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

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Abstract

Primordial germ cells migrate to the foetal 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 pre-granulosa 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 luteinising hormone (LH) in conjunction with many intraovarian growth factors and inhibitors interrelated in a complex web of regulatory balance.

The BMP signalling 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, and formation of corpora lutea after ovulation. At the anterior pituitary level, BMPs also contribute to the regulation of gonadotrophin production.

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 or egg is encapsulated by layers of follicular somatic cells that proliferate, and later differentiate and mature to form pre-ovulatory

follicles. At ovulation, the mature follicle wall ruptures and the oocyte is expelled from the follicle, and is potentially destined for fertilisation. The recruitment of follicles, their growth, and the expulsion of the oocyte are dependent on complex signalling 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 dominant follicles belong to the transforming growth factor beta (TGFβ) superfamily including the sub families of bone morphogenetic proteins (BMPs), inhibins and activins, growth differentiation factors (GDFs), and anti-Mullerian hormone (AMH) (Fig. 1) (Edson 2009, Eppig 2001, Erickson and Shimasaki 2001, Fabre, et al. 2006, Gilchrist, et al. 2004, Knight and Glister 2006, McNatty, et al. 2004, Otsuka 2013).

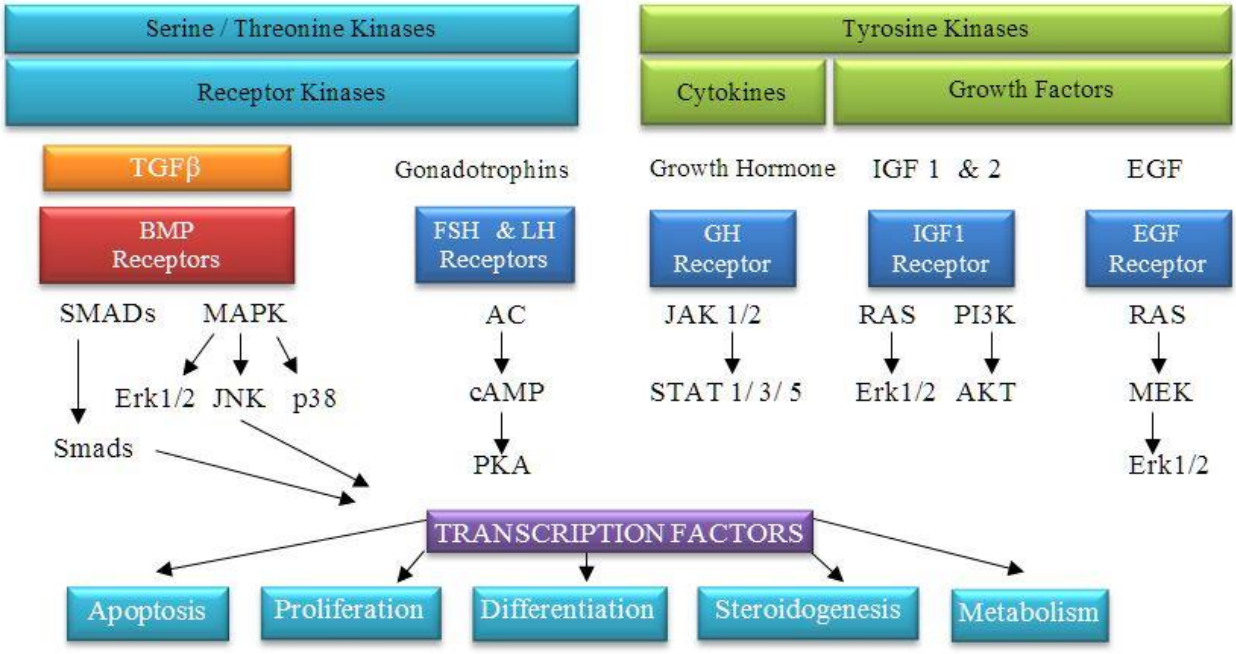

Figure 1 Overview of the TGFβ and the growth hormone kinase signalling interaction

Major Serine and Tyrosisne kinases and receptors, and singalling pathways involved in ovaian regulation (Amsterdam, et al. 2003, Fan, et al. 2009, Manna, et al. 2002, Miyazono, et al. 2010, Moore, et al. 2001, Rice, et al. 2007, Tajima, et al. 2003).

