germ cells during compensatory growth in mouse embryos. *J Embryol Exp Morphol* 64, 133-147 (1981)

8. Garcia-Castro M.I., R. Anderson, J. Heasman & C. Wylie: Interactions between germ cells and extracellular matrix glycoproteins during migration and gonad assembly in the mouse embryo. *J Cell Biol* 138, 471-480 (1997)

9. Anderson R., R. Fassler, E. Georges-Labouesse, R.O. Hynes, B.L. Bader, J.A. Kreidberg, K. Schaible, J. Heasman & C. Wylie: Mouse primordial germ cells lacking beta1 integrins enter the germline but fail to migrate normally to the gonads. *Development* 126, 1655-1664 (1999)

10. Bendel-Stenzel, M.R., M. Gomperts, R. Anderson, J. Heasman & C. Wylie: The role of cadherins during primordial germ cell migration and early gonad formation in the mouse. *Mech Dev* 91, 143-152 (2000)

11. Resnick, J.L., M. Ortiz, J.R. Keller & P.J. Donovan: Role of fibroblast growth factors and their receptors in mouse primordial germ cell growth. *Biol Reprod* 59, 1224-1229 (1998)

12. Kawase, E., H. Yamamoto, K. Hashimoto & N. Nakatsuji: Tumor necrosis factor-alpha (TNF-alpha) stimulates proliferation of mouse primordial germ cells in culture. *Dev Biol* 161, 91-95 (1994)

13. Toyoda-Ohno, H., M. Obinata & Y. Matsui: Members of the ErbB receptor tyrosine kinases are involved in germ cell development in fetal mouse gonads. *Dev Biol* 215, 399-406 (1999)

14. Pesce, M., M.G. Farrace, M. Piacentini, S. Dolci & M. De Felici: Stem cell factor and leukemia inhibitory factor promote primordial germ cell survival by suppressing programmed cell death (apoptosis). *Development* 118, 1089-1094 (1993)

15. De Felici, M & S. Dolci: Leukemia inhibitory factor sustains the survival of mouse primordial germ cells cultured on TM4 feeder layers. *Dev Biol* 147, 281-284 (1991)

16. Matsui, Y., K.M. Zsebo & B.L.M. Hogan: Embryonic expression of a haematopoietic growth factor encoded by the *Sl* locus and the ligand for c-*kit*. *Nature* 347, 667-669 (1990)

17. Keshet, E., S.D. Lyman, D.E. Williams, D.M. Anderson, N.A. Jenkins, N.G. Copeland & L.F. Parada: Embryonic RNA expression patterns of the c-kit receptor and its cognate ligand suggest multiple functional roles in mouse development. *EMBO J* 10, 2425-2435 (1991)

18. Dolci, S., D.E. Williams, M.K. Ernst, J.L. Resnick, C.I. Brannan, L.F. Lock, S.D. Lyman, H.S. Boswell & P.J. Donovan: Requirement for mast cell growth factor for primordial germ cell survival in culture. *Nature* 352, 809-811 (1991)

19. Godin, I., R. Deed, J. Cooke, K. Zsebo, M. Dexter & C.C. Wylie: Effects of the steel gene product on mouse primordial germ cells in culture. *Nature* 352, 807-809 (1991)

20. Kissel, H., I. Timokhina, M.P. Hardy, G. Rothschild, Y. Tajima, V. Soares, M. Angeles, S.R. Whitlow, K. Manova & P. Besmer: Point mutation in kit receptor tyrosine kinase reveals essential roles for kit signaling in spermatogenesis and oogenesis without affecting other kit responses. *EMBO J* 19, 1312-1326 (2000)

21. Morita, Y., T.F. Manganaro, X.J. Tao, S. Martimbeau, P.K. Donahoe & J.L. Tilly: Requirement for phosphatidylinositol-3'-kinase in cytokine-mediated germ cell survival during fetal oogenesis in the mouse. *Endocrinology* 140, 941-949 (1999)

22. Monk, M. & A. McLaren: X-chromosome activity in foetal germ cells of the mouse. *J Embryol Exp Morpho*l 63, 75-84 (1981)

23. Monk, M., M. Boubelik & S.Lehnert: Temporal and regional changes in DNA methylation in the embryonic, extraembryonic and germ cell lineages during mouse embryo development. *Development* 99, 371-382 (1987)

