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Mouse models of ovarian failure

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Ovarian failure leading to infertility can be caused by improper prenatal development of the fetal gonad or disruption of the complex postnatal process of folliculogenesis due to alterations in intragonadal or extragonadal regulation. It is critical to have physiological models that mimic events occurring during human development to understand, treat, and prevent ovarian failure in women. Many workers have chosen the mouse as the mammalian model with which to study ovarian function. This review summarizes several key events in female gonadogenesis and folliculogenesis in mice with specific emphasis on spontaneous or induced mutations yielding mouse models that have female infertility owing to ovarian failure.

Female fertility depends on the precise execution of ovarian development, regulated allocation and maturation of oocytes, and the proliferation and differentiation of the surrounding somatic cells that occur during folliculogenesis. In contrast to the continuous proliferation of male germ cells, proliferation of female oogonia occurs only prenatally in mice. At birth, the female has a finite oocyte population; some oocytes begin to grow in response to yet undiscovered intragonadal factors, while others remain quiescent until later, prolonging the female reproductive lifespan. Folliculogenesis is controlled at two levels: intragonadal factors initiate follicular growth and coordinate development of the oocyte, granulosa cell and thecal cell components of the follicle at least at early stages (Adashi, 1992), whereas extragonadal factors synchronize granulosa cell and thecal cell function later in folliculogenesis and integrate the reproductive system with the physiology of the rest of the organism (Richards, 1994).

Previous physiological, biochemical and molecular studies have provided a tremendous amount of information on hormonal regulation of follicular growth and differentiation (Greenwald and Roy, 1994). Potential intragonadal control mechanisms have been identified *in vitro*, while pharmacological manipulation and tissue ablation studies have revealed general principles of extragonadal control of folliculogenesis. However, with the development of transgenic mouse technology, it is now possible to alter the expression of a selected gene and then observe the effect on the development of a specific tissue or the entire organism (Capecchi, 1994). Use of the transgenic approach to study gonadogenesis and the regulation of folliculogenesis in mice is expanding our understanding of ovarian development and physiology, and is helping to uncover possible causes of human infertility. The mouse models presented in this review are divided into two categories on the basis of whether the mutation affects prenatal ovarian development or postnatal ovarian function. The models with postnatal ovarian defects are further divided on the basis of whether the defect affects the initiation of follicle growth, preantral follicle growth, antrum formation and later stages of follicle growth, or ovulation and corpus luteum formation.

Formation of the female gonad

The female gonad develops during early fetal life when primordial germ cells, derived from the inner cell mass of the blastocyst and residing extraembryonically, migrate to the gonadal ridges of the mesonephros of the developing embryo (Byskov and Hoyer, 1994). Mitotic proliferation of the primordial germ cells occurs during this migration, and upon reaching the gonad the germ cells form important associations with somatic cells that will last for the duration of folliculogenesis. By postnatal day 2, the oocytes have arrested in prophase of the first meiotic division and do not complete meiosis I until ovulation. Unlike spermatogenesis in males, in which spermatogonia act as stem cells and constantly divide and produce gametes, the ovary has a finite supply of oocytes. The size and the rate of depletion of this pool of oocytes determines the female reproductive lifespan. The molecular mechanisms controlling gonadogenesis require the interaction between a diverse set of regulators, including growth factors, receptors and transcription factors.

Several genetic mutations have been shown to disrupt either early germ cell development or proliferation or both (Table 1). Initial studies of the gonads of the white spotting (*W*) and Steel (*Sl*) mutant mice revealed that they contain few, if any, germ cells (Coulombre and Russell, 1954; Bennett, 1956; Mintz and Russell, 1957). Further analysis showed that the tyrosine kinase receptor, c-kit, was encoded by the *W* locus and expressed on the surface of germ cells (Manova and Bachvarova, 1991). Its ligand, stem cell factor (kit ligand), is encoded by the *Sl* locus and expressed by cells along the germ cell migratory pathway (Matsui *et al*., 1990). The interaction of c-kit with its ligand is required for the proliferation, survival and migration of the germ cells (Besmer *et al*., 1993). Similarly, a spontaneous mutation at the atrichosis (*at*) (Handel and Eppig, 1979) locus and transgene-insertion mutagenesis at the germ-cell deficient (*gcd*) locus (Pellas *et al*., 1991) cause infertility due to significantly reduced primordial germ cell populations in the developing gonad. However, the functions of the genes at the *at* and *gcd* loci remain unknown.

Knockout mice lacking *Zfx,* a gene encoding a putative zinc-finger transcription factor located on the X chromosome, have

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Table 1. Mouse models with altered gonad formation or number of germ cells

Transgenic/mutant mouse	Major reproductive findings	References
SF-1 (*Ftz-F1*) knockout	Failure of gonads to develop leading to complete agenesis; female internal and external genitalia	Luo *et al*., 1994; Ikeda *et al*., 1995
WT-1 knockout	Failure of gonadal development; normal germ cell migration	Kreidberg *et al*., 1993
Sry transgenic	Presence of Sry results in formation of testis in XX embryo; no spermatogenesis	Koopman *et al*., 1991
MT-Müllerian inhibiting substance transgenic	Females lack Müllerian duct derivatives; germ cells degenerate; feminization of high expressor males	Behringer *et al*., 1990
Ataxia telangiectasia gene (*Atm*) knockout	Male and female infertility; lack of germ cells	Barlow *et al*., 1996
White spotting (*W*) c-kit deficiency	Ovaries lack germ cells due to defect in migration and proliferation	Coulombre and Russell, 1954; Mintz and Russell, 1957
Steel (*St*)-kit ligand deficiency	Ovaries lack germ cells due to defect in migration and proliferation	Bennett, 1956
Zfx knockout	Normal germ cell migration; defect in mitotic proliferation	Luoh *et al*., 1997
Dazla knockout	Ovaries lack germ cells due to prenatal degeneration of oocytes after proliferation	Ruggiu *et al*., 1997
Germ cell deficient (*gcd*)	Transgene insertion causes marked decrease in primordial number of germ cells	Pellas *et al*., 1991
Atrichosis (*at*)	Spontaneous mutation causes marked decrease in primordial number of germ cells	Handel and Eppig, 1979

been produced by Luoh *et al*. (1997). The female mice demonstrated diminished fertility and shortened reproductive lifespan, reminiscent of the human syndrome, premature ovarian failure. The number of oocytes in the perinatal ovary was reduced to < 25% of controls, and further investigation revealed < 50% the normal number of primordial germ cells in the gonad at E11.5, although germ cell migration did not appear to be disrupted. Because of the mutant mouse phenotype, *Zfx* deficiency has been proposed as a model for premature ovarian failure, and its location on the X chromosome suggests that it is also involved in some reproductive defects seen in Turner's syndrome (45, XO). It is obvious that this period in the development and proliferation of germ cells is critical in defining the future reproductive potential of mice and other mammals.

Chromosomal sex determines gonadal sex in mice between E10 and E12.5. Specifically, Sertoli cells carrying the sex determining region Y gene (*Sry*) on the Y chromosome direct the development of the gonad into a testis, while lack of expression of *Sry* allows the default pathway of ovary formation to occur (Byskov and Hoyer, 1994). XX transgenic mice carrying a 14 kb DNA fragment containing the *Sry* gene develop testes and male secondary sex characteristics, but lack spermatozoa and are thus infertile (Koopman *et al*., 1991). This block in the transgenic *Sry*-positive XX male mice confirms that other genes on the Y chromosome are important for spermatogenesis.

