The TGF- β Family in the Reproductive Tract

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The transforming growth factor β (TGF- β) family has a profound impact on the reproductive function of various organisms. In this review, we discuss how highly conserved members of the TGF- β family influence the reproductive function across several species. We briefly discuss how TGF- β -related proteins balance germ-cell proliferation and differentiation as well as dauer entry and exit in *Caenorhabditis elegans*. In *Drosophila melanogaster*, TGF- β -related proteins maintain germ stem-cell identity and eggshell patterning. We then provide an in-depth analysis of landmark studies performed using transgenic mouse models and discuss how these data have uncovered basic developmental aspects of male and female reproductive development. In particular, we discuss the roles of the various TGF- β family ligands and receptors in primordial germ-cell development, sexual differentiation, and gonadal cell development. We also discuss how mutant mouse studies showed the contribution of TGF- β family signaling to embryonic and postnatal testis and ovarian development. We conclude the review by describing data obtained from human studies, which highlight the importance of the TGF- β family in normal female reproductive function during pregnancy and in various gynecologic pathologies.

The influence of the transforming growth factor- β (TGF- β) family on fertility and reproduction in organisms as diverse as flies and humans is impressive. In *Drosophila melanogaster*, for example, the bone morphogenetic protein (BMP)-2/4 homolog, Decapentaplegic (Dpp), is required to maintain germline stem cells in the ovary (Xie and Spradling 1998), and, at later stages, for proper egg shape and polarity (Twombly et al. 1996). In *D. melanogaster*, Dpp signaling may also be important in maintaining primordial germ-cell (PGC) identity as the

germline develops (Deshpande et al. 2014). In mammals, even the earliest stages of reproductive development, including the specification of the male and female germline, are controlled by TGF- β -related proteins, perhaps reflecting conservation that originated with insects (Donoughe et al. 2014). In the adult mammal, TGF- β -related proteins govern the growth and differentiation of somatic cells as well as germ cells within the gonads. Furthermore, TGF- β family ligands are intricately involved in the control of ovulation and fertilization, and the estab-

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lishment and maintenance of pregnancy. Several TGF- β -related growth factors also serve as endocrine hormones to integrate the reproductive status of the gonad to the physiological condition of the organism. A large number of transgenic and gene inactivation mouse models have been created that display reproductive pathologies, and highlight the importance of this family in maintaining reproductive homeostasis. These models have contributed significantly to the understanding of this protein family in reproductive processes (Chang et al. 2002; Matzuk and Lamb 2002, 2008; Pangas 2012a). This review focuses on recent progress in the study of male and female reproductive biology using genetic models for the ligands, receptors, and signaling proteins of the TGF- β family.

TGF- β -RELATED SIGNALING IN THE REPRODUCTIVE SYSTEMS OF *Caenorhabditis elegans*

On hatching, *C. elegans* develop through four larval stages, L1–L4, into adulthood (Fig. 1)

(Hubbard and Greenstein 2005). Although somatic cell and germ-cell specification occur during early embryogenesis, key developments in the reproductive potential of worms take place during the four larval stages (Hubbard and Greenstein 2005). After hatching, the fertility of *C. elegans* is heavily influenced by the environment; if the worms hatch under abundant feeding conditions, germline development continues until the end of the L1 stage and is then arrested at the L3 stage (Ren et al. 1996). However, as discussed later, this is not the case if hatching occurs in restrictive environments (Ren et al. 1996). In normal development, rapid gonadal proliferation occurs during the L3 stage in response to signals from the distal tip cells (DTC), and then again during the L4 stage (Hubbard et al. 2013). Late L4 stage is characterized by gametogenesis, when spermatogenesis occurs (Hubbard et al. 2013). Finally, oogenesis occurs during the adult stage along with meiotic maturation, ovulation, and fertilization (Hubbard and Greenstein 2005; Hubbard et al. 2013).

Figure 1. The reproductive development of *Caenorhabditis elegans* is controlled by environmental cues. *C. elegans* develop into adulthood through larval stages, where gonadal cells migrate, proliferate, and prepare for fertilization. Germ-cell proliferation is regulated by transforming growth factor β (TGF- β)-like signaling during larval development. If hatching occurs under unfavorable conditions, *C. elegans* enter a nonfeeding and nonreproductive state of arrested development known as the “dauer stage.”

In the absence of food, or under other unfavorable conditions such as high population density or elevated temperature, *C. elegans* undergo a specialized arrest in larval development and enter a dauer phase (Fig. 1) (Hu 2007). This is a special state of nonfeeding and nonreproduction that is characterized by unique changes, such as the formation of a desiccation-resistant cuticle, increased dispersal abilities, and elevated fat storage (Cassada and Russell 1975; Golden and Riddle 1984a,b). These changes allow the worms to survive up to several months under unfavorable conditions in a nonfeeding and nonreproductive state (Hu 2007). The reproductive changes that occur during the dauer phase indicate a strong association between environmental cues and germ-cell development in *C. elegans* (Ren et al. 1996). Genetic screens identified the TGF- β -related protein Daf-7 as the ligand that initiates a major signaling pathway, which coordinates the worm's entry and exit from the dauer phase (Ren et al. 1996; Hubbard et al. 2013). These studies showed that *daf-7* mutations result in constitutive entry into the dauer phase, even when hatching occurs in the presence of abundant food supply or at normal growth temperatures (Swanson and Riddle 1981; Ren et al. 1996). Similar to genes encoding ligands of the TGF- β family, *daf-7* encodes a protein with a prodomain and a ligand domain that shares 34% amino acid identity with human BMP-4, 34% with *D. melanogaster* Dpp, and 28% with human TGF- β (Ren et al. 1996). To control the posthatching decision to enter the dauer phase, Daf-7 signals through two TGF- β family type I and type II receptors, encoded by *daf-1* and *daf-4*, respectively (Georgi et al. 1990; Estevez et al. 1993). Daf-7-induced signals then converge on the expression of *daf-12* (Thomas et al. 1993) and act as a signaling mechanism that transmits environmental information to directly control the larval and reproductive development of *C. elegans* (Riddle et al. 1981; Ren et al. 1996; Antebi et al. 1998; Snow and Larsen 2000).

In addition to directing dauer entry and exit, TGF- β -related signaling also controls the proliferation and differentiation of germ cells in *C. elegans* (Hu 2007). The *daf-1*, *daf-4*, and

daf-7 genes control *C. elegans* germ-cell proliferation and differentiation (Dalfo et al. 2012). Mutation of these genes or of *daf-8* or *daf-14*, which encode Smad-like proteins, significantly decrease brood size and result in a 50% decrease of germline stem/progenitor cells (Dalfo et al. 2012). However, the TGF- β family signaling pathway that controls germ-cell proliferation is independent of the pathway that controls dauer entry and exit, and does not depend on the activity of the nuclear hormone receptor encoded by *daf-12*. Instead, the TGF- β family signaling pathway acts in a parallel but independent pathway that affects the balance of germ-cell proliferation and differentiation (Dalfo et al. 2012). Hence, TGF- β -related signaling also serves as a link between environmental signals and the reproductive germ cells of the nematode.

TGF- β -RELATED SIGNALING IN THE REPRODUCTIVE SYSTEMS OF *Drosophila melanogaster*

TGF- β -related proteins also control the reproductive development of *D. melanogaster*. In *Drosophila* ovaries, oogenesis occurs in specialized egg chambers that are arranged inside ovarioles, which are structures that contain oocytes at various developmental stages (Harris and Ashe 2011). The germaria contain the source of germ stem cells (GSCs) that differentiate into cytotoblasts, the egg precursors. Within the egg chamber, the oocyte is surrounded by hundreds of follicle cells, which give rise to the egg-shell proteins and specialized structures, including the dorsal appendages, which are respirators for the developing embryo, the operculum, an opening for larva hatching, and the micropyle, the structure specialized for sperm entry. Differentiation of the follicle cells into these specialized structures is coordinated by various growth factors, including TGF- β -related proteins.

The BMP-2/4-like ligand, Dpp, exerts substantial control over eggshell patterning and development (Fig. 2). Dpp is secreted by follicle cells during eggshell development and plays a major role in the migration and reorganization of the follicle cells surrounding the oocyte (Twombly et al. 1996; Deng and Bownes 1997).

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Figure 2. In *Drosophila*, Dpp (BMP-2/4) controls eggshell formation and germ stem-cell (GSC) maintenance. During eggshell maturation, Dpp signals arise in the follicular cells and are essential for dorsoventral specification of the eggshell. The signals are transmitted by the type I and type II receptors, Tkv and Wit, which then activate Mad by phosphorylation. Dpp-mediated Smad signaling is also important within the germ stem-cell niche, where the Dpp-induced repression of *bam* promotes GSC maintenance. pMad, Phospho-Mad; Med, Medea.

In *Drosophila*, Dpp signals through its type I receptor, Thickveins (Tkv), which is dynamically expressed in various cells within the eggshell throughout its maturation (Affolter et al. 1994; Lecuit and Cohen 1998; Mantrova et al. 1999; Berg 2005), and its type II receptor, Wishful thinking (Wit), which is expressed in follicular cells and responds to Dpp in the developing eggshell (Marmion et al. 2013).

The pool of *Drosophila* oocytes is maintained by specialized GSCs that reside within a stem-cell niche in the germarium (Harris and Ashe 2011). The stem-cell niche is composed of several somatic cell types, including the cap cells, inner germ cells, and terminal filament cells (Harris and Ashe 2011). Dpp signals that originate from these somatic cells maintain GSCs in an undifferentiated state by signaling via the type I receptors, Tkv and Saxophone (Sax), and the type II receptor, Punt (Harris and Ashe 2011). Downstream signals are transmitted through activation of Smad proteins (i.e., Mad and Medea), which then translocate into the nucleus and increase the expression of genes that maintain GSC pluripotency. *Bam* is a direct transcriptional target of Dpp signaling that is maintained in a repressed state by an activated Mad–Medea complex. As cells differentiate and exit the stem-cell niche, *Bam* expression is activated (Chen and McKearin 2003a,b). Dpp signaling originates from the cap cells and only activates canonical Dpp signaling within 1–2 cell diameters (Xie and Spradling 2000). Thus, stemness is lost and *Bam* activation is detected as the cells differentiate and migrate along the germarium, losing sensitivity to Dpp signaling (Kai and Spradling 2003).