24. Kafri, T., M. Ariel, M. Brandeis, R. Shemer, L. Urven, J. McCarrey, H. Cedar & A. Razin: Developmental pattern of gene-specific DNA methylation in the mouse embryo and germ line. *Genes Dev* 6, 705-714 (1992)

25. Brandeis, M., T. Kafri, M. Ariel, J.R. Chaillet, J. McCarrey, A. Razin & H. Cedar: The ontogeny of allele-specific methylation associated with imprinted genes in the mouse. *EMBO J* 12, 3669-3677 (1993)

26. Szabo, P.E. & J.R. Mann: Biallelic expression of imprinted genes in the mouse germ line: implications for erasure, establishment, and mechanisms of genomic imprinting. *Genes Dev* 9, 1857-1868 (1995)

27. Di Carlo, A. & M.A. De Felici: A role for E-cadherin in mouse primordial germ cell development. *Dev Biol* 226, 209-219 (2000)

28. Pesce, M., A. Di Carlo & M. De Felici: The c-kit receptor is involved in the adhesion of mouse primordial germ cells to somatic cells in culture. *Mech Dev* 68, 37-44 (1997)

29. Soyal, S.M., A. Amleh & J. Dean: FIGalpha, a germ cell-specific transcription factor required for ovarian follicle formation. *Development* 127, 4645-4654 (2000)

30. Coucouvanis, E.C., S.W. Sherwood, C. Carswell-Crumpton, E.G. Spack & P.P. Jones: Evidence that the mechanism of prenatal germ cell death in the mouse is apoptosis. *Exp Cell Res* 209, 238-247 (1993)

31. Guo, M.W., E. Mori, J.P. Xu & T. Mori: Identification of Fas antigen associated with apoptotic cell death in murine ovary. *Biochem Biophys Res Commun* 203, 1438-1446 (1994)

32. Olaso, R., C. Pairault, B. Boulogne, P. Durand & R Habert: Transforming growth factor beta1 and beta2 reduce the number of gonocytes by increasing apoptosis. *Endocrinology* 139, 733-740 (1998)

33. Ratts, V.S., J.A. Flaws, R. Kolp, C.M. Sorenson & J.L. Tilly: Ablation of bcl-2 gene expression decreases the numbers of oocytes and primordial follicles established in the post-natal female mouse gonad. *Endocrinology* 136, 3665-3668 (1995)

34. Rucker, E.B. 3rd, P. Dierisseau, K.U. Wagner, L. Garrett, A. Wynshaw-Boris, J.A. Flaws & L. Hennighausen: Bcl-x and Bax regulate mouse primordial germ cell survival and apoptosis during embryogenesis. *Mol Endocrinol* 14, 1038-1052 (2000)

35. Pelletier, J., M. Schalling, A.J. Buckler, A. Rogers, D.A. Haber & D. Housman: Expression of the Wilms' tumor gene WT1 in the murine urogenital system. *Genes Dev*. 5, 1345-1356 (1991)

36. Pritchard-Jones, K., S. Fleming, D. Davidson, W. Bickmore, D. Porteous, C. Gosden, J. Bard, A. Buckler, J. Pelletier, D.E. Housman, V. van Heyningen & N. Hastie: The candidate Wilms' tumor gene is involved in genitourinary development. *Nature* 346, 194-197 (1990)

37. Kreidberg, J.A., H. Sariola, J.M. Loring, M. Maeda, J. Pelletier, D. Housman & R. Jaenisch: WT-1 is required for early kidney development. *Cell* 74, 676-691 (1993)

38. Kim, J., D. Prawitt, N. Bardeesy, E. Torban, C. Vicaner, P. Goodyer, B. Zabel and J. Pelletier: The Wilms' tumor suppressor gene (*wt1*) product regulates Dax-1 gene expression during gonadal differentiation. *Mol Cell Biol* 19, 2289-2299 (1999)

39. Muscatelli, F., T.M. Strom, A.P. Walker, E. Zanaria, D. Recan, A. Meindl, B. Bardoni, S. Guioli, G. Zehetner, W. Rabl, H.P. Achwart, J.-C. Kaplan, G. Camerino, T. Meitinger & A.P. Monaco: Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Nature* 372, 672-676 (1994)

40. Bardoni, B., E. Zanaria, S. Guioli, G. Floridia, K.C. Worley, G. Tonini, E. Ferrante, G. Chiumello, E.R. McCabe, M. Fraccaro, O. Zuffardi & G. Camerino: A dosage sensitive locus at chromosome Xp21 is involved in male to female sex reversal. *Nat Genet* 7, 497-501 (1994)