Several other genes expressed in the urogenital ridge before gonadal sex determination also play important roles in early ovarian development. Mice lacking the Wilms' tumour associated gene, and hence the transcription factor WT-1, show normal germ cell migration but the urogenital ridge fails to develop, leading to both kidney and gonadal agenesis (Kreidberg *et al*., 1993). Steroidogenic factor-1 (SF-1), an orphan member of the nuclear receptor superfamily, is expressed in all primary steroidogenic tissues, the pituitary, hypothalamus, and the urogenital ridge at E9-9.5. Lack of SF-1 also leads to complete gonadal agenesis (Luo *et al*., 1994). Although SF-1 is known to be a key transcriptional regulator of steroidogenic enzymes, the defects in SF-1-deficient gonadal development are not due to lack of steroid production since steroid-deficient rabbit models with normal SF-1 function show normal gonad formation (Pang *et al*., 1992). Instead, SF-1 may regulate genes important in gonadal development directly, and functional SF-1 sites are present in several other genes, such as the oxytocin, prolactin receptor, and Müllerian-inhibiting substance genes (Wehrenberg *et al*., 1994; Giuili *et al*., 1997; Hu *et al*., 1997).

Müllerian-inhibiting substance (MIS), a member of the transforming growth factor β (TGF-β) superfamily, is genetically downstream from Sry and SF-1 in the testes, and prenatally causes regression of the Müllerian ducts (precursor of the oviducts, uterus and upper portion of the vagina) in males. SF-1 appears to regulate MIS directly through interaction with a binding site in the MIS promoter (Giuili *et al*., 1997). However, SRY probably regulates MIS indirectly, possibly acting through an intermediate such as the Sry-related transcription factor SOX9, which is upregulated in the male genital ridge during sex determination (McElreavey and Fellous, 1997). In females, MIS is expressed in granulosa cells of large antral follicles of the postnatal ovary. Since MIS knockout female mice have no ovarian phenotype (Behringer *et al*., 1994), MIS function in the ovary remains unknown at this time. However, metallothionein promoter-driven overexpression of human MIS in transgenic female mice leads not only to the expected Müllerian duct regression, but also to ovarian stunting and loss of germ cells (Behringer *et al*., 1990). These findings suggest that overexpression of MIS at an inappropriate time during folliculogenesis interferes with the signal transduction cascade of another member of the TGF-β superfamily.

Correct prenatal development of the ovary, including germ cell migration, proliferation and association with somatic cells, is the foundation for successful postnatal ovarian development and is crucial for fertility in adult females. In humans, cases of infertility associated with premature ovarian failure may have their origins in improper prenatal ovarian development. The mouse models presented in this section are beginning to elucidate some of the processes essential for gonad formation, and are opening new avenues of investigation. These models may offer the potential for designing novel diagnostic and treatment modalities for human infertility.

Ovarian function: folliculogenesis

The follicle is the basic functional unit of the ovary, consisting of an oocyte surrounded by granulosa cells and thecal cells. During normal folliculogenesis, there is coordination of oocyte growth and maturation, and granulosa and thecal cell proliferation and development, within each follicular unit (Hirshfield, 1991). Distinct morphological and molecular changes occurring in each of these components reflect evolving functional capabilities crucial for the continued development of the follicle and eventual successful completion of this developmental programme (Fig. 1; Box 1) Continuous, metered recruitment of follicles into the growing pool and consistent growth rates ensure that mature follicles are produced in every cycle. Follicular responsiveness to global hormonal control and the production of steroid hormones coordinates the release of the mature oocyte with alterations in female physiology conducive to mating, fertilization and support of an embryo. The various mouse models discussed in this section are summarized (Table 2, Fig. 2).

Initiation of folliculogenesis

The meiotically arrested oocytes become surrounded by somatic cells by day 2 after birth, forming primordial follicles that represent the first stage of folliculogenesis and comprise the reserve pool. Morphologically, a quiescent primordial follicle (type 2 follicle in mice) in its largest cross-section consists of a single small oocyte (< 20 mm) surrounded by two to four squamous somatic cells, called pregranulosa cells, and a basement membrane (Pedersen and Peters, 1968). While the signal that triggers recruitment of a primordial follicle into the growing pool is still unknown, initiation of folliculogenesis can be recognized by a squamous to cuboidal morphological transition of the pregranulosa cells and an increase in the number of granulosa cells in the largest follicle cross-section to five to nine granulosa cells (a type 3a follicle) (Lintern-Moore and Moore, 1979). This period of follicular growth is thought to be relatively slow as few mitotic figures are observed in the granulosa cells of this stage. Studies of the incorporation of [^{3}H]thymidine after long-term continuous infusion in rats indicate that the duration of the cell cycle of these cells may be > 7 days (Hirshfield, 1989).

Initiation of follicle growth is not restricted to sexual maturity, but in fact begins within the first week of life. Indeed, the largest number of primordial follicles in mice is recruited during the first 2 weeks after birth, after which time the rate decreases and becomes relatively stable for the rest of the reproductive lifespan (Pedersen, 1972). Initiation of follicle growth is most likely regulated by intraovarian factors, since there is little change in the numbers of growing follicles during pregnancy, gonadotrophin depletion, gonadotrophin increases or hemiovariectomy (Peters *et al*., 1973; Edwards *et al*., 1977). In addition, the number of primordial follicles in the resting pool also does not alter the rate of initiation of follicle growth. Rats exposed to busulfan *in utero* have a significantly reduced number of oocytes and demonstrate markedly shortened reproductive lifespans. However, the ovaries of these rats contain similar numbers of growing follicles compared with controls up until depletion of the primordial pool (Hirshfield, 1994). Since there is no evidence for a reserve pool of larger follicles, it appears that once a follicle enters the growing pool, it is normally committed to a programme of growth and differentiation culminating in either apoptotic death of the granulosa cells (atresia) or ovulation of the mature oocyte (Peters *et al*., 1975).

Several gene products are known to be expressed by mouse follicles during the initiation period. Coincident with the transition of the granulosa cells from squamous to cuboidal morphology and initiation of oocyte growth, the oocyte begins to secrete its unique extracellular glycoprotein matrix, the zona pellucida. The formation of the zona pellucida early in folliculogenesis suggests that it is important for oocyte–granulosa cell coupling and that it plays a role in continued follicle development (Hirshfield, 1991). However, mice deficient in ZP3, one of the three major components of the zona pellucida, show no defects in this or any subsequent stages of folliculogenesis (Liu *et al*., 1996; Rankin *et al*., 1996). Instead, ZP3-deficient female mice are infertile owing to postovulatory adherence of the oocytes to the walls of the oviducts and defects in fertilization. Similarly, it has been postulated that c-kit plays a role in oocyte growth during the initiation phase since c-kit has been demonstrated in mouse oocytes of all stages of folliculogenesis, including oocytes of primordial follicles (Manova *et al*., 1990, 1993). However, no mutations in *c-kit* that permit primordial follicle formation but prevent initiation of follicle growth have yet been identified.

With the exception of these models, very little is known about the initiation of follicular growth, as it has generally not proven amenable to extraovarian manipulation. In addition, in wild type mice, the few (< 410) granulosa cells (Pedersen, 1972) in each one-layer follicle (type 3a and 3b follicles) represent a minute fraction of the total ovarian cells, making it increasingly difficult to isolate genes and proteins involved in the earliest stages of follicular development. However, the development of mouse models in which folliculogenesis is arrested at an early stage may favour the study of factors involved in initiation of follicular growth.