PRIMORDIAL GERM-CELL DEVELOPMENT IN MAMMALS

Specification of the germ-cell lineage in mammals begins in early embryonic development. In mice, the development of germ cells begins at embryonic day 6.5 (E6.5), when the primordial germ-cell lineage is induced from the proximal epiblast (Lawson and Hage 1994). A cluster of 45 alkaline phosphatase-expressing primordial germ cells (PGCs) is noticeable at E7.5 posterior to the primitive streak in the extraembryonic mesoderm (Ginsburg et al. 1990). PGCs then proliferate and migrate through the hindgut endoderm to colonize the genital ridges and establish the primitive gonads. Complex paracrine signaling pathways control the induction, proliferation, and migration of the PGCs.

Analyses of gene inactivation mouse models for several of the BMPs and their downstream Smads have shown that the BMP system is essential for PGC formation (Table 1). Mice that lack expression of *Bmp2*, *Bmp4* or *Bmp8b* show reduced or absent PGCs (Zhang and Bradley 1996; Zhao et al. 1996; Lawson et al. 1999; Ying et al. 2000; Ying and Zhao 2001). In addition, mice with inactivated *Smad1* or *Smad5* have defects in PGC development (Chang and Matzuk 2001; Hayashi et al. 2002) and the majority of double heterozygous *Smad1*^{+/-}; *Smad5*^{+/-} embryos lack PGCs (Arnold et al. 2006). *Bmp4* and *Bmp8b* are expressed in the extraembryonic ectoderm, and *Bmp2* is expressed in the visceral endoderm. It has been proposed that BMP-4 generates inductive signals from the extraembryonic ectoderm to signal PGC formation in the epiblast (Lawson et al.

The TGF- β Family in the Reproductive Tract**Table 1.** Phenotypes of mice with inactivated genes that encode a TGF- β family signaling effector that is expressed in the gonads

Gene	Synonym	Protein function	Phenotype	References
Embryonic lethal				
<i>Acvr1l</i>	<i>Alk1</i>	Type I receptor	Vascular defects	Oh et al. 2000; Urness et al. 2000
<i>Acvr1</i>	<i>Alk2/ActRIA</i> <i>Tsk7L</i>	Type I receptor	Gastrulation defects; defective mesoderm and visceral endoderm; loss of primordial germ cells (PGCs)	Gu et al. 1999; Mishina et al. 1999; de Sousa Lopes et al. 2004
<i>Acvr1b</i>	<i>Alk4/ActRIB</i>	Type I receptor	Disrupted primitive streak	Gu et al. 1998
<i>Bmp2</i>	<i>Bmp2a</i>	Ligand	Mesoderm defects; heart defects	Zhang and Bradley 1996
<i>Bmp4</i>	<i>Bmp2b</i>	Ligand	No mesoderm differentiation; PGCs loss	Winnier et al. 1995; Dunn et al. 1997; Lawson et al. 1999
<i>Bmpr1a</i>	<i>Alk3</i>	Type I receptor	No mesoderm formation	Mishina et al. 1995; Ahn et al. 2001
<i>Bmpr2</i>	<i>BMPRII</i>	Type II receptor	Gastrulation arrest	Beppu et al. 2000
<i>Smad1</i>	<i>Madh1</i>	Signaling protein	Extraembryonic defects; no or few PGCs	Lechleider et al. 2001; Tremblay et al. 2001
<i>Smad2</i>	<i>Madh2</i>	Signaling protein	No mesoderm development; no anteroposterior axis; haploinsufficient	Nomura and Li 1998; Waldrip et al. 1998
<i>Smad4</i>	<i>Madh4/</i> <i>DPC4</i>	Signaling protein	Defective visceral endoderm	Sirard et al. 1998; Yang et al. 1998
<i>Smad5</i>	<i>Madh5</i>	Signaling protein	Defective vasculature development and cardiac development; loss of PGC development	Yang et al. 1999a; Chang et al. 2000; Chang and Matzuk 2001
<i>Tgfb1</i>		Ligand	Yolk sac defects (50%); postnatal death from inflammatory disorders	Shull et al. 1992; Kulkarni et al. 1993; Dickson et al. 1995
<i>Tgfb1</i>	<i>Alk5/TβRI</i>	Type I receptor	Vascular development defects	Larsson et al. 2001
<i>Tgfb2</i>	<i>TβRII</i>	Type II receptor	Disrupted yolk sac hematopoiesis and vasculogenesis	Oshima et al. 1996
Perinatal lethal				
<i>Acvr2b</i>	<i>ActRIIB</i>	Type II receptor	Right-left asymmetry and A-P (anterior-posterior) axis defects	Oh and Li 1997
<i>Bmp7</i>	<i>OP1</i>	Ligand	Kidney dysgenesis; skeletal patterning defects; cardiac cushion defects	Dudley et al. 1995; Luo et al. 1995; Solloway and Robertson 1999
<i>Fst</i>	<i>Follistatin</i>	Binding protein	Craniofacial defects; shiny skin; rib defects	Matzuk et al. 1995d
<i>Grem1</i>	<i>Gremlin</i>	Antagonist	Limb, lung, kidney defects; fewer germ cells at birth and meiotic delay in females	Khokha et al. 2003; Myers et al. 2011
<i>Inhba</i>		Ligand	Craniofacial defects; defective whisker, retinal, and tooth development	Matzuk et al. 1995b; Ferguson et al. 1998; Jhaveri et al. 1998; Davis et al. 2000
<i>Tgfb2</i>		Ligand	Craniofacial defects; skeletal defects; heart defects; renal defects	Sanford et al. 1997

Continued

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Table 1. *Continued*

Gene	Synonym	Protein function	Phenotype	References
<i>Tgfb3</i>		Ligand	Cleft palate; delayed lung development	Kaartinen et al. 1995; Proetzel et al. 1995
Reproductive defects				
<i>Acvr2</i>	<i>Acvr2a/ActRII</i>	Type II receptor	Pituitary defects in FSH β ; female infertility	Matzuk et al. 1995b
<i>Amh</i>	<i>MIS</i>	Ligand	Male infertility; female premature loss of primordial follicles	Behringer et al. 1990, 1994
<i>Amhr2</i>	<i>MISR2</i>	Type II receptor	Phenocopies AMH (MIS) knockout; male infertility	Mishina et al. 1996
<i>Bmp8a</i>	<i>OP2</i>	Ligand	Germ-cell degeneration (male)	Zhao and Hogan 1996; Zhao et al. 1998
<i>Bmp8b</i>	<i>OP3</i>	Ligand	Defects in PGC, germ-cell proliferation, depletion, and sterility (male)	Zhao et al. 1996
<i>Bmp15</i>	<i>Gdf9b</i>	Ligand	Female subfertility; interacts with GDF9	Yan et al. 2001
<i>Bmpr1b</i>	<i>Alk6</i>	Type I receptor	Female infertility; defects in cumulus expansion; uterine defects	Yi et al. 2001
<i>Gdf9</i>		Ligand	Female infertility; early block in folliculogenesis at primary stage	Dong et al. 1996; Carabatsos et al. 1998; Elvin et al. 1999b
<i>Inha</i>		Ligand	Gonadal and adrenal tumors	Matzuk et al. 1992, 1994
<i>Inhbb</i>		Ligand	Defects in mammary gland function; large litters; eyelid closure defects	Vassalli et al. 1994; Brown et al. 2000
<i>Smad3</i>	<i>Madh3</i>	Signaling protein	Reduced litter sizes; defective immune response; colorectal tumors; arrested follicle development	Zhu et al. 1998; Datto et al. 1999; Yang et al. 1999b; Tomic et al. 2004

1999). To test this, mutant or wild-type embryonic stem cells were injected into a recipient blastocyst to generate chimeric embryos. In chimeric embryos, the injected embryonic stem cells contribute to the epiblast to form the three embryonic germ layers and the extraembryonic mesoderm, but not the extraembryonic ectoderm or primitive endoderm. Analysis of chimeric embryos has shown that injection of *Bmp4*^{-/-} embryonic stem cells into wild-type blastocysts rescues the PGC developmental defect, although the converse experiment (i.e., injection of wild-type embryonic stem cells into *Bmp4*^{-/-} embryos) does not (Lawson et al. 1999). These experiments show that BMP-4 is required in the extraembryonic ectoderm for PGC formation. Additional experiments show that treatment of epiblasts that have been dissected away from the extraembryonic ectoderm

with recombinant BMP-4 produces PGCs (Hayashi et al. 2002).

Embryos with targeted inactivation of the *Acvr1* (also named *Alk2*) gene encoding a BMP type I receptor, lack PGCs at E7.5–E8.0, and *Acvr1*^{+/-} embryos contain fewer PGCs (de Sousa Lopes et al. 2004). *Acvr1* expression at E6.5 occurs primarily in the visceral endoderm (Gu et al. 1999), suggesting that BMP-4 might signal indirectly to the proximal epiblast during PGC formation. Expression of a constitutively active ACVR1/ALK-2 in the visceral endoderm of *Bmp4*^{-/-} embryos rescues the *Bmp4*^{-/-} phenotype (de Sousa Lopes et al. 2004). Additional studies will be necessary to address the function of the visceral endoderm in PGC formation, the distinct or overlapping roles of BMPs, their receptors, and the mechanism of PGC specification in the proximal epiblast.

Germ-cell migration from their extraembryonic origin to the genital ridges is also stimulated by BMPs. The motility of germ cells in organ cultures is inhibited by extracellular binding proteins that serve as BMP antagonists, such as noggin (Dudley et al. 2007). However, germ cells are thought to respond indirectly to the BMPs, because activated Smad1, 5, or 8 have not been detected in germ cells during migration (Dudley et al. 2007). After reaching the undifferentiated gonad, germ cells undergo another period of proliferation. In cell culture, human BMP-4 increases the number of PGCs and enhances the development of embryonic germ cells (Hiller et al. 2011). Thus, BMP signaling leads to an increase in number of PGCs; however, BMP-4 has a biphasic effect on mouse PGC proliferation in culture, with low doses increasing and high doses reducing PGC numbers. Opposing effects are also observed when cells are treated with noggin: low noggin decreases and high noggin increases the number of PGCs (Dudley et al. 2007). In this way, morphogen gradients may control developmental effects.