41. Swain, A., E. Zanaria, A. Hacker, R. Lovell-Badge & G. Camerino: Mouse Dax-1 expression is consistent with a role in sex determination as well as in adrenal and hypothalamus function. *Nat Genet* 12, 404-409 (1996)

42. Zazopoulos, E., E. Lalli, D.M. Stocco & P. Sassone-Corsi: DNA binding and transcriptional repression by DAX-1 blocks steroidogenesis. *Nature* 390, 311-315 (1997)

43. Ito, M., R. Yu & J.L. Jameson: DAX-1 inhibits SF-1-mediated transactivation via a carboxy-terminal domain that is deleted in adrenal hypoplasia congenita. *Mol Cell Biol* 17, 1476-1483 (1997)

44. Luo, X., Y. Ikeda & K.L. Parker: A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. *Cell* 77, 481-490 (1994)

45. Eppig, J.J.: Intercommunication between mammalian oocytes and companion somatic cells. *Bioessays* 13, 569-574 (1991)

46. Eppig, J.J., F. Chesnel, Y. Hirao, M.J. O'Brien, F.L. Pendola, S. Watanabe & K. Wigglesworth: Oocyte control of granulosa cell development: how and why. *Hum Reprod* 12, 127-132 (1997)

47. Eppig, J.J.: Oocyte control of ovarian follicular development and function in mammals. *Reproduction* 122, 829-838 (2001)

48. Oxberry, B.A. & G.S. Greenwald: An autoradiographic study of the binding of ^{125}I-labelled follicle-stimulating hormone, human chorionic gonadotropin, and prolactin to the hamster ovary throughout the estrous cycle. *Biol Reprod* 27, 505-516 (1982)

49. Richards, J.S.: Estradiol receptor content in rat granulosa cells during follicular development: modification by estradiol and gonadotropins. *Endocrinology* 97, 1174-1184 (1975)

50. Sicinski, P., J.L. Donaher, Y. Geng, S.B. Parker, H. Gardner, M.Y. Park, R.L. Robker, J.S. Richards, L.K. McGinnis, J.D. Biggers, J.J. Eppig, R.T. Bronson, S.J. Elledge & R.A. Weinberg: Cyclin D2 is an FSH-responsive gene involved in gonadal cell proliferation and oncogenesis. *Nature* 384, 470-474 (1996)

51. Zlotkin, T., Y. Farkash & J. Orly: Cell-specific expression of immunoreactive cholesterol side-chain cleavage cytochrome P-450 during follicular development in the rat ovary. *Endocrinology* 119, 2809-2820 (1986)

52. Bortolussi, M., G. Marini & M.L. Reolon: A histochemical study of the binding of ^{125}I-labelled HCG to the rat ovary throughout the estrus cycle. *Cell Tissue Res* 197, 213-226 (1979)

53. Markstrom, E., E.C. Svensson, R. Shao, B. Svanberg & H. Billig: Survival factors regulating ovarian apoptosis -- dependence on follicle differentiation. *Reproduction* 123, 23-30 (2002)

54. Cecconi, S. & R. Colonna: Influence of granulosa cells and of different somatic cell types on mammalian oocyte development in vitro. *Zygote* 4, 305-307 (1996)

55. Eppig, J.J. & K. Wigglesworth: Development of mouse and rat oocytes in chimeric reaggregated ovaries after interspecific exchange of somatic and germ cell components. *Biol Reprod* 63, 1014-1023 (2000)

56. Colonna, R., S. Cecconi, C. Tatone, F. Mangia & R. Buccione: Somatic cell-oocyte interactions in mouse oogenesis: stage-specific regulation of mouse oocyte protein phosphorylation by granulosa cells. *Dev Biol* 133, 305-308 (1989)

57. De La Fuente, R & J.J. Eppig: Transcriptional activity of the mouse oocyte genome: companion granulosa cells modulate transcription and chromatin remodeling. *Dev Biol* 229, 224-236 (2001)

58. Heller, D.T. & R.M. Schultz: Ribonucleoside metabolism by mouse oocytes: metabolic cooperativity between the fully grown oocyte and cumulus cells. *J Exp Zool* 214, 355-364 (1980)

59. Kumar, N.M.: Molecular biology of the interactions between connexins. *Novartis Found Symp* 219, 6-16 (1999)

60. Dekel, N.: Spatial relationship of follicular cells in the control of meiosis. *Prog Clin Biol Res* 267, 87-101 (1988)