Preantral follicle growth: intraovarian regulation

With the initiation of follicular growth, a series of characteristic morphological changes occur within the follicle: oocyte growth, granulosa cell proliferation leading to formation of multiple layers, and thecal layer formation around the follicle outside of the basement membrane. Growth of preantral follicles, corresponding to type 3b to type 5b follicles, is gonadotrophin independent and seems to be regulated primarily by intraovarian and intrafollicular mechanisms. In the FSHβ knockout mouse (Kumar *et al*., 1997) or in the *hypogonadal* (*hpg*)

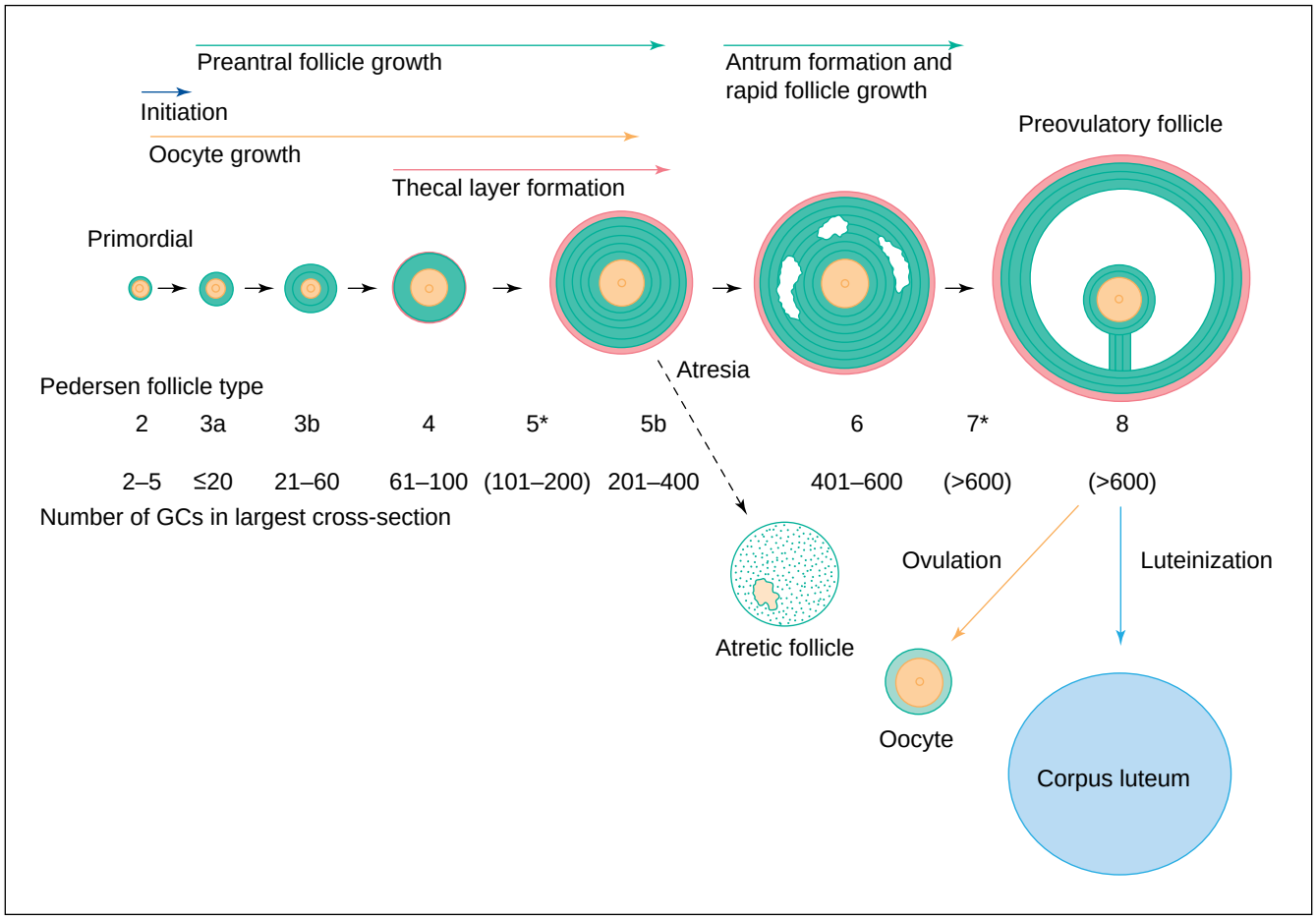

Fig. 1. Illustration of the major morphological events in folliculogenesis (adapted from Pedersen and Peters, 1968). Coloured arrows indicate the approximate onset and duration of some observable aspects of folliculogenesis: initiation, oocyte growth, granulosa cell proliferation, thecal layer formation, and antral follicle development. The normal endpoints of folliculogenesis, either atresia (apoptotic follicular death) or oocyte ovulation and follicular luteinization, are also shown. The follicle classification designation of Pedersen and Peters is indicated below each type of follicle together with the number of granulosa cells (GCs) in the largest cross-section through a follicle of that type. *Follicle class not pictured.

mouse, in which a naturally occurring mutation in the gonadotrophin releasing hormone (GnRH) gene markedly reduces synthesis of FSH and LH from the pituitary (Cattanach *et al.*, 1977), preantral follicle growth proceeds normally (see next section), confirming the gonadotrophin independence of this stage of development.

However, intrafollicular signalling from the granulosa cells to the oocyte through the kit ligand–c-kit receptor pathway is critical for preantral follicle development. Two hypomorphic alleles at the Steel (Sl) locus, Sl^{t} and Sl^{panda}, which encode the ligand for the c-kit receptor (stem cell factor; kit ligand), permit prenatal ovary development but result in growth arrest of ovarian follicles after follicular growth initiation but before the two-layer follicle stage (Beechey *et al.*, 1986; Kuroda *et al.*, 1988; Huang *et al.*, 1993). In wild type mice, kit ligand can be detected in the granulosa cells of these one-layer follicles, and expression increases preantral follicular growth. However, in Sl^{panda} homozygous mutant ovaries, kit ligand expression is extremely low (Huang *et al.*, 1993) owing to a large paracentric inversion located 115 kb 5′ of the kit ligand coding sequences (Bedell *et al.*, 1995). In addition, blocking antibodies to the c-kit receptor administered during the first 2 weeks after birth also inhibit follicular development in mice beyond the one-layer primary follicle stage (Yoshida *et al.*, 1997), supporting the essential role of kit ligand–c-kit signalling at this stage of follicular development.