SEXUAL DIFFERENTIATION AND GONADAL DEVELOPMENT

In the mouse, PGCs colonize the genital ridges at approximately E9.5–E11 (Anderson et al. 2000). Development of the bipotential gonad into a male (testis) or female (ovary) fate depends on the expression of the sex-determining

region of chromosome Y (SRY), a transcription factor that acts as the male “switch” (Fig. 3). Embryonic testis development includes the differentiation of Sertoli cells and steroidogenic Leydig cells from somatic precursor cells and the formation of testicular cords that enclose the germ cells (Ross and Capel 2005). In the developing female gonad, PGCs form germline cysts and begin to enter meiotic prophase at E13.5. Formation of ovarian follicles and differentiation of the somatic cells of the ovary (i.e., granulosa and thecal cells), do not begin until after birth.

The developing urogenital system contains the Müllerian and Wolffian ducts, which are the anlagen for the female and male reproductive tract, respectively. During sexual differentiation, the Müllerian duct regresses in males while the Wolffian duct regresses in females (Fig. 4). In males, embryonic expression of SRY leads to the expression of the TGF- β family protein anti-Müllerian hormone (AMH; also called MIS, Müllerian inhibiting substance). AMH produced by Sertoli cells causes regression of the Müllerian duct. Subsequent differentiation of Leydig cells leads to testosterone production and development of Wolffian duct derivatives. The lack of testosterone in females results in the regression of the Wolffian duct and continued development of the Müllerian duct. Male *Amh*^{-/-} mice do not show regression of the Müllerian duct and are born with uteri and oviducts in addition to male reproductive structures (Behringer et al. 1994). A similar pheno-

Figure 3. Bone morphogenetic protein (BMP) signaling induces primordial germ-cell (PGC) development in mice. BMP signaling through ALK-2 and Smad1 and Smad5 promote primordial germ-cell development during early embryonic life. BMP-2, -4, and -8b also direct the colonization of PGCs to the genital ridges during E9.5–E11. At E13.5, the expression of the sex-determining region of chromosome Y (SRY) determines the developmental fate of the bipotential gonad.

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Figure 4. The bipotential gonad differentiates into male or female in response to a signaling pathway that involves anti-Müllerian hormone/Müllerian inhibiting substance (AMH/MIS), a member of the TGF- β family. In males, the sex-determining region of chromosome Y (SRY) expression activates AMH/MIS expression, which signals via ALK-3/BMPRIA and Smad1, 5, and/or 8. This leads to differentiation of Leydig cells and expression of testosterone, resulting in the regression of the Müllerian ducts. In the absence of SRY, AMH/MIS, and testosterone, Wolffian duct regression occurs, resulting in Müllerian duct maintenance and development of a female reproductive tract.

type is seen in mice with an inactivated gene for the AMH type II receptor, *Amhr2* (Mishina et al. 1996). In humans, mutations in *AMH* or *AMHR2* results in persistent Müllerian duct syndrome—a type of male pseudohermaphroditism characterized by the presence of a uterus and Fallopian tubes in addition to the normal male reproductive tract (Josso et al. 2005).

There are seven type I receptors for members of the TGF- β family and their important roles during embryonic development are underscored by phenotypes observed in knockout mouse models. Except for *Bmpr1b* (*Alk6*) and *Acvr1c* (*Alk7*), the mouse models with global inactivation of genes encoding the TGF- β type I receptors results in embryonic lethality (Table 1). Neither the *Bmpr1b*^{-/-} nor the *Acvr1c*^{-/-} mouse model shows the *Amh*^{-/-} phenotype. Therefore, tissue-specific conditional gene inac-

tivation has been used to identify the type I receptor for AMH during Müllerian duct regression (Jamin et al. 2002). Expression of Cre recombinase from the *Amhr2* promoter results in locus-specific recombination wherever *Amhr2* is normally expressed (see below). Mice with conditional inactivation of *Bmpr1a* (*Alk3*) with *Amhr2*^{cre/+} phenocopy the *Amh*^{-/-} mutation, (i.e., 50% of male mice have uteri and oviducts) indicating that BMPRIA (ALK-3) is a component of the AMH signaling pathway in Müllerian duct regression in vivo (Jamin et al. 2002). Conditional inactivation of *Acvr1* (*Alk2*) has no effect on Müllerian duct regression, although cell culture data suggest that AMH signals through this receptor (Clarke et al. 2001; Visser et al. 2001; Zhan et al. 2006). This can be reconciled by studies showing that codeletion of *Acvr1* and *Bmpr1a* (*Alk3*) with *Amhr2*^{cre/+}

causes 100% of males to retain the Müllerian duct tissue (Orvis et al. 2008); thus, BMPRIA may be the primary receptor used by AMH, but in its absence, AMH is capable of utilizing ACVR1 (Orvis et al. 2008). The receptor-activated Smads, Smad1, Smad5, and Smad8 have also been implicated in AMH signaling, particularly Smad1 (Gouedard et al. 2000; Clarke et al. 2001; Visser et al. 2001). However, only mice with conditional deletions that include *Smad5* (for instance, *Smad1* and *Smad5*, *Smad5*, and *Smad8*, or *Smad1*, *Smad5*, and *Smad8*) in Müllerian duct tissue retain Müllerian duct derivatives, suggesting that these three transcription factors play a role in transmitting the AMH signal with some level of functional redundancy in vivo (Orvis et al. 2008).

Ovary development is often described as a “default” pathway, but newer data support the roles of some genes in an ovary-specific pathway (Menke et al. 2003; Ross and Capel 2005; Koubova et al. 2006; Liu et al. 2010). Only a handful of genes have been discovered that control ovarian development. *Wnt4* is one such gene, and genetically female (XX) *Wnt4*^{-/-} embryos are masculinized; a Müllerian duct does not form in either sex, and XX embryos retain the Wolffian duct and show massive loss of germ cells (Vainio et al. 1999). *Bmp2* expression is lost in XX gonads of *Wnt4*^{-/-} embryos, as is expression of follistatin (gene name, *Fst*) (Yao et al. 2004), a regulatory protein that inhibits activin and BMPs. It is unclear what role BMP-2 plays at this stage because the *Bmp2*^{-/-} mutation is embryonic lethal by E9.0 (Zhang and Bradley 1996). However, the importance of follistatin in gonad development has been examined using *Fst*^{-/-} mice (Matzuk et al. 1995d; Yao et al. 2004). *Fst*^{-/-} mice survive embryogenesis but die perinatally (Matzuk et al. 1995d). In gonadal development, *Fst*^{-/-} mice phenocopy *Wnt4*^{-/-} mice, and follistatin acts likely downstream of *Wnt4* signaling because *Fst* expression is lost in *Wnt4*^{-/-} embryos but *Wnt4* is expressed in *Fst*^{-/-} embryos (Yao et al. 2004). By E16.5, 90% of the germ cells in *Fst*^{-/-} XX embryos undergo apoptosis and there is an almost complete loss of germ cells by birth (Yao et al. 2004). *Fst*^{-/-} or *Wnt4*^{-/-} XX ovaries al-

so show ectopic development of a male-specific coelomic vasculature (Vainio et al. 1999; Menke and Page 2002). When the *Inhbb* gene encoding the activin/inhibin β_B subunit is deleted in either *Fst*^{-/-} or *Wnt4*^{-/-} female mice, normal ovary development resumes with no coelomic vessel formation (Yao et al. 2006), suggesting a key stimulatory role for activin B in testis development, which must be suppressed for normal ovary development.

Following sexual differentiation, at E13.5, male germ cells undergo mitotic arrest and meiosis does not begin until after birth. The arrest of male germ cells involves Sertoli cell production of the retinoic acid-degrading enzyme Cyp26b1 (Bowles et al. 2006; Koubova et al. 2006), consistent with the master regulatory signaling role of retinoid acid in the mitotic to meiotic switch (Griswold et al. 2012). Nodal, which is preferentially expressed in the developing testis, is one of the factors that promotes male germ-cell fate and suppresses the female genetic program (Souquet et al. 2012; Wu et al. 2013). Nodal acts in part by inducing expression of *Nanos2*, which encodes an RNA-binding protein in male germ cells (Wu et al. 2013). In turn, *Nanos2* controls the expression of genes involved in the initiation of meiosis, such as *Stra8* (*stimulated by retinoic acid 8*), and induces a male-specific gene expression pattern (Suzuki and Saga 2008).

In female germ cells, meiosis is initiated at E13.5 through the expression of *Stra8*, and meiosis continues until an arrest at prophase I at birth. Resumption of meiosis will not occur until the midcycle surge of luteinizing hormone that causes ovulation in the adult. There is limited information regarding the role of the TGF- β family in the embryonic ovary during mid-to-late gestation, but several lines of evidence suggest a continued function for BMPs. *Bmp2* is expressed specifically in the ovary at E11.5 (Yao et al. 2004), and *Bmp7* is expressed before E11.5 in both sexes, but its expression then becomes male-specific after E12.5 (Ross et al. 2007). BMP-7 may promote germ-cell proliferation in the embryonic gonad (Ross et al. 2007), but *BMP7* is not highly expressed in human ovaries (Childs et al. 2010), so this function is potentially performed by another ligand(s).

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Grem1^{-/-} mice that lack expression of the BMP antagonist Gremlin, show defects in oocyte development, including a reduction of oocyte numbers by birth, and a delay in meiotic progression (Myers et al. 2011). These data suggest that BMP signaling plays a key role in normal ovary development and may control the size of the ovarian reserve (Pangas 2012b).

THE TGF- β FAMILY IN MALE REPRODUCTION

The adult male testis contains germ cells organized into seminiferous tubules that also contain Sertoli cells, a somatic cell type. Outside the tubules are the Leydig cells—steroidogenic somatic cells essential for testosterone production. Spermatogenesis occurs within seminiferous tubules, which contain mitotically active spermatogonia. These cells give rise to spermatocytes that undergo meiosis to ultimately produce sperm cells. Within the testis, paracrine signaling from Sertoli cells to germ cells, as well as Leydig cells, is critical for male fertility. Also important is the endocrine feedback system between the gonads and the pituitary gland, which forms part of the hypothalamic-pituitary-gonadal axis (Fig. 5).

