CHAPTER NINE

Involvement of Bone Morphogenetic Proteins (BMP) in the Regulation of Ovarian Function

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Abstract

Primordial germ cells migrate to the fetal gonads and proliferate during gestation to generate a fixed complement of primordial follicles, the so-called ovarian reserve. Primordial follicles comprise an oocyte arrested at the diplotene stage of meiosis, surrounded by a layer of pregranulosa cells. Activation of primordial follicles to grow beyond this arrested stage is of particular interest because, once activated, they are subjected to regulatory mechanisms involved in growth, selection, maturation, and ultimately, ovulation or atresia. The vast majority of follicles succumb to atresia and are permanently lost from the quiescent or growing pool of follicles. The bone morphogenetic proteins (BMPs), together with other intraovarian growth factors, are intimately involved in regulation of follicle recruitment, dominant follicle selection, ovulation, and atresia.

Activation of primordial follicles appears to be a continuous process, and the number of small antral follicles at the beginning of the menstrual cycle provides an indirect indication of ovarian reserve. Continued antral follicle development during the follicular phase of the menstrual cycle is driven by follicle stimulating hormone (FSH) and luteinizing hormone (LH) in conjunction with many intraovarian growth factors and inhibitors interrelated in a complex web of regulatory balance.

The BMP signaling system has a major intraovarian role in many species, including the human, in the generation of transcription factors that influence proliferation, steroidogenesis, cell differentiation, and maturation prior to ovulation, as well as formation of corpora lutea after ovulation. At the anterior pituitary level, BMPs also contribute to the regulation of gonadotrophin production.

1. OVERVIEW OF FOLLICULOGENESIS

The underlying physiological processes of reproduction in females and males are similar in humans and other mammals. In the female gonad (ovary) the oocyte is encapsulated by layers of follicular somatic cells that proliferate, and later differentiate and mature to form preovulatory follicles. At ovulation, the mature follicle wall ruptures and the oocyte is expelled from the follicle and is thereafter potentially destined for fertilization. The recruitment of follicles, their growth, and the expulsion of the oocyte are dependent on complex signaling mechanisms involving the hypothalamic–pituitary–gonadal axis. Neurotransmitters and neuropeptides from the hypothalamus stimulate the release of gonadotrophin releasing hormone (GnRH) into the hypophyseal portal system, which, in turn, stimulates the anterior pituitary to release gonadotrophic hormones that act on the ovary to promote follicular growth. Several predominant growth factors that regulate the transcription of genes and control the recruitment and selection of the

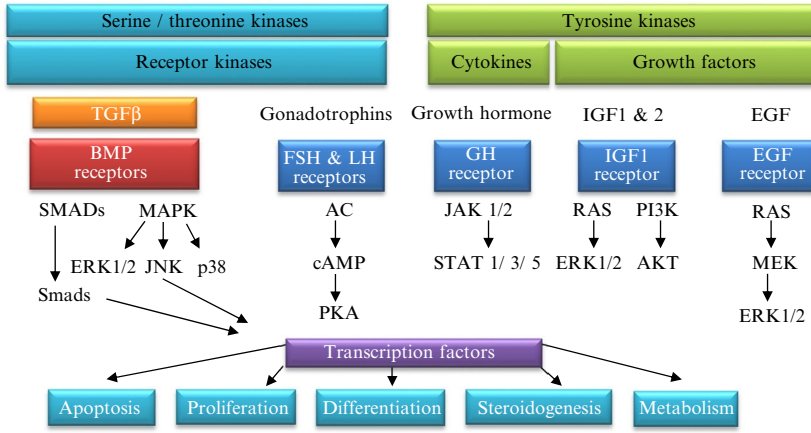

Fig. 1 Overview of the TGFβ and the growth hormone kinase signaling interaction. Major serine and tyrosine kinases and receptors, and signaling pathways involved in ovarian regulation (Amsterdam et al., 2003; Fan et al., 2009; Manna et al., 2002; Miyazono, Kamiya, & Morikawa, 2010; Moore et al., 2001; Rice, Ojha, Whitehead, & Mason, 2007; Tajima et al., 2003).

dominant follicles belong to the transforming growth factor beta (TGFβ) superfamily including the subfamilies of bone morphogenetic proteins (BMPs), inhibins, and activins, growth differentiation factors (GDFs), and anti-Müllerian hormone (AMH) (Fig. 1) (Edson, 2009; Eppig, 2001; Erickson & Shimasaki, 2001; Fabre et al., 2006; Gilchrist, Ritter, & Armstrong, 2004; Knight & Glister, 2006; McNatty et al., 2004; Otsuka, 2013).

At approximately 26 weeks of gestation in humans, the reproductive potential of the fetus is established (Childs et al., 2010). By this stage the primordial follicles are fully formed and begin a process of initial activation followed by eventual demise or ovulation and potential fertilization, over the reproductive life span of the individual (Pangas, 2012). Activated primordial follicles grow and differentiate into preantral follicles (Fig. 2). With further development, preantral follicles mature into antral follicles with the formation of a fluid-filled central compartment (Rodgers & Irving-Rodgers, 2010). At the onset of puberty, cyclic increases in gonadotrophin secretion from the anterior pituitary raise follicle-stimulating hormone (FSH) to a threshold point sufficient to rescue a growing cohort of small antral follicles and initiate cyclic recruitment (Fig. 2) (Gougeon, 1986; Richards, 1994).

Antral follicles contain an oocyte surrounded by cumulus granulosa cells that form a continuum with mural granulosa cells lining the antrum. The follicle wall is composed of granulosa and theca cells separated by the

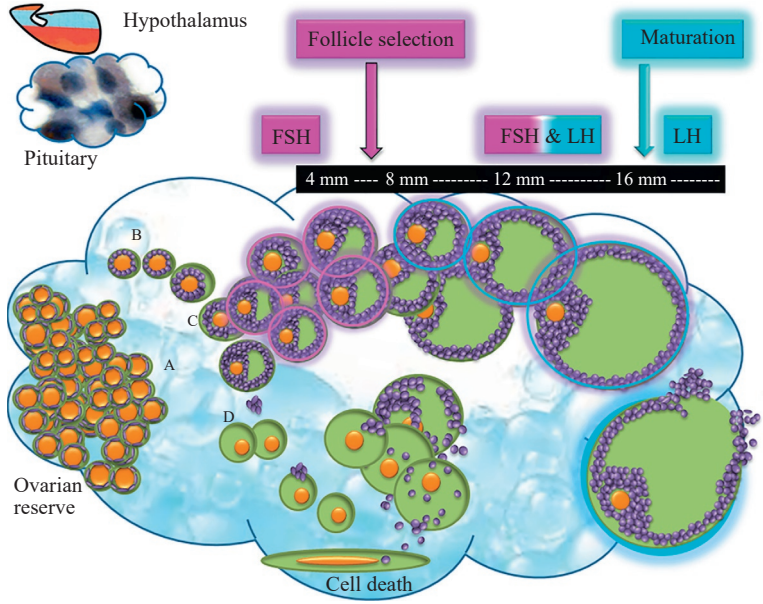

Fig. 2 Folliculogenesis: Activation of the primordial follicle, dominant follicle selection, growth and maturation before ovulation. Ovarian reserve of primordial follicle with squamous pregranulosa cells (A), activation and initial recruitment of primary follicle with cuboidal granulosa cells (B), secondary follicle with multiple layers of granulosa and no antral cavity (C), and cell death of preantral follicles (D). FSHR expression (pink rings) and FSH secretion promote antral follicle formation, followed by dominant follicle selection (pink arrow) based on LHR expression (blue rings). Proliferation and growth increase the size of the follicle. Maturation of the follicle and the LH surge differentiate the follicle cells in a complex process of luteinization. Diameter of the follicles at the respective stages of folliculogenesis is indicated in mm scale.

basal lamina. Stromal cells within a connective tissue matrix are encapsulated by a layer of epithelial cells at the ovarian surface (Erickson & Shimasaki, 2003; Rodgers & Irving-Rodgers, 2010).

Folliculogenesis involves the stage-dependent expression of intraovarian growth factors and their receptors that regulate proliferation and differentiation of granulosa and thecal cells (Erickson & Shimasaki, 2003; Gougeon, 1986). The mature follicle or preovulatory follicle completes differentiation and commences luteinization (morphological and steroidogenic capacity changes) prior to ovulation, and then ruptures, releasing the oocyte in the proximity of the opening of the fallopian tube (Ainsworth, Tsang, Downey, Marcus, & Armstrong, 1980; Rodgers & Irving-Rodgers, 2010).

The number of preovulatory follicles selected for dominance and ovulation varies according to species and is dependent on the regulation by the gonadotrophins (FSH and LH (luteinizing hormone)) and the interaction with intraovarian growth factors (Ginther et al., 2005; Gougeon, 1986). TGFβ family members, including BMPs, have been shown to play a major role in the recruitment and growth of the ovarian follicle (Edson, 2009; Erickson & Shimasaki, 2001; Fabre et al., 2006; Knight & Glister, 2006; Otsuka, 2013). There has been considerable interest in the type 1 BMP receptor (BMPR1B) which binds to the BMP ligands 2, 4, 6, 7, and 15 culminating in altered gene transcription (Miyazono, Maeda, & Imamura, 2005). A naturally occurring point mutation of the BMPR1B gene in the Booroola Merino (BB) sheep results in partial attenuation of receptor function and increases ovulation rate (Mulsant et al., 2001; Souza, MacDougall, Campbell, McNeilly, & Baird, 2001; Wilson et al., 2001). In the human clinical context, ovulation rate is increased during in vitro fertilization (IVF) treatment by administration of FSH to stimulate the growth of multiple follicles (Edwards, Lobo, & Bouchard, 1996; Edwards & Steptoe, 1983). BMPR1B-mediated signaling and its interaction with the signaling of FSH receptor (FSHR) and LH receptor (LHR) appear to have a central role in this process.

2. THE OVARIAN RESERVE

Oogonia proliferate in the ovary before commencing meiosis from approximately weeks 9 to 11 of gestation in humans (Fig. 3). Germ cell cysts (egg nests) containing multiple oogonia are infiltrated by somatic cells, forming individual primordial follicles, each with a single layer of somatic cells surrounding the oocyte (Pangas, 2012). The somatic cells differentiate into granulosa cells, and the oocyte resides in the dictyate-stage of meiotic prophase 1 until the mid-cycle LH surge triggers meiotic progression in the follicles selected for ovulation (Edwards et al., 1996). The progressive decline of the ovarian reserve is well documented and is related to chronological age (Almog, Shehata, Shalom-Paz, Tan, & Tulandi, 2011; Hansen, Hodnett, Knowlton, & Craig, 2011).

The total number of germ cells peaks at over six million at ~26 weeks gestation. At birth the number of germ cells (oocytes) has already declined by ~80%, and this decline continues inexorably throughout the reproductive life span of the individual (Fig. 3) (Monniaux et al., 2014). At puberty, the levels of gonadotrophins increase sufficiently to promote tertiary follicles to

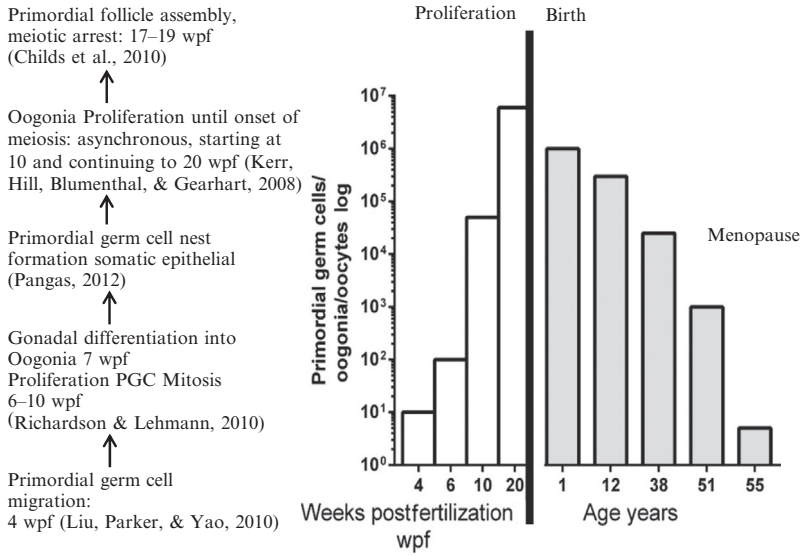

Fig. 3 Primordial germ cell proliferation and oogenesis before birth, and the loss of primordial follicles from birth to menopause. *Based on Baerwald, A., Adams, G., & Pierson, R. (2012). Ovarian antral folliculogenesis during the human menstrual cycle: A review. Human Reproduction Update, 18, 73–91; Fabre, S., Pierre, A., Mulsant, P., Bodin, L., Di Pasquale, E., Persani, L., et al. (2006). Regulation of ovulation rate in mammals: Contribution of sheep genetic models. Reproductive Biology and Endocrinology, 4, 20; Knight, P.G., & Glister, C. (2006). TGF-β superfamily members and ovarian follicle development. Reproduction, 132, 191–206; Matsuda, F., Inoue, N., Manabe, N., & Ohkura, S. (2012). Follicular growth and atresia in mammalian ovaries: Regulation by survival and death of granulosa cells. Journal of Reproduction and Development, 58, 44–50; Skinner, M.K. (2005). Regulation of primordial follicle assembly and development. Human Reproduction Update, 11, 461–471; Webb, R., & Campbell, B. K. (2007). Development of the dominant follicle: Mechanisms of selection and maintenance of oocyte quality. Society of Reproduction and Fertility Supplement, 64, 141–163.*

continue growth, and to resist apoptosis (Matsuda, Inoue, Manabe, & Ohkura, 2012). Ultrasonographic estimates of the number of small antral follicles growing (AFC) or serum levels of AMH (secretion by the small antral follicles) are strongly correlated to the ovarian reserve (Hansen et al., 2011; van Rooij et al., 2005).

