Ovarian follicle development and transgenic mouse models


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Ovarian follicle development is a complex process that begins with the establishment of what is thought to be a finite pool of primordial follicles and culminates in either the atretic degradation of the follicle or the release of a mature oocyte for fertilization. This review highlights the many advances made in understanding these events using transgenic mouse models. Specifically, this review describes the ovarian phenotypes of mice with genetic mutations that affect ovarian differentiation, primordial follicle formation, follicular growth, atresia, ovulation and corpus luteum (CL) formation. In addition, this review describes the phenotypes of mice with mutations in a variety of genes, which affect the hormones that regulate folliculogenesis. Because studies using transgenic animals have revealed a variety of reproductive abnormalities that resemble many reproductive disorders in women, it is likely that studies using transgenic mouse models will impact our understanding of ovarian function and fertility in women.

Key words: follicle development/ovary/transgenic mouse models

Introduction

The ovary is a primary functional organ of the female reproductive system, and it plays two major physiological roles. First, the ovary is responsible for the differentiation and release of a mature oocyte for fertilization (McGee and Hsueh, 2000). Second, it is responsible for synthesizing and secreting hormones that are essential for follicle development, menstrual/estrous cyclicity and maintenance of the reproductive tract and its function (Hirshfield, 1991). During the past several years, studies using transgenic mouse models have revealed novel information about the genes that control the processes of ovarian development and function. Some of these genes affect the ovary through direct mechanisms, whereas others do so through indirect mechanisms. Therefore, the purpose of this review is to highlight the advances made in understanding these events through the use of transgenic mouse models. Specifically, this review describes the ovarian phenotypes of mice with genetic mutations that directly affect ovarian differentiation, primordial follicle formation, follicular growth, atresia, ovulation and corpus luteum (CL) formation. In addition, this review describes the phenotypes of mice with mutations in a variety of genes that indirectly affect the ovary because they affect the hormones or growth factors that regulate folliculogenesis.

Mouse models with alterations in ovarian differentiation

Differentiation of a functional ovary from a bipotential gonad is a complex process (Tevosian et al., 2002). The bipotential gonad arises from the coelomic epithelium of the urogenital ridges and initially is indistinguishable in male and female mouse embryos (Cui et al., 2004). During embryonic life, the bilateral gonad undergoes a series of changes, which results in the formation of the ovary. Although relatively little information is known about the factors absolutely required for the differentiation of the ovary, some studies indicate that Podl1 (also called capsulin/epicardin/Tcf21) is an important regulator of gonadal development (Cui et al., 2004). Specifically, deletion of Podl1 disrupts proper differentiation of the ovary, because Podl1-deficient (Podl1–/–) ovarian germ cells resemble germ cells in the testes (Cui et al., 2004). This may be, in part, because of a failure to appropriately downregulate steroidogenic factor 1 (Sf1) (Hanley et al., 2000; Cui et al., 2004). Sf1 expression in the gonads has been shown to be essential for reproductive function, although wild type (WT) and Sf1–/– ovaries are indistinguishable during embryogenesis and at birth (Jeyasuria et al., 2004).

Forkhead transcription factor 2 (Foxl2) is also an important regulator of ovarian differentiation because Foxl2–/– ovaries express markers of testis determination shortly after birth (Ottolenghi et al., 2005). Other genes, such as Dax1, Wnt4 and follistatin (Fst), are important, but not absolutely required, for ovarian differentiation because their deletion does not result in complete female-to-male sex reversal (Swain et al., 1996; Vainio et al., 1999; Uda et al., 2004; Yao et al., 2004). Instead, deletion of Fst and Wnt4 leads to the appearance of a testis-specific coelomic vessel (Jeyes-Ward et al., 2003; Yao et al., 2004). Taken together, these studies suggest that Fst and Wnt4 must be expressed within the ovary to inhibit the testis differentiation pathway and promote the formation of the ovary. To date, little is known about whether...
WNT and FST play a role in ovarian differentiation in humans. A mutation in WNT4 has been shown in a female patient whose phenotype resembles Wnt4−/− mice (Baison-Lauber et al., 2004). Furthermore, abnormal FST levels have been linked to polycystic ovary syndrome (PCOS), a leading cause of infertility in women (Mason, 2000).

Mouse models with alterations in the formation of primordial germ cells

A founding population of primordial germ cells (PGCs) is allocated during gastrulation outside of the developing gonad, to which PGCs migrate by embryonic day (ED) 11 (Hirshfield, 1991; McClellan et al., 2003). The PGCs that reach the gonad, now referred to as oogonia, lose their motile characteristics and undergo extensive mitotic proliferation and apoptosis (Hirshfield, 1991). Many factors are involved in the allocation and expansion of the germ cell population. Recent studies using transgenic mouse models provide evidence that various bone morphogenetic proteins (BMPs) are involved in the allocation of the founder population of PGCs. For example, studies using Bmp4−/− embryos indicate that BMP4 is required for PGC allocation, as Bmp4−/− embryos are devoid of PGCs (Lawson et al., 1999; Ying and Zhao, 2001). Similarly, studies using Bmp8b−/− embryos demonstrate its importance for PGC allocation, although its absolute requirement depends on the genetic strain used (Ying et al., 2000).

BMPs bind to the type 1 activin-receptor-like kinase (ALK) receptors ALK2, ALK3 or ALK6 to regulate gene expression (Chen et al., 2004). ALK2−/− embryos have no detectable PGCs, suggesting that a factor induced by BMP4 signalling through ALK2 is essential for the formation of the PGC founder population (Chuva de Sousa Lopes et al., 2004). BMP signalling is mediated, in part, by SMAD5. Interestingly, Smad5−/− embryos have a significant reduction in the size of the founder population of PGCs (Chang and Matzuk, 2001). This fact, together with similar spatio-temporal expression patterns in BMPs and SMAD5, suggests that SMAD5 may be a signalling intermediate for BMP4 and BMP8b during PGC allocation (Chang and Matzuk, 2001; Chuva de Sousa Lopes et al., 2004).

Interactions between PGCs and the extracellular matrix (ECM) are thought to be important for PGC migration (Garcia-Castro et al., 1997). ECM-interacting integrins are expressed by PGCs and are necessary for colonization of the genital ridges, because nearly 70% of PGCs lacking the integrin β1 subunit are unable to enter the gonad (Anderson et al., 1999). Gap junction communication, either between PGCs themselves or between PGCs and somatic cells, may also be important for colonization of the genital ridges or PGC survival, as connexin (Cx)43-deficient (Gja1−/−) mice have a reduced number of PGCs by ED 11.5 (Juneja et al., 1999).

Stromal cell-derived factor 1 (Sdf1) and its receptor Cxcr4 have also been shown to be important for PGC migration (Ara et al., 2003; Molyneaux et al., 2003b). In both Sdf1−/− and Cxcr4−/− embryos, there is a large reduction in the number of PGCs that enter the gonad, with many PGCs remaining in the hindgut endoderm or mesentery (Ara et al., 2003; Molyneaux et al., 2003b). Naturally occurring mutations in the Steel (Sl) and Dominant white spotting (W) loci, which encode kit ligand KL and c-kit, respectively, lead to germ cell deficiency and improper PGC migration (Buehr et al., 1993). Specifically, studies have shown that some mutations in KLSL lead to a migratory defect, resulting in an absence of PGCs in mutant genital ridges at ED 11.5 in a dose-dependent manner (Zama et al., 2005).

Many factors are involved in PGC survival, including zinc-finger transcription factor (Zfx) and Oct4. Deletion of Zfx results in a 50% reduction in the number of PGCs at ED 11.5 and results in early ovarian failure (Luoh et al., 1997). Similarly, germ cell-specific deletion of Oct4 leads to massive PGC apoptosis around ED 10 and an absence of oocytes in post-natal ovaries (Kehler et al., 2004). T-cell intracellular antigen-1-related (TIAR) protein has been shown to promote the survival and the proliferative activity of PGCs. Specifically, Tiar−/− mice contain substantially fewer PGCs at ED 11.5 compared with WT mice and completely lack oogonia at ED 13.5 (Beck et al., 1998).

A factor known as proliferation of germ (pog) cells is important for maintaining germ cell proliferation (Agoulnik et al., 2002). Specifically, loss of pog cells is responsible for the germ cell-deficient (gcd) phenotype, in which animals are born with a dramatically reduced germ cell population. Therefore, females are infertile because of an absence of germ cells in adults (Agoulnik et al., 2002). This phenotype is also seen in patients with Fanconi anemia (FANCL), in which patients have an autosomal recessive disorder characterized by cellular hypersensitivity to DNA cross-linking agents. As in the human disease, Fanca−/− mice show hypogonadism (Cheng et al., 2000; Wong et al., 2003). Furthermore, at ED 8.5, Fanca−/− and WT embryos have similar numbers of PGCs, but by ED 11.5, Fanca−/− mice have 50% fewer germ cells than WT embryos (Wong et al., 2003), leading to early ovarian failure due to early depletion of oocytes (Koomen et al., 2002).