Defects in the pituitary gland have profound effects on male and female fertility. A classical feedback loop exists between the gonad, which produces inhibin, a heterodimeric TGF- β family member, and the pituitary gland, which produces gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Fig. 5). FSH is necessary for normal Sertoli cell function in the male and in granulosa cells of the female, as shown by *Fshb*^{-/-} mice, which do not express the β subunit of FSH (Kumar et al. 1997). The inhibins and closely related activins were discovered (and named) based on their ability to inhibit and activate, respectively, FSH secretion from the pituitary (Burns and Matzuk 2002; Bilezikjian et al. 2004; Namwanje and Brown 2016). The importance of activin signaling for FSH synthesis was confirmed in *Acvr2*^{-/-} mice that lack expression of one of the activin type II receptors, ActRII (also known

as ActRIIA). Male *Acvr2*^{-/-} mice have decreased FSH levels and consequently fewer Sertoli cells and smaller testes weights—a phenotype similar to *Fshb*^{-/-} mice.

Inhibin and activin are related dimeric proteins generated from combinations of α (encoded by the *Inha* gene) and β (encoded by the *Inha* gene) and β (encoded by the *Inhba* or *Inhbb* genes) subunits. Activins are homodimers or heterodimers of β subunits ($\beta_A:\beta_A$, activin A; $\beta_B:\beta_B$, activin B; $\beta_A:\beta_B$, activin AB) and inhibins are heterodimers ($\alpha:\beta_A$, inhibin A; $\alpha:\beta_B$, inhibin B) (Namwanje and Brown 2016). Targeted inactivation of each of the corresponding genes has distinct phenotypes. Mice with an inactivated *Inhba* gene die perinatally because of craniofacial defects (Matzuk et al. 1995c). *Inhbb*^{-/-} mice are viable but have eyelid defects and, in females, lactation defects (Vassalli et al. 1994). Mice with conditional deletion of *Inhba*, the gene encoding the activin A subunit, in the *Inhbb*^{-/-} background also show fertility defects. To examine the functional overlap between activin A and activin B, mice were generated with the *Inhbb* mature coding region inserted into the *Inhba* locus (Brown et al. 2000); these mice do not produce activin A or inhibin A but instead express activin B and inhibin B. Insertion of *Inhbb* into the *Inhba* locus, referred to as the *Inhba*^{BK} allele (Brown et al. 2000), rescues the perinatal lethality and craniofacial malformations of the *Inhba*^{-/-} mice, suggesting that activin A and activin B can function redundantly during embryogenesis as long as they are expressed in the same temporal and spatial pattern. However, some postnatal reproductive defects were identified in this model, suggesting that the activin isoforms have some nonoverlapping roles in male and female reproduction. Male mice with two copies of *Inhba*^{BK} have normal fertility but reduced testis volume despite increases in FSH levels. The testis volume is further reduced when the *Inhba*^{BK} allele is in trans to the null allele (*Inhba*^{BK/-}). Few female *Inhba*^{BK/BK} mice produce pups, and *Inhba*^{BK/-} female mice have smaller ovaries with fewer preantral follicles. Thus, *Inhba*^{BK} acts as a hypomorphic *Inhba* allele, possibly because of qualitative differences in activin A and

Figure 5. Role of the transforming growth factor- β (TGF- β) family in female reproductive physiology. (A) Neurons in the hypothalamus secrete gonadotropin-releasing hormone (GnRH), which binds to receptors on the anterior pituitary gland gonadotrope cells. (*Legend continues on following page.*)

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activin B activity (Brown et al. 2000, 2003). Conditional deletion of *Inhba* in the *Inhbb*^{-/-} background in granulosa cells also support this hypothesis, as these mice show dosage-dependent fertility defects (Pangas et al. 2007).

Temporal regulation of activin A expression appears to be necessary for proper germ-cell differentiation (Mithraprabhu et al. 2010). Activin A is highly expressed in the testis during the first week of postnatal life; however, as spermatogenic differentiation progresses, the levels of activin A decrease along with high expression of its inhibitors, follistatin and “BMP and activin membrane bound inhibitor” (BAMBI) (Barakat et al. 2012). In an in vivo coculture of spermatogonia and germ cells from day 4 or day 8 testes, *c-kit* mRNA expression, which marks germ-cell differentiation, was not affected by activin A on postnatal day 4, but was significantly reduced in germ cells on postnatal day 8, indicating an inhibitory role of activin A in germ-cell maturation. Activin A also plays a role in male embryonic germ-cell and Sertoli-cell proliferation (Mendis et al. 2011). In *Inhba*^{-/-} testes at E13.5 and E15.5, the number of proliferating Sertoli cells is significantly decreased compared to wild-type mice, likely as a result of impaired cell-cycle machinery activation. Similar defects in Sertoli cell proliferation are seen following conditional deletion of *Inhba* in fetal Leydig cells of *Amhr2*^{cre/+};*Inhba*^{flox/-} (Archambeault and Yao 2010). Thus, activin signaling is crucial during fetal and postnatal testis development, affecting proliferation of Sertoli cells during embryonic development and germ-cell differentiation during postnatal life.

A strikingly different phenotype is seen in male and female mice that lack a functional inhibin α gene (Matzuk et al. 1992). All male and female *Inha*^{-/-} mice develop sex-cord stromal tumors by 4–6 wk of age and die within 3–4 mo of age (Fig. 6C). *Inha*^{-/-} mice die from a cachexia-like wasting syndrome, likely caused by the high levels of activin produced in lieu of inhibin dimers. The wasting syndrome is modulated by the activin type II receptor, ActRII (Coerver et al. 1996), because mice doubly homozygous null for *Inha* and *Acvr2* do not develop cachexia and live longer. Gonadectomy does not rescue the lethality of the *Inha*^{-/-} mutation because these mice subsequently develop adrenal tumors (Matzuk and Bradley 1994). However, in males, the gonadal tumor phenotype can be rescued by expression of inhibin A using a mifepristone-inducible system (Pierson et al. 2000).

AMH and its type II receptor AMHRII also have a role in postnatal testis physiology. In the adult gonad, *Amh* and *Amhr2* are expressed in Sertoli cells of the male and the granulosa cells of the female. Male mice defective in *Amh* or *Amhr2* have Leydig cell hyperplasia suggesting that AMH suppresses Leydig cell proliferation (Behringer et al. 1994; Mishina et al. 1996). Male mice derived from genetic crosses between *Inha*^{-/-} and *Amh*^{-/-} or *Amhr2*^{-/-} mice, display Leydig cell tumors by 1 wk of age (Matzuk et al. 1995a), suggesting an interaction between the two pathways in Leydig cell function.

In the testis, *Bmp8a*, *Bmp8b*, *Bmp7*, and *Bmp4* are expressed in male germ cells, and mice deficient in expression of any of these

Figure 5. (Continued) In response, gonadotrope cells produce follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which control male and female gonadal function. FSH is stimulated by locally expressed activins that signal through the type II receptor, ACVR2 (ActRII). The gonad produces inhibin and estradiol (E_2) that repress FSH production. Multiple TGF- β -related proteins are produced in the ovary. (B) Follicle development proceeds from primordial to antral stages, which culminates in the release of an oocyte during ovulation, which is then available for fertilization. Following ovulation, the remaining cells of the follicle differentiate into the corpus luteum. Follicular blocks seen in various mouse models are indicated. (C) Conditional inactivation of *Bmp2* or *Alk2* with *Pgr*^{cre/+} results in decidualization defects and female sterility. Conditional inactivation of *Bmpr2* with *Pgr*^{cre/+} results in female infertility because of intrauterine growth restriction, abnormal spiral artery remodeling, and deficient uNK infiltration. Conditional deletion of nodal in *Nodal*^{flox/flox};*Pgr*^{cre/+} females results in subfertility as a result of intrauterine growth restriction and placental defects. uNK, Uterine natural killer cells; SA, spiral arteries; F, fetus; T, trophoblasts; L, uterine lumen.

Figure 6. Ovarian phenotypes of mice with targeted gene inactivations. (A) Schematic of the cell types in an antral stage follicle. The central oocyte is surrounded by cumulus cells. A fluid-filled antral cavity separates the cumulus cells from the mural granulosa cells. Outside a basement membrane are the steroidogenic thecal cells. (B) Gross anatomy of a normal female reproductive tract. (C) Ovarian tumors of an adult *Inha*^{-/-} mouse. (D) Histology of a wild-type adult female ovary showing multiple follicular stages including primary (PrF), secondary (SF) and antral (AnF) follicles. (E) *Fshb*^{-/-} ovary arrests before the formation of an antral cavity similar to the *Acvr2*^{-/-} ovary shown in panel F. (G,H) *Gdf9*^{-/-} ovaries have follicles arrested at the primary stage and oocytes that grow abnormally large. (I) Follicles from *Inha*^{-/-};*Gdf9*^{-/-} female mice grow to the multilaminar stage and develop a theca that is absent in *Gdf9*^{-/-} follicles (panel H). Panels are not shown to scale. Oo, Oocyte; Gr, granulosa cells; Th, theca.

genes have male reproductive phenotypes. Male *Bmp8b*^{-/-} mice are infertile because of apoptosis of spermatocytes, which eventually results in germ-cell depletion (Zhao et al. 1996). The phenotype of male *Bmp8a*^{-/-} mice is less severe, and about half of adult male *Bmp8a*^{-/-} mice show germ-cell degeneration (Zhao et al. 1998). Male *Bmp8a*^{-/-} mice also show degeneration of the epididymal epithelium, demonstrating a role for BMPs in epididymal function as well (Zhao et al. 1998). The phenotype of male *Bmp8a*^{-/-} mice can be enhanced when one copy of *Bmp7* is removed (Zhao et al. 2001).

Defects in fertility are also seen in male *Bmp4*^{+/-} mice in a C57BL/6 background; these males have lower fertility because of germ-cell loss, decreased sperm counts, and motility and epididymal defects (Hu et al. 2004). In the somatic cell compartment, there is as yet little genetic evidence for a role of BMPs in adult Sertoli and Leydig cell function. However, mouse Sertoli cells express the BMP type II receptor BMPRII, and the type I receptors ALK-2, ActRIB/ALK-4, and BMPRIA/ALK-3, encoded by *Acvr1*, *Acvr1b*, and *Bmpr1a*, respectively (*Bmpr1b* expression was not examined).