3. INTRAOVARIAN REGULATORS OF FOLLICULOGENESIS

Inducement of FSHR and LHR expression and modulation of responsiveness to gonadotrophins appear to be under the control of various intraovarian growth and development regulators (Fig. 1) (Baerwald,

Adams, & Pierson, 2012; Erickson, Wang, & Hsueh, 1979; Fan et al., 2009). BMPs, GDF9, AMH, inhibins, activins, and BMP/activin binding proteins have been implicated directly or indirectly, in vivo, by experiments that involve treatments, such as ligand infusion and active or passive immunization (Al-Samerria, Al-Ali, McFarlane, & Almahbobi, 2015; Campbell, Kendall, & Baird, 2009; Juengel, Hudson, Whiting, & McNatty, 2004; Knight, Satchell, & Glister, 2012), and by evidence from natural mutations and knockout gene models in several species (Araújo et al., 2010; Di Pasquale et al., 2006; Feary et al., 2007; McNatty et al., 2007). In vitro culture of isolated granulosa and theca cells and ovarian tissue explants has provided substantive data on the influence of these growth factors on steroidogenesis and cell proliferation (Brankin, Quinn, Webb, & Hunter, 2005; Campbell, Souza, Skinner, Webb, & Baird, 2006; Glister, Kemp, & Knight, 2004; McNatty et al., 2009; Nilsson & Skinner, 2003).

The early acquisition of granulosal FSHR and LHR facilitates dominant follicle growth in the face of declining FSH levels during the follicular phase of the cycle (Fig. 2) (LaPolt, Tilly, Aihara, Nishimori, & Hsueh, 1992; Sen et al., 2014). Acquisition of granulosal LH responsiveness supplements the FSHR-mediated conversion of androstenedione to estradiol by P450 aromatase (CYP19A1), maintaining a positive estrogen to androgen ratio in the follicle. As the antral follicle increases in size, more estrogen and antiapoptotic factors are produced to ensure the survival of the dominant ovulatory follicle (Amsterdam et al., 2003). With reduced FSHR and LHR density, the granulosa cells of subordinate follicles have a reduced capacity to convert theca-derived androgens to estrogens and are destined for atresia (Hillier, Whitelaw, & Smyth, 1994; Xu et al., 1995).

4. ROLE OF BMPS IN OVARIAN REGULATION

The body of work investigating the role of BMPs as ovarian regulators ranges from studies on primordial germ cell migration through to inducement of ovulation and corpus luteum formation (Erickson & Shimasaki, 2001, 2003; Knight & Glister, 2006; Miyazono et al., 2010; Otsuka, 2013; Pangas, 2012; Shimasaki, Moore, Otsuka, & Erickson, 2004). Various mammalian species have been used as in vivo and in vitro research models including polyovulatory rodents and pigs, monoovulatory species (sheep, cattle) and humans (Edson et al., 2010; Raz, 2003; Regan et al., 2017, 2015). The ability to create global and conditional gene knockout models in mice and to use natural and created mutations or specific cell lines to study

the effects of perturbing specific BMP pathway components has facilitated research in this area. Furthermore, treatment with BMPs as well as blocking receptors and signaling pathways has been used extensively to examine the roles of BMP signaling in ovarian function.

In human studies, in vivo and in vitro research has progressed substantially with the rise of IVF centers, providing an accessible source of follicular material from gonadotrophin-stimulated patients undergoing oocyte retrieval. However, the availability of nonpathogenic human ovarian tissue, free from exogenous gonadotrophin stimulation, is very limited and is therefore infrequently used in research (Bomsel Helmreich et al., 1979; Fowler, Sorsa, Harris, Knight, & Mason, 2001; Garcia, Jones, Acosta, & Wright, 1981; Gougeon, 1986; Klein et al., 2000; MacNaughton, Banah, McCloud, Hee, & Burger, 1992). Given the stage-specific nature of ovarian regulation and variation among species, caution should be used when interpreting results (Erickson & Shimasaki, 2003; Otsuka, 2010). In addition, the complex interactions and feedback loops between locally produced growth factors and other components of the hypothalamic–pituitary–ovarian axis complicate the interpretation of in vivo experiments exploring the intraovarian roles of specific growth factors (Zeleznik, 2001).

5. BMPs: MEMBERS OF THE TGFβ SUPERFAMILY

The TGF superfamily consists of over 40 different ligands and can be divided into several subfamilies including the BMP subfamily that is the focus of this review. As with other TGFβ superfamily members, BMP signaling pathways are operational in numerous tissues and organs across the life-course, where they exert complex inhibitory and stimulatory control over cell proliferation, apoptosis, and cell differentiation (Massagué, 2008).

There are seven TGFβ type 1 receptors, commonly referred to as ALK1 to ALK7, and six type 2 TGFβ receptors. The BMP ligands, 2, 4, 6, 7, and 15, form a receptor–ligand complex with the type 1 TGFβ receptor BMPR1B (ALK6) and a type 2 TGFβ receptor BMPR2 (Fig. 4) (Miyazono et al., 2010). The heterotetrameric receptor complex initiates phosphorylation of the intracellular substrate molecules, receptor-regulated Smads (Smads 1, 5, and/or 8 in the case of BMP signaling). The Smad forms a complex with a common mediator, Smad 4, and translocates to the nucleus. In the nucleus, allocated specific cofactors for each BMP ligand initiates transcription of genes required by the cell (Mitsui et al., 2015; Moore, Otsuka, & Shimasaki, 2003). BMP signaling is modulated at different levels by specific repressor and activator molecules in the nucleus, cytoplasm, extracellular

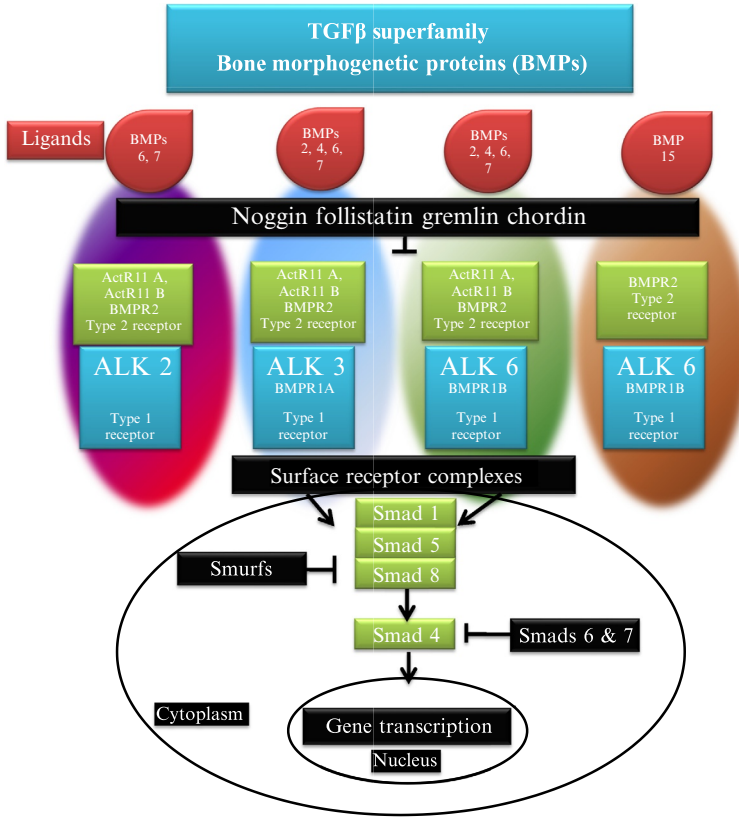

Fig. 4 BMP signaling pathway. BMP ligand signaling and the formation of hetero-tetrameric receptor complex with a type 1 and type 2 TGFβ receptor (four oval shapes). Phosphorylation of downstream signaling molecules via the receptor regulated Smads (R-Smads) via the common universal Smad 4 molecule to initiate translocation to the nucleus for gene transcription involved in cell proliferation, steroidogenesis, cell differentiation, and cell death.

fluid, and extracellular matrix. Intracellular modulators that attenuate signaling include inhibitory Smads 6 and 7 and extracellular BMP inhibitory binding proteins include follistatin, noggin, chordin, and gremlin. These nonsignaling secreted proteins sequester BMP ligands and modulate their binding to signaling receptors, generally inhibiting their actions (Miyazono et al., 2010).

In addition to activation of canonical Smad-mediated signaling pathway, BMPs can activate mitogen-activated protein kinases (MAPK) such as extracellular signal-regulated kinases 1 and 2 (ERK 1/2) (Inagaki et al., 2009). The cross talk between the Smads and the ERK 1 and ERK 2 signaling adds another dimension to the complex signaling that regulates folliculogenesis (Tajima et al., 2003).

6. OBSERVATIONS ON INTRAOVARIAN ROLES OF DIFFERENT BMPs

6.1 BMP2

Evidence suggests BMP2 is involved in oocyte endowment, primordial pool assembly, and activation of primordial follicles (Lawson et al., 1999; Ying & Zhao, 2001). The BMPs including BMP2 have been shown to be involved with the regulation of FSH synthesis through a competitive binding mechanism whereby activin increases synthesis and inhibin suppressed synthesis (Lee et al., 2007). BMP2 also increased granulosa cell estrogen and inhibin B production and FSHR expression in culture; however, it had no effect on proliferation (Shi et al., 2011; Souza, Campbell, McNeilly, & Baird, 2002). BMP2 has a relatively low affinity to bind with BMPR1B, and binds preferentially with another type 1 TGFβ receptor, BMPR1A (ALK3); however, species differences exist (Miyazono et al., 2010).

6.2 BMP4

BMP4 is produced by both theca and granulosa cells in the bovine and human model (Glister et al., 2004; Khalaf et al., 2013). BMP4 has been shown to bind precociously with BMPR1A and BMPR1B in sheep (Fabre et al., 2006), signaling via the Smad intermediate molecules to modulate ovarian function. BMP4 produced by granulosa and theca cells signals via BMPR1B to activate Smad 1 that inhibits steroidogenic acute regulatory protein (StAR) and P450 Side Chain Cleavage (CYP11A1) gene expression in the granulosa cells to progesterone synthesis during the proliferative phase of follicle development and the early onset of the LH surge and ovulation (Pierre et al., 2004). In addition, BMP4 modulates cell function via alternative signaling pathways to the TGFβ which involves the MAPK family, in particular ERK 1/2 (Fan et al., 2009; Moore et al., 2001).

In particular, BMP4 inhibits progesterone production of small antral follicles by negatively influencing cyclic adenosine monophosphate (cAMP) levels and expression of FSHR, StAR, and CYP11A1 of the granulosa cells (Fabre et al., 2003; Mulsant et al., 2001). In contrast, large antral follicles are not responsive to BMP4-induced inhibition of granulosal progesterone secretion or mitogenic growth, which indicates that BMP4 may be more involved in regulation up to dominant follicle selection (Fabre et al., 2006; Tanwar & McFarlane, 2011). While FSH-induced progesterone biosynthesis was inhibited by BMP4, it was shown to enhance FSH-induced

estrogen production in rat (Shimasaki et al., 1999) and sheep (Fabre et al., 2003; Mulsant et al., 2001). BMP4 suppressed IGF-induced progesterone production by bovine granulosa cells while enhancing estrogen, inhibin, and activin production (Glister et al., 2004).

BMP4 is also involved in oocyte endowment, primordial follicle assembly, and primordial follicle activation (Lawson et al., 1999; Lee, Otsuka, Moore, & Shimasaki, 2001; Lee et al., 2004; Nilsson & Skinner, 2003; Ying & Zhao, 2001). At the pituitary level, BMP reduces steroidogenic factor (SF-1) transcriptional activity on the LHβ promoter (Pierre et al., 2004). SF-1 is a key activator of steroidogenic endocrine function, and BMP4 and SF-1 are found in gonadotrope cells that produce LH in the anterior pituitary (Ingraham et al., 1994; Val, Lefrançois-Martinez, Veyssière, & Martinez, 2003). The direct link between BMP4 and LH synthesis via BMPR1B-induced phosphorylation of Smad 1 provides an explanation for the increase in LHRs as the BMP ligands and receptors decline during folliculogenesis, which releases their inhibitory effect (Nicol et al., 2008; Regan et al., 2017). In addition, BMP4 inhibits ovine pituitary FSHβ expression and reduces the concentration of FSH (Faure et al., 2005).