In a reciprocal signalling scenario, growth differentiation factor 9 (GDF-9), a novel, oocyte-specific member of the TGF-β superfamily of secreted growth factors, is also necessary for early preantral follicle growth. GDF-9 is first expressed by oocytes in type 3a follicles, and its expression persists in the oocyte throughout ovulation (McGrath *et al.*, 1995; J. A. Elvin and M. M. Matzuk, unpublished). The GDF-9-deficient female mice show a block in follicular development at the type 3b follicle stage. In these mice, the oocytes continue to grow to the one-layer follicle stage, but eventually die, leaving behind a ribbon of zona pellucida. The granulosa cells fail to proliferate, persist, and eventually develop an abnormal steroidogenic phenotype (Dong *et al.*, 1996). From these models, it is clear that signalling from the granulosa cells to the oocyte, as well as

Box 1. Summary of Folliculogenesis in the Mouse

Folliculogenesis is a regulated developmental sequence of growth and differentiation of the oocyte and associated somatic cells. Two days after birth, naked oocytes become associated with three to five somatic cells termed 'pre-granulosa' cells to form the primordial follicle. Primordial follicles are recruited from a quiescent state into the growing pool by an as yet unknown signal. Growth initiation is recognized first by a squamous to cuboidal transition of the granulosa cells and the beginning of oocyte growth, and subsequent production of the zona pellucida. Granulosa cell–oocyte gap junctions composed of channels of connexins form, allowing diffusion of ions and metabolites and, potentially, other signalling molecules. Normally, once follicular growth is initiated, the oocyte grows to full size and the granulosa cells proliferate to form multiple layers of granulosa cells in response to intrafollicular signals. Fibroblast-like cells are recruited from the interstitium to encircle the multi-layer preantral follicle and differentiate to form the thecal layer. Preantral follicle development encompasses approximately seven doublings of the original granulosa cells and is regulated primarily by paracrine and autocrine mechanisms.

The final stages of follicular growth are heralded by the appearance of scattered fluid-filled spaces between granulosa cells which coalesce into a single antral cavity. Accumulating follicular fluid causes rapid expansion of the antral cavity and a marked increase in follicle size. As the follicle grows, the thecal layer also undergoes morphological differentiation. Lying just outside the basement membrane of the granulosa cells, the theca interna acquires the morphological hallmarks of steroidogenesis (abundant smooth endoplasmic reticulum and mitochondria and lipid droplets). The theca externa is richly vascularized and contains macrophages, fibroblasts, and smooth muscle-like cells.

Large follicles (type 5b to type 8 follicles) are responsive to extraovarian regulation and participate actively in the hypothalamic–pituitary–gonadal axis. Unless they are rescued by increased FSH signalling, most early antral follicles suffer atresia, a degenerative process characterized by widespread apoptotic death of the granulosa cells. In follicles selected for further development, LH stimulates thecal androgen production, while FSH stimulates granulosa cell proliferation, aromatization of androgens to oestrogens, and LH receptor expression. Follicular oestrogens feedback on both the hypothalamus and pituitary to trigger the LH surge, while granulosa cell-derived inhibin decreases pituitary FSH secretion. Thus, the fluctuations of gonadotrophins essential to the oestrous cycle depend on multiple regulatory loops among follicular, pituitary and hypothalamic components.

The preovulatory follicle is recognized by a meiotically competent oocyte protruding from the follicle wall on a well-developed stalk into a large antral cavity. The LH surge triggers release of the oocyte from meiotic arrest, breakdown of the follicle wall, and extrusion of the cumulus–ooocyte complex. The oocyte completes meiosis I giving off its first polar body, and progresses to the metaphase stage of meiosis II, where it arrests again until fertilization. The granulosa cells remaining in the postovulatory follicle undergo luteinization, which involves exit of the cells from the cell cycle, cellular hypertrophy, acquisition of steroidogenic morphology, and expression of cytochrome P450 cholesterol side chain cleavage. The resulting corpus luteum is a highly vascularized, transient endocrine organ that produces progesterone, essential for uterine preparation and maintenance of pregnancy (for reviews see Hirshfield, 1991; Adashi, 1992; Greenwald and Roy, 1994; Richards, 1994).

from the oocyte to the granulosa cells, regulates preantral follicle growth and does not require extragonadal input.

The thecal layer forms when the follicle achieves two layers of granulosa cells and provides a source of aromatizable androgen to the adjacent granulosa cells crucial for follicular oestrogen production (Magoffin, 1991). It is thought that thecal cells differentiate from stromal fibroblast-like precursors adjacent to developing follicles. Studies using cultured thecal–interstitial cells support the presence of a factor secreted from rat preantral follicles with two to five layers of granulosa cells (but not one layer) that can induce theca differentiation, including expression of 17α-hydroxylase-C17-20 lyase cytochrome P450, in the absence of gonadotrophins (Magarelli *et al*., 1996). In GDF-9-deficient mice, a thecal layer fails to form despite the presence of increased FSH and LH (Dong *et al*., 1996). However, an identifiable thecal layer is formed around the multilayer preantral follicles in the FSH-deficient ovary model (Kumar *et al*., 1997). Taken together, this evidence supports the presence of a paracrine, inductive signal necessary for thecal layer development which is secreted from preantral follicles with two or more layers of granulosa cells.

Antral follicle development: sensitivity to extraovarian regulation

During follicular development in mice, the formation of a follicular antrum represents a transition from primarily intrafollicular regulation to extraovarian regulation. Follicles enter a rapid period of growth and acquire new capabilities. The hypothalamic–pituitary–gonadal axis in females integrates input from the brain, pituitary and ovary, and produces hormones that coordinate follicle maturation with sexual behaviour and physiological preparation for pregnancy. The hypothalamus produces and releases GnRH in a pulsatile manner directly into the pituitary blood supply via the pituitary portal vessels (Everett, 1994). GnRH pulse frequency is modulated by the endocrine status of the animal; oestrogen

Table 2. Mouse models of postnatal ovarian failure

Transgenic/mutant mouse	Major reproductive findings	References
Defects in preantral follicle growth		
Kit ligand - *Steel*^{t}	Infertility; defect in folliculogenesis at one-layer follicle stage	Kohrogi *et al*., 1983; Kuroda *et al*., 1988
Kit ligand - *Steel*^{panda}	Infertility; reduced number of germ cells and defect in folliculogenesis at one-layer follicle stage	Beechey *et al*., 1986; Huang *et al*., 1993
Growth differentiation factor 9 (GDF-9) knockout	Infertility; defect in folliculogenesis at one-layer follicle stage	Dong *et al*., 1996
Defects in antral follicle growth		
Extraovarian defects		
Hypogonadal (*hpg*) mouse (GnRH deletion)	Infertility; small gonads	Cattanach *et al*., 1977; Mason *et al*., 1986
Human GnRH promoter-SV40 T antigen transgenic	Infertility due to an arrest in GnRH neurone migration	Radovick *et al*., 1991
Bovine glycoprotein hormone α-subunit promoter-DT transgenic	Infertility; hypogonadal	Kendall *et al*., 1991
Common glycoprotein hormone α-subunit knockout	Infertility; hypogonadal; hypothyroid	Kendall *et al*., 1995
Follicle stimulating hormone β subunit knockout	Female infertility; folliculogenesis block before antral follicle stage; males fertile but decreased testis size	Kumar *et al*., 1997
Activin receptor type II knockout	Infertility in females; delayed fertility in males; small gonads	Matzuk *et al*., 1995
Neuronal helix–loop–helix 2 (Nhlh2) knockout	Infertility; hypogonadal; obese; females fertile if reared with males	Good *et al*., 1997
Copper/zinc superoxide dismutase (*SOD1*) knockout	Subfertility in females; decreased serum gonadotrophin concentrations and embryonic death	Ho *et al*., 1998; Matzuk *et al*., in press
Obese (*ob*/*ob*) mouse (leptin deficiency)	Infertility; perturbation of the hypothalamic–pituitary axis	Chehab *et al*., 1996
Intraovarian defects		
Insulin-like growth factor (IGF-I) knockout	Hypogonadal and infertile folliculogenesis block before antral follicle stage	Baker *et al*., 1996a; Zhou *et al*., 1997
Oestrogen receptor α (ERα) knockout	Uterine/ovarian defects in females; small testes, reduced number of spermatozoa in males	Lubahn *et al*., 1993; Korach, 1994; Couse *et al*., 1995
Cyclin D2 knockout	Female infertility secondary to granulosa cell defect; males fertile but decreased testis size	Sicinski *et al*., 1996; Robker and Richards, 1998
α-Inhibin knockout	Infertility in females; secondary infertility in males; granulosa/Sertoli cell tumours; cachexia-like syndrome	Matzuk *et al*., 1992, 1994, 1996

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increases pulse frequency and increases the sensitivity of the anterior pituitary to GnRH. In response to GnRH, the anterior pituitary releases the heterodimeric glycoprotein hormones, FSH and LH, with low pulse frequency favouring FSH release and high pulse frequency favouring LH release.