Homologues of the Drosophila RNA-binding protein, NANOS, are also thought to be regulators of PGC proliferation (Haraguchi et al., 2003; Tsuda et al., 2003). Whereas NANOS1 and NANOS2 are dispensable for female fertility, NANOS3 is essential, because Nanos3−/− females are infertile (Haraguchi et al., 2003; Tsuda et al., 2003). Normal numbers of PGCs are allocated and seen migrating at ED 9.5 in Nanos3−/− embryos, but there are no PGCs remaining in mutant gonads by ED 12.5, suggesting that Nanos3 is important for PGC maintenance or proliferation after ED 9.5 (Tsuda et al., 2003).

Although PGC proliferation appears to be maintained by extrinsic regulation, the rate of proliferation appears to be regulated by the PGCs themselves (De Felici et al., 2004). Studies using the Pin1−/− model provide evidence for this. PIN1 is a peptidyl-prolyl isomerase that affects protein stability and/or function and is also involved in cell-cycle progression (Joseph et al., 2003). Furthermore, PGCs express PIN1 through migration at least until ED 13.5, and whereas a normal number of PGCs are allocated in Pin1−/− embryos, their population expands very slowly compared with WT embryos (Atchison et al., 2003). The slow rate of expansion in the PGC population of Pin1−/− embryos appears to be because of inefficient cell-cycle progression leading to a lengthening of the cell cycle (Atchison et al., 2003).

Members of the B-cell lymphoma/leukaemia 2 (Bcl2) family have been shown to regulate apoptosis of meiotic germ cells (Morita et al., 1999). Targeted overexpression of Bcl2, an anti-apoptotic member of the Bcl2 family, in germ cells enhances germ cell survival so that females are born with a surfeit of follicles (Flaws et al., 2001). Bclxl is another anti-apoptotic member of the Bcl2 family. Studies indicate that this factor is an important
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regulator of oocyte apoptosis in the embryo because reduced Bclxl function results in a shortage of follicles at birth due to decreased oocyte survival beginning at ED 13.5 (Rucker et al., 2000). Bax, a pro-apoptotic family member, has been reported not to affect follicle endowment (Perez et al., 1999), which is surprising given that Bax deletion rescues the Bclxl reduced function phenotype (Rucker et al., 2000).

Caspases (Casps) have also been shown to regulate apoptosis in oocytes. Specifically, deletion of Casp11 (also known as Casp4) significantly reduces the number of oocytes present at birth (Morita et al., 2001). Also, studies demonstrate that oocyte loss due to cytokine insufficiency in Casp11–/– mice is dependent on the action of Casp2, because co-deletion of Casp2 restores oocyte numbers to normal (Morita et al., 2001).

Mouse models with alterations in primordial follicle formation

Oogonia cease dividing at ED 13.5 and enter meiosis to form oocytes. These oocytes are closely associated in clusters called germ cell nests (Gomperts et al., 1994; Pepling and Spradling, 1998). Germ cell nests break down shortly after birth to allow the formation of primordial follicles, a process involving apoptosis of some of the oocytes within the nests. The surviving oocytes become enveloped by somatic cells surrounding the nest, thereby producing primordial follicles (Figure 1) (Pepling and Spradling, 2001).

The primordial follicle population present at birth has long been believed to be finite (Zuckerman, 1951), although this notion has recently been challenged (Johnson et al., 2004, 2005). Establishment of this pool of primordial follicles marks the first stage of follicle development (growth of follicles from the primordial to pre-ovulatory stage), as defined by Hirshfield (1991). The end of reproductive life or ovarian senescence occurs when this pool of primordial follicles is depleted by death or through growth, followed by subsequent ovulation and/or atresia (Hirshfield, 1991). Although some primordial follicles will be stimulated to grow immediately, the majority will remain dormant, perhaps because of inhibitory stimuli, until they receive signals to enter the growing pool (McGee and Hsueh, 2000).

Although the processes of germ cell nest breakdown and primordial follicle formation are not well understood, several genetic models have identified factors important for their regulation. The Dazla gene, in particular, has been shown to be essential for the differentiation of germ cells because disruption leads to reduction in germ cell numbers embryonically and complete absence of follicles and ova in the adult ovary (Ruggiu et al., 1997; McNeilly et al., 2000). An oocyte-specific gene, factor in the germline α (Figla), is also essential for follicle formation as Figla–/– females

**Figure 1.** Germ cell nest breakdown and primordial follicle formation. During embryonic life, germ cells are closely associated in clusters called germ cell nests. Around birth, the germ cell nests break down to allow the formation of primordial follicles, a process involving apoptosis of some of the oocytes within the nests. The surviving oocytes become enveloped by somatic cells surrounding the nest, thereby producing primordial follicles.
are infertile despite the presence of normal numbers of meiotically competent germ cells in embryonic ovaries (Soyal et al., 2000). By post-natal day (PD) 1, Figlo–/+ oocytes begin to disappear and those that are present are not matured by somatic cells, suggesting that Figlo is important for regulating interactions between oocytes and granulosa cells (Soyal et al., 2000). Interestingly, Figlo has been linked to premature ovarian failure (POF) in women (Pangas and Rajkovic, 2006).

Mice lacking nerve growth factor (NGF; Ngf–/+ ) also appear to have a deficiency in germ cell nest breakdown, consequently reducing the number of growing follicles in the ovary (Dissen et al., 2001). Tyrosine kinase (Trk) receptors are receptors for NGF and may also play a role in follicle formation (Donovan et al., 1996). Specifically, studies have shown that deletion of TrkB reduces the number of follicles present in neonatal mice because of decreased oocyte survival during the period of follicle formation (Donovan et al., 1996). There is some evidence that NGF is involved in the pathogenesis of PCOS in women (Bai et al., 2004).

Studies have shown that proteins such as Spo11 [sporulation protein homologue (S. cerevisiae)], DMC1 [disrupted meiotic cDNA 1 homologue (human)] and MSH5 [mutS homologue 5 (Escherichia coli)] are also important for the formation of primary follicles (Di Giacomo et al., 2005). Spo11 is an enzyme required for the introduction of double-strand breaks during meiosis. Spo11–/+ ovaries have a reduced number of primordial follicles compared with WT ovaries due to oocyte depletion at or before follicle formation, which is independent of DNA damage arising from unrepaired recombination intermediates (Di Giacomo et al., 2005).

DMC1 is a DNA strand exchange protein that acts on double-strand breaks to catalyze strand invasion into intact homologous products, giving rise to mature recombinant products. Dmc1–/+ mice are viable but infertile because of defects in chromosome synopsis (Bannister and Schimenti, 2004). Furthermore, Dmc1–/+ ovaries are devoid of follicles, due to the elimination of oocytes before follicle formation (Di Giacomo et al., 2005). Spo11–/+ / Dmc1–/+ double-mutant mice mirror the Spo11–/+ phenotype. In these animals, the introduction of double-strand breaks is eliminated, indicating that oocytes die in Dmc1–/+ ovaries more rapidly because of a DNA damage-dependent mechanism (Di Giacomo et al., 2005).

MSH5 is another meiosis-specific protein involved in recombination. Msh5–/+ mice are sterile as a result of severe gametogenic failure (Di Giacomo et al., 2005). As in Dmc1–/+ mice, deletion of Msh5 results in reduced oocyte numbers due to a DNA damage-dependent mechanism. Spo11–/+ Msh5–/+ double knockouts are also identical to Spo11–/+ single mutants (Di Giacomo et al., 2005).

In addition to the above proteins, the atm gene [ataxia telangectasia-mutated homologue (humans)] is involved in DNA damage checkpoint control and is activated in response to double-strand breaks (Yamada and Coffman, 2005). Atm–/+ females are infertile because of abnormal chromosome synopsis and fragmentation (Barlow et al., 1996; Xu et al., 1996; Barlow et al., 1998). There is increased apoptosis of oocytes in embryonic atm–/+ ovaries compared with WT ovaries, and shortly after birth atm–/+ ovaries are devoid of follicles. Oocyte loss in atm–/+ ovaries may be because of unrepaired double-strand breaks introduced by SPO11 as Spo11–/+atm–/+ double mutants are also indistinguishable from Spo11-deficient mice (Di Giacomo et al., 2005).

