Cell. Mol. Life Sci. 63 (2006) 579–590
1420-682X/06/050579-12
DOI 10.1007/s00018-005-5394-7

Cellular and Molecular Life Sciences

Review

Genetics of early mammalian folliculogenesis

Y. Choi and A. Rajkovic*

Department of Obstetrics and Gynecology, Baylor College of Medicine, Faculty Center, 1709 Dryden Street, Suite 1100, Houston, 77030 (USA), Fax: +1 713 798 2744; e-mail: rajkovic@bcm.tmc.edu

Received 25 August 2005; received after revision 18 October 2005; accepted 21 November 2005
Online First 16 January 2006

Abstract. Early ovarian folliculogenesis begins with the breakdown of germ cell clusters and formation of primordial follicles. Primordial follicles are the smallest ovarian follicle units continuously recruited to grow into primary and more advanced ovarian follicles. Genes expressed in the germ cells such as *Figla, Nobox, Kit* and *Ntrk2*, as well as genes expressed in the surrounding somatic cells such as *Foxl2, Kitl* and *Ngf*, play critical functions during early folliculogenesis. Transgenic mice continue to provide important insights into the genetic pathways that regulate early mammalian folliculogenesis. Genes critical in early folliculogenesis are important determinants of reproductive life span and represent candidate genes for human ovarian failure.

Key words. Folliculogenesis; primordial follicle; primary follicle; germ cell clusters.

Introduction

A founder population of approximately 45 primordial germ cells (PGCs) at embryonic day 7 post conception (E7.5) gives rise to the germ cell lineage in the mouse [1, 2]. During mouse embryonic development from E9.5 to E11.5, the primordial germ cells migrate from the proximal epiblast to the urogenital ridge to form germ cell clusters also called cysts [3, 4]. Mitotic division of primordial germ cells, coupled with incomplete cytokinesis, results in clusters of oocytes around E10.5. Circa E13.5, female germ cells begin entry into the prophase I of meiosis and arrest in the diplotene stage of the first meiotic division. Oocytes arrested in meiosis I remain arrested until the time of ovulation. Mouse germ cell clusters, formed in the embryonic gonad, often contain more than 8 oocytes per cluster and break down shortly after birth as primordial follicles form (fig. 1). Primordial follicles consist of oocytes less than 20 μm in diameter individually enveloped by flat somatic cells, sometimes called pre-granulosa cells. Primordial follicles occupy the rim of the ovary with more advanced follicles located centrally. By postnatal day 7, most of oocytes in the mouse ovary are present in the primordial follicles with few germ cell clusters remaining. Around day 3 after birth, primary follicles appear in the mouse and consist of oocytes larger than 20 μm enveloped by somatic cells that are now cuboidal in shape. Primary follicles grow into larger secondary follicles, and ultimately become antral follicles, where oocytes reach a diameter of approximately 70 μm and are surrounded by highly differentiated granulosa cells.

The breakdown of germ cell clusters and formation of primordial follicles represents a critical stage in ovarian development. Transcription of numerous oocyte-specific genes is initiated, genes that are important in folliculogenesis, such as *Gdf9*, as well as maternal effect genes that affect two cell stage embryos such as *Zar1* [5–7]. Oocyte numbers decline after birth, although controversy exists whether the decline begins within the embryonic ovary or after birth [2, 4]. Regardless, not all oocytes within the germ cell clusters are thought to become primordial oocytes, and it is unclear why some become primordial oocytes and others die. The dogma has been that the ovary is endowed with finite number of oocytes, resting in primordial follicles. The primordial oocytes are continu-

* Corresponding author.

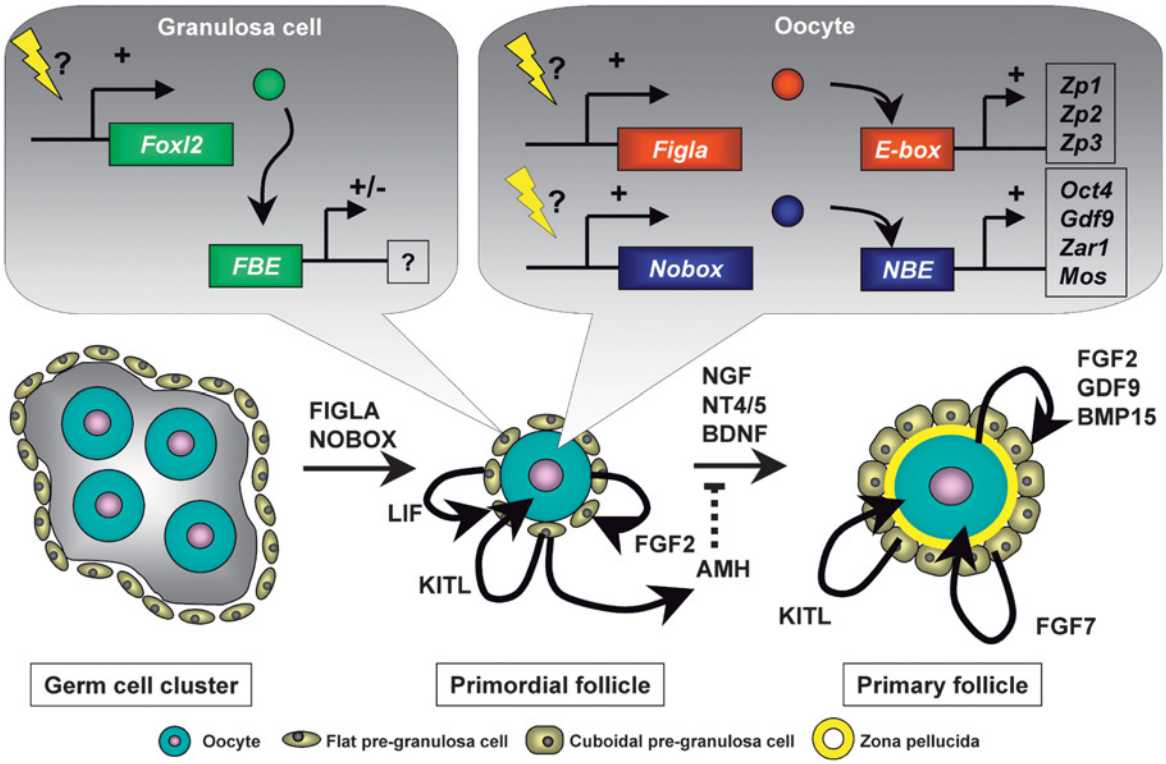

Figure 1. Genes that are important in the formation of primordial and primary follicles. The follicle structures involving the oocyte, zona pellucida, granulosa and theca cells are shown. Cell-to-cell interactions are mediated by KITL, FGF2, FGF7, GDF9, BMP15, NGF, NT4/5, BDNF, AMH and FOXL2. Transcriptional regulations are controlled by FIGLA and NOBOX. NBE is a putative NOBOX binding element. It is unknown whether NOBOX exerts direct or indirect action on *Oct4*, *Gdf9*, *Zar1* and *Mos*. FBE is a putative FOXL2 binding element. *Figla* and *Nobox* are expressed in the oocytes of germ cell clusters, primordial and more advanced follicles.

ously recruited to develop into more advanced follicular types. Since the vast majority of oocytes die during the recruitment, germ cell depletion in the ovary ultimately leads to menopause. Preliminary data suggest the existence of germline stem cells in the ovaries [8, 9]. The location of these germline stem cells is unclear, and more studies are necessary to ascertain their existence and physiologic relevance.

Follicle stimulating hormone beta, follicle stimulating hormone receptor and gonadotropin releasing hormone deficient mice can develop follicles that are beyond the primordial follicle stage, and therefore gonadotropins are not essential for early folliculogenesis [10–15]. Local factors produced by somatic and germ cells appear to play critical roles in early folliculogenesis. We will review here what is known about the molecular mechanisms of early folliculogenesis with an emphasis on knowledge derived from transgenic mouse models.

Factor in the germline alpha (*Figla*)

Factor in the germline alpha (FIGLA) is a basic helix-loop-helix transcription factor discovered in a screen to identify transcription factors that bind zona pellucida (*Zp*) promoters [16]. Mouse *Figla* is expressed as early as E13.5 in the female gonad, and appears exclusively confined to oocytes of germ cell clusters and throughout folliculogenesis. Although *Figla* is also expressed at low levels in testis, only female *Figla* knockout mice are infertile. The deficiency of *Figla* does not affect germ cell migration or proliferation, and embryonic gonads appeared normal [17]. However, oocytes rapidly disappeared after birth, and primordial follicles did not form. *Figla* was the first germ cell-specific transcription factor shown to affect primordial follicle formation. FIGLA can bind a promoter motif called E-box (CANNTG), located approximately 200 bp upstream of transcription starts sites of the zona pellucida (Zp) proteins, and interacts with the ubiquitous transcription factor E12 [16]. *Zp1*, *Zp2* and *Zp3* are major components of the extracellular zona matrix that surrounds the developing oocytes, and zona pellucida proteins are required for fertilization. Individually, *Zp1, Zp2* and *Zp3* mouse knockouts can form primordial through antral follicles [18–20]. It is not known whether the triple knockout for the *Zp* genes disrupts early folliculogenesis. *Figla* deletion does not affect the transcription of other genes preferentially expressed in the oocyte, including growth differentiation factor 9 (*Gdf9*), bone morphogenetic protein (*Bmp15*), kit receptor (*Kit*), connexin 43

and fibroblast growth factor 8. All these findings suggest that FIGLA likely regulates the expression of other downstream target genes that are critical in early folliculogenesis. Moreover, continual expression of *Figla* throughout folliculogenesis suggests that it is required to sustain transcription of its target genes that may be critical both in folliculogenesis and early embryogenesis.