6.3 BMP7

At the ovarian level, BMP7 is mostly expressed by theca cells, but with some expression by granulosa cells (Glister et al., 2004; Khalaf et al., 2013). In the rat, BMP7 has a similar biological effect as BMP4, 6, and 15 in stimulating granulosal proliferation, suppressing FSH-induced progesterone biosynthesis and increasing FSH-induced CYP19A1 expression (Lee et al., 2001; Shimasaki et al., 1999). In monoovulatory species, the effects on granulosa cells appear inconsistent in the bovine. FSH-induced hormone secretion was not altered by BMP7, whereas in the goat, BMP7 increased FSHR and decreased LHR mRNA expression. In humans, BMP7 increased FSHR expression (Shi et al., 2010; Zhu et al., 2013). Differences in response are likely due to granulosa cells either being luteinized (caused by the addition of serum during culture) or attributed to being collected from gonadotrophin-stimulated ovaries in humans.

6.4 BMP6

BMP6 is expressed by the oocyte in follicles from the primordial stage, whereas granulosa cells also express BMP6 in antral follicles in humans and other species (Glister et al., 2004; Khalaf et al., 2013; Wu et al., 2007).

BMP6 is involved in proliferation, steroidogenesis, and cytodifferentiation of granulosa cells in a species-specific manner. In the goat, BMP6 did not increase granulosal FSHR, yet it increased LHR expression (Zhu et al., 2013). In humans, BMP6 increased FSHR mRNA, whereas in the rat, its effect was exerted downstream of the FSHR (Ogura Nose et al., 2012; Otsuka, Moore, Shimasaki, et al., 2001). BMP6 has been shown to increase proliferation of granulosa cells, in contrast with BMP2 or BMP4, in cultured sheep granulosa cells. However, in the rat, BMP6 had no effect on granulosa cell proliferation (Campbell et al., 2006; Otsuka, Moore, et al., 2001). BMP6 mRNA levels in granulosa cells were significantly increased in women with polycystic ovarian syndrome (Khalaf et al., 2013). At the pituitary level, a sheep study revealed that BMP6 inhibits expression of FSHβ, which reduces the synthesis of FSH by gonadotrophs (Faure et al., 2005).

6.5 BMP15

BMP15 is exclusively secreted by the oocyte and has a strong affinity for BMPR1B (ALK6) in sheep and humans; yet it binds precociously to ALK6, ALK2, and ALK3 in mice, indicating that substantial species differences exist (Inagaki & Shimasaki, 2010; Otsuka & Shimasaki, 2002; Pulkki et al., 2012). From the primary follicle stage onward, expression of BMP15 mRNA progressively increased, peaking in sheep early antral follicles, followed by a sequential decrease in larger antral follicle (Feary et al., 2007). It is likely that a BMP15 concentration gradient is established from the oocyte via the cumulus cells to the granulosa cells. BMP15 has been shown to suppress cumulus apoptosis (Hussein, Froiland, Amato, Thompson, & Gilchrist, 2005). Oocytes surrounded by cumulus cells with greater levels of BMP15 mRNA were shown to yield an increased pregnancy rate after IVF (Li et al., 2014). Moreover, an association between high levels of BMP15 in the follicular fluid and oocyte quality has been reported (Li et al., 2014; Wu et al., 2007). Specifically, BMP15 increases proliferation of granulosa cells and cytodifferentiation, independently of FSH, yet BMP15 suppresses FSH-regulated progesterone synthesis by reducing StAR and CYP11A1 in the rat (Otsuka, Moore, et al., 2001).

It has also been shown that BMP15 decreased FSH-induced progesterone biosynthesis by decreasing FSHR mRNA, ultimately inhibiting luteinization (Moore et al., 2003; Otsuka & Shimasaki, 2002). In parallel, BMP15 promoted granulosa cell mitosis independent of the FSHR-Smad 1,5,8 pathway via activation of the ERK 1/2 pathway (Lee et al., 2001; Moore

et al., 2003; Otsuka, Yamamoto, Erickson, & Shimasaki, 2001; Shimasaki et al., 1999). Moreover, in the pituitary, BMP15 has been associated with increasing FSHβ expression (Otsuka & Shimasaki, 2002).

7. OTHER TGFβ SUPERFAMILY MEMBERS (GDF9, GDF3, AMH)

7.1 GDF9

GDF9 and GDF3 are TGFβ superfamily members with close homology to the BMPs, and signal via ALK4 and 5, and via Smad 2 and 3. GDF9 is also produced by the oocyte from primordial follicles onward and is known to suppress LHR expression along with BMP6 and 7. GDF9 gene knockout mice have arrested follicle growth at the primary stage, which indicates a very early essential role in reproduction (Kaivo-Oja et al., 2005).

7.2 GDF3

GDF3 has been detected in the cytoplasm of the oocyte of the resting primordial and primary follicles in humans, and not in the granulosa cells until antral cavity formation (Shi et al., 2012). GDF3 is found in antral granulosa cells in the human ovary, and increases LHR expression, yet it reduces the inhibitory effect of BMP6 and 7 on LHR expression (Shi et al., 2012). Around the time of dominant follicle selection, expression of BMP6 and 7 decreases and GDF3 expression increases.

7.3 AMH

Another TGFβ superfamily member that has attracted considerable interest with regard to its intraovarian role is AMH. While several BMPs have been shown to promote primordial follicle activation and increase the pool of growing follicles in cultured neonatal mouse ovaries, AMH exerts an inhibitory effect on follicle growth (Gouedard et al., 2000; Josso, di Clemente, & Gouédard, 2001). While AMH inhibits FSH-dependent follicle growth and estrogen production at a later follicle stage, several BMPs have the opposite effect. AMH signals via its own type 2 receptor (AMHR2) forming a signaling complex with either BMPR1B (ALK6) or BMPR1A (ALK3) (Josso & di Clemente, 2003). It remains unclear how AMH promotes primordial follicle activation since AMHR2 is evidently not expressed by the primordial follicle (Weenen et al., 2004).

AMH protein first appears in the activated primordial follicle, and its concentration peaks in the small antral follicles, followed by a steady decline at the time of dominant follicle selection, along with activin and BMP6 (Rice et al., 2007). In sheep and humans, AMH is not present in mural granulosa cells from large antral follicles; however, it has been reported in the cumulus granulosa cells (Campbell, Clinton, & Webb, 2012; Weenen et al., 2004). BMP6 has been shown to increase the secretion of AMH in humans which in turn has an inhibitory effect on primary follicle formation, thus preserving the ovarian reserve (Rice et al., 2007; Shi et al., 2009).

When AMH is blocked directly by immunizing sheep against AMH, their ovulation rate increases, whereas the mitogenic activity of granulosa cells remains the same (Campbell et al., 2012). AMH attenuation has, therefore, been identified as a possible contributor to the observed increase in ovulation rate of the Booroola Merino sheep carrying a mutation in BMPR1B.

8. BMPs AND PRIMORDIAL FOLLICLE ACTIVATION

Once the resting primordial follicle is assembled, it is only a matter of time before activation occurs. However, this activation process remains poorly understood. Several theories are proposed as to the activation of the primordial follicle (Fig. 5). To begin with, morphological data exist

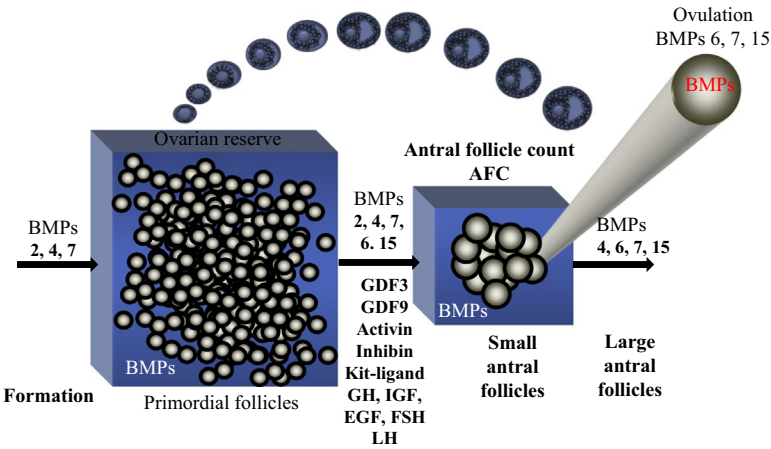

Fig. 5 BMP signaling and follicle development. The bone morphogenetic proteins (BMP) ligands 2, 4, 6, 7, and 15 via the receptor (R) BMPR1B are involved in embryonic ovarian formation of primordial follicles; activation of the primordial to primary follicle; antral follicle formation and recruitment into cyclic folliculogenesis to ovulation.

showing that the mesenchymal pericyte migrates toward the primordial follicle and aligns itself adjacent to the primordial follicle (Bukovsky, 2016). Resting primordial follicles may be in the vicinity but remain dormant, which indicates that local signaling factors initiate the primordial follicle to grow. The migration of the pericyte may be under the control of either neural or cytokine factors such as platelet-derived growth factor beta, or an immune response. However, the inhibitory gradient theory is compelling, because when resting primordial follicles are removed from the ovary, activation occurs spontaneously (Hussein et al., 2005; Suzuki et al., 2015). Furthermore, the rate of activation of the primordial follicle is proportional to the ovarian reserve (Anzalone, Hong, & LaPolt, 2001).

As the ovary ages, there is a reduction in angiogenesis, vascular endothelial growth factor, and the number of pericytes (Liu et al., 2009; Mattioli et al., 2001; Robinson et al., 2009; Taylor, Wilson, Hillier, Wiegand, & Fraser, 2007). The TGFβ super family is involved with the proliferation of pericytes (Sweeney, Ayyadurai, & Zlokovic, 2016). The pericyte has a number of known receptors, one of which is the BMPR1B (ALK 6) and ALK 5, and therefore would have the ability to respond to BMP and GDF ligands present at that time, with an affinity for the receptor.

BMP15 expression in the oocyte is not evident until the primary follicle stage in humans and sheep (Galloway et al., 2002; Li et al., 2014). Sheep with a BMP15 or BMP6 gene knockout show a much later primary to secondary follicle stage arrest in growth, resulting in infertility (Galloway et al., 2002; McNatty et al., 2007). GDF9, also secreted by the oocyte, is reported to be expressed in the primordial follicles and has been shown to increase primordial to primary follicle conversion (Vitt, Hayashi, Klein, & Hsueh, 2000). Whereas, GDF9 knockout mice are infertile due to arrested primordial to primary growth transition (Dong, Albertini, Nishimori, Kumar, & Matzuk, 1996). AMH has an inhibitory effect on primordial follicle activation in the mouse (Durlinger et al., 2002). The AMHR2 is essential for AMH responsiveness, forming a signaling complex with BMPR1B. However, in humans, AMHR2 is evidently not expressed in the primordial follicle only appearing during the primary to secondary transition (Rice et al., 2007).

Other factors implicated in primordial follicle activation include death receptor (VASA or DEAD-box4) and leukemia inhibitory factor (LIF), forkhead box O3 (Foxo 3), growth hormone, and the phosphatidylinositol 3-kinase (PI3K)-AKT signaling axis pathway (Albamonte, Albamonte, Stella, Zuccardi, & Vitullo, 2013; Castrillon, Miao, Kollipara, Horner, &

DePinho, 2003; John, Shirley, Gallardo, & Castrillon, 2007; Reddy et al., 2008; Slot et al., 2006). Phosphatase and tensin homolog (PTEN) inhibitors or AKT stimulants (including the BMP ligands) appear to influence proliferation, migration, and activation of the primordial follicle, and continued growth (Hsueh, Kawamura, Cheng, & Fauser, 2015; Kawamura et al., 2013).

In rodents, BMP4 and 7 have been reported to enhance primordial activation (Skinner, 2005) and to enhance primary to preantral growth (Lee et al., 2004). Immunization to inhibit BMP4 and BMPR1B signaling reduced the conversion of primordial follicles to primary follicles in mice, which conserved the ovarian pool of primordial follicles over time (Al-Samerria et al., 2015; Nilsson & Skinner, 2003). Similarly, Booroola sheep, with a partially attenuating mutation to the BMPR1B, retained more primordial follicles over time compared to the wild-type sheep (Ruoss, Tadros, O'Shea, McFarlane, & Almahbobi, 2009).

9. BMP RECEPTOR ACTIVITY IN THE OVARY

The BMP ligands strongly activate the BMPR1B, and their role in the regulation of gonadotrophin receptor expression has been previously reported (Miyazono et al., 2010; Shi et al., 2012, 2011, 2010; Zhu et al., 2013). The BMPR1B is first expressed in primordial follicles on the oocyte and the granulosa cells of primary follicles throughout folliculogenesis (mural and cumulus) (Abir et al., 2008). Androgen receptors are first expressed in the transitional follicle between the primordial and primary stage, and are early regulators of ovarian development, particularly the inducement of FSHR on the granulosa cell (Fig. 6) (Erickson et al., 1979; Nielsen et al., 2011; Rice et al., 2007; Sen & Hammes, 2010; Sen et al., 2014).