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(Puglisi et al. 2004), suggesting that these cells are competent to respond to BMP ligands.

TGF- β FAMILY SIGNALING IN OVARIAN FUNCTION

During ovary formation, somatic cells, which eventually form the granulosa cells, surround oocytes to form nongrowing primordial ovarian follicles. These “pregranulosa cells” are derived in part from cells that ingress from the coelomic epithelium in multiple waves that begin in late gestation and cease a few days after birth (Mork et al. 2011). In the mouse, primordial follicle formation begins just before birth such that newborn mouse ovaries contain mostly germ-cell cysts and a few primordial follicles. Subsequent folliculogenesis coordinates oocyte development, granulosa cell proliferation, and thecal cell steroidogenesis (Fig. 6A). Initially, these events appear to be orchestrated primarily by the oocytes (Eppig et al. 2002; Matzuk et al. 2002), although subsequent development is likely bidirectional. Two key signaling proteins are growth and differentiation factor (GDF)-9 and BMP-15 oocyte-specific members of the TGF- β family (McGrath et al. 1995; Dong et al. 1996; Dube et al. 1998; Elvin et al. 2000). GDF-9 and BMP-15 are essential for fertility in many animals including mice, humans, and sheep (Dong et al. 1996; Galloway et al. 2000; Wilson et al. 2001; Juengel et al. 2002; Di Pasquale et al. 2004). In mice, *Gdf9* and *Bmp15* are coexpressed during folliculogenesis starting approximately at the newborn stage and until the late antral stage in adults (Dube et al. 1998; Elvin et al. 1999b; Rajkovic et al. 2004). Female *Gdf9*^{-/-} mice have follicles arrested at the primary stage with a single layer of granulosa cells, no associated thecal cell layer and defects in oocyte meiotic competence and growth (Fig. 6G,H) (Dong et al. 1996; Carabatsos et al. 1998).

Granulosa cells from *Gdf9*^{-/-} ovaries show increased expression of a number of genes, including the gene encoding inhibin α (Elvin et al. 1999b). Excess inhibin is partly responsible for follicular arrest, and lack of inhibin α expression in *Gdf9*^{-/-} mice, in *Inha*^{-/-}; *Gdf9*^{-/-} mice

allows follicles to progress to the multilaminar stage, although the follicles are abnormal (Fig. 6I) (Wu et al. 2004; Myers et al. 2013). The ovaries of *Inha*^{-/-}; *Gdf9*^{-/-} mice contain additional defects not found in *Gdf9*^{-/-} mice. For example, a distinct theca layer forms in double mutant mice but does not become functional (i.e., steroidogenic). This is not understood, but may relate to the loss of LH receptor expression in thecal cells (Wu et al. 2004). Interestingly, *Inha*^{-/-} ovaries show precocious follicle development, with overgrowth of granulosa cells, more follicles in the growth phase, and an increase in activin expression (Myers et al. 2009, 2013; Nagaraja et al. 2010). Deletion of the *Gdf9* gene in an *Inha*^{-/-} genetic background partly rescues some of the follicle growth at the initial stages of *Inha*^{-/-} follicle development, perhaps in part because of decreased levels of activin B in the double mutant ovaries. However, at later follicle stages, the double mutant ovaries look similar to *Inha*^{-/-} mutant ovaries, with high levels of activin and suppressed levels of kit ligand, which promote granulosa cell proliferation although suppressing oocyte growth. These data suggest that GDF-9 and activins have sequential and partly nonredundant roles in folliculogenesis (Myers et al. 2013).

GDF-9 and BMP-15 play important roles in cumulus cells (i.e., the cells directly adjacent to the oocyte), during ovulation. During ovulation, cumulus cells become embedded in a hyaluronan-rich matrix that binds them to the oocyte. Extracellular matrix synthesis is critical for proper ovulation and fertilization (Hizaki et al. 1999; Varani et al. 2002; Fulop et al. 2003) and oocytes are known to induce cumulus expansion in the presence of FSH (Buccione et al. 1990). Through analysis in cell culture, mouse GDF-9 has been shown to play a key role in this process by inducing the expression of genes necessary for cumulus expansion including those encoding hyaluronan synthase 2 (*Has2*), prostaglandin-endoperoxide synthase 2 (*Ptgs2*, *Cox2*), pentraxin 3 (*Ptx3*), and tumor necrosis factor α -induced protein 6 (*Tnfaip6*), while repressing others, such as those encoding luteinizing hormone/choriogonadotropin receptor (*Lhcgr*) and urokinase-type plasminogen acti-

vator (*Plau*) (Elvin et al. 1999a; Matzuk 2000; Varani et al. 2002). The phenotypes of double crosses of *Gdf9* and *Bmp15* mutant mice, in comparison with the single gene deficiencies, indicate an interaction of the two proteins during late stages of follicle development and ovulation. Although *Bmp15*^{-/-} females are subfertile, female *Gdf9*^{+/-};*Bmp15*^{-/-} mice are infertile in a 129/SvEv inbred background, and subfertile in a mixed hybrid background (C57Bl/6/129/SvEv) (Yan et al. 2001). Eggs of *Gdf9*^{+/-};*Bmp15*^{-/-} mice lack associated cumulus cells, both within follicles and in oviducts, suggesting that the cumulus cell-oocyte communication has been disrupted, and that cumulus cells no longer produce a stable extracellular matrix during ovulation.

Additional oocyte and cumulus cell defects have been found in *Gdf9*^{+/-};*Bmp15*^{-/-} mice. The ability of cumulus cells to undergo expansion can be tested using a cell culture assay: cumulus-oocyte complexes that have the oocyte removed (oocyctomized; OOX) undergo expansion when cocultured with fully grown oocytes in the presence of FSH (Buccione et al. 1990; Vanderhyden et al. 1992). Wild-type oocytes expand wild-type OOX complexes, but oocytes from *Gdf9*^{+/-};*Bmp15*^{-/-} mice cannot expand wild-type OOX complexes to the same degree (Su et al. 2004). Wild-type oocytes also have a reduced ability to expand *Gdf9*^{+/-};*Bmp15*^{-/-} OOX complexes. Because the addition of wild-type oocytes cannot rescue the defect in mutant cumulus cells, the problem is likely intrinsic to the cumulus cells. These data support the hypothesis that GDF-9 and BMP-15 are necessary for proper development of cumulus cells throughout many stages of folliculogenesis (Su et al. 2004).

In sheep, homozygous or heterozygous mutations in the *BMP15* gene result in sterile (homozygous) or superfertile (heterozygous) females (Davis et al. 1991; Galloway et al. 2000). Additionally, mutations in *GDF9* and *ALK6/BMPRII*, which encode a type I receptor for BMP-15, also affect fertility in sheep (Otsuka et al. 2011). GDF-9 and BMP-15 are known to cooperate and heterodimerize, although the functional roles of the GDF-9:BMP-15 hetero-

dimers have been poorly defined (Liao et al. 2003). BMP heterodimers were first isolated from bovine bone and shown to potently induce bone formation in vivo (Wang et al. 1988). Compared to BMP homodimers, BMP heterodimers show enhanced activity (Guo and Wu 2012). Accordingly, GDF-9:BMP-15 heterodimers also show enhanced bioactivity (Peng et al. 2013a). Studies using recombinant human and mouse GDF-9:BMP-15 heterodimers show that, relative to their respective homodimers, the heterodimer potently induces cumulus cell expansion. This is also reflected in the striking increases of the cumulus cell expansion genes *Ptx3*, *Has2*, and *Ptgs2*. Furthermore, these studies point to species-specific differences in the bioactivity of these ligands; mouse GDF-9 homodimers strongly activate cumulus cell expansion, although human GDF-9 homodimers are inactive. Similarly, mouse BMP-15 homodimers are inactive when compared to mouse GDF-9 homodimers, although human BMP-15 homodimers have low bioactivity (Peng et al. 2013b). These results show that the heterodimer conformation of these key oocyte-secreted factors may be a potent modulator of ovarian function in mice and humans. Currently, a recombinantly produced heterodimer of human BMP-15 and GDF-9 termed “cumulin,” is being tested to boost success rates of some artificial reproductive technologies such as in vitro maturation of oocytes (Mottershead et al. 2015).

Few mutations causal for infertility in humans have been identified. *BMP15*, located on the X chromosome, is a candidate gene for female infertility. A human mutation in *BMP15* from two sisters causes hypergonadotropic ovarian failure and ovarian dysgenesis (Di Pasquale et al. 2004). Loss of function mutations in *NOBOX*, a gene encoding a key transcription factor that regulates *GDF9* expression (Rajkovic et al. 2004), are found at high frequency in women with primary ovarian insufficiency, also called premature ovarian failure (Bouilly et al. 2011), which affects approximately 1% of women under the age of 40 and is associated with infertility. Because of their role in PGC function and gonadal development, further examination of members of the BMP family will

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be necessary to establish any pathophysiology associated with improper germ-cell development in humans.

Many other BMP ligands are expressed in the ovary during folliculogenesis, and there is much to be learned about the *in vivo* contributions of the BMP system to female fertility. Both *Bmp4* and *Bmp7* are expressed in thecal cells, but homozygous null mutations in either gene in mice results in embryonic or postnatal lethality, respectively, and these have yet to be analyzed by conditional mouse models in the ovary. Inactivation of *Bmp6*, expressed in the oocyte, does not result in an overt reproductive phenotype (Solloway et al. 1998). Instead, *Bmp6*^{-/-} female mice have subtle fertility defects, including a 22% decrease in litter size and reduced numbers of ovulated oocytes with decreased developmental competence (Sugiura et al. 2010). *Bmp2* has been reported to be expressed in rat granulosa cells (Erickson and Shimasaki 2003), but *Bmp2*^{-/-} embryos die at E7.5–E9.0 (Zhang and Bradley 1996), leaving questions unanswered regarding its specific reproductive function in granulosa cells.