Collectively, transgenic models with mutations in meiotic genes provide evidence that several meiotic genes play an essential role in meiotic chromosome synopsis and gametogenesis (Mandon-Pepin et al., 2002). Their phenotypes are supported clinically, because mutations in DMC1 have been found in patients with POF (Mandon-Pepin et al., 2002). Furthermore, many of the mouse models discussed in this section may shed light on the recent claims that oogenesis continues in the post-natal ovary. Specifically, the deletion of Dazla, Bcl-xl and Zfx refute these claims as these knockout mice all experience POF, apparently due to prenatal germ cell depletion.

Mouse models with alterations in primary follicle development

Once in the growing pool, the primordial follicle enlarges because of an increase in size of the oocyte and conversion of the squamous granulosa cells into cuboidal granulosa cells. At this time, the follicle is known as a primary follicle (Figure 2) (Hirshfield, 1991). One striking characteristic of the primary follicle is the presence of a zona pellucida, which surrounds the oocyte and is maintained throughout growth until the oocyte is ovulated (Rankin et al., 1996, 1999, 2001). The extracellular zona pellucida matrix is composed of three glycoproteins (ZP1, ZP2 and ZP3) (Rankin et al., 1999). These proteins have been shown to affect folliculogenesis in that Zp1–/+ mice have loosely organized zonae pellucidae and reduced litter sizes compared with WT mice (Rankin et al., 1999, 2001). Zp2–/+ mice have a thin zona pellucida surrounding oocytes and are sterile (Rankin et al., 2001). Zp3–/+ mice do not form a zona pellucida and are sterile (Rankin et al., 1996). Interestingly, genetic variations in ZPs have also been associated with fertilization failure in patients undergoing IVF (Mannikko et al., 2005).

An oocyte-specific factor, NOBOX, is important for primary follicle formation as nobox–/– mice have accelerated post-natal oocyte loss, and their follicles do not transition from the primordial stage into the growing follicle pool (Rajkovic et al., 2004). Foxl2 has also been shown to play a role in folliculogenesis at the primary follicle stage. In Foxl2–/– ovaries, granulosa cell differentiation is blocked at the squamous to the cuboidal transition; thus, no primary follicles are formed (Schmidt et al., 2003). Foxo3a has also been identified as being a major activator of follicular development as Foxo3a–/– ovaries have a tremendous increase in the number of early growing follicles with enlarged oocytes at PD 14 (Castrillon et al., 2003). Furthermore, there appears to be an apparent lack of co-ordination between oocytes and granulosa cells in these follicles because oocyte enlargement is not accompanied by a transition of granulosa cells from flattened to cuboidal shape (Castrillon et al., 2003). Thus, the early activation of primordial follicles leads to premature exhaustion of the follicle pool (Castrillon et al., 2003). To our knowledge, there is no clinical evidence that genetic alterations in Foxo3a cause POF in women. Mutations in Foxl2 and NOBOX, however, have been linked to POF (Gersak et al., 2004; Pangas and Rajkovic, 2006).

Mouse models with alterations in pre-antral follicle development

The primary follicle transitions into a pre-antral follicle as the granulosa cells proliferate to form multiple layers (Figure 2). In addition, the pre-antral follicle acquires an outer layer of thecal...
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Atretic Follicle

Figure 2. Ovarian follicle development. Folliculogenesis begins with the establishment of a finite pool of primordial follicles. Primordial follicles must grow to the primary, pre-antral and antral stages before they reach the pre-ovulatory stage and are capable of releasing an oocyte for fertilization. After ovulation, the remaining granulosa and thecal cells differentiate into a structure known as the corpus luteum (CL). At the antral stage, however, 99.9% of antral follicles will undergo atresia (follicle death).

Germ Cells

Primordial

Primary

Pre-antral

Antral

Somatic Cells

Corpus Luteum

Pre-ovulatory

Germ Cells Primary Antral Atretic Follicle

Primordial Pre-antral

Somatic Cells Corpus Luteum Pre-ovulatory

Prenatal Life Postnatal Life

cells. At the end of this stage, the pre-antral follicle has several layers of granulosa cells and has acquired an extensive network of gap junctions (Hirshfield, 1991).

Gap junctions are intercellular membrane channels that allow nutrients, inorganic ions, second messengers and small metabolites to pass from cell to cell (Kidder and Mhawi, 2002). Each of these intercellular channels is composed of CXS, a family of proteins that connect adjacent cells (Kidder and Mhawi, 2002). Studies using mice lacking Cx43 (Gja1–/–) and Cx37 (Gja4–/–) provide evidence that gap junctions are important for follicular development to the pre-antral stage (Simon et al., 1997; Ackert et al., 2001). Cx43, the most abundant CX in the ovary, is expressed in the granulosa cells from the start of folliculogenesis. In Gja1–/– mice, folliculogenesis is arrested at the primary stage and the follicles contain meiotically incompetent oocytes (Carabatsos et al., 2000; Ackert et al., 2001). Furthermore, gap junctions are extremely rare in Gja1–/– granulosa cells, although these cells are still able to form gap junctions with WT granulosa cells (Gittens et al., 2003, 2005). This suggests that CX43 is not the only CX expressed during the early stages of folliculogenesis (Gittens et al., 2003, 2005). Whereas Cx43 is required for the earliest stages of development (Juneja et al., 1999), Cx37 has also been shown to be important (Simon et al., 1997; Juneja, 2003). Cx37 is expressed mainly between the oocyte and surrounding granulosa cells (Simon et al., 1997). In Gja4–/– mice, folliculogenesis is arrested at the pre-antral stage and the oocytes are not meiotically competent (Carabatsos et al., 2000).

Glutathione (GSH), which plays a role in protecting cells from oxidative stress and cellular injury, may also be involved in regulating folliculogenesis to the pre-antral follicle stage (Kumar et al., 2000). γ-Glutamyl transpeptidase (GGT) is responsible for converting secreted GSH into cysteinyl-glycine and γ-glutamic acid (Kumar et al., 2000). Transgenic mice deficient in GGT are infertile because of a block in folliculogenesis at the pre-antral stage and an increased number of degenerating oocytes compared with WT mice (Kumar et al., 2000).

Mouse models with alterations in antral follicle development

The next phase of folliculogenesis, the antral stage, is marked by the appearance of small fluid-filled spaces, which eventually form
a single antral cavity (Figure 2). The follicular fluid that fills the antral cavity contains water, electrolytes, serum proteins and high concentrations of steroid hormones secreted by the granulosa cells (Hirshfield, 1991). At the antral stage, most of the follicles will undergo atresia, whereas the remaining antral follicles will survive under the influence of FSH and grow to the pre-ovulatory stage (Hirshfield, 1991).

Atresia is an apoptotic process that is highly regulated by pro-apoptotic and anti-apoptotic factors. Members of the Bcl2 family are important for regulating the atretic degradation of antral follicles. Specifically, studies have shown that deletion of Bcl2 reduces healthy follicle numbers and increases abnormal follicle numbers compared with WT ovaries (Ratts et al., 1995). Furthermore, targeted overexpression of Bcl2 to granulosa cells of growing follicles results in reduced apoptosis of granulosa cells, more follicles that spontaneously ovulate when given exogenous pregnant mare’s serum gonadotrophin (PMSG) and larger litter sizes (Knudson et al., 1995) and reduces healthy follicle numbers and increases abnormal follicle numbers compared with WT ovaries (Ratts et al., 1995). In the mouse ovary, both eNOS and iNOS are present in the oocytes and thecal cells (Mitchell et al., 2004). Studies using eNos–/– mice have demonstrated that they have reduced fertility due to impaired ovulatory efficiency, abnormalities in meiotic maturation, increased oocyte apoptosis and altered estrous cyclicity compared with their WT littermates (Jablonska-Shariff and Olson, 1998; Drazen et al., 1999; Heffler and Gregg, 2002). Studies using iNos–/– mice, however, show that iNos does not alter ovulatory capacity, but it may play a role in fertilization (Yang et al., 2005). Conditional nNos–/– mice are infertile because of ovulation defects as well as altered hormonal regulation (Gyurko et al., 2002).

Macrophage-stimulating factor 1 receptor (Mstr1) or Ron is involved in the regulation of iNOS and the production of NO during inflammatory responses (Hess et al., 2003). Specifically, RON inhibition has been shown to increase NO activity (Chen et al., 1998; Waltz et al., 2001). Transgenic mice deficient in Ron are infertile because of abnormalities in ovulation (Waltz et al., 2001). The ovulation defect in these mice is due to elevated levels of NO caused by increased iNOS levels (Hess et al., 2003).