Dominant follicle selection takes place when the androgen receptor expression reduces and estrogen production increases. BMPR1B and FSHR expression has also been shown to decrease at this time, followed by an increase in LHR expression (Fig. 7) (Regan et al., 2016, 2017). At the time of maturation of the follicle, downregulation of BMPR1B, FSHR, and LHR expression is associated with reduced proliferation and a shift from estrogen to progesterone synthesis in the ovulatory follicles in both human and animal models (Figs. 6 and 7) (Regan et al., 2016, 2017, 2015; Rice et al., 2007). This shift in steroidogenesis requires the progesterone-suppressive BMP signaling to be downregulated in the largest follicles (Regan et al., 2016, 2017). In addition, medium to large antral follicles require substantial androgen substrate to generate estrogen, and a reduction

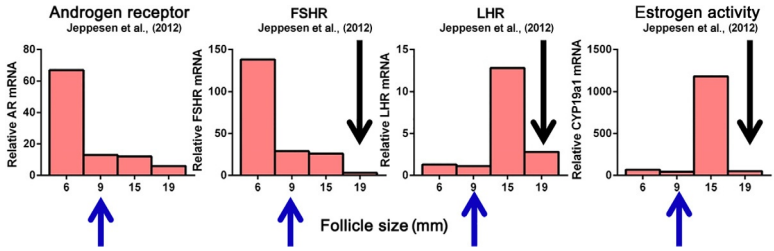

Fig. 6 The stage-specific relationship between granulosal receptor expression and estrogen activity during folliculogenesis in a natural cycle. Dominant follicle selection took place when the androgen receptor and FSHR expression decreased, and LHR expression increased (indicated by the *blue upwards-arrow*). Downregulation of FSHRs, LHRs, and the cessation of proliferation occurs in the preovulatory follicles in humans and animals (indicated by the *black downwards-arrow*). *CYP19a1* is the gene that encodes aromatase, essential for the production of estrogen. *Based on Gasperin, B.G., Ferreira, R., Rovani, M.T., Bordignon, V., Duggavathi, R., Buratini, J., et al. (2014). Expression of receptors for BMP15 is differentially regulated in dominant and subordinate follicles during follicle deviation in cattle*. Animal Reproduction Science, 144, *72–78; Jeppesen, J.V., Kristensen, S.G., Nielsen, M.E., Humaidan, P., Dal Canto, M., Fadini, R., et al. (2012). Lh-receptor gene expression in human granulosa and cumulus cells from antral and preovulatory follicles*, The Journal of Clinical Endocrinology and Metabolism, 97, *E1524–E1531*.

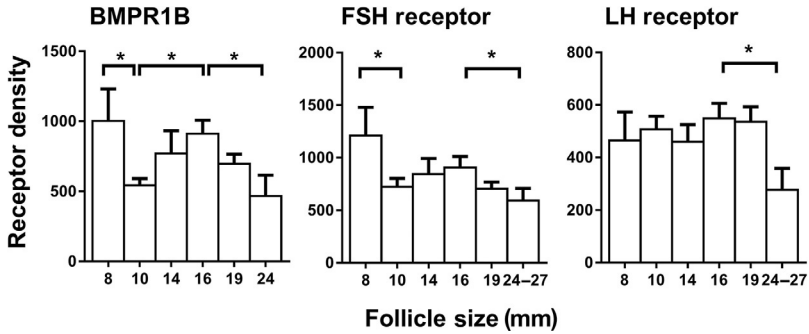

Fig. 7 The stage-specific relationship between granulosal receptor expression during folliculogenesis in human IVF cycles. Granulosal BMPR1B, FSHR, and LHR protein density and follicle size profile of young patients with a typical ovarian reserve for the age group. The patients were 23–30 years old and stimulated with gonadotrophins during an IVF cycle. Values in graphs are means ± SEM, and differences were considered significant if $^*P < 0.05$ (Regan et al., 2016, 2017).

in BMP signaling reflects the ability of BMPs to regulate thecal androgen production (Glister, Richards, & Knight, 2005).

Granulosa cells are unique in the ovary because they express FSHRs, which are required for the synthesis of estrogen expression (Fig. 8) (Miller & Auchus, 2011). Theca cells express LHRs and synthesize androgens, which are used by the granulosa cells as substrate for estrogen synthesis.

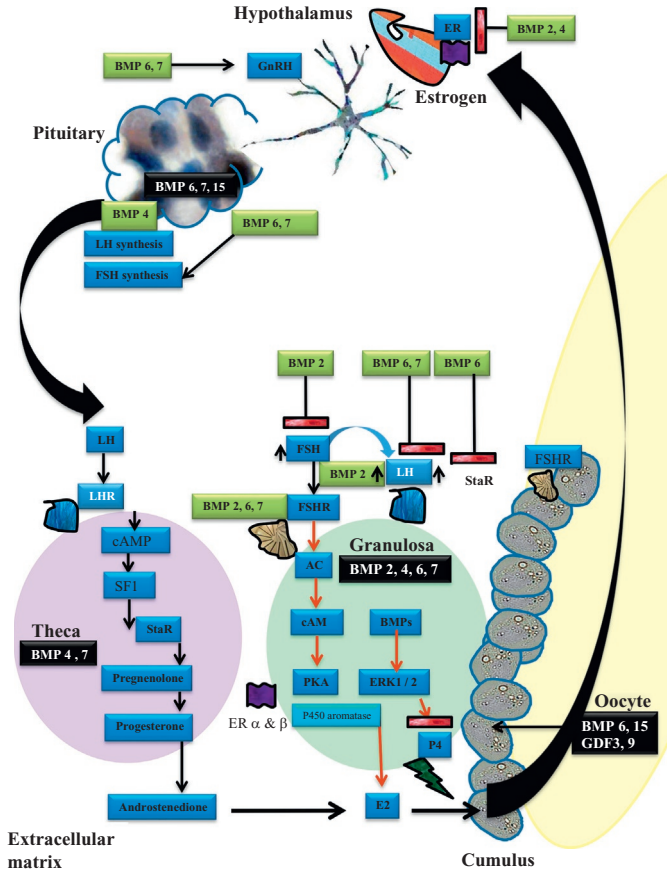

Fig. 8 Hypothalamic–pituitary–ovarian axis of regulation and the granulosa and theca cell interaction. Hypothalamic, pituitary control of ovarian growth and differentiation of the ovarian follicle comprised of the theca (*circle on left*), granulosa (*circle on center*), cumulus granulosa cells (attached to oocyte), and oocyte (*yellow oval on right*) surrounded by extracellular matrix. BMPs production by cell type is indicated in *black*. BMP signaling activity (*green*) and inhibition (*red bar*). Delayed expression of LHR (*white text*) on granulosa compared to the theca cells; estroen receptor (ER). *Based on Bao, B., Garverick, H. A., Smith, G. W., Smith, M. F., Salfen, B. E., & Youngquist, R. S. (1997). Changes in messenger ribonucleic acid encoding luteinizing hormone receptor, cytochrome P450-side chain cleavage, and aromatase are associated with recruitment and selection of bovine ovarian follicles. Biology of Reproduction, 56, 1158–1168; Chen, A.Q., Yu, S., Wang, Z., Xu, Z., & Yang, Z. (2008). Stage-specific expression of bone morphogenetic protein type I and type II receptor genes: Effects of follicle-stimulating hormone on ovine antral follicles,* Animal Reproduction Science, 111, *391–399; Dijke, P., Korchynskyi, O., Valdimarsdottir, G., & Goumans, M.-J. (2003). Controlling cell fate by bone morphogenetic protein receptors. Molecular and Cellular Endocrinology, 211, 105–113; Feary, E., Juengel, J., Smith, P., French, M., O'Connell, A., Lawrence, S., et al. (2007). Patterns of expression of messenger RNAs encoding GDF9, BMP15, TGFBR1, BMPR1B, and BMPR2 during follicular development*

and characterization of ovarian follicular populations in ewes carrying the woodlands FecX2W mutation. Biology of Reproduction, 77, *990–998; Fitzpatrick, S. L., Carlone, D. L., Robker, R. L., & Richards, J. S. (1997). Expression of aromatase in the ovary: Down-regulation of mRNA by the ovulatory luteinizing hormone surge. Steroids, 62, 197–206; Hillier, S., Whitelaw, P., & Smyth, C. (1994). Follicular oestrogen synthesis: The 'two-cell, two-gonadotrophin' model revisited.* Molecular and Cellular Endocrinology, 100, *51–54; Hussein, T., Froiland, D., Amato, F., Thompson, J., and Gilchrist, R. (2005). Oocytes prevent cumulus cell apoptosis by maintaining a morphogenic paracrine gradient of bone morphogenetic proteins.* Journal of Cell Science, 118, *5257–5268; Kayani, A. R., Glister, C., & Knight, P. G. (2009). Evidence for an inhibitory role of bone morphogenetic protein(s) in the follicular–luteal transition in cattle. Reproduction, 137, 67–78; Miller, W., & Bose, H. (2011). Early steps in steroidogenesis: Intracellular cholesterol trafficking.* Journal of Lipid Research, 52*, 2111–2135; Miyazono, K., Maeda, S., & Imamura, T. (2005). BMP receptor signaling: Transcriptional targets, regulation of signals, and signaling cross-talk.* Cytokine & Growth Factor Reviews, 6, *251–263; Miyoshi, T., Otsuka, F., Inagaki, K., Otani, H., Takeda, M., Suzuki, J., et al. (2007). Differential regulation of steroidogenesis by bonemorphogenetic proteins in granulosa cells: Involvement of extracellularly regulated kinase signaling and oocyte actions in follicle-stimulating hormone-induced estrogen production. Endocrinology, 148, 337–345; Moore, R.K., Otsuka, F., & Shimasaki, S. (2001). Role of ERK1/2 in the differential synthesis of progesterone and estradiol by granulosa cells.* Biochemical and Biophysical Research Communications, 289*, 796–800; Nicol, L., Faure, M.O., McNeilly, J.R., Fontaine, J., Taragnat, C., and McNeilly A.S. (2008). Bone morphogenetic protein-4 interacts with activin and GnRH to modulate gonadotrophin secretion in LbetaT2 gonadotrophs.* Journal of Endocrinology, 196*, 497–507; Pierre, A., Pisselet, C., Dupont, J., Mandon-Pepin, B., Monniaux, D., Monget P., et al. (2004). Molecular basis of bone morphogenetic protein-4 inhibitory action on progesterone secretion by ovine granulosa cells.* Journal of Molecular Endocrinology, 33*, 805–814; Rice, S., Ojha, K., Whitehead, S., & Mason H. (2007). Stage-specific expression of androgen receptor, follicle-stimulating hormone receptor, and anti-Müllerian hormone type II receptor in single, isolated, human preantral follicles: Relevance to polycystic ovaries.* The Journal of Clinical Endocrinology and Metabolism, 92*, 1034–1040; Seger, R., Hanoch, T., Rosenberg, R., Dantes, A., Merz, W. E., Strauss, J. F., et al. (2001). The ERK signaling cascade inhibits gonadotropin-stimulated steroidogenesis. Journal of Biological Chemistry, 276, 13957–13964; Sugawara, T., Saito, M., & Fujimoto, S. (2000). Sp1 and SF-1 interact and cooperate in the regulation of human steroidogenic acute regulatory protein gene expression. Endocrinology, 141, 2895–2903; Sullivan, M. W., Stewart Akers, A., Krasnow, J. S., Berga, S. L., & Zeleznik, A. J. (1999). Ovarian responses in women to recombinant follicle-stimulating hormone and luteinizing hormone (LH): A role for LH in the final stages of follicular maturation. The Journal of Clinical Endocrinology and Metabolism, 84, 228–232; Takeda, M., Otsuka, F., Takahashi, H., Inagaki, K., Miyoshi, T., Tsukamoto, N., et al. (2012). Interaction between gonadotropin-releasing hormone and bone morphogenetic protein-6 and -7 signaling in LβT2 gonadotrope cells. Molecular and Cellular Endocrinology, 348, 147–154; ten Dijke, P., Korchynskyi, O., Valdimarsdottir, G., & Goumans, M. (2003). Controlling cell fate by bone morphogenetic protein receptors. Molecular and Cellular Endocrinology, 211, 105–113; Yamamoto, T., Saatcioglu, F., & Matsuda, T. (2002). Cross-talk between bone morphogenic proteins and estrogen receptor signaling. Endocrinology, 143, 2635–2642; Yuan, W., Bao, B., Garverick, H. A., Youngquist, R. S., & Lucy, M. C. (1998). Follicular dominance in cattle is associated with divergent patterns of ovarian gene expression for insulin-like growth factor (IGF)-I, IGF-II, and IGF binding protein-2 in dominant and subordinate follicles. Domestic Animal Endocrinology, 15(1), 55–63.*

The receptor density of BMPRIB on granulosal cells fluctuates biphasically during menstrual cycle in unison with the FSHR in young women and Merino sheep (Regan et al., 2016, 2015).

As pituitary FSH secretion is reduced, the follicles with granulosal LHRs have the capacity to supplement the FSH-dependent synthesis of estrogen. The follicles with granulosal-expressed LHR continue to grow and become the selected dominant follicles (Fig. 2). The extent of androstenedione conversion to estrogen continues to increase, which creates a positive estrogen feedback loop to the hypothalamic–pituitary complex, leading to further GnRH release (Fig. 8) (Faure et al., 2005).