AMH plays a role in follicle development in the adult female. Female mice that lack AMH expression are fertile with normal litter sizes, and show premature infertility (Durlinger et al. 1999). Ovaries from adult *Amh*^{-/-} mice show an approximately threefold greater number of small growing follicles and concomitant loss of primordial follicles compared to wild-type littermates (Durlinger et al. 1999). Combined with cell culture evidence that recombinant AMH inhibits primordial follicle growth (Durlinger et al. 2002), it has been suggested that AMH plays an important role in the recruitment phase of folliculogenesis (Visser et al. 2006). This has also led to the proposal that AMH levels might be useful markers of ovarian reserve in humans during aging or disease (van Rooij et al. 2002; Visser et al. 2006). In a study of female patients undergoing *in vitro* fertilization, serum AMH levels correlate highly with number of antral follicles and oocytes retrieved, and reduced serum AMH is associated with a poor response during *in vitro* fertilization (van Rooij et al. 2002). In other studies,

serum levels of AMH predict menopause (van Rooij et al. 2004, 2005). Detailed studies in mice show that, although AMH levels do not change in individual growing follicles of aging mice, serum AMH levels decline with age (Kevenaar et al. 2006). In addition, in mice, there is a correlation between growing follicles and numbers of primordial follicles, as well as a strong correlation between primordial follicles and serum AMH levels (Kevenaar et al. 2006). Thus, the use of serum AMH holds significant promise for monitoring fertility during the female reproductive lifespan (Visser et al. 2006, 2012; Anderson et al. 2012).

The inhibin-activin system in female mice is critical for both follicle development and regulation of the estrous cycle (Fig. 5). FSH is a dimeric protein consisting of an α and β subunit, and FSH is required for antral follicle development in female mice (Kumar et al. 1999; Burns and Matzuk 2002). Pituitary-derived activins regulate the expression of the FSH β subunit (*Fshb*), thereby controlling FSH production (Carroll et al. 1989, 1991; Bilezikjian et al. 2004). The phenotypes of *Fshb*^{-/-} and *Acrv2*^{-/-} female mice are very similar; both mouse models have impaired follicle development before the full development of the antral cavity (Fig. 6). In addition, the follicular block in both *Fshb*^{-/-} and *Acrv2*^{-/-} female mice can be rescued by treatment with exogenous gonadotropins, suggesting that lack of FSH is the principal cause of the phenotype. Mice conditionally null for *Smad4* in gonadotrope cells (i.e., the FSH- and LH-producing cells in the anterior pituitary), are hypogonadal and FSH-deficient, although subfertile (Fortin et al. 2014a). However, *Smad4* deletion in combination with deletion of *Foxl2*, which encodes a transcription factor known to regulate *Fshb* (Vidal et al. 1998; Corpuz et al. 2010; Lamba et al. 2010), results in sterility with no FSH production (Fortin et al. 2014b). These mice highlight the importance of TGF- β family signaling (like-ly activin) in FSH synthesis.

Activin and TGF- β signal through Smad2 and Smad3. *Smad2*^{-/-} embryos fail to gastrulate, have defective mesoderm formation and anteroposterior axis defects, and die at embry-

onic day E6.5–E8.5 (Nomura and Li 1998; Waldrup et al. 1998). Mice engineered to express either a splice variant of *Smad2* that lacks exons 3, or engineered to replace the *Smad2* coding sequence with *Smad3*, are viable and fertile, suggesting that the short form of *Smad2*, or *Smad3*, can regulate all essential *Smad2* functions (Dunn et al. 2005). By comparison, *Smad3*^{-/-} mice are viable (Zhu et al. 1998; Yang et al. 1999b), but a targeted allele that removes exon 8 (encoding the carboxy-terminal phosphorylation site) has reproductive defects (Tomic et al. 2002, 2004). Female *Smad3*^{-/-} mice have reduced fertility, reduced numbers of large pre-antral and antral follicles, and a larger primordial follicle pool during adulthood (Tomic et al. 2002). Serum analysis in these mice shows high levels of FSH and low levels of estradiol, and, in ovaries, reduced expression of inhibin α , cyclin D2, estrogen receptor- β but not of the FSH receptor (Tomic et al. 2004). The low estradiol level and decreased inhibin expression are consistent with the loss of antral follicles and the subsequent lack of negative feedback control in the pituitary. However, *Smad2* and *Smad3* have been shown to compensate in mouse knockout models, and are also important for pituitary function. Thus, there was a need to generate additional conditional mutants using loxP-flanked alleles and *Amhr2*^{cre/+} to address these issues (see next paragraph on conditional mutations). Single conditional deletion of *Smad2* or *Smad3* in granulosa cells results in normal fertility of female mice (Li et al. 2008). In contrast, combined deletion of *Smad2* and *Smad3* in granulosa cells results in reproductive defects. These mice show disrupted follicle development, increased follicular atresia, reduced ovulation, and attenuated cumulus cell expansion, consistent with their function in activin and GDF-9 signaling (Li et al. 2008).

To circumvent the embryonic or perinatal lethality of homozygous null mutants, many researchers conditionally inactivate genes using the cre/loxP recombination system (Sauer and Henderson 1989). Several mouse lines express Cre recombinase in various cell types in reproductive tissues (Table 2). In female reproduc-

tion, the *Amhr2*^{cre/+} mouse is useful to direct recombination of floxed alleles in granulosa cells of the postnatal ovary (Jorgez et al. 2004). *Amhr2*^{cre/+} mice have been used to generate mice with a conditional allele for *Bmpr1a* (*Alk3*) (see above), *Smad4* (see below) or *Fst*. Conditional inactivation of the *Fst* gene in granulosa cells (*Fst* cKO) results in decreased fertility, increased levels of FSH and LH, and reduced testosterone (Jorgez et al. 2004). In many ways, the phenotype of adult female mice with a conditionally inactivated *Fst* gene is reminiscent of premature ovarian failure in women. Premature ovarian failure is characterized by amenorrhea, infertility, and elevated gonadotropins in women before the age of 40. Its etiology is unknown but is likely heterogeneous. Therefore, these mice may be seen as a model to study premature ovarian failure. The mechanism of their rapid follicular loss is unknown but possibly related to unregulated activin activity in early follicular recruitment and growth (Jorgez et al. 2004).

The roles of the TGF- β family in follicle development have also been analyzed by a conditional inactivation of *Smad4* in the ovary (Pangas et al. 2006). These mice are initially subfertile, but are infertile at 4–6 mo of age. Folliculogenesis is disrupted because granulosa cells in growing follicles undergo precocious differentiation (i.e., luteinization), in response to stimulation by pituitary gonadotropins. In addition, severe cumulus cell defects occur during folliculogenesis and cumulus expansion, confirming the critical role of the TGF- β family in the function of these cells. *SMAD4* is a tumor suppressor gene in humans (Hahn et al. 1996), and mice with conditionally inactivated *Smad4* in the epidermis and mammary gland develop carcinomas (Li et al. 2003; Yang et al. 2005; Qiao et al. 2006). However, no tumor development is seen as a result of lack of ovarian *Smad4* expression, and conditional loss of *Smad4* results in a phenotype that is very similar to the phenotype resulting from inactivation of both *Smad2* and *Smad3*, which also results in premature granulosa cell luteinization (Li et al. 2008). Possibly, granulosa cell tumor formation is prevented by the premature luteinization in *Smad4*^{-/-} granulosa cells (Pangas 2012a).

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Table 2. Mouse lines expressing *Cre* recombinase for analyses of reproductive function

Cre Line	Promoter	Type	Tissue	References
<i>Amh-Cre</i>	Anti-Müllerian hormone	Transgene	Sertoli cells (male), granulosa cells (female)	Lécureuil et al. 2002
<i>Amhr2-Cre</i>	Anti-Müllerian hormone receptor 2	Knock-in	Granulosa cells (female), Leydig cells (male), Müllerian duct	Jamin et al. 2002; Jorgez et al. 2004
<i>Alpha GSU-Cre</i>	α Subunit glycoprotein hormone	Transgene	Gonadotropes, thyrotropes of anterior pituitary	Cushman et al. 2000
<i>Cyp19-cre</i>	Aromatase	Transgene	Granulosa cells (antral follicles)	Fan et al. 2008
<i>Gdf9-iCre</i>	Growth differentiation factor-9	Transgene	Oocytes	Lan et al. 2004
<i>GnRH-iCre</i>	Gonadotropin-releasing hormone	Transgene	GnRH neurons	Shimshek et al. 2002
<i>GnRHR-cre</i>	Gonadotropin-releasing hormone receptor	Knock-in	Gonadotropes of the anterior pituitary	Wen et al. 2008
<i>Inha-Cre</i>	Inhibin α	Transgene	Sertoli, Leydig cells (male), granulosa, theca (female)	Jorgez et al. 2006
<i>Pgr-Cre</i>	Progesterone receptor	Knock-in	Anterior pituitary gland, uterus, oviduct, ovary, mammary gland	Soyal et al. 2005
<i>Prm1-Cre</i>	Protamine-1	Transgene	Spermatogenic cells	O'Gorman et al. 1997
<i>PrP-Cre-ER^T</i>	Prion protein (inducible)	Transgene	Spermatogonia, spermatocytes	Weber et al. 2003
<i>Sycp1-Cre</i>	Synaptonemal complex protein-1	Transgene	Spermatogonia	Vidal et al. 1998
<i>Tnap-cre</i>	Tissue-nonspecific alkaline phosphatase	Knock-in	Primordial germ cells	Kehler et al. 2004
<i>Zp-Cre</i>	Zona pellucida 3	Transgene	Oocytes	Lewandoski et al. 1997; de Vries et al. 2000