FSH, in conjunction with locally produced oestradiol, functions primarily to promote follicular growth (Rao *et al*., 1978), causing the mitotic index of the late preantral to early antral granulosa cells to peak (that is, the final two to four rounds of granulosa cell division) (Pedersen, 1972; Hirshfield, 1991), and to induce the granulosa cell gene expression necessary for follicle maturation. Binding of FSH to the G-protein-coupled FSH receptor, expressed exclusively on granulosa cells, activates adenylyl cyclase leading to an increase in cAMP, activation of tissue-specific isoforms of protein kinase A, and phosphorylation of the transcription factor cAMP-response element-binding protein (CREB) (Richards *et al*., 1995). The FSH-triggered signal transduction cascade synergizes with oestradiol to activate expression of cytochrome P450 aromatase (CYP19), the α and β subunits of inhibin, as well as LH receptor (Richards, 1994). Concurrently, tonic LH concentrations stimulate maximum thecal cell androgen production. Feedback mechanisms include the peptide hormones, activin and inhibin, which stimulate or inhibit, respectively, pituitary FSH biosynthesis and release (Vale *et al*., 1994). Activins and inhibins are produced in many tissues, including the pituitary and the ovary, and can function in autocrine and paracrine signalling or as endocrine factors. Defects in the hypothalamic–pituitary–gonadal axis have a marked effect on antral follicle development.

Mutations leading to gonadotrophin deficiency, disregulation or insensitivity disrupt antral follicle development. Loss of pituitary stimulation by hypothalamic GnRH owing to a gene

Table 2. (*continued*)

Transgenic/mutant mouse	Major reproductive findings	References
Defects in ovulation/corpus luteum formation		
Extraovarian defects		
Transcription factor NGFI-A knockout	Infertility; luteinizing hormone suppression causing no corpora lutea	Lee *et al*., 1996
Bovine α promoter-bLHβ-CTP transgenic	Infertility; polycystic ovaries; granulosa cell tumours	Risma *et al*., 1995, 1997
Prolactin (*PRL*) knockout	Infertility; irregular and prolonged oestrus cycles	Horseman *et al*., 1997
Intraovarian defects		
Transcription factor NGFI-A-LacZ knock-in	Infertility; LH deficiency; LH receptor suppression, causing no ovulation and no corpora lutea	Topilko *et al*., 1998
Connexin 37 knockout	Infertility; defect in folliculogenesis at the Graafian follicle stage	Simon *et al*., 1997
Cyclooxygenase II (prostaglandin endoperoxide synthase-2) knockout	Largely infertile; absence of corpora lutea owing to apparent ovulation defect	Dinchuk *et al*., 1995
C/EBPβ (CCAAT/enhancer-binding protein β) knockout	Infertility; reduced ovulations and block in 'mature' corpora lutea formation	Sterneck *et al*., 1997
$p27^{Kip1}$ CDK inhibitory protein knockout	Female infertility; corpora lutea defects; males fertile but increased testis size	Fero *et al*., 1996; Kiyokawa *et al*., 1996
Progesterone receptor knockout	Infertility; defects in all reproductive tissues; no ovulation but corpora lutea formation	Lydon *et al*., 1995
Prolactin receptor (PRLR) knockout	Infertility; reduced ovulation and fertilization; blocked preimplantation development	Ormandy *et al*., 1997
Non-follicular fertility defects (selected)		
mZP3 knockout	Infertility; no zona pellucida	Liu *et al*., 1996; Rankin *et al*., 1996
c-*mos* knockout	Decreased fertility in females only; ovarian cysts and teratomas	Colledge *et al*., 1994; Hashimoto *et al*., 1994
MLH1 DNA mismatch repair enzyme knockout	Male and female infertility; defective meiosis at pachytene stage (males) and failure to complete meiosis II (females)	Edelmann *et al*., 1996; Baker *et al*., 1996b
Osteopetrotic (colony stimulating factor-1 mutant) mice	Male and female mice subfertile; reduced testosterone (males); implantation and lactation defects (females)	Cohen *et al*., 1997
Leukemia inhibitory factor (LIF) knockout	Infertility; implantation defect	Stewart *et al*., 1992
Steroid 5α-reductase type I knockout	Reduced litter size; parturition defects (fetal death owing to excess oestrogens)	Mahendroo *et al*., 1996, 1997

bLHβ-CTP, bovine luteinizing hormone β subunit–human chorionic gonadotrophin carboxyl-terminal peptide fusion; C/EBP, CCAAT/enhancer-binding protein β; DT, diphtheria toxin A chain; GnRH, gonadotrophin releasing hormone; MT, mouse metallotheionein I promoter; NGFI-A, nerve growth factor induced-A.

deletion, as in the hypogonadal (*hpg*) mouse (Mason *et al*., 1986), or owing to migration arrest of GnRH neurones and tumour formation, as in the GnRH-SV40 T antigen transgenic mouse (Radovick *et al*., 1991), prevents release of FSH (and LH). In the GnRH mouse models and the FSHβ-deficient mouse model (Kumar *et al*., 1997), follicles progress normally to the multilayer preantral follicle stage, but are unable to form antra, confirming the pivotal role of FSH in mouse antral follicle development. Mutations in both the FSH receptor (Aittomäki *et al*., 1995) and the GnRH receptor (Layman *et al*., 1998) have also been identified in humans as causes of recessively inherited hypogonadism, primary amenorrhoea and infertility.

Activins and inhibins are dimeric members of the TGF-β superfamily, in which the ratio of α subunits to β subunits produced by the cell determines whether the hormonal output is FSH stimulating (activin) or FSH suppressing (inhibin) (Meunier *et al*., 1988). The α inhibin-deficient and the activin receptor type II-deficient (ActRII) mice emphasize the importance of gonad-produced peptide hormone feedback and the intrapituitary effects of these peptides on FSH regulation. The α-inhibin knockout mice demonstrate increased serum FSH, confirming the known role of inhibin in decreasing pituitary FSH release. However, fertilizable oocytes were recovered from the oviducts of pregnant mares' serum gonadotrophin (PMSG)–hCG primed immature inhibin-deficient females, although in greatly reduced numbers compared with wild type (Matzuk *et al*., 1996). This finding indicates that, although folliculogenesis and ovulation can occur, overall fertility is reduced by the absence of inhibin. In addition, generation of this knockout mouse revealed an important antiproliferative and tumour suppressive role for inhibin in the gonads. Essentially 100% of inhibin-deficient mice develop early onset, rapidly growing granulosa–Sertoli cell

tumours that cause death secondary to a cancer cachexia-like syndrome mediated by activins secreted from the tumours (Matzuk *et al*., 1992, 1994; Coerver *et al*., 1996). The predisposition of inhibin-deficient mice to develop gonadal tumours identifies inhibin as a novel, secreted, tumour suppressor.