Proliferation of the granulosa and theca cells continues as the rise in estrogen level promotes proliferation, until a threshold level is reached, which culminates in the generation of the LH surge (Austin et al., 2001; Ginther et al., 2005). In the event that reduced conversion of androstenedione to estrogen occurs, androgen levels rise, creating an androgen dominant follicle. Greater androstenedione-to-estrogen ratios have been shown to result in an elevated level of granulosa cell apoptosis and follicle demise (Yuan & Giudice, 1997).

10. BMPs AND DOMINANT FOLLICLE SELECTION

In monovular species such as humans and cattle, follicle divergence (i.e., selection of a dominant follicle from a pool of growing 4–8 mm antral follicles) occurs at a stage of the cycle when pituitary FSH secretion reduces and LH secretion increases (Fig. 2) (Austin et al., 2001; Edwards et al., 1996). During a natural cycle, the follicles with a higher density of gonadotrophin receptors are presumed to be more responsive to the gonadotrophins and continue to increase in size (Bächler, Menshykau, De Geyter, & Iber, 2014; Gougeon, 1986; LaPolt et al., 1992).

FSHRs are expressed exclusively by granulosa cells that respond to pituitary-derived FSH by proliferating and increasing estrogen output. In turn, this promotes expression of LHR by granulosa cells of the selected dominant follicle, enabling them to respond to LH pulses and survive the fall in FSH (Fig. 2).

Downregulation of granulosal BMPR1B and FSHR expression has been observed at the stage of cyclic dominant follicle selection, occurring between ~8–10 mm in the human and ~1–1.7 mm in the Merino sheep (Regan et al., 2016, 2015). As mentioned earlier, follicles are selected as a consequence of the decline in pituitary FSH and only follicles with the newly acquired LHR can sustain estrogen production during the

preovulatory phase. Suppression of granulosal progesterone synthesis, in favor of FSH-dependent estrogen production, appears to be governed by the action of the BMPs (Knight & Glister, 2006; Moore et al., 2001).

At this stage in follicle development, BMP15 and inhibins increase to stimulate proliferation and further growth of the follicle and oocyte (Feary et al., 2007; Yding Andersen, 2017). The ability of the follicle to reach the FSH–estrogen threshold before ovulation with sufficient granulosal LHR appears to be of paramount importance to the survival of the selected dominant follicle. Alternatively, follicular regression proceeds followed by atresia (Campbell, Dobson, Baird, & Scaramuzzi, 1999; Ginther et al., 2012; Luo, Gumen, Haughian, & Wiltbank, 2011; Picton & McNeilly, 1991).

11. BMPs AND OVULATION RATE

The number of preovulatory follicles that develop can be artificially enhanced by exogenous rFSH stimulation, such as that used in IVF treatment cycles, or by a naturally occurring mutation-induced increase in responsiveness, such as that seen in the Booroola Merino sheep (Fig. 9) (Mulsant et al., 2001; Souza et al., 2001; Wilson et al., 2001).

Fig. 9 Booroola Merino and wild-type Merino sheep, Armidale NSW. The Booroola (red number on sheep backs) sheep have a naturally occurring gene mutation that partially attenuates the BMPR1B receptor signaling and increases the ovulation rate to ~5. Wild-type Merino (blue numbers). University of New England, breeding program, 1964 to 2010, Dr Tim O'Shea (deceased).

The TGFβ type 1 receptor BMPR1B has been localized on sheep granulosa cells from the primordial follicle stage onward (Al-Samerria & Almahbobi, 2014; Anthony et al., 2015; Chen, Yu, Wang, Xu, & Yang, 2008; Erickson & Shimasaki, 2003; Gasperin et al., 2014). The level of expression increased sequentially from primordial to antral follicles in sheep. BMPR1B is expressed mainly on granulosa cells and the oocyte in the bovine and human model, and to a lesser degree in their theca cells (Abir et al., 2008; Glister et al., 2004). It has also been demonstrated that BMP ligands are produced in a stage-specific manner by follicular cells in animals and humans (Gasperin et al., 2014; Glister et al., 2004; Regan et al., 2016, 2017, 2015).

The Booroola Merino, with a naturally occurring point mutation of the BMPR1B gene, has an increased ovulation rate (Fabre et al., 2006; Mulsant et al., 2001; Souza et al., 2001; Wilson et al., 2001). This increase is likely due to the follicles being more sensitive to FSH at an earlier follicle size (Baird & Campbell, 1998; McNatty et al., 1985).

The Booroola sheep follicles contained significantly fewer granulosa cells than the normal wild-type (Campbell et al., 2006; McNatty et al., 1985). Studies conducted on the granulosa cells show that, when stimulated in vitro by LH or FSH, they produced more cAMP, estrogen, and androstenedione from the same number of cells from the large antral follicle (Campbell et al., 2006). An increased cellular capacity to produce estrogen would compensate for the reduced number of granulosa cells. Taken together, it is apparent that the Booroola sheep produce multiple follicles because of the greater density of receptors for FSH and LH due to the attenuated BMPR1B signal (Regan et al., 2015).

Recently, compelling data show that the expression of mature surface granulosa receptors for FSHR, LHR, and BMPR1B is significantly elevated in the Booroola compared to the young wild-type Merino sheep (Regan et al., 2015). In another study, conflicting results were observed; however, the Booroola sheep were much older (6–10 years compared to 4 years). In addition, mRNA expression was measured rather than the mature expressed protein itself. It is plausible that the Booroola mutation of the BMPR1B signal may partially eliminate the BMP ligand-induced suppression of FSHR and LHR expression. Partial attenuation of BMP action may thus lead to an upregulation of FSHR, earlier acquisition of LHR, and an increase of BMPR1B itself in the Booroola sheep. The increased signaling resulted in multiple ovulations and an increased birth rate of ∼5 (Otsuka,

Yamamoto, et al., 2001; Regan et al., 2015). The findings clearly show the effect of the repression exerted by the BMPs in regulating ovulation rate.

Evidence indicates a strong connection between the role of BMPs, AMH, and the gonadotropin-dependent regulation of ovulation rate. BMP4, 6, and 15 increase the transcriptional activity of the AMH promotor activity via SF-1 (Anthony et al., 2015). Yet it still remains unclear as to why immunization against AMH increased the ovulation rate but did not reduce proliferation of granulosa cells, as reported in the Booroola mutation (Campbell et al., 2012). A possible explanation may be related to the stage-specific downregulation of AMH after dominant follicle selection. The greatest mitogenic activity of granulosa cells occurs after this divergence when AMH is low, whereas dominant follicle selection occurs when AMH is high (Austin et al., 2001). Immunization against AMH alone would, therefore, increase ovulation rate but not granulosa cell proliferation governed by other BMPs. Whereas immunization against BMPR1B or an attenuating mutation in BMPR1B, such as that in Booroola sheep, would affect both proliferation and ovulation rate.

Identification of the BMP ligand responsible for the increased ovulation rate or decreased proliferation of the granulosa cells has not been achieved. BMP15 has been associated with an increase in ovulation rate in sheep with specific mutations in the BMP15 gene (Hanrahan et al., 2004; McNatty et al., 2009). Heterozygous Inverdale sheep with an inactivation mutation for BMP15 exhibit an increase in ovulation rate, whereas in homozygous carriers, follicle development did not progress past the primary follicle stage (Braw-Tal et al., 1993; McNatty et al., 2009). In sheep, short-term immunization against BMP15 increased the ovulation rate from 1–2 to $\geq$3 without affecting plasma progesterone concentration (Juengel et al., 2004, 2011).

In another study, complete neutralization of BMP15 prevented exogenous FSH-induced follicle rescue resulting in anovulation, which indicates that BMP15 is required for FSHR transcription (McNatty et al., 2009). Sheep with ovarian infusion of BMP6 showed a reduced cycle length and size of the preovulatory follicles (Campbell et al., 2009). Although the effect of the infusion was short-lived, the estrogen and androstenedione increased with no change to the ovulation rate. Findings from the Booroola sheep indicate that a combination of BMP15 and BMP6 signal attenuation (via BMPR1B) may be responsible for the reduced mitogenic activity of granulosa cells and the increased ovulation rate, with BMP2 and 4 influencing primordial to preantral follicle development.

12. BMPs AND THE TERMINAL STAGE OF FOLLICULOGENESIS

Granulosa cell proliferation continues, and the ovarian follicle increases in size from 10 to 20+mm in the human, producing large quantities of estrogen that reach a critical level, triggering the release of LH from the pituitary and the onset of the LH surge. The release of LH surge initiates numerous events, changing the granulosa cells and the oocyte in preparation for the expulsion of the oocyte from the follicle, and corpus luteum formation.

The cells of the follicle differentiate morphologically, inducing cytoskeletal reorganization, expansion of the granulosa cell, and cessation of mitogenic proliferation. Resumption of meiosis and oocyte maturation takes place. The cumulus cells expand away from the oocyte-cumulus complex, severing the morphological cumulus gap junctions where cross talk linkages and concentration gradient of BMP15 and 6 radiating from the oocyte are disrupted (Hussein et al., 2005). Angiogenic cells infiltrate the degenerating basal lamina in preparation for blood vessel formation in the developing corpus luteum.

The BMP-induced suppression of progesterone synthesis by the granulosa cells is released. The granulosa cell acquires the ability to synthesize large amounts of progesterone (Westergaard, Christensen, & McNatty, 1986). The BMPR1B expression density on the cell surface is reduced in the largest follicles either by the degradation of receptors or by reduced BMPR1B mRNA production (Menon & Menon, 2014; Regan et al., 2016, 2015; Zhang & Roy, 2004).

This process, collectively referred to as luteinization, appears to be associated with BMPR1B, FSHR, and LHR downregulation in the leading dominant follicles (Fan et al., 2009; Izadyar, Zeinstra, & Bevers, 1998; Regan et al., 2017, 2015). Furthermore, LHR density peaks in the preovulatory follicle in the wild type and the Booroola followed by a significant reduction in the leading dominant follicle during the LH surge coincident with a reduced mitogenic index and reduced estrogen levels (Jeppesen et al., 2012; LaPolt et al., 1992; Ophir et al., 2014; Regan et al., 2017, 2015).

In women reaching the end of their reproductive life span, dysregulation of granulosal BMPR1B (Fig. 10) and FSHR occurs (Regan et al., 2016, 2017). As the ovarian pool of primordial follicles depletes in women, receptor density continues to increase with follicle growth.

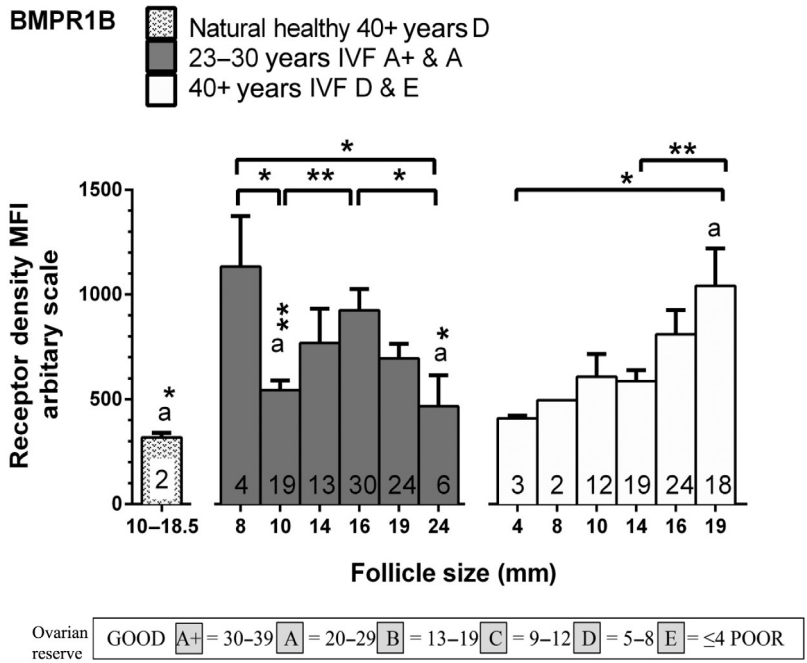

Fig. 10 Granulosal BMPR1B density from follicles of different sizes collected from young and older IVF patients compared to an unstimulated natural healthy cycle. Granulosal BMPR1B protein density and follicle size profile of a natural healthy unstimulated patient of 41 years with an AFC of D, before the LH surge (*patterned bar*). Patients, 23–30 years stimulated, IVF cycle with an AFC of A+ and A (*gray bar*). Patients, 40+ years stimulated IVF cycle with an AFC of D and E (*white bar*). IVF patients were grouped according to ovarian reserve measured indirectly by the antral follicle count (AFC). The antral follicle count, is defined as the number of follicles between 2 and 10 mm in diameter, combining the number collected from both ovaries; that were present on ~day 5 of a preliminary assessment cycle, without rFSH. Group A+ = 30–39 small follicles; group A = 20–29 small follicles; group B = 13–19 small follicles; group C = 9–12 small follicles, group D = 5–8 small follicles; group E = ≤4 small follicles. Mean fluorescent intensity (MFI) was obtained using an average of ~ 8000 granulosa cells per follicle for the direct measurement of receptor protein expression. The data were subjected to statistical verification using one-way ANOVA with an uncorrected Fisher's LSD. Values in graphs are means ± SEM, and differences were considered significant if $^{*}P < 0.05$ and $^{**}P < 0.01$. The letter, "a" signifies a statistical difference to the matching letter with an attached asterisk(s) (a*, a**). The number within the column represents the number of follicles analyzed for that group (Regan et al., 2016).