The cyclooxygenase (COX) pathway, which responsible for in prostaglandin (PG) synthesis, is very similar to the NO pathway in that both pathways are important regulators of inflammatory responses and both have constitutive and inducible isozymes (McGarry et al., 2005). Furthermore, both pathways are able to crosstalk with other pathways. Specifically, there is crosstalk between NO and PGs and/or iNOS and COX (Clancy et al., 2000). PGs are common mediators of many inflammatory responses, including ovulation. Female mice lacking PGE receptor 2 (Ep2) have defects in ovulation due to defects in cumulus–oocyte complex (COC) expansion (Hizaki et al., 1999).

The two COX isozymes responsible for the synthesis of PGs are COX1 and COX2. COX1 is the constitutive form, whereas COX2 is inducible by a variety of factors including growth factors, cytokines, mitogens and tumour promoters (Smith and Langenbach, 2001). In the ovary, LH/hCG induces COX2 in the mural granulosa cells (Matsumoto et al., 2001). Although Cox1–/– mice have normal fertility, except for some defects in parturition, Cox2–/– females are infertile and exhibit abnormalities in ovulation due to PG deficiency (Matsumoto et al., 2001). Furthermore, Cox2–/– and Ep2–/– mice show decreased expression of tumour necrosis factor-induced protein-6 (TNFIP6 or TSG6), which is a hyaluronan-binding protein involved in COC expansion (Ochsner et al., 2003). Interestingly, Tnfi6–/– mice are sterile because of the inability of the cumulus cells to assemble their hyaluronan-rich ECM (Fulop et al., 2003). Collectively, these results indicate that COX2 is a critical stimulator of PG signalling and that COX2-derived PGS interact with their cognate receptor, EP2, to promote ovulation (Matsumoto et al., 2001).
Inter-α-trypsin inhibitors (ITIs) have been shown to inhibit inflammatory responses by blocking the induction of pro-inflammatory cytokines (Suzuki et al., 2004). Bikunin, commonly referred to as urinary trypsin inhibitor (UTI), is a member of the ITI family (Sato et al., 2001). Bikunin-deficient mice are infertile because of severe ovulation defects (Sato et al., 2001). Granulocyte macrophage colony-stimulating factor (GM-CSF) is also a member of the cytokine family. Studies indicate that Gmcsf<sup>−/−</sup> mice have normal ovulation rates but decreased numbers of activated macrophages in the stromal and thecal cells during ovulation (Jasper et al., 2000). These results suggest that GM-CSF plays a role in regulating steroidogenesis in the theca by regulating local macrophage populations (Gilchrist et al., 2000).

The plasminogen activator (PA) system plays a role in the proteolytic degradation of the follicle wall at the time of ovulation (Ny et al., 1997) and in the formation/degradation of the CL (Liu et al., 2003). Studies of gonadotrophin-induced ovulation reveal that mice with single deficiencies of tPA, uPA or PAI-1 have normal ovulation, whereas tPA<sup>−/−</sup>uPA<sup>−/−</sup> double-mutant mice have impaired ovulation (Ny et al., 1997).

Oocyte maturation is an important event in ovulation (Jamnongjit and Hammes, 2005). During the process of oocyte maturation, the oocyte resumes meiosis and progresses from prophase I to metaphase II (Jamnongjit and Hammes, 2005). Shortly before follicle rupture, the nucleus of the oocyte, or germinal vesicle (GV), undergoes a series of changes that involve GV breakdown (GVBD), chromatin condensation, chromosome segregation and extrusion of the first polar body (Wiersma et al., 1998). Studies using transgenic mouse models indicate that alterations in the factors that regulate these events result in defects in maturation/ovulation as well as other female reproductive phenotypes. For example, phosphodiesterase 3A (PDE3A) is primarily responsible for oocyte cAMP hydrolysis and plays an important role in resuming meiosis in mammalian oocytes. Pde3a<sup>−/−</sup> mice are completely infertile because their oocytes remain arrested in meiotic prophase I, as demonstrated by the persistence of the nuclear GV, suggesting an inability to resume meiosis (Masciarelli et al., 2004). This supports the notion that cAMP is a maturation-inhibiting factor.

The Erc1 (excision repair cross complementation group 1) gene is required for successful completion of meiosis in oocytes (Selfridge et al., 2001). Specifically, Erc1<sup>−/−</sup> mice die by 3 weeks of age because of a liver problem that can be corrected by use of a liver-specific transgene (Selfridge et al., 2001). Erc1<sup>−/−</sup> mice, with the liver-specific transgene, die by 12 weeks of age and are infertile because of a reduced number of oocytes compared with WT mice (Hsia et al., 2003). Furthermore, oocytes that are present often seem to be in the process of degeneration (Hsia et al., 2003).

Notch gene family members are also thought to regulate meiosis (Hahn et al., 2005). The lunatic fringe gene (Lfrng) is an important regulator of the Notch-signalling pathway. Lfrng<sup>−/−</sup> mice are infertile and have many aberrant follicles (Hahn et al., 2005). Furthermore, Lfrng<sup>−/−</sup> mice can ovulate when induced, but the oocytes cannot be fertilized because they are incapable of completing meiotic maturation (Hahn et al., 2005). Lfrng<sup>−/−</sup> follicles can undergo cumulus expansion, suggesting a disconnection between cumulus expansion and GVBD, and completion of meiotic maturation (Hahn et al., 2005).

Heat shock factors (Hsfs) are also required for normal progression of meiosis (Christians et al., 2000). HSFI is required for the development of the embryo to the 2-cell stage as Hsfl<sup>−/−</sup> females are infertile (Christians et al., 2000). Hsf2<sup>−/−</sup> females are subfertile and exhibit ovulation defects due to large haemorrhagic follicles with trapped oocytes (Kallio et al., 2002).

Regulators of cell-cycle progression, such as cyclin-dependent kinases (CDKs), cyclin kinase (CK) proteins and G protein-coupled receptor 3 (GPR3), have also been shown to be important for meiotic oocyte maturation (Ortega et al., 2003; Spruck et al., 2003). Specifically, Cdk2<sup>−/−</sup> mice are infertile, in part, because of improper localization of centromeres and synaptonemal complex protein 3 (SCP3) (Berthet et al., 2003; Ortega et al., 2003). Cdk4<sup>−/−</sup> mice are also infertile due to impaired progesterone production by the CL (Moons et al., 2002). CK proteins are homologues of CDKs and, thus, are important for oocyte maturation. Ck2<sup>−/−</sup> mice are sterile because of an arrest of the oocyte at metaphase I (Spruck et al., 2003). GPR3 is endowed with constitutive Gs-signalling activity during the cell cycle (Mehlmann et al., 2004). Gpr3<sup>−/−</sup> ovaries show a decrease in the percentage of GV-stage oocytes, suggesting that GPR3 is required to maintain meiotic arrest of the oocyte (Ledent et al., 2005). Cytokines may also play an essential role in oocyte maturation. Specifically, Gp130 is the shared receptor for members of the IL6 family of cytokines (Molyneaux et al., 2003a). Female mice with a germ cell-specific ablation of Gp130 have fertility defects due to abnormal oocyte maturation/ovulation (Molyneaux et al., 2003a).

Transgenic models with alterations in certain transcription factors also provide evidence that improper gene transcription can alter ovulation. Specifically, the Ahr may be involved in regulating events involved in ovulation as Ahr<sup>−/−</sup> mice have reduced numbers of CL compared with WT mice (Benedict et al., 2000). Mice deficient in the Zfx, Krox24 (also known as Egr1), do not ovulate and, thus, are infertile (Topliko et al., 1998). Members of the CCAAT/enhancer-binding protein (CEBP) family could be involved in regulating ovulation, because Cebpβ<sup>−/−</sup> mice lack CL and are completely infertile (Sterneck et al., 1997).

Mouse models with alterations in steroid hormones or steroid receptors that control follicle development

The process of ovarian follicle development requires the coordinated actions of steroid hormones. Steroid hormones also play an important role in the maintenance of reproductive capacity and secondary sex characteristics (Hirshfield, 1991; Richards, 1994). Steroid hormones produced by the ovary (progestins, androgens and estrogens) are synthesized in a sequential manner by thecal and granulosa cells of follicles (Drummond et al., 2002). The steroidogenesis pathway begins with the precursor cholesterol binding to low-density lipoprotein (LDL) receptors on the plasma membrane of granulosa cells. Steroidogenic acute regulatory (STAR) protein then promotes the transport of cholesterol from the outer membrane to the inner membrane of the mitochondria. This step is considered to be one of the rate-limiting steps in the steroidogenesis pathway.