At approximately 26 weeks of gestation in humans, the reproductive potential of the foetus 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 lifespan of the individual (Pangas 2012). Activated primordial follicles grow and differentiate into pre-antral follicles (Fig. 2). With further development, pre-antral follicles mature into antral follicles with the formation of a fluid filled central compartment (Rodgers and Irving-Rodgers 2010). At the onset of puberty, cyclic increases in gonadotrophin secretion from the anterior pituitary raise 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).

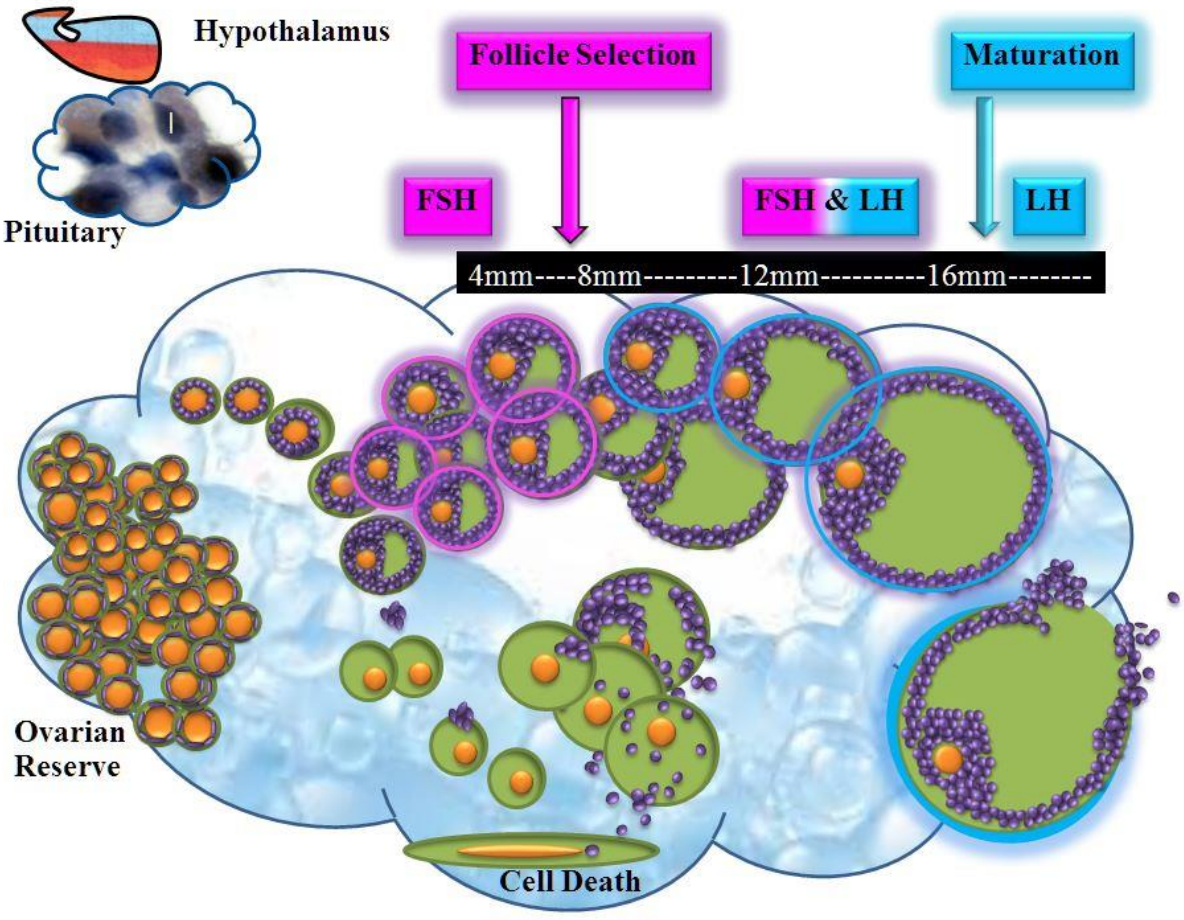

Figure 2 Folliculogenesis: Activation of the primordial follicle, dominant follicle selection, growth and maturation before ovulation

Ovarian reserve of primordial follicle with squamous pre-granulosa 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 luteinisation. Diameter of the follicles at the respective stages of folliculogenesis is indicated in mm scale.