61. Juneja, S.C., K.J. Barr, G.C. Enders & G.M. Kidder: Defects in the germ line and gonads of mice lacking connexin43. *Biol Reprod* 60, 1263-1270 (1999)

62. Orr-Urtreger, A., A. Avivi., Y. Zimmer, D. Givol, Y. Yarden & P. Lonai: Developmental expression of c-*kit*, a proto-oncogene encoded by the *W* locus. *Development* 109, 911-923 (1990)

63. Manova, K., K. Nocka, P. Besmer & R.F. Bachvarova: Gonadal expression of c-*kit* encoded at the *W* locus of the mouse. *Development* 110, 1057-1069 (1990)

64. Horie, K., K. Takakura, S. Taii, K. Narimoto, Y. Noda, S. Nishikawa, H. Nakayama, J. Fujita & T. Mori: The expression of c-*kit* protein during oogenesis and early embryonic development. *Biol Reprod* 45, 547-552 (1991)

65. Horie, K., J. Fujita, K. Takakura, H. Kanzaki, H. Suginami, M. Iwai, H. Nakayama & T. Mori: The expression of c-*kit* protein in human adult and fetal tissues. *Human Reprod* 8, 1955-1962 (1993)

66. Ismail, R., J. Okawara, J.N. Fryer & B.C. Vanderhyden: Hormonal regulation of the ligand for c-*kit* in the ovary and its effects on spontaneous oocyte meiotic maturation. *Mol Reprod Dev* 43, 458-469 (1996)

67. Manova, K., E.J. Huang, M. Angeles, V. De Leon, S. Sanchez, S.M. Pronovost, P. Besmer & R.F. Bachvarova: The expression pattern of the c-*kit* ligand in gonads of mice supports a role for the c-*kit* receptor in oocyte growth and in proliferation of spermatogonia. *Dev Biol* 157, 85-99 (1993)

68. Motro, B. & A. Bernstein: Dynamic changes in ovarian c-*kit* and *Steel* expression during the estrous reproductive cycle. *Dev Dynamics* 197, 69-79 (1993)

69. Laitinen, M., E.M. Rutanen & O. Ritvos: Expression of *c-kit* ligand messenger ribonucleic acids in human ovaries and regulation of their steady state levels by gonadotropins in cultured granulosa-luteal cells. *Endocrinology* 136, 4407-4414 (1995)

70. Ismail, R.S., M. Cada & B.C. Vanderhyden: Transforming growth factor-ß regulates Kit ligand expression in rat ovarian surface epithelial cells. *Oncogene* 18, 4734-4741 (1999)

71. Packer, A.I., Y.C. Hsu, P. Besmer & R.F. Bachvarova: The ligand of the c-kit receptor promotes oocyte growth. *Dev Biol* 161, 194-205 (1994)

72. Kuroda, H., N. Terada, H. Nakayama, K. Matsumoto & Y. Kitamura: Infertility due to growth arrest of ovarian follicles in *Sl/Sl^t* mice. *Dev Biol* 126, 71-79 (1988)

73. Huang, E.J., K. Manova, A.I. Packer, S. Sanchez, R.F. Bachvarova & P. Besmer: The murine steel panda mutation affects kit ligand expression and growth of early ovarian follicles. *Dev Biol* 157, 100-109 (1993)

74. Yoshida, H., N. Takakura, H. Kataoka, T. Kunisada, H. Okamura & S.I. Nishikawa: Stepwise requirement of c-kit tyrosine kinase in mouse ovarian follicle development. *Dev Biol* 184, 122-137 (1997)

75. Parrott, J.A. & M.K. Skinner: Kit-ligand/stem cell factor induces primordial follicle development and initiates folliculogenesis. *Endocrinology* 140, 4262-4271 (1999)

76. Klinger, F.G. & M. De Felici: *In vitro* development of growing oocytes from fetal mouse oocytes: stage-specific regulation by stem cell factor and granulosa cells. *Dev Biol* 244, 85-95 (2002)

77. Huang, E.J., K.H. Nocka, J. Buck & P. Besmer: Differential expression and processing of two cell associated forms of the Kit-ligand: KL-1 and KL-2. *Mol Biol Cell* 3, 349-362 (1992)