Once cholesterol reaches the inner membrane of the mitochondria, it undergoes enzymatic cleavage of its side chain by an enzyme known as cytochrome P450 side-chain cleavage (CYP11a1) to
form pregnenolone. Pregnenolone is converted to estradiol (E$_2$) via two pathways (Suter, 2004). In the first pathway, pregnenolone is converted to dehydroepiandrosterone (DHEA) by CYP17a1. DHEA is converted to androstenedione (ASD) by 3β-hydroxysteroid dehydrogenase (3β-HSD). In the second pathway, pregnenolone is converted to progesterone by 3β-HSD. Progesterone is then converted to ASD by CYP17a1. Here, the pathways converge, and ASD is converted to testosterone by 17β-HSD. In the final step, testosterone is converted to E$_2$ by aromatase (Suter, 2004). Once E$_2$ is produced, it can stimulate the growth of ovarian follicles by inducing proliferation of granulosa cells (Suter, 2004).

Several recent studies have used transgenic mouse models to examine the roles of steroidogenic factors in the process of ovarian folliculogenesis, female reproduction and disease. For example, investigators have shown that transgenic mice deficient in Star mimic patients with congenital lipid adrenal hyperplasia, an autosomal recessive disorder in which patients have impaired adrenal and gonadal steroidogenesis (Miller, 2005). Studies conducted with Star$^{-/-}$ (StARKO) mice reveal that after birth, the animals fail to grow normally and die within 1–2 days after birth because of adrenocortical insufficiency (Caron et al., 1997). Further studies, in which StARKO mice were treated with corticosteroids to keep them alive into adulthood, revealed that StARKO ovaries have impaired folliculogenesis and contain lipid deposits in the stromal cells (Hasegawa et al., 2000). These mice have high levels of CYP11a1 along with decreased levels of progesterone (Hasegawa et al., 2000). Taken together, these studies suggest that Star deficiency has direct consequences on the steroidogenic capacity of the ovary (Caron et al., 1997; Hasegawa et al., 2000).

To our knowledge, there are no transgenic or knockout mouse models with alterations in any of the CYP450 enzymes in the steroidogenic pathway except for the CYP19 (aromatase) knockout (ArKO) model. ArKO mice, which lack the capacity to produce estrogen, are infertile because of impaired folliculogenesis at the antral stage and an inability to ovulate (Fisher et al., 1998). In addition, the antral follicles in ArKO ovaries become haemorrhagic and cystic with advancing age (Britt et al., 2000). The reasons for the abnormal follicle growth in the ArKO model may be because of abnormal levels of hormones including FSH, LH, E$_2$ and testosterone (Britt et al., 2001).

Although there are limited models with alterations in steroidogenic CYP450 enzymes, there are several models with alterations in either steroid hormones or steroid hormone receptors. For example, several studies have been conducted using mice with alterations in the ability to respond to progesterone (Lydon et al., 1995; Conneely et al., 2001). Progesterone plays an important role in the establishment and maintenance of pregnancy. The actions of progesterone are mediated via specific interactions with its corresponding receptors. Progesterone receptors are expressed in two isoforms, PR-A and PR-B, in the granulosa cells of mature antral follicles and CL (Conneely et al., 2001). Mice lacking both PR isoforms (PRKO) are infertile because they fail to ovulate, even though follicles grow to the antral stage (Lydon et al., 1995). Similar to PRKO ovaries, ovaries from mice deficient in only the PR-A isoform (PRAKO) contain numerous mature, arrested anovulatory follicles (Conneely et al., 2001). In addition, PRAKO mice have a severely impaired ability to respond to hormone treatments during ovulation induction (Conneely et al., 2001). Taken together, these data indicate that PR-B alone cannot support normal folliculogenesis and that PR-A is primarily responsible for follicle rupture. These data also suggest that events involved in ovulation could be mediated by heterodimeric interactions between PR-A and PR-B (Conneely et al., 2001).

Progesterone can be converted to androgens, such as testosterone. Testosterone binds to the androgen receptor (AR) and promotes gene transcription. Studies have shown that abnormalities in the AR can cause a variety of disorders including androgen insensitivity disorder (AIS) in humans and testicular feminization disorder (Tfm), the equivalent in mice (Couse and Korach, 1998). There is some uncertainty, however, about the role of androgens in female reproduction (Ohno et al., 1973; Lyon and Glenister, 1974). This uncertainty is partially due to the fact that all androgen-insensitive mice are genetically male despite their female phenotype (Matsumoto et al., 2005). Studies using a very small number of Tfm homozygous mutants show that androgens are not essential for female reproduction (Lyon and Glenister, 1974). In contrast, studies using the Cre-loxP system to delete the Ar have shown that important regulators of folliculogenesis are under the transcriptional control of the Ar (Shiina et al., 2006). Thus, the Ar is thought to be required for female reproduction, particularly folliculogenesis.

Testosterone is aromatized to E$_2$ in granulosa cells via aromatase. E$_2$ plays a role in the feedback regulation of gonadotrophin secretion as well as a role in folliculogenesis by stimulating granulosa cell proliferation. The actions of E$_2$ are mediated via the binding of estrogen receptors (Estrs), which exist in two forms, Esr1 (α) and Esr2 (β) (Couse and Korach, 1998). Studies using mice deficient in Esr1 (ERKO), Esr2 (BERKO) and both Esr1 and Esr2 (ERαβKO) have all confirmed that E$_2$ is required for normal follicular growth (Lubahn et al., 1993; Krege et al., 1998; Dupont et al., 2000). ERKO mice are acyclic, infertile and possess hyperaemic ovaries devoid of CL (Lubahn et al., 1993). In addition, folliculogenesis in ERKO mice is arrested at the early antral stage, with large secondary follicles becoming cystic and haemorrhagic within 3 weeks of birth (Couse and Korach, 1999). Furthermore, LH receptor (Lhr) mRNA expression is high in the granulosa and thecal cells of all antral follicles in ERKO compared with WT ovaries (Schomberg et al., 1999). BERKO ovaries have follicles ranging from the primordial to the antral stage and a reduced number of CL compared with WT ovaries (Krege et al., 1998). Also, BERKO follicles do not progress from the early antral to the antral stage (Emmen et al., 2005). ERαβKO mice are completely resistant to estrogenic function, and their ovaries exhibit follicular transdifferentiation to structures resembling seminiferous tubules of the testis (Couse et al., 1999). ERαβKO ovaries of prepubertal mice contain adult-like follicles with a defined antrum, with absent or degenerating oocytes (Couse et al., 1999). Granulosa cells in ERαβKO are also either absent or pyknotic (Couse et al., 1999). The presence of elevated AMH, SOX9 and sulphated glycoprotein-2 (SGP2) in ERαβKO ovaries suggests sex reversal in these mice (Couse et al., 1999). Interestingly, alterations in the expression of ESR1 and ESR2 have been shown in patients with PCOS (Jakimiuk et al., 2002).

**Mouse models with alterations in growth factors that control folliculogenesis**

The process of follicle growth requires the co-ordinated action of both endocrine hormones and locally derived growth factors.
(Richards, 1994). Among these, gonadal factors are members of the transforming growth factor β (TGFβ) superfamily. The TGFβ family of proteins consists of multi-functional cytokines that modulate a wide variety of cellular functions including proliferation, differentiation, adhesion and migration (Hu et al., 1998; Dunker and Krieglstein, 2000; Ingman and Robertson, 2002). Furthermore, the TGFβ family is involved in regulating many stages of ovarian development, folliculogenesis and female fertility. Because several previous reviews have described the roles of TGFβ in female reproduction in detail (Dunker and Krieglstein, 2000; Chang et al., 2001, 2002; Pangas and Matzuk, 2004), this current review presents only a brief overview of their many roles.

The TGFβ family consists of more than 35 members in vertebrates, and several TGFβ family members are expressed in the ovary (Chang et al., 2001, 2002; Pangas and Matzuk, 2004). Growth-differentiation factor 9 (GDF9), BMP6 and BMP15 are expressed in oocytes, whereas other TGFβ family members are expressed in granulosa cells [anti-Müllerian hormone (AMH), inhibins, activins, TGFβ1, TGFβ2 and TGFβ3] and theca cells (BMP4 and BMP7) within the ovary (Chang et al., 2002; Pangas and Matzuk, 2004).