Newborn ovary homeobox gene (*Nobox*)

Newborn ovary homeobox protein (NOBOX) is another germ cell-specific transcription factor critical in early folliculogenesis. *Nobox* was originally discovered by *in silico* subtraction of expressed sequence tags (ESTs) derived from the newborn ovaries [21, 22]. *Nobox* expression is detectable as early as E13.5, although its expression is significantly higher in E15.5 embryonic ovaries and beyond [23]. *Nobox* RNA and protein are preferentially expressed in oocytes of germ cell clusters and in primordial and growing oocytes throughout different stages of folliculogenesis [21, 23]. *Nobox* deletion causes postnatal oocyte loss and abolishes the transition from primordial to growing follicles in mice [23]. Newborn ovary histology in *Nobox* knockout and wild-type mice is grossly similar, but differs significantly at the molecular level. Numerous genes preferentially expressed in the oocytes, including *Gdf9*, *Bmp15*, *Mos* and *Oct4*, are downregulated in oocytes that lack *Nobox*. *Nobox* therefore directly or indirectly regulates transcription of cri-tical oocyte-specific genes. The onset of molecular changes in the *Nobox* knockout ovaries is likely to occur in the embryonic gonad, and microarray analysis of knockout and wild-type embryonic gonads at different times during the development will be useful. *Nobox* deficiency did not significantly affect expression of all oocyte-specific genes. *Figla*, *Zp1, Zp2,* and *Zp3* were similarly expressed in the *Nobox* knockout and wild-type newborn ovaries. Whether FIGLA directly regulates *Nobox* is unknown.

Transcriptional regulators upstream of *Figla* and *Nobox* are currently unknown, but their discovery will help better understand molecular pathways that govern germ cell development in mammals and hopefully shed a light on genes necessary to establish a female germline. It is likely that multiple other, yet unknown, oocyte-specific transcription factors are involved. Moreover, the role of others, such as *Pou5f1* (POU domain, class 5, transcription factor 1, *Oct4*), needs to be clarified. *Pou5f1* is expressed throughout folliculogenesis and is detectable in primordial oocytes, but its role in early and later folliculogenesis is unclear.

Kit-ligand (*Kitl*) and kit receptor (*Kit*)

Kit ligand (KITL) and Kit receptor (KIT) were originally found by studying mutations of the *steel* (*Sl*) and *dominant white spotting* (*W*) loci in mice, respectively. The mutation at both alleles causes various deficiencies in pigmentation, hematogenesis as well as defects in germ cell migration and proliferation [24–29]. The *Sl* locus encodes a growth factor, kit ligand (*Kitl*), and the *W* locus encodes the kit receptor, *Kit* [30–36]. KITL was the first granulosa cell-derived growth factor that can directly stimulate theca cell growth in the bovine ovary [37]. Theca cells in human fetal ovaries do not stain for KIT and the role of KITL in human theca cell growth is therefore unclear [38]. KITL is also known in the literature as steel factor [26], stem cell factor [39], and mast cell factor [40]. Alternative splicing generates two membrane-bound forms of kit ligand, KITL1 and KITL2, and it is not clear whether the two forms have different effects in early folliculogenesis [26]. *Kitl* messenger RNA (mRNA) is present as early as E9.5 in the fetal ovary and continues to be expressed in the 'pre-granulosa' and granulosa cells throughout folliculogenesis [41–43]. KITL signals through its receptor, KIT, which is a member of the family of type III transmembrane tyrosine kinase receptors. *Kit* expression becomes detectable in germ cells from E7.5, but is not detectable between E13.5 and E15.5, at the time when female germ cells enter meiosis [44]. *Kit* is expressed in the oocytes of primordial and growing follicles [37, 41–43].

There are 45 phenotypic alleles in the *Kitl* locus, and 87 phenotypic alleles in the *Kit* locus. Phenotypes differ depending on the mutation in the *Kitl* allele. The Sl^{d} allele infertility results from the deficiency of the germ cells in the ovary [45]. The Sl^{pan}, Sl^{con} and Sl^{t} mutant females have defects in postnatal ovarian follicle development [46–48] that affect early folliculogenesis. KITL addition to *in vitro* culture of the 4-day-old rat ovaries accelerates the transition from the primordial-to-primary follicles [49]. This effect is slight but statistically significant [49]. The importance of the KITL in primordial-to-primary follicle transition was demonstrated by using antibodies(ACK2) against KITL [49, 50]. ACK2 did not affect primordial follicle formation *in vitro* and *in vivo* but blocked primordial-to-primary follicle development [50]. These results implicate KITL in the primordial-to-primary follicle transition, but the role of KITL in the breakdown of germ cell clusters into primordial follicles is not clear.

Anti-Müllerian hormone (*Amh*)

The dimeric glycoprotein anti-Müllerian hormone (AMH) is a member of the transforming growth factor-beta (TGFβ) superfamily known also as Müllerian-inhibiting substance (MIS). AMH was originally found in degenerating Müllerian (female) ducts during male sexual differentiation [51]. *Amh* is also expressed in the mesenchymal cells adjacent to the epithelium of somatic and

granulosa cells of the fetal and adult female gonads [52–55]. AMH and its receptor AMHR2 are postnatally present in the granulosa cells of primary and growing follicles of the ovary in mice [51, 53, 56–60]. AMH appears to be the only natural ligand of the AMHR2 receptor, as the phenotype of *Amh* and *Amhr2* null mice are indistinguishable from each other in contrast to the diverse signaling pathways of other TGFβ gene family signaling pathways [61]. Female *Amh* null mice are fertile and have normal litter size [52]. However, the ovaries from 4-month-old and 13-month-old *Amh* knockout mice contained significantly less primordial follicles than corresponding wild-type mice [62]. AMH may have an inhibitory effect on follicle growth by attenuating the sensitivity of ovarian follicles to FSH in the sexually mature ovary [63]. *In vitro* experiments with postnatal day 2 mouse ovaries treated with AMH [59] showed fewer growing follicles, and these experiments support claims that AMH inhibits the growth of primordial follicles. The experiments above appear to exclude the possibility that AMH deficiency negatively affects the total number of female germ cell endowment in the embryonic ovary. It is interesting that this is exactly what occurs in mice that overexpress human *Amh* under the control of the mouse metallothionein promoter. *Amh* over-expressing mice are infertile, contain fewer germ cells in the ovary at birth and lose germ cells within 2 weeks of birth [64]. The molecular mechanisms whereby overexpression of *Amh* leads to the rapid loss of germ cells remain unclear. It is possible that overexpression of *Amh* at an inappropriate time during folliclulogenesis has a dominant negative effect on other TGFβ family members that in turn disrupts folliculogenesis. AMH may have dual functions in folliculogenesis: one that regulates the number of germ cells during embryogenesis, and the other that controls the size of the growing follicle pool by affecting the rate of recruitment.

Forkhead box L2 (*Foxl2*)

Forkhead box L2 (FOXL2) is a member of the forkhead (FKH)/hepatocyte nuclear factor 3 (HNF3) gene family of transcription factors [65]. The FKH/HNF3 family of transcription factors contains a conserved winged helix domain important for DNA binding to a common DNA motif in the promoter of target genes. Forkhead transcription factors bind to a 7-bp DNA binding motif (G/A) (T/C) (C/A) A A (C/T) A [66–70]. Mutations in the *Foxl2* gene cause type I blepharophimosis ptosis epicanthus inversus syndrome (BPES) and type II BPES in humans [65]. Unlike type II BPES, type I BPES women present with premature ovarian failure.

In mice, *Foxl2* is preferentially expressed in the pre-granulosa cells within the ovary [71, 72]. Ovaries of *Foxl2* null mice contain oocytes surrounded by flat granulosa cells [73, 74], but no advanced follicular structures are present. *Foxl2* deficiency aborts the proliferation of granulosa cells as well as the transition of flat squamous cells that surround primordial oocyte to cuboidal granulosa cells that surround primary oocytes. FOXL2 targets are not known. The deficiency of *Foxl2* has no effect on the expression of other oocyte-specific genes such as *Figla, Gdf9* and *Kit*, but the expressions of *Activin βA* and *Amh* were reduced in the ovaries of *Foxl2* null mice [59, 60, 75–77]. FOXL2 binding DNA motif is present upstream of the steroidogenic acute regulatory (*Star*) gene [78]. FOXL2 repressed the activity of the *Star* promoter, and engineered dominant negative mutations within *Foxl2* abolished wild-type FOXL2 repression activity of *Star in vitro*. *Star* may be a direct downstream target for *Foxl2*. *Star* is expressed in steroidogenic tissues, including granulosa and theca cells of the ovary, and plays a role in mobilization and delivery of cholesterol precursors to the inner mitochondrial membrane. However, derepression of STAR activity in *Foxl2* mice probably does not disrupt early follicle formation, and a more comprehensive search for FOXL2 downstream targets that affect early folliculogenesis is necessary.