It is proposed that the lack of receptor downregulation results in a reduced response to the LH surge and reduced output of progesterone, and is associated with poor oocyte quality and pregnancy rates (Regan et al., 2016, 2017).

REFERENCES

Abir, R., Ben-Haroush, A., Melamed, N., Felz, C., Krissi, H., & Fisch, B. (2008). Expression of bone morphogenetic proteins 4 and 7 and their receptors IA, IB, and II in human ovaries from fetuses and adults. *Fertility and Sterility*, *89*, 1430–1440.

Ainsworth, L., Tsang, B. K., Downey, B. R., Marcus, G. J., & Armstrong, D. T. (1980). Interrelationships between follicular fluid steroid levels, Gonadotropic stimuli, and oocyte maturation during preovulatory development of porcine follicles. *Biology of Reproduction*, *23*, 621–627.

Albamonte, M. I., Albamonte, M. S., Stella, I., Zuccardi, L., & Vitullo, A. D. (2013). The infant and pubertal human ovary: Balbiani's body-associated VASA expression, immunohistochemical detection of apoptosis-related BCL2 and BAX proteins, and DNA fragmentation. *Human Reproduction*, *28*, 698–706.

Almog, B., Shehata, F., Shalom-Paz, E., Tan, S. L., & Tulandi, T. (2011). Age-related normogram for antral follicle count: McGill reference guide. *Fertility and Sterility*, *95*, 663–666.

Al-Samerria, S., Al-Ali, I., McFarlane, J. R., & Almahbobi, G. (2015). The impact of passive immunisation against BMPRIB and BMP4 on follicle development and ovulation in mice. *Reproduction*, *149*, 403–411.

Al-Samerria, S., & Almahbobi, G. (2014). Three-dimensional image analysis to quantify the temproro-spacial expression of cellular receptors. *Journal of Medical and Bioengineering*, *3*, 179–182.

Amsterdam, A., Sasson, R., Keren Tal, I., Aharoni, D., Dantes, A., Rimon, E., et al. (2003). Alternative pathways of ovarian apoptosis: Death for life. *Biochemical Pharmacology*, *66*, 1355–1362.

Anthony, E., Pierre, A., Clemente, N. d., Picard, J.-Y., Jarrier, P., Mansanet, C., et al. (2015). Anti-Müllerian hormone regulation by the bone morphogenetic proteins in the sheep ovary: Deciphering a direct regulatory pathway. *Endocrinology*, *156*, 301–313.

Anzalone, C. R., Hong, L.-S., Lu, J. K. H., & LaPolt, P. S. (2001). Influences of age and ovarian follicular reserve on estrous cycle patterns, ovulation, and hormone secretion in the Long-Evans rat. *Biology of Reproduction*, *64*, 1056–1062.

Araújo, V. R., Silva, C. M. G., Magalhães, D. M., Silva, G. M., Báo, S. N., Silva, J. R. V., et al. (2010). Effect of bone morphogenetic protein-7 (BMP-7) on in vitro survival of caprine preantral follicles. *Pesquisa Veterinária Brasileira*, *30*, 305–310.

Austin, E. J., Mihm, M., Evans, A. C. O., Knight, P. G., Ireland, J. L. H., Ireland, J. J., et al. (2001). Alterations in intrafollicular regulatory factors and apoptosis during selection of follicles in the first follicular wave of the bovine estrous cycle. *Biology of Reproduction*, *64*, 839–848.

Bächler, M., Menshykau, D., De Geyter, C., & Iber, D. (2014). Species-specific differences in follicular antral sizes result from diffusion-based limitations on the thickness of the granulosa cell layer. *Molecular Human Reproduction*, *20*, 208–221.

Baerwald, A., Adams, G., & Pierson, R. (2012). Ovarian antral folliculogenesis during the human menstrual cycle: A review. *Human Reproduction Update*, *18*, 73–91.

Baird, D., & Campbell, B. (1998). Follicle selection in sheep with breed differences in ovulation rate. *Molecular and Cellular Endocrinology*, *145*, 89–95.

Bomsel Helmreich, O., Gougeon, A., Thebault, A., Saltarelli, D., Milgrom, E., Frydman, R., et al. (1979). Healthy and atretic human follicles in the preovulatory phase: Differences in evolution of follicular morphology and steroid content of follicular fluid. *The Journal of Clinical Endocrinology and Metabolism*, *48*, 686–694.

Brankin, V., Quinn, R. L., Webb, R., & Hunter, M. G. (2005). BMP-2 and -6 modulate porcine theca cell function alone and co-cultured with granulosa cells. *Domestic Animal Endocrinology*, *29*, 593–604.

Braw-Tal, R., McNatty, K., Smith, P., Heath, D., Hudson, N., Phillips, D., et al. (1993). Ovaries of ewes homozygous for the X-linked Inverdale gene (FecXI) are devoid of secondary and tertiary follicles but contain many abnormal structures. *Biology of Reproduction*, *49*, 895–907.

Bukovsky, A. (2016). Involvement of blood mononuclear cells in the infertility, age-associated diseases and cancer treatment. *World Journal of Stem Cells*, *8*, 399–427.

Campbell, B., Clinton, M., & Webb, R. (2012). The role of anti-mullerian hormone (AMH) during follicle development in a monovulatory species (sheep). *Endocrinology*, *153*, 4533–4543.

Campbell, B. K., Dobson, H., Baird, D. T., & Scaramuzzi, R. J. (1999). Examination of the relative role of FSH and LH in the mechanism of ovulatory follicle selection in sheep. *Journal of Reproduction and Fertility*, *117*, 355–367.

Campbell, B. K., Kendall, N. R., & Baird, D. T. (2009). Effect of direct ovarian infusion of bone morphogenetic protein 6 (BMP6) on ovarian function in sheep. *Biology of Reproduction*, *81*, 1016–1023.

Campbell, B. K., Souza, C. J. H., Skinner, A. J., Webb, R., & Baird, D. T. (2006). Enhanced response of granulosa and theca cells from sheep carriers of the FecB mutation in vitro to gonadotropins and bone morphogenic protein-2, -4, and -6. *Endocrinology*, *147*, 1608–1620.

Castrillon, D. H., Miao, L., Kollipara, R., Horner, J. W., & DePinho, R. A. (2003). Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. *Science*, *301*, 215–218.

Chen, A. Q., Yu, S., Wang, Z., Xu, Z., & Yang, Z. (2008). Stage-specific expression of bone morphogenetic protein type I and type II receptor genes: Effects of follicle-stimulating hormone on ovine antral follicles. *Animal Reproduction Science*, *111*, 391–399.

Childs, A. J., Bayne, R. A. L., Murray, A. A., Silva, S. J. M. D., Collins, C. S., Spears, N., et al. (2010). Differential expression and regulation by activin of the neurotrophins BDNF and NT4 during human and mouse ovarian development. *Developmental Dynamics*, *239*, 1211–1219.

Di Pasquale, E., Rossetti, R., Marozzi, A., Bodega, B., Borgato, S., Cavallo, L., et al. (2006). Identification of new variants of human BMP15 gene in a large cohort of women with premature ovarian failure. *The Journal of Clinical Endocrinology and Metabolism*, *91*, 1976–1979.

Dong, J., Albertini, D., Nishimori, K., Kumar, T., Lu, N., & Matzuk, M. (1996). Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature*, *383*, 531–535.

Durlinger, A. L. L., Gruijters, M. J. G., 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.

Edson, M. A. (2009). The mammalian ovary from genesis to revelation. *Endocrine Reviews*, *30*, 624–712.

Edson, M. A., Nalam, R. L., Clementi, C., Franco, H. L., DeMayo, F. J., Lyons, K. M., et al. (2010). Granulosa cell-expressed BMPR1A and BMPR1B have unique functions in regulating fertility but act redundantly to suppress ovarian tumor development. *Molecular Endocrinology*, *24*, 1251–1266.

Edwards, R. G., Lobo, R., & Bouchard, P. (1996). Time to revolutionize ovarian stimulation. *Human Reproduction*, *11*, 917–919.

Edwards, R. G., & Steptoe, P. C. (1983). Current status of in-vitro fertilisation and implantation of human embryos. *Lancet (London, England)*, *2*, 1265–1269.

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

Erickson, G., & Shimasaki, S. (2001). The physiology of folliculogenesis: The role of novel growth factors. *Fertility and Sterility*, *76*, 943–949.

Erickson, G., & Shimasaki, S. (2003). The spatiotemporal expression pattern of the bone morphogenetic protein family in rat ovary cell types during the estrous cycle. *Reproductive Biology and Endocrinology*, *1*(9), 1–20.

Erickson, G. F., Wang, C., & Hsueh, A. J. W. (1979). FSH induction of functional LH receptors in granulosa cells cultured in a chemically defined medium. *Nature*, *279*, 336–338.

Fabre, S., Pierre, A., Mulsant, P., Bodin, L., Di Pasquale, E., Persani, L., et al. (2006). Regulation of ovulation rate in mammals: Contribution of sheep genetic models. *Reproductive Biology and Endocrinology*, *4*, 20.

Fabre, S., Pierre, A., Pisselet, C., Mulsant, P., Lecerf, F., Pohl, J., et al. (2003). The Booroola mutation in sheep is associated with an alteration of the bone morphogenetic protein receptor-IB functionality. *The Journal of Endocrinology*, *177*, 435–444.

Fan, H., Liu, Z., Shimada, M., Sterneck, E., Johnson, P. F., Hedrick, S. M., et al. (2009). MAPK3/1 (ERK1/2) in ovarian granulosa cells are essential for female fertility. *Science*, *324*, 938–941.

Faure, M. O., Nicol, L., Fabre, S., Fontaine, J., Mohoric, N., McNeilly, A., et al. (2005). BMP-4 inhibits follicle-stimulating hormone secretion in ewe pituitary. *The Journal of Endocrinology*, *186*, 109–121.

Feary, E., Juengel, J., Smith, P., French, M., O'Connell, A., Lawrence, S., et al. (2007). Patterns of expression of messenger RNAs encoding GDF9, BMP15, TGFBR1, BMPR1B, and BMPR2 during follicular development and characterization of ovarian follicular populations in ewes carrying the woodlands FecX2W mutation. *Biology of Reproduction*, *77*, 990–998.

Fowler, P. A., Sorsa, T., Harris, W. J., Knight, P. G., & Mason, H. D. (2001). Relationship between follicle size and gonadotrophin surge attenuating factor (GnSAF) bioactivity during spontaneous cycles in women. *Human Reproduction*, *16*, 1353–1358.

Galloway, S., Gregan, S., Wilson, T., McNatty, K., Juengel, J., Ritvos, O., et al. (2002). Bmp15 mutations and ovarian function. *Molecular and Cellular Endocrinology*, *191*, 15–18.

Garcia, J., Jones, G. S., Acosta, A. A., & Wright, G. L. (1981). Corpus Luteum function after follicle aspiration for oocyte retrieval. *Fertility and Sterility*, *36*, 565–572.

Gasperin, B. G., Ferreira, R., Rovani, M. T., Bordignon, V., Duggavathi, R., Buratini, J., et al. (2014). Expression of receptors for BMP15 is differentially regulated in dominant and subordinate follicles during follicle deviation in cattle. *Animal Reproduction Science*, *144*, 72–78.

Gilchrist, R., Ritter, L., & Armstrong, D. (2004). Oocyte-somatic cell interactions during follicle development in mammals. *Animal Reproduction Science*, *82–83*, 431–446.

Ginther, O. J., Beg, M. A., Gastal, E. L., Gastal, M. O., Baerwald, A. R., & Pierson, R. A. (2005). Systemic concentrations of hormones during the development of follicular waves in mares and women: A comparative study. *Reproduction*, *130*, 379–388.

Ginther, O. J., Khan, F. A., Hannan, M. A., Rodriguez, M. B., Pugliesi, G., & Beg, M. A. (2012). Role of LH in luteolysis and growth of the ovulatory follicle and estradiol regulation of LH secretion in heifers. *Theriogenology*, *77*, 1442–1452.

Glister, C., Kemp, C., & Knight, P. (2004). Bone morphogenetic protein (BMP) ligands and receptors in bovine ovarian follicle cells: Actions of BMP-4, -6 and 7 on granulosa cells and differential modulation of Smad-1 phosphorylation by follistatin. *Reproduction*, *127*, 239–254.

Glister, C., Richards, S., & Knight, P. (2005). Bone morphogenetic proteins (BMP) -4, -6, and -7 potently suppress basal and luteinizing hormone-induced androgen production by bovine theca interna cells in primary culture: Could ovarian hyperandrogenic dysfunction be caused by a defect in thecal BMP signaling? *Endocrinology*, *146*, 1883–1892.

Gouedard, L., Chen, Y. G., Thevenet, L., Racine, C., Borie, S., Lamarre, I., et al. (2000). Engagement of bone morphogenetic protein type IB receptor and Smad1 signaling by anti-Mullerian hormone and its type II receptor. *The Journal of Biological Chemistry*, *275*.