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 basal lamina. Stromal cells within a connective tissue matrix are encapsulated by a layer of epithelial cells at the ovarian surface (Erickson and Shimasaki 2003, Rodgers and 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 and Shimasaki 2003, Gougeon 1986). The mature follicle or pre-ovulatory follicle completes differentiation and commences luteinisation (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, et al. 1980, Rodgers and Irving-Rodgers 2010).

The number of pre-ovulatory follicles selected for dominance and ovulation varies according to species, and is dependent on the regulation by the gonadotrophins (FSH and LH) 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 and Shimasaki 2001, Fabre, et al. 2006, Knight and 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, et al.

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, et al. 2001, Wilson, et al. 2001). In the human clinical context, ovulation rate is increased during in vitro fertilisation (IVF) treatment by administration of FSH to stimulate the growth of multiple follicles (Edwards, et al. 1996, Edwards and Steptoe 1983). BMPR1B-mediated signalling and its interaction with the signalling of FSH receptor (FSHR) and LH (LHR) appear to have a central role in this process.

The ovarian reserve

Oogonia proliferate in the ovary before commencing meiosis from approximately week 9-11 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 follicle(s) selected for ovulation (Edwards, et al. 1996). The progressive decline of the ovarian reserve is well documented, and is related to chronological age (Almog, et al. 2011, Hansen, et al. 2011).

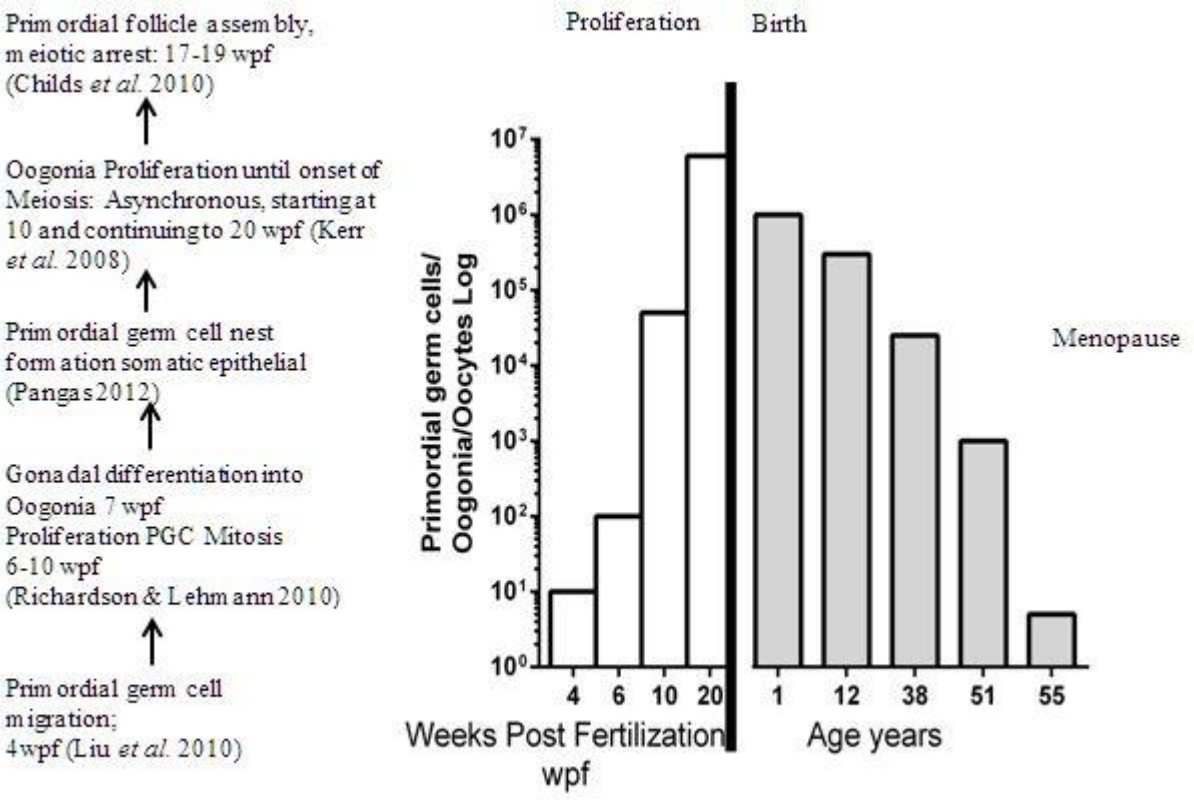

Figure 3 Primordial germ cell proliferation and oogenesis before birth, and the loss of primordial follicles from birth to menopause.