Interestingly, TGFβ family members have very different roles in regulating female fertility. Tgfb1–/– mice (Dickson et al., 1995), Tgfb2+/– (TGFβ-receptor type 2) mice (Oshima et al., 1996) and Bmp4+/– mice (Winnier et al., 1995) die during embryogenesis, whereas deficiencies of Tgfb2 (Sanford et al., 1997), Tgfb3 (Kaartinen et al., 1995) and Bmp7 (Dudley et al., 1995) are perinatally lethal. Thus, little is known about the function of these members in the adult ovary. Mice with a deletion of Bmp6 are viable and have minor skeletal defects but do not show any ovarian defects (Solloway et al., 1998). In contrast, mice with genetic alterations in Amh, Gdf9, Bmp15, activin and inhibin have altered the regulation of ovarian follicle development and fertility as described below.

The principal function of AMH, also known as Müllerian inhibiting substance (MIS), is to induce regression of the Müllerian ducts during male sex differentiation (Durlinger et al., 2002). Whereas AMH is not expressed in the ovary before birth, it is expressed in granulosa cells of growing follicles during post-natal life (Ueno et al., 1989; Munsterberg and Lovell-Badge, 1991; Hirobe et al., 1992). Moreover, the generation of transgenic mice that overexpress Amh (Behringer et al., 1990) or are deficient in Amh (AMHKO) (Behringer et al., 1994) reveals that AMH plays an important role during ovarian folliculogenesis and fertility. Mice overexpressing Amh are infertile, have a blind-ending vagina and contain ovaries devoid of germ cells that develop cord-like structures within 2 weeks after birth (Behringer et al., 1990). AMHKO mice are fertile and give birth to normal-sized litters but have enhanced follicle recruitment and depletion of primordial follicles at an early age (Behringer et al., 1994; Durlinger et al., 1999, 2001). Additional studies of AMHKO mice have revealed that AMH may be important in regulating the responsiveness of growing follicles to FSH (Durlinger et al., 2001). Taken together, these studies suggest that AMH may participate in two critical selection points of follicle development: it inhibits the recruitment of primordial follicles into the pool of growing follicles and decreases the responsiveness of growing follicles to FSH.

GDF9 and BMP15 are oocyte-specific TGFβ family members that have an identical pattern of expression. In the mouse ovary, these factors are expressed in growing oocytes but not in oocytes of quiescent primordial follicles (McGrath et al., 1995). Gdf9+/– mice exhibit primary infertility due to failed ovarian follicular development (Dong et al., 1996). Gdf9+/– ovaries contain numerous primordial and primary follicles but no normal follicles past the one-layer follicle stage (Dong et al., 1996; Carabatos et al., 1998). Furthermore, Gdf9+/– ovaries exhibit a block in the growth and proliferation of granulosa cells, an absence of thecal layer formation and defects in oocyte meiotic competence (Dong et al., 1996; Carabatos et al., 1998; Elvin et al., 1999b). Moreover, GDF9 may promote the progression of early primary, but not primordial, follicle development, in part, by altering kit-ligand expression as seen in bovine and rat granulosa cells (Nilsson and Skinner, 2002). Additional studies using recombinant GDF9 reveal that GDF9 regulates multiple cumulus granulosa cell functions in the pre-ovulatory period including hyaluronic acid synthesis and cumulus expansion (Elvin et al., 1999a). Furthermore, a downstream protein in the GDF9 signal transduction cascade, pentraxin 3 (PTX3), plays an important role in cumulus cell–oocyte interaction as Ptx3+/+ mice are sub fertile because of defects in the integrity of the COC (Varani et al., 2002).

In contrast to Ggf9+/– mice, Bmp15+/– mice are subfertile with reduced litter sizes and reduced ovulation and fertilization rates (Yan et al., 2001). Interestingly, missense mutations in BMP15 have been associated with POF (Dixit et al., 2006). Additional studies show that there is an important dosage-sensitive synergistic interaction between GDF9 and BMP15 in the mouse ovary (Yan et al., 2001). Specifically, double homozygous females (Gdf9–/– Bmp15–/–) display oocyte loss, develop cysts and resemble Gdf9+/– mice (Yan et al., 2001). In contrast, Gdf9+/– Bmp15+/– female mice have more severe fertility defects than Bmp15+/– females, and these defects are due to abnormalities in ovarian folliculogenesis, COC expansion and fertilization (Yan et al., 2001). Thus, the dosage of intact Bmp15 and Gdf9 alleles might directly influence the destiny of the oocyte during folliculogenesis and the periovulatory period (Yan et al., 2001).

Inhibins and activins are dimeric protein members of the TGFβ superfamily that were initially recognized as gonadal peptides that, respectively, inhibit or stimulate FSH production by the pituitary gland (Vale et al., 1986; Welt et al., 2002). The inhibins are dimers with either βA or βB subunits paired with an α subunit [inhibin A (α:βA) and inhibin B (α:βB)], whereas the activins are dimers with two β subunits [activin A (βA:βA), activin B (βB:βB) and activin AB (βA:βB)]. The inhibin α subunit is primarily expressed in the ovary, testis, adrenal and pituitary, whereas the two β subunits are expressed in multiple tissues during embryonic and adult development (Meunier et al., 1988). Studies have shown that mice lacking the inhibin α subunit develop mixed or incompletely differentiated gonadal stromal tumours, adrenal cortical tumours and a cachexia-like wasting syndrome and are infertile (Matzuk et al., 1992, 1994). Furthermore, mice overexpressing the inhibin α subunit have reduced fertility, reduced FSH levels, increased LH levels and a decreased ovulation rate (Cho et al., 2001). In addition, treatment of transgenic females with exogenous gonadotrophins results in ovulation rates similar to that of stimulated WT animals, suggesting that altered gonadotrophin levels may be responsible for the decreased ovulation rates in mice overexpressing the inhibin α subunit (Cho et al., 2001). Furthermore, ovaries of inhibin α-overexpressing mice contain polyovular follicles,
have fewer mature antral follicles and CL compared with controls and develop large fluid-filled ovarian cysts as early as 3 months of age (McMullen et al., 2001). In fact, McMullen et al. (2001) report that by 12 months, more than 92% of female transgenic mice develop ovarian cysts. Mice lacking the activin βA subunit die in the perinatal period, whereas mice lacking activin βB subunits are viable and fertile but have large litters and a delay in delivery and fail to nurse their offspring (Schreve et al., 1994; Vassalli et al., 1994). In additional studies using knockin technology, Brown et al. (2000) have shown that activin βB knockin to the activin βA locus can rescue the activin βA−/− neonatal lethality but that activin βB cannot compensate for activin βA during all stages of folliculogenesis in adult mice.

Activins initiate a signal transduction cascade by binding to serine/threonine kinase type 1 and 2 receptors (ACVR) (Hu et al., 1998; Chang et al., 2001). Acvr2−/− mice are infertile and have thin uteri, low levels of FSH and small ovaries with an increased incidence of follicle atresia and fewer CL compared with controls (Matzuk et al., 1995). FST is an activin-binding protein and activin antagonist in vivo that can regulate the action of activins (Shimokawa et al., 1991; Schneyer et al., 1994). Studies using both Fst conditional knockout mice (Jorgez et al., 2004) and mice overexpressing Fst (Guo et al., 1998) suggest an important role of FST in the adult mammalian ovary. Fst conditional knockout mice have reduced litter sizes, defects in ovulation and fertilization, impaired ovarian folliculogenesis, and elevated levels of serum FSH and LH (Jorgez et al., 2004). Mice overexpressing Fst are viable but have extensive reproductive defects including a block in folliculogenesis at either the pre-antral or antral stage (Guo et al., 1998).

Recently, the SMAD proteins have been shown to mediate the TGF-signalling pathway through a cascade of ligand-induced phosphorylation (Hu et al., 1998; Zimmerman and Padgett, 2000; Chang et al., 2002). Homozygous disruptions of Smad1 (Lechleider et al., 2001; Tremblay et al., 2001; Hayashi et al., 2002), Smad2 (Nomura and Li, 1998; Waldrip et al., 1998; Weinstein et al., 1998), Smad4 (Srirad et al., 1998; Yang et al., 1998), Smad5 (Chang et al., 1999, 2000; Yang et al., 1999; Chang and Matzuk, 2001) and Smad6 (Galvin et al., 2000) in mice are embryonically lethal, and thus, little is known about their function in the adult mammalian ovary. Mice that harbour a deletion of the Smad3 gene are viable and survive to adulthood (Zhu et al., 1998; Datto et al., 1999; Tomic et al., 2002, 2004). Smad3−/− ovaries have impaired follicular growth, increased apoptosis and altered differentiation (Tomic et al., 2002, 2004). Moreover, studies show that deletion of Smad3 alters folliculogenesis by affecting the expression of genes that control cell-cycle progression (Cdk4 and Ccnd2), cell survival (Bcl2 family of protooncogenes) and cell differentiation (Esr1, Esr2 and inhibit α subunits) (Tomic et al., 2004). Furthermore, a recent study indicates that absence of the Smad3 gene induces cellular proliferation in the ovarian surface epithelium (Symonds et al., 2003). Interestingly, a mouse model with lineage in the TGFβ family has been generated to understand ovarian endosalpingiosis, a condition in women associated with pelvic pain and cystic spaces lined by cells that resemble a tubal-type epithelium (Bristol-Gould et al., 2005). This model expresses a dominant-negative form of Smad2 (Smad2-dn) in the ovary (Bristol-Gould et al., 2005). Smad2-dn female mice have an ovarian endosalpingioal phenotype, are subfertile, give birth to reduced numbers of pups and exhibit decreased breeding frequency (Bristol-Gould et al., 2005). Furthermore, Smad2-dn mice develop fluid-filled ovarian cysts, which are similar to those seen in mice overexpressing inhibin α (Bristol-Gould et al., 2005). Thus, downstream SMAD-signalling proteins might be essential determinants of the different actions of TGFβ superfamily members in the ovary.