Neurotrophins and their receptors

Neurotrophins have a wide-ranging role in the development of both the nervous system and the development of non-neuronal systems, including the cardiovascular, endocrine, reproductive and immune systems [79]. Nerve growth factor (NGF) is one of the neurotrophins, and the neurotrophin family includes brain-derived neurotrophic factor (BDNF), neurotrophin 3 (*Ntf3*) and neurotrophin 5 (*Ntf5*). *Ngf* expression in the somatic cells and oocytes of the ovary precedes follicle formation [80–84]. Neurotrophin receptors *Ngfr* (*p75*), *Ntrk1* (*trkA*), *Ntrk2* (*trkB*) and *Ntrk3* (*trkC*) are all expressed in the oocytes as well as the somatic cells of the ovary [81, 85–87]. Ovarian expression of *Ntf5* and its receptor *Ntrk2* was increased, *Ngf* and its receptor *Ntrk1* mRNA expression was reduced, whereas *Ntf3* and *Ntrk3* (*trkC)* mRNAs did not change at the time of early folliculogenesis [81]. These findings suggest that each neurotrophin and its respective receptor play different roles in the proliferation and differentiation of somatic cells as well as early folliculogenesis.

The role of neurotrophins and their receptors has been examined in transgenic knockout mice. *Ngf*-deficient ovaries contain a reduced number of primary and secondary follicles and an increased number of oocytes enclosed within the germ cell clusters in the ovary [88, 89]. In addition, the proliferation of somatic cells was reduced in the ovaries of *Ngf*-deficient mice compared with wild-type mice. These results suggest that NGF plays a role in the formation of follicles by affecting differentiation of

flat squamous cells, which surround primordial oocytes, into cubodal pre-granulosa cells that surround primary oocytes. The reduction in the number of secondary follicles indicates that GDF9 and KITL may interact with NGF to induce proliferation and differentiation of somatic cells surrounding the oocyte. NGF signals through the tyrosine kinase receptors *Ngfr* and *Ntrk1*. These two receptors are expressed in the ovaries of wild-type and *Ngf* null mice [88]. The lack of *Ngfr* does not affect the formation of primordial, primary or secondary follicles, and *Ngfr* null mice are fertile [90]. *Ntrk1* deficiency causes death at or shortly after birth [91, 92]. The function of these receptors in the ovary remains unclear and conditional, ovary-specific knockout of *Ntrk1*, will be useful to assess *Ntrk1* contribution to early folliculogenesis. It is also possible that NGF signals through receptors other than NGFR and NTRK1.

Ntf5 and BDNF, the other two neurotrophins, preferentially signal through NTRK2 receptors. Mice deficient in both *Ntf5* and *Bdnf* have a significantly lower number of primary and secondary follicles compared with wild-type [89, 91, 93]. *Ntrk2* null mice suffer from neurologic deficits and die within the first week of life. As expected, if *Ntrk2* functions as a preferential receptor for *Ntf5* and *Bdnf*, *Ntrk2*-deficient seven-day-old ovaries contained a reduced number of secondary follicles [93], and the block appeared to be in the transition from primary to secondary follicles, partially reminiscent of *Gdf9* null ovaries [94, 95]. The presence of *Gdf9* and *Kitl* in *Ntrk2* null ovaries suggests that *Ntf5* and *Bdnf* may affect the transition of primary into secondary follicles by a different pathway. Quantitative reverse-transcription polymerase chain reaction (RT-PCR) shows that the expression of follicle-stimulating hormone receptor (FSHR) was significantly reduced in *Ntrk2* null mice as compared with wild-type mice, but this may be just a reflection of the decreased number of secondary follicles in *Ntrk2* null ovaries.

Ntrk2 null ovaries were also transplanted under the kidney capsule to study postnatal ovarian development, and these studies showed rapid loss of oocytes in *Ntrk2* null ovaries. Neurotrophins clearly play an important role in nonneuronal targets, and alongside *Gdf9* and *Kitl*, are critical in early stages of folliculogenesis through unknown molecular mechanisms. Use of conditional, ovary-specific knockouts will be very useful to study the effects of neurotrophins and their receptors in mouse models affected by perinatal lethality.

Growth differentiation factor 9 (*Gdf9*) and bone morphogenetic protein 15 (*Bmp15*)

Growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15, also known as GDF9b) are members of the transforming growth factor-β superfamily [5, 6, 96, 97]. *Gdf9* and *Bmp15* are preferentially expressed in oocytes [6, 97]. In mice, *Bmp15* null females are subfertile , with decreased ovulation and fertilization rate and normal gross histologic appearance of the ovary [98], while *Gdf9* null female are infertile, and follicle growth arrests at the primary follicle stage [94] *Gdf9* and *Bmp15* do not appear critical in the formation of primordial follicles, and *Gdf9* in mice is essential for the growth and differentiation of the surrounding granulosa cells [95, 99, 100], antral follicle growth and ovulation [101-104]. The subtle phenotype in *Bmp15* knockout mice was surprising.

However, the importance of BMP15 in ovarian function was shown in the study of Inverdale and Hanna sheep, which carry naturally occurring missense and nonsense mutations in the Bmp15 coding region [105, 106]. These mutations increase fertility in the heterozygotes but cause infertility in homozygotes [105]. Interestingly, the ovarian phenotype in *Bmp15* sheep mutants is phenotypically similar to that in *Gdf9* null mice, but not in *Bmp15* null mice. The *Bmp15* deletion mutation engineered in mice causes null phenotype and no adverse effects on GDF9 action, while nonsense and missense mutations in sheep are likely to cause dominant negative effects on GDF9 action [107, 108]. It would be of great interest to generate mutant mice that carry sheep mutations and study whether such mutations will disrupt rodent folliculogenesis. The dominant negative role of *Bmp15* mutations in granulosa cell signaling may also account for infertility in humans [109]. Two female siblings of an Italian family had primary amenorrhea, ovaries that lacked follicles, a normal 46 XX karyotype, and no evidence of autoimmune disease or consanguinity. A search for candidate genes revealed a mutation in the human BMP15 that involved an A-to-G transition at nucleotide 704, changing a tyrosine (Y) at position 235 to a cysteine (C). The mutant Y235C protein was unable to stimulate incorporation of ^{3}H-thymidine into human granulosa cells, and it also inhibited wild-type BMP15 ability to stimulate ^{3}H-thymidine incorporation into granulosa cells. In sheep, mutated BMP15 likely act in a dominant negative manner to block GDF9. When sheep were immunized with GDF9 peptide, BMP15 peptide or ovine BMP15 mature protein, normal follicular development was arrested at the transitory or primary stage [110]. Above experiments clearly indicate that GDF9 and BMP15 are important in follicular growth in mice, sheep and humans.

Fibroblast growth factor 2 (*Fgf2*)

Fibroblast growth factor 2 (FGF2) is a member of a family of fibroblast growth factors. *Fgf2* is involved in cell differentiation, migration and angiogenesis in many tissues [111, 112]. *Fgf2* is primarily localized in the oocyte of primordial and growing follicles as well as the granulosa cells in the ovary [113–116]. FGF2 receptor is de-

tected in granulosa cells surrounding the ovary [117, 118]. FGF2 can induce an increase in the transition of primordial to growing follicles in FGF2-treated rat ovaries [114], and antibody against FGF2 slightly decreased follicle development. FGF2 can stimulate the growth of theca and stroma cells. Ovaries treated with FGF2 appear to express higher levels of *Kitl* mRNA [119]. These observations suggest that one function of oocyte-derived FGF2 may be to increase granulosa-derived *Kitl* expression, and that both KITL and FGF2 are required to optimally promote primordial-to-primary follicle transition. Futhermore, FGF2 suppresses apoptosis in granulosa cells [120]. Therefore, FGF2 produced by the oocyte in early follicles appears to regulate a variety of ovarian functions, including cell growth, development of the primordial follicles and stabilization of the follicle. *Fgf2* knockout mice are viable and fertile [121], although detailed examination of *Fgf2* knockout ovaries has not been reported.

It would be of interest to examine more closely the reproductive function in *Fgf2* null mice, since subtle fertility defects may be missed. In addition, these mice may be useful to identify which growth factors may compensate *in vivo* for the lack of *Fgf2*.