Gougeon, A. (1986). Dynamics of follicular growth in the human: A model from preliminary results. *Human Reproduction*, *1*, 81–87.
Hanrahan, J., Gregan, S., Mulsant, P., Mullen, M., Davis, G., Powell, R., et al. (2004). Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (Ovis aries). *Biology of Reproduction*, *70*, 900–909.
Hansen, K. R., Hodnett, G. M., Knowlton, N., & Craig, L. B. (2011). Correlation of ovarian reserve tests with histologically determined primordial follicle number. *Fertility and Sterility*, *95*, 170–175.
Hillier, S., Whitelaw, P., & Smyth, C. (1994). Follicular oestrogen synthesis: The 'two-cell, two-gonadotrophin' model revisited. *Molecular and Cellular Endocrinology*, *100*, 51–54.
Hsueh, A. J. W., Kawamura, K., Cheng, Y., & Fauser, B. C. J. M. (2015). Intraovarian control of early folliculogenesis. *Endocrine Reviews*, *36*, 1–24.
Hussein, T., Froiland, D., Amato, F., Thompson, J., & Gilchrist, R. (2005). Oocytes prevent cumulus cell apoptosis by maintaining a morphogenic paracrine gradient of bone morphogenetic proteins. *Journal of Cell Science*, *118*, 5257–5268.
Inagaki, K., Otsuka, F., Miyoshi, T., Yamashita, M., Takahashi, M., Goto, J., et al. (2009). p38-mitogen-activated protein kinase stimulated steroidogenesis in granulosa cell-oocyte cocultures: Role of bone morphogenetic proteins 2 and 4. *Endocrinology*, *150*, 1921–1930.
Inagaki, K., & Shimasaki, S. (2010). Impaired production of BMP-15 and GDF-9 mature proteins derived from proproteins WITH mutations in the proregion. *Molecular and Cellular Endocrinology*, *328*, 1–7.
Ingraham, H. A., Lala, D. S., Ikeda, Y., Luo, X., Shen, W. H., Nachtigal, M. W., et al. (1994). The nuclear receptor steroidogenic factor 1 acts at multiple levels of the reproductive axis. *Genes & Development*, *8*, 2302–2312.
Izadyar, F., Zeinstra, E., & Bevers, M. M. (1998). Follicle-stimulating hormone and growth hormone act differently on nuclear maturation while both enhance developmental competence of in vitro matured bovine oocytes. *Molecular Reproduction and Development*, *51*, 339–345.
Jeppesen, J. V., Kristensen, S. G., Nielsen, M. E., Humaidan, P., Dal Canto, M., Fadini, R., et al. (2012). Lh-receptor gene expression in human granulosa and cumulus cells from antral and preovulatory follicles. *The Journal of Clinical Endocrinology and Metabolism*, *97*, E1524–E1531.
John, G. B., Shirley, L. J., Gallardo, T. D., & Castrillon, D. H. (2007). Specificity of the requirement for Foxo3 in primordial follicle activation. *Reproduction*, *133*, 855–863.
Josso, N., & Clemente, N. d. (2003). Transduction pathway of anti-Müllerian hormone, a sex-specific member of the TGF-β family. *Trends in Endocrinology and Metabolism*, *14*, 91–97.
Josso, N., di Clemente, N., & Gouédard, L. (2001). Anti-Müllerian hormone and its receptors. *Molecular and Cellular Endocrinology*, *179*, 25–32.
Juengel, J., Hudson, N., Whiting, L., & McNatty, K. (2004). Effects of immunization against bone morphogenetic protein 15 and growth differentiation factor 9 on ovulation rate, fertilization, and pregnancy in ewes. *Biology of Reproduction*, *70*, 557–561.
Juengel, J., Quirke, L., Lun, S., Heath, D., Johnstone, P., & McNatty, K. (2011). Effects of immunizing ewes against bone morphogenetic protein 15 on their responses to exogenous gonadotrophins to induce multiple ovulations. *Reproduction*, *142*, 565–572.
Kaivo-Oja, N., Mottershead, D. G., Mazerbourg, S., Myllymaa, S., Duprat, S., Gilchrist, R. B., et al. (2005). Adenoviral gene transfer allows Smad-responsive gene promoter analyses and delineation of type I receptor usage of transforming growth factor-β family ligands in cultured human granulosa luteal cells. *The Journal of Clinical Endocrinology and Metabolism*, *90*, 271–278.

Kawamura, K., Cheng, Y., Suzuki, N., Deguchi, M., Sato, Y., Takae, S., et al. (2013). Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 17474–17479.

Kerr, C. L., Hill, C. M., Blumenthal, P. D., & Gearhart, J. D. (2008). Expression of pluripotent stem cell markers in the human fetal ovary. *Human Reproduction*, *23*, 589–599.

Khalaf, M., Morera, J., Bourret, A., Reznik, Y., Denoual, C., Herlicoviez, M., et al. (2013). BMP system expression in GCs from polycystic ovary syndrome women and the in vitro effects of BMP4, BMP6, and BMP7 on GC steroidogenesis. *European Journal of Endocrinology*, *168*, 437–444.

Klein, N. A., Battaglia, D. E., Woodruff, T. K., Padmanabhan, V., Giudice, L. C., Bremner, W. J., et al. (2000). Ovarian follicular concentrations of Activin, Follistatin, Inhibin, insulin-like growth factor I (IGF-I), IGF-II, IGF-binding Protein-2 (IGFBP-2), IGFBP-3, and vascular endothelial growth factor in spontaneous menstrual cycles of normal women of advanced reproductive age. *The Journal of Clinical Endocrinology and Metabolism*, *85*, 4520–4525.

Knight, P. G., & Glister, C. (2006). TGF-β superfamily members and ovarian follicle development. *Reproduction*, *132*, 191–206.

Knight, P. G., Satchell, L., & Glister, C. (2012). Intra-ovarian roles of activins and inhibins. *Molecular and Cellular Endocrinology*, *359*, 53–65.

LaPolt, P. S., Tilly, J. L., Aihara, T., Nishimori, K., & Hsueh, A. J. (1992). Gonadotropin-induced up- and down-regulation of ovarian follicle-stimulating hormone (FSH) receptor gene expression in immature rats: Effects of pregnant mare's serum gonadotropin, human chorionic gonadotropin, and recombinant FSH. *Endocrinology*, *130*, 1289–1295.

Lawson, K. A., Dunn, N. R., Roelen, B. A., Zeinstra, L. M., Davis, A. M., Wright, C. V., et al. (1999). Bmp4 is required for the generation of primordial germ cells in the mouse embryo. *Genes & Development*, *13*, 424–436.

Lee, K., Khivansara, V., Santos, M., Lamba, P., Yuen, T., Sealfon, S., et al. (2007). Bone morphogenetic protein 2 and activin A synergistically stimulate follicle-stimulating hormone beta subunit transcription. *Journal of Molecular Endocrinology*, *38*, 315–330.

Lee, W., Otsuka, F., Moore, R., & Shimasaki, S. (2001). Effect of bone morphogenetic protein-7 on folliculogenesis and ovulation in the rat. *Biology of Reproduction*, *65*, 994–999.

Lee, W.-S., Yoon, S.-J., Yoon, T.-K., Cha, K.-Y., Lee, S.-H., Shimasaki, S., et al. (2004). Effects of bone morphogenetic protein-7 (BMP-7) on primordial follicular growth in the mouse ovary. *Molecular Reproduction and Development*, *69*, 159–163.

Li, Y., Li, R.-Q., Ou, S.-B., Zhang, N.-F., Reng, L., Wei, L.-N., et al. (2014). Increased GDF9 and BMP15 mRNA levels in cumulus granulosa cells correlate with oocyte maturation, fertilization, and embryo quality in humans. *Reproductive Biology and Endocrinology*, *12*, 81.

Liu, Z., Kobayashi, K., van Dinther, M., van Heiningen, S. H., Valdimarsdottir, G., van Laar, T., et al. (2009). VEGF and inhibitors of TGF{beta} type-I receptor kinase synergistically promote blood-vessel formation by inducing {alpha}5-integrin expression. *Journal of Cell Science*, *122*, 3294–3302.

Liu, C.-F., Parker, K., & Yao, H. H. C. (2010). WNT4/β-catenin pathway maintains female germ cell survival by inhibiting activin βB in the mouse fetal ovary. *PLoS ONE*, *5*, e10382.

Luo, W., Gumen, A., Haughian, J., & Wiltbank, M. (2011). The role of luteinizing hormone in regulating gene expression during selection of a dominant follicle in cattle. *Biology of Reproduction*, *84*, 369–378.

MacNaughton, J., Banah, M., McCloud, P., Hee, J., & Burger, H. (1992). Age related changes in follicle stimulating hormone, luteinizing hormone, oestradiol and immunoreactive inhibin in women of reproductive age. *Clinical Endocrinology*, *36*, 339–345.

Manna, P., Dyson, M., Eubank, D., Clark, B., Lalli, E., Sassone Corsi, P., et al. (2002). Regulation of steroidogenesis and the steroidogenic acute regulatory protein by a member of the cAMP response-element binding protein family. *Molecular Endocrinology*, *16*, 184–199.

Massagué, J. (2008). A very private TGF-[beta] receptor embrace. *Molecular Cell*, *29*, 149–150.

Matsuda, F., Inoue, N., Manabe, N., & Ohkura, S. (2012). Follicular growth and atresia in mammalian ovaries: Regulation by survival and death of granulosa cells. *Journal of Reproduction and Development*, *58*, 44–50.

Mattioli, M., Barboni, B., Turriani, M., Galeati, G., Zannoni, A., Castellani, G., et al. (2001). Follicle activation involves vascular endothelial growth factor production and increased blood vessel extension. *Biology of Reproduction*, *65*, 1014–1019.

McNatty, K., Heath, D., Hudson, N., Lun, S., Juengel, J., & Moore, L. (2009). Gonadotrophin-responsiveness of granulosa cells from bone morphogenetic protein 15 heterozygous mutant sheep. *Reproduction*, *138*, 545–551.

McNatty, K. P., Hudson, N. L., Whiting, L., Reader, K. L., Lun, S., Western, A., et al. (2007). The effects of immunizing sheep with different BMP15 or GDF9 peptide sequences on ovarian follicular activity and ovulation rate. *Biology of Reproduction*, *76*, 552–560.

McNatty, K. P., Henderson, K. M., Lun, S., Heath, D. A., Ball, K., Hudson, N. L., et al. (1985). Ovarian activity in Booroola X Romney ewes which have a major gene influencing their ovulation rate. *Journal of Reproduction and Fertility*, *73*, 109–120.

McNatty, K., Moore, L., Hudson, N., Quirke, L., Lawrence, S., Reader, K., et al. (2004). The oocyte and its role in regulating ovulation rate: A new paradigm in reproductive biology. *Reproduction*, *128*, 379–386.

Menon, K. M. J., & Menon, B. (2014). Regulation of luteinizing hormone receptor expression by an RNA binding protein: Role of ERK signaling. *The Indian Journal of Medical Research*, *140*, 112–119.

Miller, W. L., & Auchus, R. J. (2011). The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocrine Reviews*, *32*(1), 81–151.

Mitsui, Y., Hirata, H., Arichi, N., Hiraki, M., Yasumoto, H., Chang, I., et al. (2015). Inactivation of bone morphogenetic protein 2 may predict clinical outcome and poor overall survival for renal cell carcinoma through epigenetic pathways. *Oncotarget*, *6*, 9577–9591.

Miyazono, K., Kamiya, Y., & Morikawa, M. (2010). Bone morphogenetic protein receptors and signal transduction. *Journal of Biochemistry*, *147*, 35–51.

Miyazono, K., Maeda, S., & Imamura, T. (2005). BMP receptor signaling: Transcriptional targets, regulation of signals, and signaling cross-talk. *Cytokine & Growth Factor Reviews*, *6*, 251–263.

Monniaux, D., Clément, F., Dalbiès-Tran, R., Estienne, A., Fabre, S., Mansanet, C., et al. (2014). The ovarian reserve of primordial follicles and the dynamic reserve of antral growing follicles: What is the link? *Biology of Reproduction*, *90*, 85. 81–11.

Moore, R. K., Otsuka, F., & Shimasaki, S. (2001). Role of ERK1/2 in the differential synthesis of progesterone and estradiol by granulosa cells. *Biochemical and Biophysical Research Communications*, *289*, 796–800.

Moore, R. K., Otsuka, F., & Shimasaki, S. (2003). Molecular basis of bone morphogenetic protein-15 signaling in granulosa cells. *The Journal of Biological Chemistry*, *278*, 304–310.

Mulsant, P., Lecerf, F., Fabre, S., Schibler, L., Monget, P., Lanneluc, I., et al. (2001). Mutation in bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola Mérino ewes. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 5104–5109.

Nicol, L., Faure, M. O., McNeilly, J. R., Fontaine, J., Taragnat, C., & McNeilly, A. S. (2008). Bone morphogenetic protein-4 interacts with activin and GnRH to modulate gonadotrophin secretion in LbetaT2 gonadotrophs. *Journal of Endocrinology*, *196*, 497–507.

Nielsen, M. E., Rasmussen, I. A., Kristensen, S. G., Christensen, S. T., Møllgård, K., Wreford Andersen, E., et al. (2011). In human granulosa cells from small antral follicles, androgen receptor mRNA and androgen levels in follicular fluid correlate with FSH receptor mRNA. *Molecular Human Reproduction*, *17*, 63–70.