### Mouse models with alterations in the pituitary control of folliculogenesis

The anterior pituitary gland is known to secrete at least six different hormones: LH, FSH, growth hormone (GH), prolactin (PRL), thyroid-stimulating hormone (TSH) and pro-opiomelanocortin-α (POMC1). During the past several years, investigators have generated mouse models that synthesize abnormally high levels of some of these pituitary hormones, lack the capacity to produce some of these pituitary hormones or lack the ability to respond to some of these pituitary hormones. Several of these mouse models have defects in folliculogenesis and ovarian function and, thus, are discussed below. To our knowledge, there are no reports of mouse models that overexpress or lack TSH and POMC1, and thus, they will not be discussed here.

Investigators have developed mouse models that either synthesize abnormally high levels of gonadotrophins (mice overexpressing LH or FSH), low levels of gonadotrophins (Fshb−/− mice) or lack the ability to normally respond to gonadotrophins (Lhrh−/− mice, Fshr−/− mice or mice with chronic Lhr activation) (Risma et al., 1995, 1997, 1999; Kumar et al., 1999; Abel et al., 2000; Nilson et al., 2000; Burns et al., 2001; Lei et al., 2001; Zhang et al., 2001; Huhtaniemi et al., 2002; Abel et al., 2003; Meehan et al., 2005; Pakarainen et al., 2005a,b). Mice that maintain chronically elevated serum LH via the expression of a chimeric LHb subunit in pituitary gonadotrophs (LH-carboxyl terminal peptide (CTP) mice) have LH levels that are two- to three-fold higher than those in WT mice (Risma et al., 1995). As a result, these mice have elevated levels of progesterone, testosterone and E2 (Risma et al., 1995). At 3 weeks, LH-CTP mice have normal numbers of primordial follicles. By 5 weeks, however, LH-CTP mice have significantly fewer primordial and primary follicles than WT controls (Flaws et al., 1997). In addition, LH-CTP ovaries contain blood-filled cysts, pockets of blood and luteinized cells (Risma et al., 1995, 1997; Flaws et al., 1997; Nilson et al., 2000). LH-CTP mice also develop granulosa and thecal cell tumours (Risma et al., 1995, 1997; Keri et al., 2000; Nilson et al., 2000). Furthermore, LH-CTP mice are infertile primarily because of anovulation but partially because of defects in uterine receptivity and midgestation pregnancy failure (Risma et al., 1995; Mann et al., 1999). Collectively, these data indicate that excessive levels of LH serve to rapidly deplete the primordial follicle pool, increase sex steroid hormone levels, produce ovarian cysts and tumours and cause infertility in mice. These data are supported clinically as women diagnosed with PCOS have high levels of LH accounting for 75% of anovulatory infertility (Mann et al., 1999).

A recent study described the phenotype of mice with chronic Lhr activation (Meehan et al., 2005). These mice were generated using a constitutively active yoked hormone–receptor complex in which a fusion protein of hCG was covalently linked to the N-terminus of the rat LHR (Meehan et al., 2005). Female mice with chronic LHR activation have precocious puberty, early vaginal opening and enhanced follicular development, including the presence
of CL at a young age (Meehan et al., 2005). At 12 weeks, the ovaries from mice with chronic LHR activation have excessive interstitial tissue, hypertropic and luteinized cells, degenerating follicles and haemorrhagic cysts (Meehan et al., 2005). Collectively, these data indicate that chronic LHR activation results in a phenotype that is similar in many ways to the phenotype of mice that chronically overexpress LH. Furthermore, these data indicate that chronic gonadotrophin-induced LHR activation leads to early sex development.

Several investigators have described the ovarian phenotype present in mice that lack the ability to respond to LH (Themmen and Huhtaniemi, 2000; Lei et al., 2001; Zhang et al., 2001; Huhtaniemi et al., 2002; Hirst et al., 2004; Bachelot and Binart, 2005; Chudgar et al., 2005; Pakarainen et al., 2005a,b). For instance, Lhr–/– mice contain ovaries that are dramatically reduced in size compared with WT ovaries (Zhang et al., 2001). Furthermore, Lhr–/– ovaries contain primordial, primary and antral follicles but completely lack pre-ovulatory follicles and CL. In addition, Lhr–/– ovaries contain lower levels of key enzymes required for steroid biosynthesis (STAR, CYP11a1 and CYP17a1) than WT ovaries (Lei et al., 2001; Zhang et al., 2001). As a result, E2 and progesterone levels are decreased in Lhr–/– mice compared with WT mice (Zhang et al., 2001). In recent experiments, studies examined whether the phenotype in Lhr–/– mice could be rescued by FSH treatment and whether the ovarian phenotype in the Lhr–/– mice was due to direct or indirect effects of LH deletion on the ovary (Pakarainen et al., 2005a,b). It was determined that high doses of FSH did not induce follicular development and ovulation in the absence of Lh expression (Pakarainen et al., 2005a). Additional studies reveal that Lhr deletion has a direct effect on the ovary as Lhr–/– ovaries transplanted into WT mice still contain follicles arrested at the antral stage (Pakarainen et al., 2005b). Taken together, these data indicate that the very last steps of follicular maturation, as well as ovulation and normal sex steroid production, do not occur without LH action in the ovary.

Although hCG is a placental hormone and not a pituitary hormone, it deserves mention in this section because it is a member of the heterodimeric glycoprotein hormone superfamily that includes FSH and LH. It also contains an α subunit, which is identical to the α subunits present in FSH, LH and TSH. Furthermore, hCG has similar properties to LH and it binds the LHR. A transgenic mouse model that overexpresses the β subunit of hCG has been developed (Rulli et al., 2002, 2003). These mice have enhanced ovarian steroidogenesis and infertility (Rulli et al., 2002, 2003). In addition, mice that overexpress either one or both subunits of hCG in multiple tissues have also been developed (Matzuk et al., 2003). Mice overexpressing the α subunit of hCG are fertile, but mice overexpressing the β subunit of hCG are infertile and develop cystic ovaries (Matzuk et al., 2003). Furthermore, mice overexpressing both subunits of hCG are infertile, although they have elevated levels of E2 and develop haemorrhagic, cystic ovaries with thecal cell enlargement and stromal cell proliferation (Matzuk et al., 2003). Collectively, these data indicate that excessive hCG, like excessive LH and FSH, results in abnormal ovarian steroidogenesis and structure.

Transgenic mice that ectopically overexpress human FSH (hFSH) in multiple tissues have also been generated (Kumar et al., 1999). Similar to LH-overexpressing mice, female mice that overexpress hFSH have haemorrhagic and cystic ovaries, elevated levels of E2 and progesterone and are infertile (Kumar et al., 1999). Unlike LH-overexpressing mice, female mice that overexpress hFSH do not develop gonadal tumours (Kumar et al., 1999). When mice that overexpress hFSH are mated with inhibin-deficient mice, however, the offspring develop tumours (Kumar et al., 1999). In subsequent studies, mice that overexpress hFSH were used to characterize the definitive actions of FSH alone, distinct from LH effects, by combining transgenic FSH expression with the hypogonadal (hpg) mouse (Allan et al., 2001). Ovaries from hFSH-overexpressing mice crossed with hpg mice showed increased follicle recruitment and development to the antral stage compared with hpg mice (Allan et al., 2001). Taken together, these data indicate that excessive levels of FSH serve to increase steroid hormone levels, produce ovarian cysts and cause infertility in mice. Furthermore, these data indicate that elevated levels of FSH alone are not sufficient to cause ovarian tumours, but they may be an important modifying factor for gonadal tumorigenesis in inhibin-deficient mice. In addition, these data indicate that excessive FSH can rescue the phenotype of hpg mice.