Fibroblast growth factor 7 (*Fgf7*)

Fibroblast growth factor 7 (FGF7, also known as keratinocyte growth factor, Kgf) is like FGF2, a member of the fibroblast growth factor family. *Fgf7* was originally found as a human mitogen with preferential action in the epithelial cells, and plays an important role in mesenchymal stimulation of normal epithelial cell proliferation [122, 123]. FGF7 mediates mesenchymal-epithelial cell interactions in many tissues including the ovary [124, 125], placenta [126], bladder [127], hair follicle [128], stomach [129], lung [130, 131], ventral prostate [132], and seminal vesicle [133]. *Fgf7* mRNA is detected in thecal cells, and its receptor is expressed in granulosa cells in the ovary [124, 134]. FGF7 produced by thecal cells stimulates proliferation of granulosa cells during follicular development in the ovary [125]. Primordial-to-primary follicle transition was increased from 45% to 65% with the addition of FGF7 to the 4-day-old rat ovary organ culture system [135]. FGF7 may act as a mesenchymal factor that promotes primordial-to-primary follicle development. However, *in vivo* knockout data to support this view is lacking. *Fgf7* knockout mice display abnormal hair development but no mention is made with regards to fertility [128].

Leukemia inhibitory factor (*Lif*)

Leukemia inhibitory factor (LIF) is a multifunctional glycoprotein cytokine. LIF is produced in blastocysts [136] and several tissues including the uterus [137, 138], thymus and lung [139], hypophysis [140], cardiac muscle [141], kidney [142], and in the skin [143]. LIF signals through its heterodimeric membrane receptor composed of a low-affinity LIF-specific receptor and the gp130 receptor chain [144]. LIF is present in follicular fluid, and its levels rise around the time of ovulation, indicating that LIF may play a role in ovulatory events, embryonic development and implantation [138, 145–149]. LIF protein is detected in the granulosa and somatic cells of primordial and primary follicles in the mouse ovary [150]. Exogenous treatment of the 4-day-old rat ovaries with LIF increased the primordial-to-primary follicle transition relative to the unstimulated controls [150]. Like FGF2, LIF treatment increased expression of *Kitl* mRNA in cultured granulosa cells, but LIF did not affect the proliferation of granulosa cells. These studies suggest that LIF may indirectly play a role in promoting the transition of primordial-to-primary follicles through induction of *Kitl* expression. *Lif* knockout mice are viable and fertile [147, 151], although detailed examination of ovaries lacking LIF has not been done.

In vitro effects observed on the primordial-to-primary follicle transition with LIF and other growth factors may not be relevant *in vivo*. However, functional redundancy may exist whereby deficiency of one growth factor is compensated by other growth factors. *In vivo* effects of FGF2, FGF7 and LIF on the primordial-to-primary follicle transition may require generation of double or triple knockout mice.

Steroids in early folliculogenesis

Steroids may play a role in early folliculogenesis. In mice primordial follicle formation occurs perinatally, when a precipitous drop in estrogen and progesterone occurs. Aromatase knockout mice provides an excellent model to study the effects of complete estrogen deficiency on follicular development. Ovaries that lack aromatase can develop primordial, primary, secondary and antral follicles. Estrogen deficiency is therefore not critical for early folliculogenesis. However, a closer look at aromatase knockouts revealed that the number of primordial follicles was approximately 40% less than in the wild-type ovaries [152, 153]. The number of primary follicles was statistically not significantly different between aromatase knockout and wild-type animals. Germ cell numbers in the newborn and embryonic aromatase knockout ovaries are not known, so the possibility remains that aromatase deficiency disrupts embryonic germ cell development. Primordial follicle numbers were also reduced in the newborns of pregnant baboons treated with an aromatase inhibitor [154]. It is unclear if the aromatase inhibitor effect is due to the direct effect on the developing ovary

Table 1. Mouse models affecting early folliculogenesis.

Gene	Phenotype of transgenic or knockout mouse	References
Figla	knockout; infertile; oocyte loss by postnatal day 2; unable to form primordial follicles	[17]
Nobox	knockout; infertile; most oocytes lost by postnatal day 14; primordial to primary follicles transition disrupted	[23]
Sl^{d}	spontaneous mutation; infertile; lack of germ cells	[45]
$Sl^{t, pan, con}$	spontaneous mutation; infertile; defect in folliculogenesis at primordial follicle stage; reduced number of germ cells	[46-48]
MT-Amh	*Amh* transgenic overexpression; infertile; lack of Müllerian duct derivatives; rapid loss of germ cells by postnatal day 14	[64]
Foxl2	knockout; infertile; block at stage of the primordial and primary follicle	[73, 74]
Ngf	knockout; reduced number of primary and secondary follicles; reduced proliferation of somatic cells;	[88, 89]
Ntf4/5 & Bdnf	double knockout; reduced number of primary and secondary follicles	[91, 93]
Ntrk2	knockout; failed transition from primary to secondary follicle	[93]
Gdf9	knockout; infertile; arrest of follicle growth at the primary follicle stage	[94]
Bmp15	knockout; subfertile; defects in ovulation and fertilization	[98]

or whether aromatase inhibitors exert subtle effects on the embryonic vasculature resulting in the smaller endowment of germ cells.

In vivo and *in vitro* studies on rat ovaries suggest that progesterone may inhibit primordial follicle formation from germ cell clusters [155]. *In vitro* rat studies also suggest that estrogen and progesterone may inhibit primordial-to-primary follicle transition. Progesterone receptor knockout mice have apparently normal follicular development until ovulation [156], and observed progesterone effects in rat ovaries may be artifactual, or progesterone may act via a nonnuclear receptor. The rapid decline in estrogen and progesterone concentrations after birth was hypothesized to be in part responsible for the breakdown of germ cell clusters and formation of primordial follicles in mammals. It is interesting to note, however, that primordial follicle formation occurs in humans during the time of continual rise in estrogen and progesterone levels, around 17–19 weeks of gestation [157–159]. In summary, the effects of estrogen and progesterone on early folliculogenesis are not critical, as shown by mouse knockout models, and further research is necessary to determine whether the observed effects documented by *in vitro* and *in vivo* approaches are physiologically relevant.

Conclusion

The breakdown of germ cell clusters and the formation of primordial follicles is poorly understood. These early steps in folliculogenesis are critical, as primordial follicles are considered the fundamental reproductive units of the ovary that give rise to all dominant follicles. Transcription of numerous genes, essential for both folliculogenesis and embryogenesis, is initiated during early folliculogenesis [23]. Both naturally occurring mutations as in the case of *Kit* and *Kitl*, and targeted disruption of critical genes such as *Figla* and *Nobox*, have helped elucidate genes critical in early folliculogenesis. Recent studies have focused on identifying the molecular and cellular mechanisms whereby follicle formation is regulated. Analysis of mouse newborn ovary and embryonic gonad transcriptomes as well as the use of microarrays will help identify other critical genes in early folliculogenesis [160, 161]. Mouse transgenic and knockout models for growth factors, cytokine, cell surface factors and transcriptional factors that affect early folliculogenesis will allow better functional understanding of genetic networks that are critical in early folliculogenesis (table 1). It is clear that oocyte-specific and somatic genes are critical for early folliculogenesis. Somatic growth factors such as KITL and NGF are clearly important and bind oocyte specific receptors KIT and NTRK2, respectively, to effect primordial follicle formation. In mice, oocytes from secondary follicles accelerate somatic cell differentiation and proliferation [162, 163]. It is possible that germ cell clusters and primordial oocytes manufacture growth factors involved in early signaling with surrounding somatic cells. Such oocyte-specific growth factors may play an important role in the transition from primordial-to-primary follicles, just as GDF9 is essential for primary-to-secondary follicle transition. Oocyte-specific transcription factors

such as FIGLA and NOBOX do not appear to affect primordial germ cell migration and proliferation, but the precise onset of molecular pathology is unclear. It is likely that *Figla* and *Nobox* deficiencies disrupt genetic pathways in the embryonic gonad, probably after germ cells enter meiosis. Transcriptome differences between knockout and wild-type embryonic ovaries may exist, and these differences should be assessed with microarray technologies.

The integration of genomic technologies into reproductive biology assisted and will continue to assist in uncovering critical genetic components in early folliculogenesis. Similarly, new developments in the stem cell biology field may affect our view about the earliest stages of follicle development. Recently, it has been proposed that germline stem cells exist within mouse ovaries [9] or bone marrow [8] and give rise to new oocytes in the adult ovaries. The existence of mammalian germline stem cells challenges the dogma that the initial primordial follicle pool gives rise to all mature eggs. However, data supporting ovarian germline stem cells in mammals is relatively new and warrants further investigation. The definitive experiment for adult ovarian germline stem cells (i.e. birth of offspring carrying the genetic markers of the bone marrow donor) has not been accomplished as of yet. Even so, it is possible that oocyte regeneration during adulthood is a rare event, but as such, it may be physiologically irrelevant to overall reproductive success. Whatever the outcome, a re-examination of primordial follicle physiology is likely to reveal additional important mechanisms in follicle development.

Acknowledgements. This work was supported in part by NIH grant HD44858, and grant no. 6-FY05-70 from the March of Dimes Birth Defects Foundation to A.R. We thank Dr. Stephanie Pangas for helpful comments.