Although mice with inactivation of *Smad4* or inactivation of *Smad2* and *Smad3* in ovarian granulosa cells show similarities in their phenotype, inactivation of genes encoding *Smad1* and *Smad5*, which transduce signals for AMH and BMPs, results in a phenotype that is completely different. *Smad1* and *Smad5* are coexpressed in mouse granulosa cells, and deletion of both genes is required to produce a phenotype in granulosa cells (Pangas et al. 2008). Single deletion of either *Smad1* or *Smad5* in granulosa cells has no effect on female reproductive function, suggesting functionally redundant activities in particular cell types (Pangas et al. 2008). Functional redundancy between *Smad1* and *Smad5* may also occur during Müllerian duct regression in the

mouse (Orvis et al. 2008). Combined deletion of *Smad1* and *Smad5* using the *Amhr2^{cre/+}* allele causes development of granulosa cell tumors in females or Sertoli–Leydig cell tumors in males in 100% of the mice (Pangas et al. 2008). These tumors develop around 8 wk of age and progress to metastatic tumors at older ages leading to decreased survival (Middlebrook et al. 2009). Granulosa cell tumor development is also seen in granulosa cell-specific deletion of the genes encoding the BMP type I receptors, *Bmpr1a* and *Bmpr1b* (Edson et al. 2010), suggesting that loss of the BMP-activated Smad signaling is likely the cause of the tumors. These tumors phenocopy the juvenile form of granulosa cell tumors in humans based on histological, molecular, and hormonal

characteristics (Middlebrook et al. 2009). One characteristic shared by the *Smad1*^{-/-}; *Smad5*^{-/-} granulosa cell tumors and primary samples of human juvenile granulosa cell tumors, is the activation of Smad2 and Smad3 signaling. Although the role of active Smad2 and Smad3 signaling in granulosa cell tumors has yet to be determined, a number of mouse mutants suggest that in granulosa cells, Smad2 and Smad3 signaling promotes cell proliferation and tumor growth. For instance, loss of activin or loss of Smad2 and Smad3 in the somatic cells of the follicle results in infertility, but not tumor formation (Pangas et al. 2007; Li et al. 2008). In contrast, loss of inhibin expression causes sex-cord stromal tumors producing supraphysiological levels of activin (Matzuk et al. 1992, 1994; Li et al. 2007a,b). Combined with the phenotypes of granulosa cell Smad mutants, it is likely that signaling by Smad1 and/or Smad5 in response to BMPs or AMH, and Smad2 and/or Smad3 in response to TGF- β or activin, controls the balance of cell proliferation and differentiation in granulosa cells. Disruption of this balance can lead to either formation of ovarian tumors (i.e., in the *Inha*^{-/-} or conditional *Smad1*^{-/-}; *Smad5*^{-/-} mice), or infertility (i.e., after *Smad2* and *Smad3* as well as *Smad4* conditional inactivation). In support of this hypothesis, triple conditional inactivation of *Smad1*, *Smad5*, and *Smad4*, which should additionally inhibit all Smad4-dependent signaling within the *Smad1* and *Smad5* granulosa cell tumors, remarkably slows tumor growth and metastasis development (Mansouri-Attia et al. 2014).

TGF- β FAMILY SIGNALING IN THE UTERUS AND PREGNANCY

The endometrial layer of the uterus is continuously remodeled throughout the menstrual cycle in response to ovarian hormones and various growth factors to coordinate embryo implantation (Fig. 5C) (Cha et al. 2012). Estradiol (E₂) induces the proliferative phase of the endometrium, while progesterone (P₄) and several growth factors stimulate endometrial stromal cell decidualization during the secretory phase

of the cycle (Cha et al. 2012). Decidualization, a process of stromal cell differentiation, prepares the uterus for pregnancy by transforming the stromal cells of the endometrium into secretory cells that nurture the embryo during early pregnancy (Ramathal et al. 2010). Ultimately, the endometrium regresses through menstruation in the absence of pregnancy, or continues decidualizing if pregnancy occurs. In the mouse, decidualization is also coordinated by growth factors and steroid hormones, but occurs only in response to an implanting embryo (Ramathal et al. 2010). In mice, artificial decidualization can be induced with a mechanical stimulus to the uterine wall followed by estrogen and progesterone treatments that mimic pregnancy (Fig. 7B,C) (Lydon et al. 1995). Both mice and humans establish hemochorial placentation, where the trophoblasts are in direct contact with the maternal blood, making the mouse a suitable model to study pregnancy (Schlafke and Enders 1975; Carson et al. 2000).

Various components of TGF- β family signaling pathway are expressed in the uterus, and their expression changes throughout pregnancy. TGF- β ligands and their receptors are expressed at the fetal-maternal interface (Tamada et al. 1990; Selić et al. 1994; Godkin and Dore 1998). *Tgfb2* mRNA is expressed in the luminal and glandular epithelium during the mouse peri-implantation period, and in decidual cells following implantation (Das et al. 1992). In mice, *Tgfb3* transcripts are detected in myometrial cells and in the vascular smooth muscle cells during the peri- and postimplantation periods (Das et al. 1992). Furthermore, E₂ or diethylbesterol (DES) treatments increase the expression of uterine *Tgfb1*, *Tgfb2*, and *Tgfb3* (Das et al. 1992; Takahashi et al. 1994). Similar dynamic expression patterns of TGF- β 1, - β 2, and - β 3 are observed in the human uterus. *TGFB1*, *TGFB2*, and *TGFB3* mRNAs are detected in the three cell types of the uterus, with maximal expression observed in the luminal and glandular epithelium during the late proliferative to early secretory phase, suggesting progesterone-mediated regulation (Chegini et al. 1994). *TGFB1*, *TGFB2*, and *TGFB3* expression is also dynamically expressed during pregnancy,

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Figure 7. Bone morphogenetic protein (BMP) signaling via ALK-2 promotes endometrial stromal cell decidualization in the mouse and human uterus. (A) BMPs signal through the BMP type I receptor, ALK-2 and activate Smad1, Smad5 or Smad8 to increase *CEBPB* expression. C/EBP β then increases *Pgr* (progesterone receptor) expression, which is necessary for endometrial stromal cell decidualization. (B,C) Gross anatomy of a normal uterus and a uterus after an artificial decidual stimulus. Decidualization is assessed by increased uterine weight and histologically by the presence of proliferative and decidual cells. Following the induction of an artificial decidual stimulus that mimics embryo implantation, the stromal cells of a healthy uterus proliferate and decidualize into specialized secretory cells. (D,E) Conditional deletion of *Acvr1/Alk2* or *Bmpr2* with *Pgr^{cre/+}* results in mouse sterility. E8.5 uteri of control and *Alk2^{flox/flox}-Pgr^{cre/+}* mice. *Alk2^{flox/flox}-Pgr^{cre/+}* females show postimplantation and decidualization defects. uh, Undecidualized horn; dh, decidualized horn.

and TGF- β is localized to cytotrophoblasts, intermediate trophoblasts, and villous cytotrophoblasts, as well as in the stroma and glands of the maternal decidua (Selick et al. 1994).

An increasing number of mouse models have examined the *in vivo* contribution of TGF- β signaling in the female reproductive tract using conditional gene deletion strategies (Table 2) to overcome the embryonic lethality of global mutations. Typically, these models use the deleter strain, *Amhr2^{cre/+}*, to target the uterine myometrium and stroma (i.e., mesenchymal cells originating from the Müllerian duct), or they use the *progesterone receptor cre* (*Pgr^{cre/+}*) to target the uterine endometrium. Female mice with conditional deletion of *Tgfb1* (*Alk5*), thus lacking the expression of TGF- β type I receptor, or ALK-5, generated using *Amhr2^{cre/+}* are sterile. The primary defect appears to be in the oviduct and uterine myometrium. Mutant ovi-

ducts develop oviductal diverticula, which could impede sperm transport and/or capacitation, and reduce transit of embryos through the oviduct (Li et al. 2011). These mutant mice also show defective smooth muscle development in the uterus, with uterine cyst formation and abnormal masses of tissue forming at 8 months of age. Thus, *Tgfb1* has a key role in the development of the muscular architecture of the oviduct and uterus (Li et al. 2011; Gao et al. 2014, 2015).

Tgfb1 has also been conditionally inactivated using *Pgr^{cre/+}* to target cells in the endometrial compartments of the uterus (Peng et al. 2015b). Conditional deletion of *Tgfb1* with *Pgr^{cre/+}* results in subfertility because of defects throughout gestation, including abnormal embryo implantation, decreased uterine natural killer (uNK) cell infiltration, defective uterine spiral artery remodeling, and placental

defects (Peng et al. 2015b). Thus, observations from these two different mouse models (*Tgfb β 1^{flox/bgal}-Amhr2^{cre/+}* and *Tgfb β 1^{flox/bgal}-Pgr^{cre/+}*) show the unique roles of *Tgfb β 1* in the different cell types of the female reproductive tract.

Nodal signaling is important during the process of embryo implantation and throughout pregnancy in the mouse (Park and Dufort 2011). Conditional inactivation of *Nodal* in progesterone receptor-expressing tissues (with *Pgr^{cre/+}*) results in subfertility and postimplantation defects, such as intrauterine growth restriction (Park et al. 2012). Placental defects include abnormal giant cell morphology that extends into the mesometrial and conceptus sites. Additionally, females with conditional inactivation of *Nodal* have a defective and hemorrhagic decidua basalis (i.e., the endometrial cells that will become the maternal part of the placental) at day 10.5 of pregnancy, with decreased proliferation and increased apoptosis (Park et al. 2012). Recent studies suggest that uterine *Acvr1b* (encoding the activin A receptor, type IB or ALK-4) is the nodal receptor throughout gestation (Peng et al. 2015a). Female mice with conditional inactivation of *Acvr1b* with *Pgr^{cre/+}* are subfertile and show defects during gestation that include intrauterine growth restriction and placental abnormalities (Peng et al. 2015a). Reminiscent of the *Nodal^{flox/flox}-Pgr^{cre/+}* females, mice with conditional *Acvr1b* deletion display abnormal trophoblast giant cell expansion and decreased placental spongiotrophoblast and labyrinth layers. These studies show the interplay between nodal and its receptor, ALK-4, in placental development and throughout gestation.

Activin signaling also uses ALK-4, but the role of activin in the uterus is unclear. No genes have been conditionally inactivated to specifically study activin function in vivo. Activin expression is detected in primary human placental cells, and activin stimulates the production of gonadotropin-releasing hormone (GnRH), progesterone, and human chorionic gonadotropin (hCG) (Petraglia et al. 1989; Debieve et al. 2000). Activin A expression increases in the serum and placentas of preeclamptic wom-

en, and is associated with increased apoptosis at the maternal–fetal interface (Table 3) (Yu et al. 2012). In addition, genome-wide association studies indicate linkage to chromosome 2q22 in families affected with preeclampsia or eclampsia, and point to *ACVR2A* (*ACVR2*) as the candidate susceptibility gene (Table 3) (Moses et al. 2006; Fitzpatrick et al. 2009; Roten et al. 2009).