Several investigators have characterized the phenotype of mice that lack the ability to synthesize or respond to FSH (Kumar et al., 1997; Dierich et al., 1998; Abel et al., 2000, 2003; Matzuk, 2000; Burns et al., 2001; Danilovich et al., 2002). In general, the phenotypes of Fshb–/– mice and Fshr–/– mice are similar (Kumar et al., 1997; Abel et al., 2003; Hirst et al., 2004). Both of these mouse models are infertile because of a block in folliculogenesis before antral follicle formation (Kumar et al., 1997; Matzuk, 2000; Abel et al., 2003). The phenotype of mice that lack both Fshr and Fshb (Fshb–/–/Fshb–/–) has also been described (Abel et al., 2003). These double mutants are infertile, and the ovaries contain abnormal structures. Specifically, >90% of the double-mutant ovaries contain structures resembling oviduct epithelium, some of which contain fluid-filled cysts (Abel et al., 2003). Furthermore, the Fshl Follistatin receptor-null (FORKO) mice are sterile and acyclic and contain underdeveloped ovaries (Danilovich et al., 2001, 2004). FORKO mice also have chronic depletion of estrogen, POF and ovarian tumours and cysts (Danilovich et al., 2001, 2004). Additional studies show that FORKO ovaries contain abnormal follicle numbers compared with WT ovaries (Balla et al., 2003). Specifically, at PD 2, FORKO ovaries contain 50% fewer naked oocytes than WT ovaries, and at PD 10, FORKO ovaries contain 20% fewer primary and 60% fewer pre-antral follicles than WT ovaries. At PD 24, FORKO ovaries lack antral follicles, the diameter of primary follicles is smaller than the diameter of WT primary follicles, granulosa cells infiltrate the oocytes, and the surface epithelium is abnormally thick (Balla et al., 2003). Collectively, these data indicate that FSH is required for normal follicular growth to the antral and pre-ovulatory stages, and is required for normal fertility.

Mouse models have been developed and characterized that either overexpress or fail to respond to non-gonadotrophin pituitary hormones such as GH (Bartke et al., 1988; Mayerhofer et al., 1990; Chandrashekar and Bartke, 1993; Ikeda et al., 1997; Sasaki et al., 1997; Danilovich et al., 1999; Zhu et al., 2002). Specifically, investigators have generated mice that overexpress human or bovine GH (Mayerhofer et al., 1990; Ikeda et al., 1997; Danilovich et al., 2000). Mice that overexpress human GH contain ovaries with significantly fewer pre-antral follicles and more antral follicles than WT ovaries (Mayerhofer et al., 1990). Furthermore, mice that overexpress human GH are infertile primarily because of
luteal and hypothalamic defects (Bartke et al., 1988; Mayerhofer et al., 1990; Cecim et al., 1995; Sasaki et al., 1997). Mice that overexpress bovine GH are infertile because of luteal failure from PRL deficiency and have an abnormal response to GnRH treatment (Cecim et al., 1995; Chandrashekar and Bartke, 1996). Growth hormone receptor (Ghr)-deficient mice have a reduced number of CL compared with WT mice (Bachelot et al., 2002; Zaczek et al., 2002). In addition, Ghr–/– mice have a reduced number of pre-ovulatory follicles compared with WT mice. Furthermore, Ghr–/– mice are fertile, but their age at first conception is delayed, and their average litter size is reduced compared with WT mice (Bachelot et al., 2002). Additional studies show that insulin-like growth factor 1 (IGF1) treatment does not restore ovulation rate in Ghr–/– mice (Bachelot et al., 2002). Taken together, these data suggest that GH plays an important role in follicle growth, ovulation and luteal function. In addition, the results suggest that IGF1 alone does not mediate the effects of GH on ovulation.

PRL is another pituitary hormone that is known to affect ovarian function (Grosdemouge et al., 2003), and it also plays a major role in regulating implantation (Ormandy et al., 1997; Binart et al., 2000; Baran et al., 2002; Grosdemouge et al., 2003). Follicular development, the ovulation process and the subsequent expression of enzymes involved in steroid production were examined in Prl–/– and WT mice (Grosdemouge et al., 2003). Prl–/– mice ovulate similar numbers of oocytes and contain follicles in all stages of development. Furthermore, the CL in Prl–/– mice exhibit increased regression, increased disorganization, low levels of vascularization markers and reduced expression of Lhr, Star, Cyp11a1, Cdkn1b (p27) and Ccnd2. In addition, Prl–/– mice have reduced levels of progesterone compared with WT mice. Collectively, these data indicate that the CL is formed in Prl–/– mice, but it contains an excess of apoptotic cells and lacks normal angiogenesis. In turn, these modifications lead to a decrease of Lhr expression and consequently to a loss of the enzymatic cascades necessary to produce adequate levels of progesterone, which are required for the maintenance of pregnancy (Grosdemouge et al., 2003).

### Conclusions

The use of transgenic mouse models has provided a great deal of information with regard to the mechanisms underlying the formation of the ovary, follicular growth and fertility. Some of the genes involved at various stages of follicular development, as determined by transgenic mouse models, are shown in Figures 3 and 4. Furthermore, the use of transgenic mouse models has provided evidence that mutations in many genes affect the synthesis of hormones and growth factors and/or responsiveness of hormones and growth factors, which in turn indirectly affect ovarian development, function and fertility. The data obtained from transgenic mouse models may provide clues about the reasons for abnormal

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**Figure 3.** Factors involved in ovarian germ cell nest breakdown and primordial follicle formation. Studies using transgenic mouse models have elucidated that many genes regulate germ cell nest breakdown and primordial follicle formation. This schematic illustrates the major sites of action of the regulators described in the text.
Follicle development in transgenic mice

reproductive conditions in humans, because multiple genes shown to regulate folliculogenesis and fertility in mice have been shown to play a role in human reproduction.

For example, studies investigating the roles of several genes have led to a greater understanding of POF. POF is a heterogeneous condition associated with amenorrhea before the age of 40 years (Christin-Maitre et al., 1998). The exact aetiology of POF is unknown. Some studies, however, show that mutations in the FSHR, INHa, FOXL2 and BMP15 have been associated with POF in women (Dixit et al., 2006). Therefore, mouse models of POF promise to be of significant value in understanding the complexity of this condition.

Transgenic mouse models are also useful for understanding human physiology as they mimic many human syndromes (Christin-Maitre et al., 1998). As mentioned earlier, FANCL patients exhibit POF, which is mimicked by Fanca<sup>−/−</sup> mice and Gcd mice (Agoulnik et al., 2002). The Zfx<sup>−/−</sup> mouse mimics the ovarian dysgenesis phenotype that occurs in Turner’s syndrome (Luoh et al., 1997; Christin-Maitre et al., 1998). In addition, Foxl2<sup>−/−</sup> and atm<sup>−/−</sup> mice display ovarian failure similar to that in patients with blepharophimosis–ptosis syndrome (BPES) (Christin-Maitre et al., 1998; Di Giacomo et al., 2005). Aire<sup>−/−</sup> mice are also infertile, which is consistent with the infertility manifested in autoimmune polyglan-
dular candidasis ectodermal dystrophy (APECED) patients (Christin-Maitre et al., 1998). Although a reduction in follicle numbers has not been shown in Aire<sup>−/−</sup> mice, the infertility in these patients has been linked to ovarian atrophy (Ramsey et al., 2002).

The understanding of PCOS has also been enhanced through the use of transgenic mouse models. PCOS is a common heterogeneous endocrine disorder associated with amenorrhea and high LH levels. Furthermore, PCOS is the leading cause of infertility in women. As in POF, the exact cause of PCOS is unknown. Several genes have been implicated in its pathogenesis. As mentioned earlier, some evidence has linked NGF and FST to PCOS (Bai et al., 2004). Members of the TGFβ family also may be associated

Figure 4. Factors involved in ovarian follicle development. Studies using transgenic mouse models have elucidated that many genes regulate follicle development from the primordial to pre-ovulatory stages and corpus luteum (CL) formation. This schematic illustrates the major sites of action of the regulators described in the text.
with PCOS, because Gdf9 mRNA is decreased in oocytes in women with PCOS (Teixeira Filho et al., 2002). Although ArKO mice present with cystic ovaries, mutations in the aromatase gene or its promoter were not found to be the cause of PCOS in a sample of women (Soderlund et al., 2005).

In conclusion, studies investigating the roles of factors that affect the formation of the ovary, follicle development, ovulation or hormone production may lead to a better understanding of the preventive measures needed to combat infertility as well as lead to the development of novel treatments for reproductive disorders or the development of new contraceptive agents.

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