1 Lawson K. A. and Hage W. J. (1994) Clonal analysis of the origin of primordial germ cells in the mouse. Ciba. Found. Symp. **182:** 68–84; discussion 84–91
2 McClellan K. A., Gosden R. and Taketo T. (2003) Continuous loss of oocytes throughout meiotic prophase in the normal mouse ovary. Dev. Biol. **258:** 334–348
3 Wylie C. (1999) Germ cells. Cell **96:** 165–174
4 Pepling M. E. and Spradling A. C. (2001) Mouse ovarian germ cell cysts undergo programmed breakdown to form primordial follicles. Dev. Biol. **234:** 339–351
5 Elvin J. A., Yan C. and Matzuk M. M. (2000) Oocyte-expressed TGF-beta superfamily members in female fertility. Mol. Cell. Endocrinol. **159:** 1–5
6 McGrath S. A., Esquela A. F. and Lee S. J. (1995) Oocyte-specific expression of growth/differentiation factor-9. Mol. Endocrinol. **9:** 131–136
7 Wu X., Viveiros M. M., Eppig J. J., Bai Y., Fitzpatrick S. L. and Matzuk M. M. (2003) Zygote arrest 1 (Zar1) is a novel maternal-effect gene critical for the oocyte-to-embryo transition. Nat. Genet. **33:** 187–191
8 Johnson J., Bagley J., Skaznik-Wikiel M., Lee H. J., Adams G. B., Niikura Y. et al. (2005) Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood. Cell **122:** 303–315
9 Johnson J., Canning J., Kaneko T., Pru J. K. and Tilly J. L. (2004) Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature **428:** 145–150
10 Abel M. H., Wootton A. N., Wilkins V., Huhtaniemi I., Knight P. G. and Charlton H. M. (2000) The effect of a null mutation in the follicle-stimulating hormone receptor gene on mouse reproduction. Endocrinology **141:** 1795–1803
11 Cattanach B. M., Iddon C. A., Charlton H. M., Chiappa S. A. and Fink G. (1977) Gonadotrophin-releasing hormone deficiency in a mutant mouse with hypogonadism. Nature **269:** 338–340
12 Dierich A., Sairam M. R., Monaco L., Fimia G. M., Gansmuller A., LeMeur M. et al. (1998) Impairing follicle-stimulating hormone (FSH) signaling *in vivo*: targeted disruption of the FSH receptor leads to aberrant gametogenesis and hormonal imbalance. Proc. Natl. Acad. Sci. USA **95:** 13612–13617
13 Kendall S. K., Samuelson L. C., Saunders T. L., Wood R. I. and Camper S. A. (1995) Targeted disruption of the pituitary glycoprotein hormone alpha-subunit produces hypogonadal and hypothyroid mice. Genes Dev. **9:** 2007–2019
14 Kendall S. K., Saunders T. L., Jin L., Lloyd R. V., Glode L. M., Nett T. M. et al. (1991) Targeted ablation of pituitary gonadotropes in transgenic mice. Mol. Endocrinol. **5:** 2025–2036
15 Kumar T. R., Wang Y., Lu N. and Matzuk M. M. (1997) Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. Nat. Genet. **15:** 201–204
16 Liang L., Soyal S. M. and Dean J. (1997) FIGalpha, a germ cell specific transcription factor involved in the coordinate expression of the zona pellucida genes. Development **124:** 4939–4947
17 Soyal S. M., Amleh A. and Dean J. (2000) FIGalpha, a germ cell-specific transcription factor required for ovarian follicle formation. Development **127:** 4645–4654
18 Rankin T., Talbot P., Lee E. and Dean J. (1999) Abnormal zonae pellucidae in mice lacking ZP1 result in early embryonic loss. Development **126:** 3847–3855
19 Rankin T. L., O'Brien M., Lee E., Wigglesworth K., Eppig J. and Dean J. (2001) Defective zonae pellucidae in Zp2-null mice disrupt folliculogenesis, fertility and development. Development **128:** 1119–1126
20 Rankin T. L., Tong Z. B., Castle P. E., Lee E., Gore-Langton R., Nelson L. M. et al. (1998) Human ZP3 restores fertility in Zp3 null mice without affecting order-specific sperm binding. Development **125:** 2415–2424
21 Suzumori N., Yan C., Matzuk M. M. and Rajkovic A. (2002) Nobox is a homeobox-encoding gene preferentially expressed in primordial and growing oocytes. Mech. Dev. **111:** 137–141
22 Ko M. S., Kitchen J. R., Wang X., Threat T. A., Wang X., Hasegawa A. et al. (2000) Large-scale cDNA analysis reveals phased gene expression patterns during preimplantation mouse development. Development **127:** 1737–1749
23 Rajkovic A., Pangas S. A., Ballow D., Suzumori N. and Matzuk M. M. (2004) NOBOX deficiency disrupts early folliculogenesis and oocyte-specific gene expression. Science **305:** 1157–1159
24 Bernstein A., Chabot B., Dubreuil P., Reith A., Nocka K., Majumder S. et al. (1990) The mouse W/c-kit locus. Ciba. Found. Symp. **148:** 158–166; discussion 166–172
25 Besmer P. (1991) The kit ligand encoded at the murine Steel locus: a pleiotropic growth and differentiation factor. Curr. Opin. Cell. Biol. **3:** 939–946
26 Besmer P., Manova K., Duttlinger R., Huang E. J., Packer A., Gyssler C. et al. (1993) The kit-ligand (steel factor) and its receptor c-kit/W: pleiotropic roles in gametogenesis and melanogenesis. Dev. Suppl.: 125–137
27 Galli S. J., Zsebo K. M. and Geissler E. N. (1994) The kit ligand, stem cell factor. Adv. Immunol. **55:** 1–96