Several mouse models have been generated to examine BMP signaling in the uterus using *Pgr^{cre/+}*, including *Bmp2*, the type II receptor *Bmpr2*, and the type I receptors *Acvr1* and *Bmpr1a*, as well as a global knockout of *Bmpr1b* (Table 1). These mouse models show critical BMP roles at multiple stages, including implantation, decidualization, and placentation. Conditional inactivation of *Bmp2* using *Pgr^{cre/+}* resulted in the first model for BMP signaling in the uterus (Lee et al. 2007). Female mice with conditional *Bmp2* inactivation were infertile, with impaired uterine decidualization (Lee et al. 2007). In contrast, conditional ablation of the BMP type II receptor, *Bmpr2* with *Pgr^{cre/+}* shows defects in the later stages of gestation (Nagashima et al. 2013). These midgestation defects include defective spiral artery remodeling and placental hemorrhage, which leads to restricted embryo growth and fetal death. Females with conditional ablation of *Bmpr2* are sterile (Nagashima et al. 2013). Conditional deletion of *Acvr1* (the gene encoding ALK-2) using *Pgr^{cre/+}* shares some similarity with the *Bmp2-Pgr^{cre/+}* model, with defects in implantation and decidualization (Clementi et al. 2013). The defects may be due in part to loss of induction of the gene encoding the key transcription factor, CCAAT/enhancer-binding protein, β (*CEBPB*) by Smad1 and Smad5 (Clementi et al. 2013). *CEBPB* activates progesterone receptor expression, and both are required for endometrial cell decidualization (Fig. 7) (Mantena et al. 2006).

The earliest pregnancy phenotype for mouse models of the BMP signaling pathway was discovered through conditional deletion of the BMP type I receptor *Bmpr1a* (*ALK3*) using *Pgr^{cre/+}* (Monsivais et al. 2015). Female mice with conditional *Bmpr1a* inactivation are sterile with a nonreceptive endometrium char-

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Table 3. Alterations in the TGF- β family signaling pathways in female reproductive diseases

Gene	Disease	Description	References
<i>ACVR2A</i>	Preeclampsia	GWAS identifies <i>ACVR2A</i> as the candidate susceptibility gene in families affected with preeclampsia	Moses et al. 2006; Fitzpatrick et al. 2009; Roten et al. 2009
<i>BMP6</i>	Endometriosis	BMP-6 is expressed in ectopic endometriotic lesions	Athanasios et al. 2012
<i>BMPR1B</i>	Endometriosis	Deactivating polymorphism in miR-125b enhances <i>BMPR1B</i> production in women with endometriosis	Chang et al. 2013
<i>INHBA</i>	Preeclampsia	Women with preeclampsia have increased serum and placental levels of activin A	Yu et al. 2012
<i>LEFTY</i>	Infertility	Lefty is overexpressed in the endometrium of women with unexplained infertility	Tabibzadeh et al. 2000
<i>TGFB1</i>	Endometriosis	Women with endometriosis have elevated peritoneal fluid concentrations of TGFB1	Oosterlynck et al. 1994; Kupker et al. 1998; Sotnikova et al. 2010
<i>TGFB3</i>	Uterine fibroids	<i>TGFB3</i> mRNA is increased in uterine fibroids	Arici and Sozen 2000
<i>TGFB1</i> , <i>TGFB2</i> , <i>TGFB3</i>	Uterine fibroids	Treatment with leuprolide acetate decreases <i>TGFB1</i> , <i>TGFB2</i> , and <i>TGFB3</i> mRNA in uterine fibroids	Dou et al. 1996

acterized by elevated microvilli density in the luminal uterine epithelium, enhanced response to estrogen, and decreased sensitivity to progesterone. Furthermore, mice with *Bmpr1a* conditional inactivation showed an integration of BMP- and progesterone-mediated signaling at the promoter of *Klf15* (Monsivais et al. 2015). KLF15 is a critical transcription factor during the window on implantation that programs the luminal epithelium of the endometrium into a state receptive for embryo implantation (Ray and Pollard 2012).

Mouse models for canonical Smad signaling have only been examined in the reproductive tract using *Amhr2^{cre/+}* to conditionally inactivate the Smad gene of interest. Many of these models do not appear to show reproductive tract defects, including single conditional deletion of *Smad1*, *Smad2*, *Smad3*, *Smad4*, and *Smad5* (Pangas et al. 2006, 2008; Li et al. 2008). Surprisingly, triple conditional deletion of *Smad1*, *Smad5*, and *Smad4* using *Amhr2^{cre/+}* not only affects granulosa cell tumor development (see previous section), but also causes sterility because of defects in the development of the oviduct and uterus (Rodriguez et al. 2016). These mice have abnormal smooth muscle de-

velopment with the oviduct that may disrupt the transport of embryos or sperm (Rodriguez et al. 2016). These mice also show defects within myometrium and endometrium, resulting in pregnancy loss by midgestation (Rodriguez et al. 2016). Thus, inactivation of ligands, receptors and the downstream effectors of the BMP signaling pathway show its fundamental role in the development and function of the female reproductive system.

TGF- β FAMILY SIGNALING AND FEMALE REPRODUCTIVE TRACT DISEASES

Endometriosis is an estrogen-dependent gynecological disease that affects about 10% of women of reproductive age, and is characterized by extrauterine growth of endometrial tissues (Giudice and Kao 2004; Bulun 2009). Endometriosis is associated with chronic pelvic pain and infertility and has only a few effective therapeutic options (Valle and Sciarra 2003; Giudice 2010). Although most therapies include suppression of ovarian hormones using oral contraceptives, GnRH-analogs, or aromatase inhibitors as nonhormonal interventions are desirable (Valle and Sciarra 2003). Several stud-

ies indicate a correlation between TGF- β signaling and progression of endometriosis. Compared to healthy women, TGF- β levels are elevated in the peritoneal fluid of women with endometriosis (Oosterlynck et al. 1994; Kupker et al. 1998); elevated TGF- β induces fibrosis, increasing the formation of endometriotic adhesions in the peritoneum (Chegini 2008). *TGFB2* mRNA levels are increased in the endometriotic lesions of women and in a rat model of endometriosis (Sotnikova et al. 2010). Further evidence shows that TGF- β signaling increases endometriotic lesion growth by transcriptional activation of KLF11 (Correa et al. 2016). Genetic evidence shows that a deactivating polymorphism in miR-125b results in enhanced BMPRII expression in women with endometriosis (Chang et al. 2013). Furthermore, BMP-6 expression is increased in endometriotic lesions (Athanasios et al. 2012), suggesting a role for BMP signaling pathways in endometriosis.

TGF- β signaling has also been widely explored in the pathogenesis of uterine fibroids. Uterine fibroids, or leiomyomas, are benign smooth muscle tumors of the uterus that are associated with uterine bleeding, anemia, pelvic discomfort, and recurrent pregnancy loss (Okolo 2008; Bulun 2013). Compared to normal myometrial tissue, uterine fibroids have increased extracellular matrix deposition with disorganized collagen fibril formation (Leppert et al. 2004). Increased collagen deposition is characteristic of other diseases with altered TGF- β signaling such as pulmonary fibrosis, scleroderma, and liver cirrhosis (Border and Noble 1994; Blobe et al. 2000). Compared to healthy myometrium, *TGFB3* expression is increased in uterine fibroids and correlates with increased fibronectin and increased cell proliferation (Arici and Sozen 2000). TGF- β 3 also increases the expression of versican in myometrial and leiomyoma-derived cells (Norian et al. 2009). Various studies have tested the proliferative effects of TGF- β on cultured myometrial and leiomyoma-derived smooth muscle cells (Tang et al. 1997; Arici and Sozen 2000; Lee and Nowak 2001). These studies show that TGF- β signaling increases leiomyoma cell proliferation (Tang et al. 1997), and this response is

affected by the phase of the menstrual cycle (Arici and Sozen 2003). In other studies, TGF- β 3, but not TGF- β 1 or TGF- β 2, significantly stimulates leiomyoma cell proliferation (Lee and Nowak 2001). The GnRH agonist leuprolide acetate significantly decreases *TGFB1*, *TGFB3*, and *TGFBR2* expression in leiomyoma tissues (Dou et al. 1996). Overall, these studies show that TGF- β increases extracellular matrix formation and cell proliferation in uterine fibroids.

CONCLUSION

In vivo studies using mouse models have contributed significantly to our understanding of the physiology and pathophysiology of the reproductive system that may not have been discovered through other experimental analyses. Such was the case for the novel tumor suppressor functions of inhibin α , because inhibin was best known for its role in regulating pituitary gonadotrope function. Mice null for *Gdf9* and *Bmp15*, oocyte-specific members of the TGF- β family, highlight the importance of the oocyte in controlling follicular growth; both have become candidate genes for infertility research. Mutations in *BMP15* have been identified that cause ovarian failure in women (Di Pasquale et al. 2004). In addition, GDF-9 and BMP-15 may form highly potent heterodimers, which may have clinically useful activities. However, most of the TGF- β family proteins and their signaling systems control critical stages in embryonic development or the function of multiple organ systems. As such, mice with targeted gene inactivation display embryonic or perinatal lethality that precludes their use in studying their reproduction function. To circumvent this issue, mice with conditional gene inactivation have been generated. *Bmp2* and *Acvr1* conditional inactivations result in early pregnancy failure, and conditional deletion of *Bmpr2* or *Nodal* disrupts signaling pathways during late pregnancy. Translational studies using human tissues, cells, and genome-wide association studies also implicate abnormal TGF- β signaling in female reproductive diseases, such as endometriosis, uterine fibroids, and preeclampsia. In the end, mice with tissue-specific gene inac-

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tivation and mice with global gene inactivation ultimately provide a basis for understanding infertility and disease in humans.

ACKNOWLEDGMENTS

Studies on TGF- β signaling pathways have been supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development grants R01-HD033438 and R01-HD032067 (to M.M.M.) and R01-HD085994 and R01-HD076980 (to S.A.P.) and by the Institutional Research and Academic Career Development Award (IRACDA) K12-GM084897 (to D.M.). D.M. holds a Postdoctoral Enrichment Program Award from the Burroughs Wellcome Fund.

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Cold Spring Harb Perspect Biol 2017; doi: 10.1101/cshperspect.a022251 originally published online February 13, 2017

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