In contrast to the α-inhibin-deficient mice, mice lacking one of the activin receptors, activin receptor type II (ActRII), exhibit markedly suppressed serum and pituitary concentrations of FSH, indicating that ActRII is the major pituitary receptor through which activins affect FSH synthesis and secretion (Matzuk *et al*., 1995). ActRII-deficient ovaries display a block in folliculogenesis at a slightly later developmental stage compared with the ovaries of FSH-deficient mice. This finding suggests that the block in folliculogenesis is due only to the decreased FSH concentrations (that is, the phenotype is similar to an FSH hypomorphic allele) and not to the lack of paracrine signalling through ActRII in the ovary. Consistent with this role of ActRII in the pituitary, ActRII-deficient ovaries transplanted into ovariectomized immunocompatible wild type hosts (that is, females with normal FSH concentrations) resumed normal folliculogenesis, including formation of ovulatory follicles and corpora lutea, and restored fertility in the host mice (K. A. Coerver, Q. Guo and M. M. Matzuk, unpublished). Thus, lack of signalling through ActRII in the pituitary leads to suppressed serum FSH concentration which causes the impaired folliculogenesis in the ActRII-deficient mice.

Mice overexpressing follistatin, the activin-binding protein, using the metallothionein promoter have been generated to understand further the roles of activins and other members of the TGF-β superfamily (Guo *et al*., 1998). Female mice from two of the transgenic lines with the highest expression of the follistatin transgene often had blocks in folliculogenesis at the preantral and antral follicle stage, resulting in infertility in the most affected mice. These results suggest that follistatin can exert its effects in the ovaries as a local regulator of activin and possibly other TGF-β family members.

The insulin-like growth factor I (IGF-I) knockout mice (Baker *et al*., 1996a; Zhou *et al*., 1997) have an almost identical ovarian phenotype to the FSH knockout mice. IGF-I and IGF-I receptor are expressed in follicles that appear healthy, and may be markers for follicular selection since IGF-I has been shown to enhance proliferation of many cell types. IGF-I expression is not dependent upon gonadotrophins, as IGF-I is still expressed in FSH-deficient follicles (Zhou *et al*., 1997). Instead, IGF-I expression is thought to be initiated by intrafollicular signalling from the oocyte. However, expression of the FSH receptor (FSH-R) is augmented by both FSH and IGF-I, as evidenced by a 50% decrease in FSH-R concentration in the IGF-I-deficient ovaries or after elimination of gonadotrophins by hypophysectomy. The decrease in FSH-R concentration leading to relative gonadotrophin insensitivity in the IGF-I-deficient follicles is proposed as a mechanism for follicular arrest in the IGF-I-deficient model. As opposed to FSH-deficient follicles that show obvious thecal layer development, the largest follicles in the IGF-I-deficient ovaries are apparently surrounded by fibroblast-like cells devoid of steroidogenic morphology. Thus, IGF-I may also have a direct role in inducing thecal layer development.

The production of oestrogens is critical for the establishment of a positive feedback loop on the pituitary to cause the LH surge and augmentation of FSH regulated gene expression, including induction of LH receptors on the granulosa cells of the preovulatory follicle (Richards, 1994). The enzymes necessary for the synthesis of oestradiol, the main steroid hormone product of the preovulatory follicle, are divided between the cells of the thecal and granulosa compartments, demonstrating the importance of coordinated development of follicular components. Cytochrome P450 aromatase, induced in granulosa cells by FSH, catalyses the conversion of androgens to oestrogens. Thecal cells constitutively express LH receptors, and LH binding stimulates expression of the steroidogenic enzymes, cholesterol side chain cleavage cytochrome P450 and 17α-hydroxylase cytochrome P450, which catalyse androgen synthesis. These androgens diffuse across the basement membrane into the granulosa cells, where aromatase converts them into oestradiol.

Oestrogens require interaction with oestrogen receptors in target cells to exert their effects. Two different oestrogen receptors, ERα and ERβ, have been cloned and are expressed in ovarian cells. The ERα knockout mouse (ERKO) has been shown to be infertile due to a failure to ovulate, but unexpectedly the development of large antral follicles is preserved (Lubahn *et al*., 1993). The well-documented synergy between FSH and oestrogens necessary for antral follicle development is probably mediated through ERβ, which is specifically expressed in the granulosa cells of small, growing and preovulatory follicles, and is downregulated by the preovulatory LH surge (Byers *et al*., 1997). Signalling of oestrogen through ERβ in the ovary of the ERKO mouse may explain the mild ovarian phenotype, and may be masking important physiological functions of oestrogens in other tissues in addition.

Although FSH has long been recognized to induce proliferation of granulosa cells, only recently has the molecular mechanism of its mitogenic action been suggested. Granulosa cell proliferation appears to be mediated by FSH and oestradiol induction of cyclin D2 (Robker and Richards, 1998). Studies of rat granulosa cells cultured *in vitro* showed that forskolin induced cyclin D2 comparably with FSH, indicating that FSH signal transduction through the cAMP–protein kinase A pathway activated cyclin D2 expression (Sicinski *et al*., 1996). Increased expression and gene amplification of cyclin D2 were detected in a variety of human granulosa cell tumours, emphasizing its potential clinical importance in regulating granulosa cell proliferation.

Cyclin D2-deficient mice provide definitive evidence that FSH and oestradiol induction of cyclin D2 is functionally significant for granulosa cell proliferation (Sicinski *et al*., 1996). Although the number of oocytes and follicles in cyclin D2-deficient ovaries was normal, there was an obvious reduction in the number of granulosa cells surrounding each oocyte. Mutant ovaries showed minimal response to FSH administration in contrast to the rapid FSH-induced proliferation of the granulosa cell layer in wild type ovaries. This was particularly apparent in cyclin D2-deficient antral follicles, which rarely had more than four layers of granulosa cells, compared with controls containing up to ten layers. The gonadotrophin signal transduction cascade was shown to be intact, as cyclin D2-deficient follicles produce oestradiol in response to FSH, and expressed luteal cell markers, prostaglandin synthase-2, cholesterol side chain cleavage cytochrome P450 and progesterone receptor in response to LH. However, the cyclin D2-deficient