28 Russell E. S. (1979) Hereditary anemias of the mouse: a review for geneticists. Adv. Genet. **20:** 357–459
29 Witte O. N. (1990) Steel locus defines new multipotent growth factor. Cell **63:** 5–6
30 Chabot B., Stephenson D. A., Chapman V. M., Besmer P. and Bernstein A. (1988) The proto-oncogene c-kit encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus. Nature **335:** 88–89
31 Copeland N. G., Gilbert D. J., Cho B. C., Donovan P. J., Jenkins N. A., Cosman D. et al. (1990) Mast cell growth factor maps near the steel locus on mouse chromosome 10 and is deleted in a number of steel alleles. Cell **63:** 175–183
32 Geissler E. N., Ryan M. A. and Housman D. E. (1988) The dominant-white spotting (W) locus of the mouse encodes the c-kit proto-oncogene. Cell **55:** 185–192
33 Huang E., Nocka K., Beier D. R., Chu T. Y., Buck J., Lahm H. W. et al. (1990) The hematopoietic growth factor KL is encoded by the Sl locus and is the ligand of the c-kit receptor, the gene product of the W locus. Cell **63:** 225–233
34 Leslie R. D., Isaacs A. J., Gomez J., Raggatt P. R. and Bayliss R. (1978) Hypothalamo-pituitary-thyroid function in anorexia nervosa: influence of weight gain. Br. Med. J. **2:** 526–528
35 Williams D. E., Eisenman J., Baird A., Rauch C., Van Ness K., March C. J. et al. (1990) Identification of a ligand for the c-kit proto-oncogene. Cell **63:** 167–174
36 Zsebo K. M., Williams D. A., Geissler E. N., Broudy V. C., Martin F. H., Atkins H. L. et al. (1990) Stem cell factor is encoded at the Sl locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. Cell **63:** 213–224
37 Parrott J. A. and Skinner M. K. (1997) Direct actions of kit-ligand on theca cell growth and differentiation during follicle development. Endocrinology **138:** 3819–3827
38 Hoyer P. E., Byskov A. G. and Mollgard K. (2005) Stem cell factor and c-Kit in human primordial germ cells and fetal ovaries. Mol. Cell. Endocrinol. **234:** 1–10
39 Zsebo K. M., Wypych J., McNiece I. K., Lu H. S., Smith K. A., Karkare S. B. et al. (1990) Identification, purification and biological characterization of hematopoietic stem cell factor from buffalo rat liver – conditioned medium. Cell **63:** 195–201
40 Anderson D. M., Lyman S. D., Baird A., Wignall J. M., Eisenman J., Rauch C. et al. (1990) Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. Cell **63:** 235–243
41 Joyce I. M., Pendola F. L., Wigglesworth K. and Eppig J. J. (1999) Oocyte regulation of kit ligand expression in mouse ovarian follicles. Dev. Biol. **214:** 342–353
42 Manova K., Huang E. J., Angeles M., De Leon V., Sanchez S., Pronovost S. M. et al. (1993) The expression pattern of the c-kit ligand in gonads of mice supports a role for the c-kit receptor in oocyte growth and in proliferation of spermatogonia. Dev. Biol. **157:** 85–99
43 Manova K., Nocka K., Besmer P. and Bachvarova R. F. (1990) Gonadal expression of c-kit encoded at the W locus of the mouse. Development **110:** 1057–1069
44 Manova K. and Bachvarova R. F. (1991) Expression of c-kit encoded at the W locus of mice in developing embryonic germ cells and presumptive melanoblasts. Dev. Biol. **146:** 312–324
45 Brannan C. I., Lyman S. D., Williams D. E., Eisenman J., Anderson D. M., Cosman D. et al. (1991) Steel-Dickie mutation encodes a c-kit ligand lacking transmembrane and cytoplasmic domains. Proc. Natl. Acad. Sci. USA **88:** 4671–4674
46 Bedell M. A., Brannan C. I., Evans E. P., Copeland N. G., Jenkins N. A. and Donovan P. J. (1995) DNA rearrangements located over 100 kb 5′ of the Steel (Sl)-coding region in Steel-panda and Steel-contrasted mice deregulate Sl expression and cause female sterility by disrupting ovarian follicle development. Genes Dev. **9:** 455–470
47 Huang E. J., Manova K., Packer A. I., Sanchez S., Bachvarova R. F. and Besmer P. (1993) The murine steel panda mutation affects kit ligand expression and growth of early ovarian follicles. Dev. Biol. **157:** 100–109
48 Kuroda H., Terada N., Nakayama H., Matsumoto K. and Kitamura Y. (1988) Infertility due to growth arrest of ovarian follicles in Sl/Slt mice. Dev. Biol. **126:** 71–79
49 Parrott J. A. and Skinner M. K. (1999) Kit-ligand/stem cell factor induces primordial follicle development and initiates folliculogenesis. Endocrinology **140:** 4262–4271
50 Yoshida H., Takakura N., Kataoka H., Kunisada T., Okamura H. and Nishikawa S. I. (1997) Stepwise requirement of c-kit tyrosine kinase in mouse ovarian follicle development. Dev. Biol. **184:** 122–137
51 Weenen C., Laven J. S., Von Bergh A. R., Cranfield M., Groome N. P., Visser J. A. et al. (2004) Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol. Hum. Reprod. **10:** 77–83
52 Behringer R. R., Finegold M. J. and Cate R. L. (1994) Mullerian-inhibiting substance function during mammalian sexual development. Cell **79:** 415–425
53 Hirobe S., He W. W., Lee M. M. and Donahoe P. K. (1992) Mullerian inhibiting substance messenger ribonucleic acid expression in granulosa and Sertoli cells coincides with their mitotic activity. Endocrinology **131:** 854–862
54 Munsterberg A. and Lovell-Badge R. (1991) Expression of the mouse anti-mullerian hormone gene suggests a role in both male and female sexual differentiation. Development **113:** 613–624
55 Taketo T., Saeed J., Manganaro T., Takahashi M. and Donahoe P. K. (1993) Mullerian inhibiting substance production associated with loss of oocytes and testicular differentiation in the transplanted mouse XX gonadal primordium. Biol. Reprod. **49:** 13–23
56 Ueno S., Kuroda T., Maclaughlin D. T., Ragin R. C., Manganaro T. F. and Donahoe P. K. (1989) Mullerian inhibiting substance in the adult rat ovary during various stages of the estrous cycle. Endocrinology **125:** 1060–1066
57 Rajpert-De Meyts E., Jorgensen N., Graem N., Muller J., Cate R. L. and Skakkebaek N. E. (1999) Expression of anti-Mullerian hormone during normal and pathological gonadal development: association with differentiation of Sertoli and granulosa cells. J. Clin. Endocrinol. Metab. **84:** 3836–3844
58 Baarends W. M., Hoogerbrugge J. W., Post M., Visser J. A., De Rooij D. G., Parvinen M. et al. (1995) Anti-mullerian hormone and anti-mullerian hormone type II receptor messenger ribonucleic acid expression during postnatal testis development and in the adult testis of the rat. Endocrinology **136:** 5614–5622
59 Durlinger A. L., Gruijters M. J., Kramer P., Karels B., Ingraham H. A., Nachtigal M. W. et al. (2002) Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. Endocrinology **143:** 1076–1084
60 Durlinger A. L., Visser J. A. and Themmen A. P. (2002) Regulation of ovarian function: the role of anti-Mullerian hormone. Reproduction **124:** 601–609
61 Mishina Y., Rey R., Finegold M. J., Matzuk M. M., Josso N., Cate R. L. et al. (1996) Genetic analysis of the Mullerian-inhibiting substance signal transduction pathway in mammalian sexual differentiation. Genes Dev. **10:** 2577–2587
62 Durlinger A. L., Kramer P., Karels B., de Jong F. H., Uilenbroek J. T., Grootegoed J. A. et al. (1999) Control of primordial follicle recruitment by anti-Mullerian hormone in the mouse ovary. Endocrinology **140:** 5789–5796
63 Durlinger A. L., Gruijters M. J., Kramer P., Karels B., Kumar T. R., Matzuk M. M. et al. (2001) Anti-Mullerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology **142:** 4891–4899
64 Behringer R. R., Cate R. L., Froelick G. J., Palmiter R. D. and Brinster R. L. (1990) Abnormal sexual development in trans-

genic mice chronically expressing mullerian inhibiting substance. Nature **345:** 167–170
65 Crisponi L., Deiana M., Loi A., Chiappe F., Uda M., Amati P. et al. (2001) The putative forkhead transcription factor FOXL2 is mutated in blepharophimosis/ptosis/epicanthus inversus syndrome. Nat. Genet. **27:** 159–166
66 Kaufmann E. and Knochel W. (1996) Five years on the wings of fork head. Mech. Dev. **57:** 3–20
67 Kaufmann E., Muller D. and Knochel W. (1995) DNA recognition site analysis of Xenopus winged helix proteins. J. Mol. Biol. **248:** 239–254
68 Overdier D. G., Porcella A. and Costa R. H. (1994) The DNA-binding specificity of the hepatocyte nuclear factor 3/forkhead domain is influenced by amino-acid residues adjacent to the recognition helix. Mol. Cell. Biol. **14:** 2755–2766
69 Pierrou S., Hellqvist M., Samuelsson L., Enerback S. and Carlsson P. (1994) Cloning and characterization of seven human forkhead proteins: binding site specificity and DNA bending. EMBO J. **13:** 5002–5012
70 Roux J., Pictet R. and Grange T. (1995) Hepatocyte nuclear factor 3 determines the amplitude of the glucocorticoid response of the rat tyrosine aminotransferase gene. DNA Cell Biol. **14:** 385–396
71 Loffler K. A., Zarkower D. and Koopman P. (2003) Etiology of ovarian failure in blepharophimosis ptosis epicanthus inversus syndrome: FOXL2 is a conserved, early-acting gene in vertebrate ovarian development. Endocrinology **144:** 3237–3243
72 Wang D., Kobayashi T., Zhou L. and Nagahama Y. (2004) Molecular cloning and gene expression of Foxl2 in the Nile tilapia, Oreochromis niloticus. Biochem. Biophys. Res. Commun. **320:** 83–89
73 Schmidt D., Ovitt C. E., Anlag K., Fehsenfeld S., Gredsted L., Treier A. C. et al. (2004) The murine winged-helix transcription factor Foxl2 is required for granulosa cell differentiation and ovary maintenance. Development **131:** 933–942
74 Uda M., Ottolenghi C., Crisponi L., Garcia J. E., Deiana M., Kimber W. et al. (2004) Foxl2 disruption causes mouse ovarian failure by pervasive blockage of follicle development. Hum. Mol. Genet. **13:** 1171–1181
75 Bath L. E., Wallace W. H., Shaw M. P., Fitzpatrick C. and Anderson R. A. (2003) Depletion of ovarian reserve in young women after treatment for cancer in childhood: detection by anti-Mullerian hormone, inhibin B and ovarian ultrasound. Hum. Reprod. **18:** 2368–2374
76 Gruijters M. J., Visser J. A., Durlinger A. L. and Themmen A. P. (2003) Anti-Mullerian hormone and its role in ovarian function. Mol. Cell. Endocrinol. **211:** 85–90
77 Salmon N. A., Handyside A. H. and Joyce I. M. (2004) Oocyte regulation of anti-Mullerian hormone expression in granulosa cells during ovarian follicle development in mice. Dev. Biol. **266:** 201–208
78 Pisarska M. D., Bae J., Klein C. and Hsueh A. J. (2004) Forkhead l2 is expressed in the ovary and represses the promoter activity of the steroidogenic acute regulatory gene. Endocrinology **145:** 3424–3433
79 Tessarollo L. (1998) Pleiotropic functions of neurotrophins in development. Cytokine Growth Factor Rev. **9:** 125–137
80 Dissen G. A., Hill D. F., Costa M. E., Les Dees C. W., Lara H. E. and Ojeda S. R. (1996) A role for trkA nerve growth factor receptors in mammalian ovulation. Endocrinology **137:** 198–209
81 Dissen G. A., Hirshfield A. N., Malamed S. and Ojeda S. R. (1995) Expression of neurotrophins and their receptors in the mammalian ovary is developmentally regulated: changes at the time of folliculogenesis. Endocrinology **136:** 4681–4692
82 Ernfors P., Wetmore C., Olson L. and Persson H. (1990) Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. Neuron **5:** 511–526
83 Hallbook F., Ibanez C. F. and Persson H. (1991) Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in Xenopus ovary. Neuron **6:** 845–858
84 Lara H. E., Hill D. F., Katz K. H. and Ojeda S. R. (1990) The gene encoding nerve growth factor is expressed in the immature rat ovary: effect of denervation and hormonal treatment. Endocrinology **126:** 357–363
85 Klein R., Parada L. F., Coulier F. and Barbacid M. (1989) trkB, a novel tyrosine protein kinase receptor expressed during mouse neural development. EMBO J. **8:** 3701–3709
86 Lamballe F., Klein R. and Barbacid M. (1991) trkC, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3. Cell **66:** 967–979
87 Dissen G. A., Hill D. F., Costa M. E., Ma Y. J. and Ojeda S. R. (1991) Nerve growth factor receptors in the peripubertal rat ovary. Mol. Endocrinol. **5:** 1642–1650
88 Dissen G. A., Romero C., Hirshfield A. N. and Ojeda S. R. (2001) Nerve growth factor is required for early follicular development in the mammalian ovary. Endocrinology **142:** 2078–2086
89 Ojeda S. R., Romero C., Tapia V. and Dissen G. A. (2000) Neurotrophic and cell-cell dependent control of early follicular development. Mol. Cell. Endocrinol. **163:** 67–71
90 Lee K. F., Li E., Huber L. J., Landis S. C., Sharpe A. H., Chao M. V. et al. (1992) Targeted mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral sensory nervous system. Cell **69:** 737–749
91 Liebl D. J., Klesse L. J., Tessarollo L., Wohlman T. and Parada L. F. (2000) Loss of brain-derived neurotrophic factor-dependent neural crest-derived sensory neurons in neurotrophin-4 mutant mice. Proc. Natl. Acad. Sci. USA **97:** 2297–2302
92 Smeyne R. J., Klein R., Schnapp A., Long L. K., Bryant S., Lewin A. et al. (1994) Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene. Nature **368:** 246–249
93 Paredes A., Romero C., Dissen G. A., DeChiara T. M., Reichardt L., Cornea A. et al. (2004) TrkB receptors are required for follicular growth and oocyte survival in the mammalian ovary. Dev. Biol. **267:** 430–449
94 Dong J., Albertini D. F., Nishimori K., Kumar T. R., Lu N. and Matzuk M. M. (1996) Growth differentiation factor-9 is required during early ovarian folliculogenesis. Nature **383:** 531–535
95 Elvin J. A., Yan C., Wang P., Nishimori K. and Matzuk M. M. (1999) Molecular characterization of the follicle defects in the growth differentiation factor 9-deficient ovary. Mol. Endocrinol. **13:** 1018–1034
96 Chang H., Brown C. W. and Matzuk M. M. (2002) Genetic analysis of the mammalian transforming growth factor-beta superfamily. Endocr. Rev. **23:** 787–823
97 Dube J. L., Wang P., Elvin J., Lyons K. M., Celeste A. J. and Matzuk M. M. (1998) The bone morphogenetic protein 15 gene is X-linked and expressed in oocytes. Mol. Endocrinol. **12:** 1809–1817
98 Yan C., Wang P., DeMayo J., DeMayo F. J., Elvin J. A., Carino C. et al. (2001) Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. Mol. Endocrinol. **15:** 854–866
99 Elvin J. A., Clark A. T., Wang P., Wolfman N. M. and Matzuk M. M. (1999) Paracrine actions of growth differentiation factor-9 in the mammalian ovary. Mol. Endocrinol. **13:** 1035–1048
100 Elvin J. A. and Matzuk M. M. (1998) Mouse models of ovarian failure. Rev. Reprod. **3:** 183–195
101 Elvin J. A., Yan C. and Matzuk M. M. (2000) Growth differentiation factor-9 stimulates progesterone synthesis in granulosa cells via a prostaglandin E2/EP2 receptor pathway. Proc. Natl. Acad. Sci. USA **97:** 10288–10293