78. Tajima, Y., H. Onoue, Y. Kitamura & Y. Nishimune: Biologically active kit ligand growth factor is produced by mouse Sertoli cells and is defective in Sl^d mutant mice. *Development* 113, 1031-1035 (1991)

79. Allard, E.K., K.T. Blanchard & K. Boekelheide: Exogenous stem cell factor (SCF) compensates for altered SCF expression in 2,5-hexanedione-induced testicular atrophy in rats. *Biol Reprod* 55, 185-193 (1996)

80. El-Fouly, M.A., B. Cook, M. Nekola & A.V. Nalbandov: Role of the ovum in follicular luteinization. *Endocrinology* 87, 288-293 (1970)

81. Hubbard, G.M. * G.F. Erickson: Luteinizing hormone-dependent luteinization and ovulation in the hypophysectomized rat: a possible role for the oocyte? *Biol Reprod* 39, 183-1194 (1988)

82. Nekola, M.V. & A.V. Nalbandov: Morphological changes of rat follicular cells as influenced by oocytes. *Biol Reprod* 4, 154-160 (1971)

83. Buccione, R., B.C. Vanderhyden, P.J. Caron & J.J. Eppig: 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 (1990)

84. Vanderhyden, B.C., J.N. Cohen & P. Morley: Mouse oocytes regulate granulosa cell steroidogenesis. *Endocrinology* 133, 423-426 (1993)

85. Vanderhyden, B.C. & A.M. Tonary. Differential regulation of progesterone and estradiol production by mouse cumulus and mural granulosa cells by a factor(s) secreted by the oocyte. *Biol Reprod* 53, 1243-1250 (1995)

86. Vanderhyden, B.C. & E.A. Macdonald: Mouse oocytes regulate granulosa cell steroidogenesis throughout follicular development. *Biol Reprod* 59, 1296-1301 (1998)

87. Eppig, J.J., K. Wigglesworth, F. Pendola & Y. Hirao: Murine oocytes suppress expression of luteinizing hormone receptor messenger ribonucleic acid by granulosa cells. *Biol Reprod* 56, 976-894 (1997)

88. Vanderhyden, B.C., E.E. Telfer & J.J. Eppig: ouse oocytes promote proliferation of granulosa cells from preantral and antral follicles *in vitro*. *Biol Reprod* 46,1196-1204 (1992)

89. Vanderhyden, B.C., P.J. Caron, R. Buccione & J.J. Eppig: 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 (1990)

90. McGrath, S.A., A.F. Esquela & S.J. Lee: Oocyte-specific expression of growth/differentiation factor-9. *Mol Endocrinol* 9, 131-136 (1995)

91. Dube, J.L., P. Wang, J. Elvin, K.M. Lyons, A.J. Celeste & M.M. Matzuk: The bone morphogenetic protein 15 gene is X-linked and expressed in oocytes. *Mol Endocrinol* 12, 1809-1817 (1998)

92. Otsuka, F., Z. Yao, T. Lee, S. Yamamoto, G.F. Erickson & S. Shimasaki: Bone morphogenetic protein-15. Identification of target cells and biological functions. *J Biol Chem* 275, 39523-39528 (2000)

93. Vitt, U.A., M. Hayashi, C. Klein & A.J. Hsueh: Growth differentiation factor-9 stimulates proliferation but suppresses the follicle-stimulating hormone-induced differentiation of cultured granulosa cells from small antral and preovulatory rat follicles. *Biol Reprod* 262, 370-377 (2000)

94. Elvin, J.A., A.T. Clark, P. Wang, N.M. Wolfman & M.M. Matzuk: Paracrine actions of growth differentiation factor-9 in the mammalian ovary. *Mol Endocrinol* 13, 1035-1048 (1999)

95. Elvin, J.A., C. Yan, P. Wang, K. Nishimori & M.M. Matzuk: Molecular characterization of the follicle defects in the growth differentiation factor 9-deficient ovary. *Mol Endocrinol* 13, 1018-1034 (1999)

96. Yan, C., P. Wang, J. DeMayo, F.J. DeMayo, J.A. Elvin, C. Carino, S.V. Prasad, S.S. Skinner, B.S. Dunbar, J.L. Dube, A.J. Celeste & M.M. Matzuk: Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. *Mol Endocrinol* 15, 854-866 (2001)

97. Galloway, S.M., K.P. McNatty, L.M. Cambridge, M.P. Laitinen, J.L. Juengel, T.S. Jokiranta, R.J. McLaren, K. Luiro, K.G. Dodds, G.W. Montgomery, A.E. Beattie, G.H. Davis & O. Ritvos: Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nat Genet* 25, 279-283 (2000)