Nilsson, E. E., & Skinner, M. K. (2003). Bone morphogenetic protein-4 acts as an ovarian follicle survival factor and promotes primordial follicle development. *Biology of Reproduction*, *69*, 1265–1272.

Ogura Nose, S., Yoshino, O., Osuga, Y., Shi, J., Hiroi, H., Yano, T., et al. (2012). Anti-Mullerian hormone (AMH) is induced by bone morphogenetic protein (BMP) cytokines in human granulosa cells. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, *164*, 44–47.

Ophir, L., Yung, Y., Maman, E., Rubinstein, N., Yerushalmi, G. M., Haas, J., et al. (2014). Establishment and validation of a model for non-luteinized human mural granulosa cell culture. *Molecular and Cellular Endocrinology*, *384*, 165–174.

Otsuka, F. (2010). Multiple endocrine regulation by bone morphogenetic protein system. *Endocrine Journal*, *57*, 3–14.

Otsuka, F. (2013). Multifunctional bone morphogenetic protein system in endocrinology. *Acta Medica Okayama*, *67*, 75–86.

Otsuka, F., Moore, R. K., & Shimasaki, S. (2001). Biological function and cellular mechanism of bone morphogenetic Protein-6 in the ovary. *The Journal of Biological Chemistry*, *276*, 32889–32895.

Otsuka, F., & Shimasaki, S. (2002). A novel function of bone morphogenetic protein-15 in the pituitary: Selective synthesis and secretion of FSH by gonadotropes. *Endocrinology*, *143*, 4938–4941.

Otsuka, F., Yamamoto, S., Erickson, G., & Shimasaki, S. (2001). Bone morphogenetic protein-15 inhibits follicle-stimulating hormone (FSH) action by suppressing FSH receptor expression. *The Journal of Biological Chemistry*, *276*, 11387–11392.

Pangas, S. A. (2012). Regulation of the ovarian reserve by members of the transforming growth factor beta family. *Molecular Reproduction and Development*, *79*, 666–679.

Picton, H. M., & McNeilly, A. S. (1991). Evidence to support a follicle-stimulating hormone threshold theory for follicle selection in ewes chronically treated with gonadotrophin-releasing hormone agonist. *Journal of Reproduction and Fertility*, *93*, 43–51.

Pierre, A., Pisselet, C., Dupont, J., Mandon-Pepin, B., Monniaux, D., Monget, P., et al. (2004). Molecular basis of bone morphogenetic protein-4 inhibitory action on progesterone secretion by ovine granulosa cells. *Journal of Molecular Endocrinology*, *33*, 805–814.

Pulkki, M., Mottershead, D., Pasternack, A., Muggalla, P., Ludlow, H., van Dinther, M., et al. (2012). A covalently dimerized recombinant human bone morphogenetic protein-15 variant identifies bone morphogenetic protein receptor type 1B as a key cell surface receptor on ovarian granulosa cells. *Endocrinology*, *153*, 1509–1518.

Raz, E. (2003). Primordial germ-cell development: The zebrafish perspective. *Nature Reviews. Genetics*, *4*, 690–700.

Reddy, P., Liu, L., Adhikari, D., Jagarlamudi, K., Rajareddy, S., Shen, Y., et al. (2008). Oocyte-specific deletion of Pten causes premature activation of the primordial follicle pool. *Science*, *319*, 611–613.

Regan, S. L. P., Knight, P. G., Yovich, J., Stanger, J., Leung, Y., Arfuso, F., et al. (2016). Dysregulation of granulosal bone morphogenetic protein receptor 1B density is associated with reduced ovarian reserve and the age-related decline in human fertility. *Molecular and Cellular Endocrinology*, *425*, 84–93.

Regan, S. L. P., Knight, P. G., Yovich, J. L., Stanger, J. D., Leung, Y., Arfuso, F., et al. (2017). Infertility and ovarian follicle reserve depletion are associated with dysregulation of the FSH and LH receptor density in human antral follicles. *Molecular and Cellular Endocrinology*, *446*, 40–51.

Regan, S. L. P., McFarlane, J. R., O'Shea, T., Andronicos, N., Arfuso, F., Dharmarajan, A., et al. (2015). Flow cytometric analysis of FSHR, BMPR1B, LHR and apoptosis in granulosa cells and ovulation rate in merino sheep. *Reproduction*, *150*, 151–163.

Rice, S., Ojha, K., Whitehead, S., & Mason, H. (2007). Stage-specific expression of androgen receptor, follicle-stimulating hormone receptor, and anti-müllerian hormone type II receptor in single, isolated, human preantral follicles: Relevance to polycystic ovaries. *The Journal of Clinical Endocrinology and Metabolism*, *92*, 1034–1040.

Richards, J. S. (1994). Hormonal control of gene expression in the ovary. *Endocrine Reviews*, *15*, 725–751.

Richardson, B. E., & Lehmann, R. (2010). Mechanisms guiding primordial germ cell migration: Strategies from different organisms. *Nature Reviews. Molecular Cell Biology*, *11*, 37–49.

Robinson, R. S., Woad, K. J., Hammond, A. J., Laird, M., Hunter, M. G., & Mann, G. E. (2009). Angiogenesis and vascular function in the ovary. *Reproduction*, *138*, 869–881.

Rodgers, R. J., & Irving-Rodgers, H. F. (2010). Formation of the ovarian follicular antrum and follicular fluid. *Biology of Reproduction*, *82*, 1021–1029.

Ruoss, C., Tadros, A., O'Shea, T., McFarlane, J., & Almahbobi, G. (2009). Ovarian follicle development in Booroola sheep exhibiting impaired bone morphogenetic protein signalling pathway. *Reproduction*, *138*, 689–696.

Sen, A., & Hammes, S. (2010). Granulosa cell-specific androgen receptors are critical regulators of ovarian development and function. *Molecular Endocrinology*, *24*, 1393–1403.

Sen, A., Prizant, H., Light, A., Biswas, A., Hayes, E., Lee, H.-J., et al. (2014). Androgens regulate ovarian follicular development by increasing follicle stimulating hormone receptor and microRNA-125b expression. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(8), 3008–3013.

Shi, J., Yoshino, O., Osuga, Y., Akiyama, I., Harada, M., Koga, K., et al. (2012). Growth differentiation factor 3 is induced by bone morphogenetic protein 6 (BMP-6) and BMP-7 and increases luteinizing hormone receptor messenger RNA expression in human granulosa cells. *Fertility and Sterility*, *97*, 979–983.

Shi, J., Yoshino, O., Osuga, Y., Koga, K., Hirota, Y., Hirata, T., et al. (2009). Bone morphogenetic protein-6 stimulates gene expression of follicle-stimulating hormone receptor, inhibin/activin beta subunits, and anti-Müllerian hormone in human granulosa cells. *Fertility and Sterility*, *92*, 1794–1798.

Shi, J., Yoshino, O., Osuga, Y., Koga, K., Hirota, Y., Nose, E., et al. (2011). Bone morphogenetic protein-2 (BMP-2) increases gene expression of FSH receptor and aromatase and decreases gene expression of LH receptor and StAR in human granulosa cells. *American Journal of Reproductive Immunology*, *1989*(65), 421–427.

Shi, J., Yoshino, O., Osuga, Y., Nishii, O., Yano, T., & Taketani, Y. (2010). Bone morphogenetic protein 7 (BMP-7) increases the expression of follicle-stimulating hormone (FSH) receptor in human granulosa cells. *Fertility and Sterility*, *93*, 1273–1279.

Shimasaki, S., Moore, R., Otsuka, F., & Erickson, G. (2004). The bone morphogenetic protein system in mammalian reproduction. *Endocrine Reviews*, *25*, 72–101.

Shimasaki, S., Zachow, R. J., Li, D., Kim, H., Iemura, S.-I., Ueno, N., et al. (1999). A functional bone morphogenetic protein system in the ovary. *Proceedings of the National Academy of Sciences of the United States of America*, *96*, 7282–7287.

Skinner, M. K. (2005). Regulation of primordial follicle assembly and development. *Human Reproduction Update*, *11*, 461–471.

Slot, K. A., Kastelijn, J., Bachelot, A., Kelly, P. A., Binart, N., & Teerds, K. J. (2006). Reduced recruitment and survival of primordial and growing follicles in GH receptor-deficient mice. *Reproduction*, *131*, 525–532.

Souza, C., Campbell, B., McNeilly, A., & Baird, D. (2002). Effect of bone morphogenetic protein 2 (BMP2) on oestradiol and inhibin A production by sheep granulosa cells, and localization of BMP receptors in the ovary by immunohistochemistry. *Reproduction*, *123*, 363–369.

Souza, C., MacDougall, C., Campbell, B., McNeilly, A., & Baird, D. (2001). The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (BMPR1B) gene. *The Journal of Endocrinology*, *169*, R1–R6.

Suzuki, N., Yoshioka, N., Takae, S., Sugishita, Y., Tamura, M., Hashimoto, S., et al. (2015). Successful fertility preservation following ovarian tissue vitrification in patients with primary ovarian insufficiency. *Human Reproduction*, *30*, 608–615.

Sweeney, M. D., Ayyadurai, S., & Zlokovic, B. V. (2016). Pericytes of the neurovascular unit: Key functions and signaling pathways. *Nature Neuroscience*, *19*, 771–783.

Tajima, K., Dantes, A., Yao, Z., Sorokina, K., Kotsuji, F., Seger, R., et al. (2003). Down-regulation of steroidogenic response to gonadotropins in human and rat preovulatory granulosa cells involves mitogen-activated protein kinase activation and modulation of DAX-1 and steroidogenic factor-1. *The Journal of Clinical Endocrinology and Metabolism*, *88*, 2288–2299.

Tanwar, P., & McFarlane, J. (2011). Dynamic expression of bone morphogenetic protein 4 in reproductive organs of female mice. *Reproduction*, *142*, 573–579.

Taylor, P. D., Wilson, H., Hillier, S. G., Wiegand, S. J., & Fraser, H. M. (2007). Effects of inhibition of vascular endothelial growth factor at time of selection on follicular angiogenesis, expansion, development and atresia in the marmoset. *Molecular Human Reproduction*, *13*, 729–736.

Val, P., Lefrançois-Martinez, A. M., Veyssière, G., & Martinez, A. (2003). SF-1 a key player in the development and differentiation of steroidogenic tissues. *Nuclear Receptor*, *1*, 8.

van Rooij, I. A. J., Broekmans, F. J. M., Scheffer, G. J., Looman, C. W. N., Habbema, J. D. F., de Jong, F. H., et al. (2005). Serum antimüllerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: A longitudinal study. *Fertility and Sterility*, *83*, 979–987.

Vitt, U., Hayashi, M., Klein, C., & Hsueh, A. (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. *Biology of Reproduction*, *62*, 370–377.

Weenen, C., Laven, J. S. E., von Bergh, A. R. M., 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. *Molecular Human Reproduction*, *10*, 77–83.

Westergaard, L., Christensen, I. J., & McNatty, K. P. (1986). Steroid levels in ovarian follicular fluid related to follicle size and health status during the normal menstrual cycle in women. *Human Reproduction*, *1*, 227–232.

Wilson, T., Wu, X.-Y., Juengel, J. L., Ross, I. K., Lumsden, J. M., Lord, E. A., et al. (2001). Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. *Biology of Reproduction*, *64*, 1225–1235.

Wu, Y.-T., Tang, L., Cai, J., X-E, L., Xu, J., Zhu, X.-M., et al. (2007). High bone morphogenetic protein-15 level in follicular fluid is associated with high quality oocyte and subsequent embryonic development. *Human Reproduction*, *22*, 1526–1531.

Xu, Z., Garverick, H. A., Smith, G. W., Smith, M. F., Hamilton, S. A., & Youngquist, R. S. (1995). Expression of follicle-stimulating hormone and luteinizing hormone receptor messenger ribonucleic acids in bovine follicles during the first follicular wave. *Biology of Reproduction*, *53*, 951–957.

Yding Andersen, C. (2017). Inhibin-B secretion and FSH isoform distribution may play an integral part of follicular selection in the natural menstrual cycle. *MHR: Basic Science of Reproductive Medicine*, *23*, 16–24.

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

Yuan, W., & Giudice, L. (1997). Programmed cell death in human ovary is a function of follicle and corpus luteum status. *The Journal of Clinical Endocrinology and Metabolism*, *82*, 3148–3155.

Zeleznik, A. J. (2001). Follicle selection in primates: "many are called but few are chosen". *Biology of Reproduction*, *65*, 655–659.

Zhang, Y.-M., & Roy, S. K. (2004). Downregulation of follicle-stimulating hormone (fsh)-receptor messenger rna levels in the hamster ovary: Effect of the endogenous and exogenous FSH. *Biology of Reproduction*, *70*, 1580–1588.

Zhu, G., Cui, Y., Qinglin-Wang, Yonggang-Kang, Yanzhi-Lv, Wang, G., et al. (2013). Bone morphogenetic proteins (BMP) 2, 4, 6 and 7 affect ovarian follicular development through regulation of follicle-stimulating hormone receptor (FSHR) and luteinizing hormone receptor (LHR) expression in goat granulosa cells. *Journal of Cell Biology and Genetics*, *3*(1), 14–21.