102 Matzuk M. M. (2000) Revelations of ovarian follicle biology from gene knockout mice. Mol. Cell. Endocrinol. **163:** 61–66

103 Vitt U. A., Hayashi M., Klein C. and Hsueh A. J. (2000) Growth differentiation factor-9 stimulates proliferation but suppresses the follicle-stimulating hormone-induced differentiation of cultured granulosa cells from small antral and preovulatory rat follicles. Biol. Reprod. **62:** 370–377

104 Vitt U. A. and Hsueh A. J. (2001) Stage-dependent role of growth differentiation factor-9 in ovarian follicle development. Mol. Cell. Endocrinol. **183:** 171–177

105 Galloway S. M., McNatty K. P., Cambridge L. M., Laitinen M. P., Juengel J. L., Jokiranta T. S. et al. (2000) Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. Nat. Genet. **25:** 279–283

106 McNatty K. P., Juengel J. L., Wilson T., Galloway S. M. and Davis G. H. (2001) Genetic mutations influencing ovulation rate in sheep. Reprod. Fertil. Dev. **13:** 549–555

107 Liao W. X., Moore R. K., Otsuka F. and Shimasaki S. (2003) Effect of intracellular interactions on the processing and secretion of bone morphogenetic protein-15 (BMP-15) and growth and differentiation factor-9. Implication of the aberrant ovarian phenotype of BMP-15 mutant sheep. J. Biol. Chem. **278:** 3713–3719

108 Liao W. X., Moore R. K. and Shimasaki S. (2004) Functional and molecular characterization of naturally occurring mutations in the oocyte-secreted factors bone morphogenetic protein-15 and growth and differentiation factor-9. J. Biol. Chem. **279:** 17391–17396

109 Di Pasquale E., Beck-Peccoz P. and Persani L. (2004) Hypergonadotropic ovarian failure associated with an inherited mutation of human bone morphogenetic protein-15 (BMP15) gene. Am. J. Hum. Genet. **75:** 106–111

110 Juengel J. L., Hudson N. L., Heath D. A., Smith P., Reader K. L., Lawrence S. B. et al. (2002) Growth differentiation factor 9 and bone morphogenetic protein 15 are essential for ovarian follicular development in sheep. Biol. Reprod. **67:** 1777–1789

111 Slavin J. (1995) Fibroblast growth factors: at the heart of angiogenesis. Cell. Biol. Int. **19:** 431–444

112 Gospodarowicz D. (1989) Fibroblast growth factor. Crit. Rev. Oncog **1:** 1–26

113 van Wezel I. L., Umapathysivam K., Tilley W. D. and Rodgers R. J. (1995) Immunohistochemical localization of basic fibroblast growth factor in bovine ovarian follicles. Mol. Cell. Endocrinol. **115:** 133–140

114 Nilsson E., Parrott J. A. and Skinner M. K. (2001) Basic fibroblast growth factor induces primordial follicle development and initiates folliculogenesis. Mol. Cell. Endocrinol. **175:** 123–130

115 Quennell J. H., Stanton J. A. and Hurst P. R. (2004) Basic fibroblast growth factor expression in isolated small human ovarian follicles. Mol. Hum. Reprod. **10:** 623–628

116 Yamamoto S., Konishi I., Tsuruta Y., Nanbu K., Mandai M., Kuroda H. et al. (1997) Expression of vascular endothelial growth factor (VEGF) during folliculogenesis and corpus luteum formation in the human ovary. Gynecol. Endocrinol. **11:** 371–381

117 Shikone T., Yamoto M. and Nakano R. (1992) Follicle-stimulating hormone induces functional receptors for basic fibroblast growth factor in rat granulosa cells. Endocrinology **131:** 1063–1068

118 Wandji S. A., Pelletier G. and Sirard M. A. (1992) Ontogeny and cellular localization of 125I-labeled basic fibroblast growth factor and 125I-labeled epidermal growth factor binding sites in ovaries from bovine fetuses and neonatal calves. Biol. Reprod. **47:** 807–813

119 Nilsson E. E. and Skinner M. K. (2004) Kit ligand and basic fibroblast growth factor interactions in the induction of ovarian primordial to primary follicle transition. Mol. Cell. Endocrinol. **214:** 19–25

120 Tilly J. L., Billig H., Kowalski K. I. and Hsueh A. J. (1992) Epidermal growth factor and basic fibroblast growth factor suppress the spontaneous onset of apoptosis in cultured rat ovarian granulosa cells and follicles by a tyrosine kinase-dependent mechanism. Mol. Endocrinol. **6:** 1942–1950

121 Ortega S., Ittmann M., Tsang S. H., Ehrlich M. and Basilico C. (1998) Neuronal defects and delayed wound healing in mice lacking fibroblast growth factor 2. Proc. Natl. Acad. Sci. USA **95:** 5672–5677

122 Rubin J. S., Osada H., Finch P. W., Taylor W. G., Rudikoff S. and Aaronson S. A. (1989) Purification and characterization of a newly identified growth factor specific for epithelial cells. Proc. Natl. Acad. Sci. USA **86:** 802–806

123 Finch P. W., Rubin J. S., Miki T., Ron D. and Aaronson S. A. (1989) Human KGF is FGF-related with properties of a paracrine effector of epithelial cell growth. Science **245:** 752–755