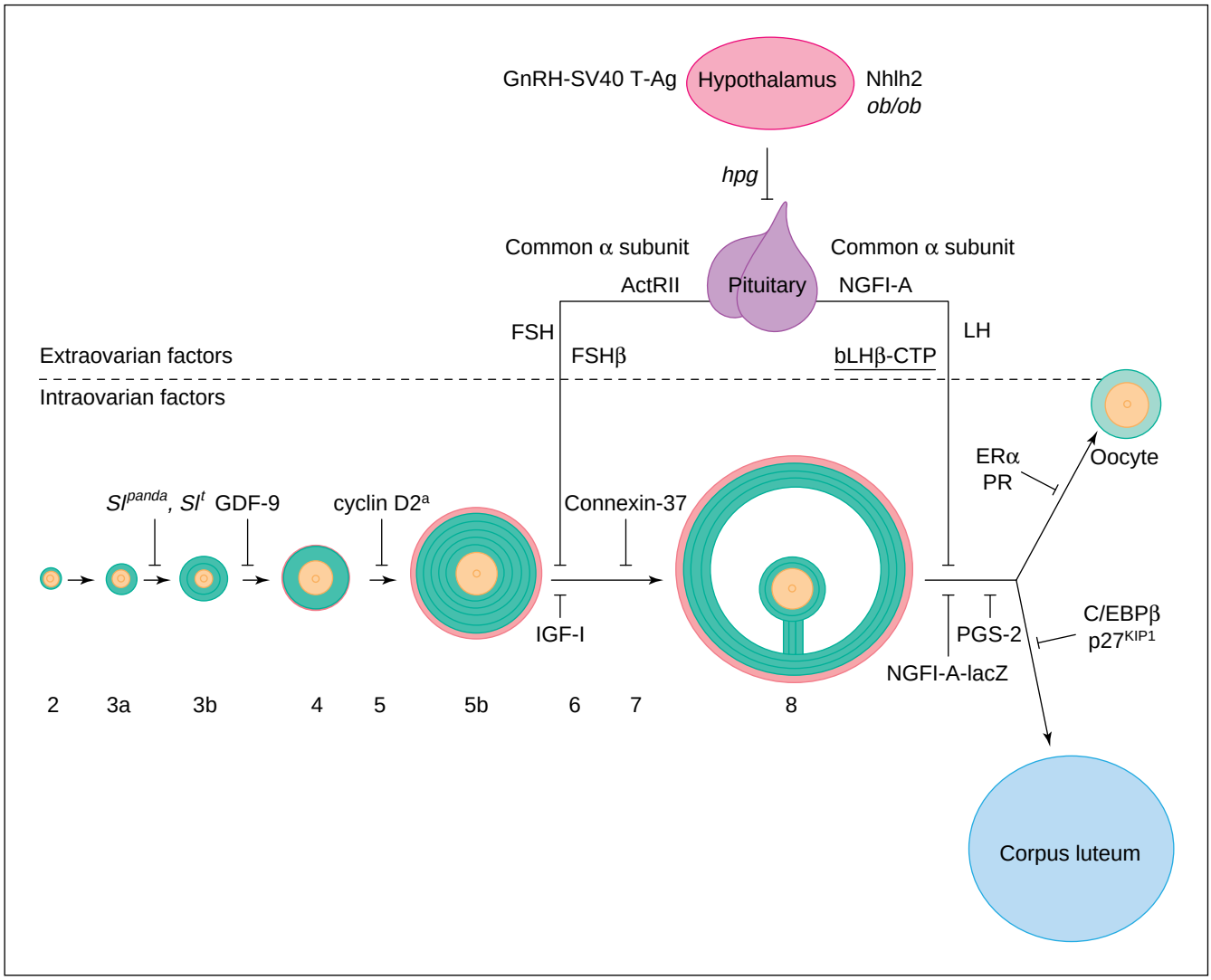

Fig. 2. Illustration of the stage of folliculogenesis blocked in selected mouse models. Mouse models of intraovarian factors are listed below the dashed line, while those altering ovarian function secondary to hypothalamic or pituitary defects are listed next to the affected organ above the dashed line. Knockout models are in bold type, spontaneous mutations in italics, transgenics are underlined, and blocks are denoted by ⊥. The follicle designation of Pedersen and Peters is listed below each type of follicle for reference. ActRII, activin type II receptor; bLHβ-CTP, bovine LHβ–hCG carboxyl-terminal peptide fusion; C/EBPβ, CCAAT/enhancer binding protein β; common α subunit, glycoprotein hormone common α subunit; ERα, oestrogen receptor α; GDF-9, growth differentiation factor 9; IGF-I, insulin-like growth factor I; MLH, MutL homologue; NGFI-A, nerve growth factor induced-A; Nhlh2, neuronal helix–loop–helix 2; PGS-2, prostaglandin synthase 2; PR, progesterone receptor. [a]Cyclin D2-deficient follicles develop antra, but granulosa cell proliferation is essentially blocked at this stage.

mice failed to ovulate in response to LH, and instead corpora lutea were formed with oocytes trapped inside. The cause of the ovulation defect is still unclear, but this phenotype emphasizes the importance of the coordination of growth and differentiation for successful completion of folliculogenesis.

During follicular growth, the oocyte grows and matures, first acquiring competence to undergo germinal vesicle (oocyte nucleus) breakdown (GVBD) and then competence to complete meiosis I. Connexin 37 is a component of the oocyte–granulosa cell gap junction, and connexin 37-deficient ovaries demonstrate a lack of junctional communication between the oocyte and granulosa cells, but not among granulosa cells. Oocyte growth is reduced in these mutant ovaries compared with controls, and > 90% of the oocytes are incompetent to resume meiosis (Simon *et al*., 1997). In addition, there are defects in the later stages of follicular development, including a failure to ovulate. This finding suggests that gap junction-mediated communication is important for the later stages of both oocyte and follicle development.

Ovulation and formation of corpora lutea

The preovulatory follicle responds to the LH surge by releasing the oocyte and undergoing a series of functional and

morphological changes in a process known as luteinization. The granulosa cells of the preovulatory follicle express LH receptors at high concentrations, enabling them to sense the LH surge. LH, like FSH, stimulates adenylyl cyclase to produce cAMP and activate PKA, and may also increase inositol triphosphate and activate protein kinase C (Richards *et al*., 1995). Activation of this additional second messenger system may explain why the follicular response to the LH surge is so radically different from its response to FSH. P450 aromatase expression is abolished; cell division is halted; and genes responsible for breakdown of the follicular wall and basement membrane begin to be expressed. Disruption of pituitary LH synthesis, LH receptor binding, or its downstream signalling, are likely to block ovulation, luteinization, or both.

Regulation of the LHβ subunit in the pituitary occurs during transcription, polyadenylation and glycosylation of the protein. The LHβ promoter contains binding sites for SF-1, ERα, CREB, and the zinc-finger transcription factor, NGFI-A (Lee *et al*., 1996). Whereas either SF-1 or NGFI-A can activate relatively low LH expression, together they have been shown to activate high LHβ expression synergistically. Female mice carrying a targeted disruption of NGFI-A are infertile due to a block in ovulation (Lee *et al*., 1996). In this model, serum LH concentration demonstrated a sexually dimorphic response to NGFI-A disruption: LH was decreased in males, and undetectable in females. Administration of exogenous LH to the NGFI-A females resulted in normal ovulation and corpus luteum formation, indicating that the critical defect causing ovarian failure was LH deficiency. In contrast, a second model in which a lacZ marker gene was inserted into the NGFI-A exon 1 to disrupt gene function showed a deficiency of LH synthesis in both males and females (Topilko *et al*., 1997). LacZ staining was observed in the corpora lutea and granulosa cells of the mature antral follicles, and the oocytes of mice heterozygous for the NGFI-A-LacZ knock-in. The anovulatory phenotype in the homozygous NGFI-A-LacZ knock-in females could not be rescued by pharmacological replacement of LH, possibly due to the significantly reduced LH-R in the granulosa cells of preovulatory follicles. These findings suggest that NGFI-A regulates expression of LH receptors in granulosa cells, as well as the synthesis of LH in the anterior pituitary.