98. Solloway, M.J., A.T. Dudley, E.K. Bikoff, K.M. Lyons, B.L. Hogan, E.J. Robertson: Mice lacking Bmp6 function. *Dev Genet* 22, 321-339 (1998)

99. Teixeira Filho, F.L., E.C. Baracat, T.H. Lee, C.S. Suh, M. Matsui, R.J. Chang, S. Shimasaki & G.F. Erickson: Aberrant expression of growth differentiation factor-9 in oocytes of women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 87, 1337-1344 (2002)

100. Takebayashi, K., K. Takakura, H. Wang, F. Kimura, K. Kasahara & Y. Noda: Mutation analysis of the growth differentiation factor-9 and -9B genes in patients with premature ovarian failure and polycystic ovary syndrome. *Fertil Steril* 74, 976-979 (2000)

101. Hreinsson, J.G., J.E. Scott, C. Rasmussen, M.L. Swahn, A.J. Hsueh & O. Hovatta: Growth differentiation factor-9 promotes the growth, development, and survival of human ovarian follicles in organ culture. *J Clin Endocrinol Metab* 87, 316-321 (2002)

102. Joyce, I.M., A.T. Clark, F.L. Pendola & J.J.Eppig: 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 (2000)

103. Otsuka, F. & S. Shimasak: A negative feedback system between oocyte bone morphogenetic protein 15 and granulosa cell kit ligand: Its role in regulating granulosa cell mitosis. *Proc Natl Acad Sci USA* 99, 8060-8065 (2002)

104. Gore-Langton, R.E. & D.T. Armstrong: Follicular steroidogenesis and its control. In: The Physiology of Reproduction. Eds: Knobil E, Neill J, Raven Press, New York 331-385 (1988)

105. Parrott, J.A. & M.K. Skinner: Direct actions of kit-ligand on theca cell growth and differentiation during follicle development. *Endocrinology* 138, 3819-3827 (1997)

106. Parrott, J.A. & M.K. Skinner: Kit ligand actions on ovarian stromal cells: effects on theca cell recruitment and steroid production. *Mol Reprod Dev* 55, 55-64 (2000)

107. Parrott, J.A. & M.K. Skinner: Thecal cell-granulosa cell interactions involve a positive feedback loop among keratinocyte growth factor, hepatocyte growth factor, and Kit ligand during ovarian follicular development. Endocrinology 139, 2240-2245 (1998)

108. Ito, M., T. Harada, M. Tanikawa, A. Fujii, G. Shiota, N. Terakawa: Hepatocyte growth factor and stem cell factor involvement in paracrine interplays of theca and granulosa cells in the human ovary. *Fertil Steril* 75, 973-979 (2001)

109. Solovyeva, E.V., M. Hayashi, K. Margi, C. Barkats, C. Klein, A. Amsterdam, A.J. Hsueh & A. Tsafriri: Growth differentiation factor-9 stimulates rat theca-interstitial cell androgen biosynthesis. *Biol Reprod* 63, 1214-1218 (2000)

110. Yamamoto, N., L.K. Christenson, J.M. McAllister, J.F. Strauss 3rd: Growth differentiation factor-9 inhibits 3'5'-adenosine monophosphate-stimulated steroidogenesis in human granulosa and theca cells. *J Clin Endocrinol Metab* 87, 2849-2856 (2002)

111. Eppig, J.J. & A.C. Schroeder: Capacity of mouse oocytes from preantral follicles to undergo embryogenesis and development to live young after growth, maturation, and fertilization *in vitro*. *Biol Reprod* 41, 268-76 (1989)

112. Cho, W.K., S. Stern & J.D. Biggers: Inhibitory effect of dibutyryl cAMP on mouse oocyte maturation *in vitro*. *J Exp Zool* 187, 183-186 (1974)

113. Dekel, N. & W.H. Beers: Rat oocyte maturation *in vitro*: relief of cAMP inhibition by gonadotropins. *Proc Natl Acad Sci* 75, 4369-4373 (1978)

114. Eppig, J.J., P.F. Ward-Bailey& D.L. Coleman: Hypoxanthine and adenosine in murine ovarian follicular fluid: concentrations and activity in maintatining oocyte meiotic arrest. *Biol Reprod* 33, 1041-1049 (1985)