124 Parrott J. A. and Skinner M. K. (1998) Developmental and hormonal regulation of keratinocyte growth factor expression and action in the ovarian follicle. Endocrinology **139:** 228–235

125 Parrott J. A., Vigne J. L., Chu B. Z. and Skinner M. K. (1994) Mesenchymal-epithelial interactions in the ovarian follicle involve keratinocyte and hepatocyte growth factor production by thecal cells and their action on granulosa cells. Endocrinology **135:** 569–575

126 Izumi S., Slayden O. D., Rubin J. S. and Brenner R. M. (1996) Keratinocyte growth factor and its receptor in the rhesus macaque placenta during the course of gestation. Placenta **17:** 123–135

127 Baskin L. S., Hayward S. W., Sutherland R. A., DiSandro M. J., Thomson A. A., Goodman J. et al. (1996) Mesenchymal-epithelial interactions in the bladder. World J. Urol. **14:** 301–309

128 Guo L., Degenstein L. and Fuchs E. (1996) Keratinocyte growth factor is required for hair development but not for wound healing. Genes Dev. **10:** 165–175

129 Matsubara Y., Ichinose M., Tatematsu M., Ichinose M., Oka M., Yahagi N. et al. (1996) Stage-specific elevated expression of the genes for hepatocyte growth factor, keratinocyte growth factor and their receptors during the morphogenesis and differentiation of rat stomach mucosa. Biochem. Biophys. Res. Commun. **222:** 669–677

130 Post M., Souza P., Liu J., Tseu I., Wang J., Kuliszewski M. et al. (1996) Keratinocyte growth factor and its receptor are involved in regulating early lung branching. Development **122:** 3107–3115

131 Shiratori M., Oshika E., Ung L. P., Singh G., Shinozuka H., Warburton D. et al. (1996) Keratinocyte growth factor and embryonic rat lung morphogenesis. Am. J. Respir. Cell. Mol. Biol. **15:** 328–338

132 Sugimura Y., Foster B. A., Hom Y. K., Lipschutz J. H., Rubin J. S., Finch P. W. et al. (1996) Keratinocyte growth factor (KGF) can replace testosterone in the ductal branching morphogenesis of the rat ventral prostate. Int. J. Dev. Biol. **40:** 941–951

133 Alarid E. T., Rubin J. S., Young P., Chedid M., Ron D., Aaronson S. A. et al. (1994) Keratinocyte growth factor functions in epithelial induction during seminal vesicle development. Proc. Natl. Acad. Sci. USA **91:** 1074–1078

134 Koji T., Chedid M., Rubin J. S., Slayden O. D., Csaky K. G., Aaronson S. A. et al. (1994) Progesterone-dependent expression of keratinocyte growth factor mRNA in stromal cells of the primate endometrium: keratinocyte growth factor as a progestomedin. J. Cell Biol. **125:** 393–401

135 Kezele P., Nilsson E. E. and Skinner M. K. (2005) Keratinocyte growth factor acts as a mesenchymal factor that promotes ovarian primordial to primary follicle transition. Biol. Reprod. (in press)

136 Conquet F. and Brulet P. (1990) Developmental expression of myeloid leukemia inhibitory factor gene in preimplantation blastocysts and in extraembryonic tissue of mouse embryos. Mol. Cell. Biol. **10:** 3801–3805
137 Song H., Lim H., Das S. K., Paria B. C. and Dey S. K. (2000) Dysregulation of EGF family of growth factors and COX-2 in the uterus during the preattachment and attachment reactions of the blastocyst with the luminal epithelium correlates with implantation failure in LIF-deficient mice. Mol. Endocrinol. **14:** 1147–1161
138 Vogiagis D. and Salamonsen L. A. (1999) Review: The role of leukaemia inhibitory factor in the establishment of pregnancy. J. Endocrinol. **160:** 181–190
139 Fukada K., Korsching S. and Towle M. F. (1997) Tissue-specific and ontogenetic regulation of LIF protein levels determined by quantitative enzyme immunoassay. Growth Factors **14:** 279–295
140 Chesnokova V. and Melmed S. (2000) Leukemia inhibitory factor mediates the hypothalamic pituitary adrenal axis response to inflammation. Endocrinology **141:** 4032–4040
141 Ancey C., Corbi P., Froger J., Delwail A., Wijdenes J., Gascan H. et al. (2002) Secretion of IL-6, IL-11 and LIF by human cardiomyocytes in primary culture. Cytokine **18:** 199–205
142 Morel D. S., Taupin J. L., Potier M., Deminiere C., Potaux L., Gualde N. et al. (2000) Renal synthesis of leukaemia inhibitory factor (LIF), under normal and inflammatory conditions. Cytokine **12:** 265–271
143 Bonifati C., Mussi A., D'Auria L., Carducci M., Trento E., Cordiali-Fei P. et al. (1998) Spontaneous release of leukemia inhibitory factor and oncostatin-M is increased in supernatants of short-term organ cultures from lesional psoriatic skin. Arch. Dermatol. Res. **290:** 9–13
144 Metcalf D. (2003) The unsolved enigmas of leukemia inhibitory factor. Stem Cells **21:** 5–14
145 Kholkute S. D., Katkam R. R., Nandedkar T. D. and Puri C. P. (2000) Leukaemia inhibitory factor in the endometrium of the common marmoset Callithrix jacchus: localization, expression and hormonal regulation. Mol. Hum. Reprod. **6:** 337–343
146 Senturk L. M. and Arici A. (1998) Leukemia inhibitory factor in human reproduction. Am. J. Reprod. Immunol. **39:** 144–151
147 Stewart C. L. and Cullinan E. B. (1997) Preimplantation development of the mammalian embryo and its regulation by growth factors. Dev. Genet. **21:** 91–101
148 Coskun S., Uzumcu M., Jaroudi K., Hollanders J. M., Parhar R. S. and al-Sedairy S. T. (1998) Presence of leukemia inhibitory factor and interleukin-12 in human follicular fluid during follicular growth. Am. J. Reprod. Immunol. **40:** 13–18
149 Ozornek M. H., Bielfeld P., Krussel J. S., Hirchenhain J., Jeyendran R. S. and Koldovsky U. (1999) Epidermal growth factor and leukemia inhibitory factor levels in follicular fluid. Association with *in vitro* fertilization outcome. J. Reprod. Med. **44:** 367–369
150 Nilsson E. E., Kezele P. and Skinner M. K. (2002) Leukemia inhibitory factor (LIF) promotes the primordial to primary follicle transition in rat ovaries. Mol. Cell. Endocrinol. **188:** 65–73
151 Stewart C. L., Kaspar P., Brunet L. J., Bhatt H., Gadi I., Kontgen F. et al. (1992) Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature **359:** 76–79
152 Britt K. L., Drummond A. E., Dyson M., Wreford N. G., Jones M. E., Simpson E. R. et al. (2001) The ovarian phenotype of the aromatase knockout (ArKO) mouse. J. Steroid Biochem. Mol. Biol. **79:** 181–185
153 Fisher C. R., Graves K. H., Parlow A. F. and Simpson E. R. (1998) Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the cyp19 gene. Proc. Natl. Acad. Sci. USA **95:** 6965–6970
154 Zachos N. C., Billiar R. B., Albrecht E. D. and Pepe G. J. (2002) Developmental regulation of baboon fetal ovarian maturation by estrogen. Biol. Reprod. **67:** 1148–1156
155 Kezele P. and Skinner M. K. (2003) Regulation of ovarian primordial follicle assembly and development by estrogen and progesterone: endocrine model of follicle assembly. Endocrinology **144:** 3329–3337
156 Lydon J. P., DeMayo F. J., Funk C. R., Mani S. K., Hughes A. R., Montgomery C. A. Jr. et al. (1995) Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. Genes Dev. **9:** 2266–2278
157 Forabosco A., Sforza C., De Pol A., Vizzotto L., Marzona L. and Ferrario V. F. (1991) Morphometric study of the human neonatal ovary. Anat. Rec. **231:** 201–208
158 Kurilo L. F. (1981) Oogenesis in antenatal development in man. Hum. Genet. **57:** 86–92
159 Reynaud K., Cortvrindt R., Verlinde F., De Schepper J., Bourgain C. and Smitz J. (2004) Number of ovarian follicles in human fetuses with the 45,X karyotype. Fertil. Steril. **81:** 1112–1119
160 Small C. L., Shima J. E., Uzumcu M., Skinner M. K. and Griswold M. D. (2005) Profiling gene expression during the differentiation and development of the murine embryonic gonad. Biol. Reprod. **72:** 492–501
161 Rajkovic A., Yan M. S. C., Klysik M. and Matzuk M. (2001) Discovery of germ cell-specific transcripts by expressed sequence tag database analysis. Fertil. Steril. **76:** 550–554
162 Eppig J. J. (2001) Oocyte control of ovarian follicular development and function in mammals. Reproduction **122:** 829–838
163 Eppig J. J., Wigglesworth K. and Pendola F. L. (2002) The mammalian oocyte orchestrates the rate of ovarian follicular development. Proc. Natl. Acad. Sci. USA **99:** 2890–2894