Ovulation is frequently compared with an inflammatory response. Follicular hyperaemia and oedema occur within a few hours of the gonadotrophin surge and are probably mediated by vasoactive agents such as histamine, kinins and prostaglandins. In response to the gonadotrophin surge and inflammatory mediators, serine proteases and metalloproteinases, such as plasminogen activator and collagenases, also increase in ovulatory follicles, suggesting a biochemical mechanism for follicular rupture (Espey and Lipner, 1994). Indomethacin, a potent non-steroidal anti-inflammatory agent, can block ovulation potentially through inhibition of prostaglandin synthesis. Prostaglandin synthase 2 (cyclooxygenase 2; PGS-2) is one of two isoforms of the enzyme that catalyse the formation of prostaglandins from arachidonic acid, and has been shown to be rapidly, but transiently, induced in granulosa cells of the preovulatory follicle by the LH surge (Richards, 1994). Normally, concentrations of mRNA encoding PGS-2 reach peak values 4 h after administration of hCG and return to almost undetectable concentrations by 6–8 h after treatment (Sirois *et al*., 1992). In PGS-2-deficient ovaries, folliculogenesis progresses normally to the preovulatory stage, but the ovaries lack corpora lutea, resulting in infertility in the majority of the female mice (Dinchuk *et al*., 1995). These studies reinforce the importance of the proposed functional role of prostaglandins in the ovulatory process.

The PGS-2 promoter has a binding site for the LH surge-induced transcription factor, CCAAT/enhancer-binding protein β (C/EBPβ), suggesting that C/EBPβ controls PGS-2 transcription (Sirois and Richards, 1993). As demonstrated by *in situ* hybridization, expression of C/EBPβ is high in granulosa cells of late antral follicles by 7 h after hCG injection, suggesting that it is important in late follicular development. C/EBPβ-deficient mice demonstrate a significant decrease in ovulation efficiency and an absolute block in 'mature' corpus luteum formation (Sterneck *et al*., 1997). In C/EBPβ-deficient ovaries, PGS-2 was still induced by the LH surge, eliminating the possibility that the phenotypic similarities between C/EBPβ and PGS-2 knockouts were due simply to the absence of PGS-2. In contrast, both PGS-2 and cytochrome P450 aromatase persisted at least 7 h after the LH surge, indicating that C/EBPβ may mediate the transcriptional attenuation of PGS-2 and aromatase. Wild type, immunocompatible females retaining one wild type ovary and one transplanted C/EBPβ-deficient ovary were mated with wild type males and produced heterozygous pups, albeit at a much lower frequency than wild type pups, confirming that successful ovulation and fertilization of mutant oocytes can occur. Corpora lutea were never seen in the C/EBPβ-deficient ovaries even after confirmed ovulations had occurred, suggesting that the pregnancy was supported entirely by corpora lutea of the wild type ovary. The absolute requirement for C/EBPβ in luteal maturation may reflect a role in transcriptional attenuation of preovulatory genes or activation of other unknown genes. It will be of interest to determine whether the phenotype of the C/EBPβ knockout mice is secondary to the persistent expression of key preovulatory genes.

Coincident with inactivation of oestrogen biosynthesis through P450 aromatase loss, the LH surge activates progesterone biosynthesis by stimulating expression of cholesterol side chain cleavage cytochrome P450 in granulosa cells. Progesterone is known to play an essential role in preparing the uterus for pregnancy and allowing implantation of the embryo, and may also have physiological functions in the mammary gland, brain and ovary. These effects are mediated through binding to the progesterone receptor (PR), a member of the nuclear-receptor superfamily of transcription factors. PR is induced by ovulatory concentrations of LH in granulosa cells in culture and *in vivo* (Natraj and Richards, 1993; Park-Sarge and Mayo, 1994). The generation of the PR-deficient mouse (PRKO) has confirmed that progesterone plays an essential physiological role as an ovarian modulator (Lydon *et al*., 1995). PRKO females are infertile even though the ovaries exhibit normal folliculogenesis through the preovulatory stage. However, even pharmacological treatment with PMSG and hCG to induce superovulation fails to yield ovulated oocytes. Instead, histological examination of the ovary reveals many unruptured follicles containing oocytes surrounded by cumulus cells that have undergone expansion. This observation demonstrates that PR is required for follicular rupture, suggesting

that progesterone is not only necessary for postfertilization events but is also an essential regulator of ovulation.

Luteinization is the terminally differentiated state of granulosa cells and is accompanied by cell cycle arrest. $p27^{KIP1}$ is a cell cycle regulatory protein that controls cell cycle progression by binding to and inactivating cyclin–cyclin-dependent kinase (CDK) complexes in response to extracellular, anti-mitogenic signals. $p27^{KIP1}$ is widely expressed in nonproliferating cells, including the cells of the corpora lutea, but was not detectable in non-luteinized granulosa cells. In addition, $p27^{KIP1}$ has been shown to be induced in granulosa cells by LH (Robker and Richards, 1998).

Two different approaches were taken to generate a $p27^{KIP1}$ knockout mouse, yielding slightly different phenotypes (Fero *et al*., 1996; Kiyokawa *et al*., 1996). In both cases, $p27^{KIP1}$-deficient females had prolonged oestrous cycles and were unable to form corpora lutea after ovulation under physiological conditions. In one model, a neomycin cassette in the opposite transcription orientation was inserted into the first exon, allowing synthesis of immunodetectable carboxy-terminal p27 protein (Kiyokawa *et al*., 1996). However, no binding of the truncated p27 to cyclinE–CDK2 or cyclinD–CDK4 complexes could be detected by co-immunoprecipitation. In this model, superovulatory stimulation with PMSG–hCG yielded fertilizable oocytes but no corpora lutea. In contrast, in the model where both coding exons were replaced by a neomycin cassette yielding a definitive null allele, superovulation could produce corpora lutea (Fero *et al*., 1996). These results suggest that $p27^{KIP1}$ deficiency decreases the sensitivity of granulosa cells to luteinizing signals, consistent with a role in cell cycle arrest associated with luteal differentiation, and synthesis of a truncated $p27^{KIP1}$ protein may actively block luteinization.

The LH surge stimulates the mature oocyte to undergo GVBD and progression through meiosis I. The ovulated oocyte enters meiosis II, where it arrests in metaphase of meiosis II until fertilization. The protein product of c-*mos*, $pp39^{mos}$, plays an important role in this process, as c-*mos*-deficient oocytes fail to maintain meiotic arrest after maturation. Deficiency in c-*mos* leads to decreased fertility due to parthenogenic activation of ovulated oocytes, which renders them incapable of fertilization. The small numbers of offspring that do arise from c-*mos*-deficient mothers are presumed to be derived from fertilization of eggs shortly after maturation and before parthenogenic activation occurs.

Conclusions

Currently, more than 30 mouse models are available to study ovarian failure at different stages of development and regulation. Mouse models for studying prenatal ovarian development and the earliest stages of folliculogenesis are particularly important, as these periods of development have been relatively resistant to other methods of investigation. The transgenic mouse approach is also providing *in vivo* evidence to support *in vitro* studies of intraovarian and extraovarian regulators of later stages of folliculogenesis, as well as defining novel regulators of ovarian function. However, conventional knockout models only define the first essential function of that gene product in ovarian development or folliculogenesis, while often expression occurs at many stages. Generation of stage-specific, tissue-specific and inducible knockout models will be useful in further elucidation of the multiple roles played by ovarian regulators. Clearly, transgenic mice will continue to be useful *in vivo* models with which to study female reproductive development and function.

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