115. Takahashi, M., S.S. Koide & P.K. Donahoe: Müllerian inhibiting substance as oocyte meiosis inhibitor. *Mol Cell Endocrinol* 47, 225-234 (1986)

116. Richard, F.J., A. Tsafriri & M. Conti: Role of phosphodiesterase type 3A in rat oocyte maturation. *Biol Reprod* 65, 1444-51 (2001)

117. Thomas, R.E., D.T. Armstrong & R.B. Gilchrist: Differential effects of specific phosphodiesterase isoenzyme inhibitors on bovine oocyte meiotic maturation. *Dev Biol* 244, 215-225 (2002)

118. Mayes, M.A. & M.A. Sirard: Effect of type 3 and type 4 phosphodiesterase inhibitors on the maintenance of bovine oocytes in meiotic arrest. *Biol Reprod* 66, 180-184 (2002)

119. Abrieu, A., M. Doree & D. Fisher: The interplay between cyclin-B-Cdc2 kinase (MPF) and MAP kinase during maturation of oocytes. *J Cell Sci* 114, 257-267 (2001)

120. Hampl, A. & J.J. Eppig: Analysis of the mechanism(s) of metaphase I arrest in maturing mouse oocytes. *Development* 121, 925-933 (1995)

121. Winston, N.J.: Stability of cyclin B protein during meiotic maturation and the first mitotic cell division in mouse oocytes. *Biol Cell* 89, 211-219 (1997)

122. Chesnel, F. & J.J. Eppig: Synthesis and accumulation of p34cdc2 and cyclin B in mouse oocytes during acquisition of competence to resume meiosis. *Mol Reprod Dev* 40, 503-508 (1995)

123. Lincoln, A.J., D. Wickramasinghe, P. Stein, R.M. Schultz, M.E. Palko, M.P. De Miguel, L. Tessarollo, P.J. Donovan : Cdc25b phosphatase is required for resumption of meiosis during oocyte maturation. *Nat Genet* 30, 446-449 (2002)

124. Masui,Y.: From oocyte maturation to the *in vitro* cell cycle: the history of discoveries of Maturation-Promoting

Factor (MPF) and Cytostatic Factor (CSF). *Differentiation* 69, 1-17 (2001)

125. Ismail, R.S., M. Dube & B.C. Vanderhyden: Hormonally regulated expression and alternative splicing of kit ligand may regulate kit-induced inhibition of meiosis in rat oocytes. *Dev Biol* 184, 333-342 (1997)

126. Motro, B. & A. Bernstein: Dynamic changes in ovarian c-*kit* and *Steel* expression during the estrous reproductive cycle. *Dev. Dynamics* 197, 69-79 (1993)

127. Bennett, D.: Developmental analysis of a mutation with pleiotropic effects in the mouse. *J Morphol* 98, 199-233 (1956)

128. Thibault, C.: Are follicular maturation and oocyte maturation independent processes? *J Reprod Fertil* 51, 1-15 (1977)

129. Vanderhyden, B.C. & D.T. Armstrong: Role of cumulus cells and serum on the in vitro maturation, fertilization, and subsequent development of rat oocytes. *Biol Reprod* 40, 720-728 (1989)

130. Armstrong, D.T., X. Zhang, B.C. Vanderhyden & F. Khamsi: Hormonal actions during oocyte maturation influence fertilization and early embryonic development. *Ann NY Acad Sci* 626, 137-158 (1991)

131. Ka, H.H., K. Sawai, W.H. Wang, K.S. Im & K. Niwa: Amino acids in maturation medium and presence of cumulus cells at fertilization promote male pronuclear formation in porcine oocytes matured and penetrated in vitro. *Biol Reprod* 57, 1478-1483 (1997)

132. Chen, L., P.T. Russell & W.J. Larsen: Functional significance of cumulus expansion in the mouse: roles for the preovulatory synthesis of hyaluronic acid within the cumulus mass. *Mol Reprod Dev* 34, 87-93 (1993)

133. Hess, K.A., L. Chen, W.J. Larsen: Inter-alpha-inhibitor binding to hyaluronan in the cumulus extracellular matrix is required for optimal ovulation and development of mouse oocytes. *Biol Reprod* 61, 436-443 (1999)

134. Lim, H., B.C. Paria, S.K. Das, J.E. Dinchuk, R. Langenbach, J.M. Trzaskos & S.K. Dey: Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* 91, 197-208 (1997)

135. Davis, B.J., D.E. Lennard, C.A. Lee, H.F. Tiano, S.G. Morham, W.C. Wetsel & R. Langenbach: Anovulation in cyclooxygenase-2-deficient mice is restored by prostaglandin E2 and interleukin-1beta. *Endocrinology* 140, 2685-2695 (1999)

136. Hizaki, H., E. Segi, Y. Sugimoto, M. Hirose, T. Saji, F. Ushikubi, T. Matsuoka, Y. Noda, T. Tanaka, N. Yoshida, S. Narumiya & A.Ichikawa: Abortive expansion of the cumulus and impaired fertility in mice lacking the prostaglandin E receptor subtype EP(2). *Proc Natl Acad Sci USA* 96, 10501-10506 (1999)

137. Salustri, A., S. Ulisse, M. Yanagishita & V.C. Hascall: Hyaluronic acid synthesis by mural granulosa cells and cumulus cells in vitro is selectively stimulated by a factor produced by oocytes and by transforming growth factor-beta. *J Biol Chem* 265, 19517-19523 (1990)

138. Tirone, E., C. D'Alessandris, V.C. Hascall, G. Siracusa & A. Salustri: Hyaluronan synthesis by mouse cumulus cells is regulated by interactions between follicle-stimulating hormone (or epidermal growth factor) and a soluble oocyte factor (or transforming growth factor beta1). *J Biol Chem* 272, 4787-4794 (1997)

139. Canipari, R., O. Epifano, G. Siracusa & A. Salustri: Mouse oocytes inhibit plasminogen activator production by ovarian cumulus and granulosa cells. *Dev Biol* 167, 371-378 (1995)

140. Vanderhyden. B.C., E. Nagyova and A. Dhawan: Are members of the TGF-beta superfamily the oocyte factors that control cumulus expansion and steroidogenesis? *Reproduction*, in press

141. Clark, B.J., J. Wells, S.R. King & D.M. Stocco: The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR). *J Biol Chem* 269, 28314-28322 (1994)

142. Clark, B.J. & D.M. Stocco: Expression of the steroidogenic acute regulatory (StAR) protein: a novel LH-induced mitochondrial protein required for the acute regulation of steroidogenesis in mouse Leydig tumor cells. *Endocr Res* 21, 243-257 (1995)

143. Lubahn, D.B., J.S. Moyer, T.S. Golding, J.F. Couse, K.S. Korach & O. Smithies: Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. *Proc Natl Acad Sci USA* 90, 11162-11166 (1993)

144. Robker, R.L. & J.S. Richards: Hormonal control of the cell cycle in ovarian cells: proliferation versus differentiation. *Biol Reprod* 59, 476-482 (1998)

145. Hillensjo, T., C. Magnusson, U. Svensson & H. Thelander. Effect of LH and FSH on steroid synthesis by cultured rat cumulus cells. In: Dynamics of ovarian function. Raven Press, New York 105-110 (1981)

146. Seifer, D.B., M.R. Freeman, A.C. Gardiner, G.A. Hill, A.L. Schneyer & B.C. Vanderhyden: Autologous granulosa cell co-culture demonstrates zygote suppression of granulosa cell steroidogenesis. *Fert Steril* 66, 425-429 (1996)

147. Coskun, S., M. Uzumcu, Y.C. Lin, C.I. Friedman & B.M. Alak: Regulation of cumulus cell steroidogenesis by the porcine oocyte and preliminary characterization of oocyte-produced factor(s). *Biol Reprod* 53, 670-675 (1995)

148. Tischkau, S.A. & J.M. Bahr: Avian germinal disc region secretes factors that stimulate proliferation and inhibit progesterone production by granulosa cells. *Biol Reprod* 53, 670-675 (1996)

149. Sretarugs, P. & R.A. Wallace: The developing Xenopus oocyte specifies the type of gonadotropin-stimulated steroidogenesis performed by its associated follicle cells. *Develop Growth Differ* 39, 87-97 (1997)

150. Otsuka, F., R.K. Moore & S. Shimasaki: Biological function and cellular mechanism of bone morphogenetic protein-6 in the ovary. *J Biol Chem* 276, 32,889-32,895 (2001)

151. Otsuka, F., S. Yamamoto, G.F. Erickson & S. Shimasaki: Bone morphogenetic protein-15 inhibits follicle-stimulating hormone (FSH) action by suppressing FSH receptor expression. *J Biol Chem* 276, 11,387-11,392 (2001)

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