Based on (Baerwald, et al. 2012, Fabre, et al. 2006, Knight and Glister 2006, Matsuda, et al. 2012, Skinner 2005, Webb 2007)

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 lifespan of the individual (Fig. 3) (Monniaux, et al. 2014). At puberty, the levels of gonadotrophins increase sufficiently to promote tertiary follicles to continue growth, and to resist apoptosis (Matsuda, et al. 2012). Ultrasonographic estimates of the number of small antral follicles growing (AFC) or serum levels of AMH (secretion by the small antral follicles) is strongly correlated to the ovarian reserve (Hansen, et al. 2011, van Rooij, et al. 2005).

Intra-ovarian regulators of folliculogenesis

Inducement of FSHR and LHR expression and modulation of responsiveness to gonadotrophins appears to be under the control of various intraovarian growth and development regulators (Fig. 1) (Baerwald, et al. 2012, Erickson, et al. 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 immunisation (Al-Samerria, et al. 2015, Campbell, et al. 2009, Juengel, et al. 2004, Knight, et al. 2012), and by evidence from natural mutations and knockout gene models in several species (Araújo 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, et al. 2005, Campbell, et al. 2006, Glister, et al. 2004b, McNatty, et al. 2009, Nilsson and 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, et al. 1992, Sen, et al. 2014). Acquisition of granulosal LH-responsiveness supplements the FSHR-mediated conversion of androstenedione to oestradiol by P450 Aromatase (CYP19A1), maintaining a positive oestrogen to androgen ratio in the follicle. As the antral follicle increases in size, more oestrogen and anti-apoptotic 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 oestrogens, and are destined for atresia (Hillier, et al. 1994, Xu, et al. 1995).

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 and Shimasaki 2001, 2003, Knight and Glister 2006, Miyazono, et al. 2010, Otsuka 2013, Pangas 2012, Shimasaki, et al. 2004). Various mammalian species have been used as in vivo and in

vitro research models including poly-ovulatory rodents and pigs, mono-ovulatory species (sheep, cattle) and humans (Edson, et al. 2010, Raz 2003, Regan, et al. 2017, Regan, et al. 2015b). 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, in vitro, and to a lesser extent, in vivo treatment with BMPs and the blocking of receptors and signalling pathways provide have been used extensively to examine the roles of BMP signalling in ovarian function.

In human studies, in vivo and in vitro research has progressed substantially with the rise of IVF centres, providing an accessible source of follicular material from gonadotrophin-stimulated patients undergoing oocyte retrieval. However, the availability of non-pathogenic human ovarian tissue, free from exogenous gonadotrophin stimulation, is very limited, and is therefore infrequently used in research (Bomsel Helmreich, et al. 1979, Fowler, et al. 2001, Garcia, et al. 1981, Gougeon 1986, Klein, et al. 2000, MacNaughton, et al. 1992). Given the stage-specific nature of ovarian regulation and variation amongst species, caution should be used when interpreting results (Erickson and 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, complicates the interpretation of in vivo experiments exploring the intraovarian roles of specific growth factors (Zeleznik 2001)

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 signalling 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 hetero-tetrameric receptor complex initiates phosphorylation of the intracellular substrate molecules, receptor-regulated Smads (Smads 1, 5 and/or 8 in the case of BMP signalling). The Smad forms a complex with a common mediator, Smad 4, and translocates to the nucleus. In the nucleus, allocated specific co-factors for each BMP ligand initiates transcription of genes required by the cell (Mitsui, et al. 2015, Moore, et al. 2003). BMP signalling is modulated at different levels by specific repressor and activator molecules in the nucleus, cytoplasm, extracellular fluid and extracellular matrix. Intracellular modulators that attenuate signalling include inhibitory Smads 6 and 7 and extracellular BMP inhibitory binding proteins include follistatin, noggin, chordin and gremlin. These non-signalling secreted proteins sequester BMP ligands and modulate their binding to signalling receptors, generally inhibiting their actions (Miyazono, et al. 2010).

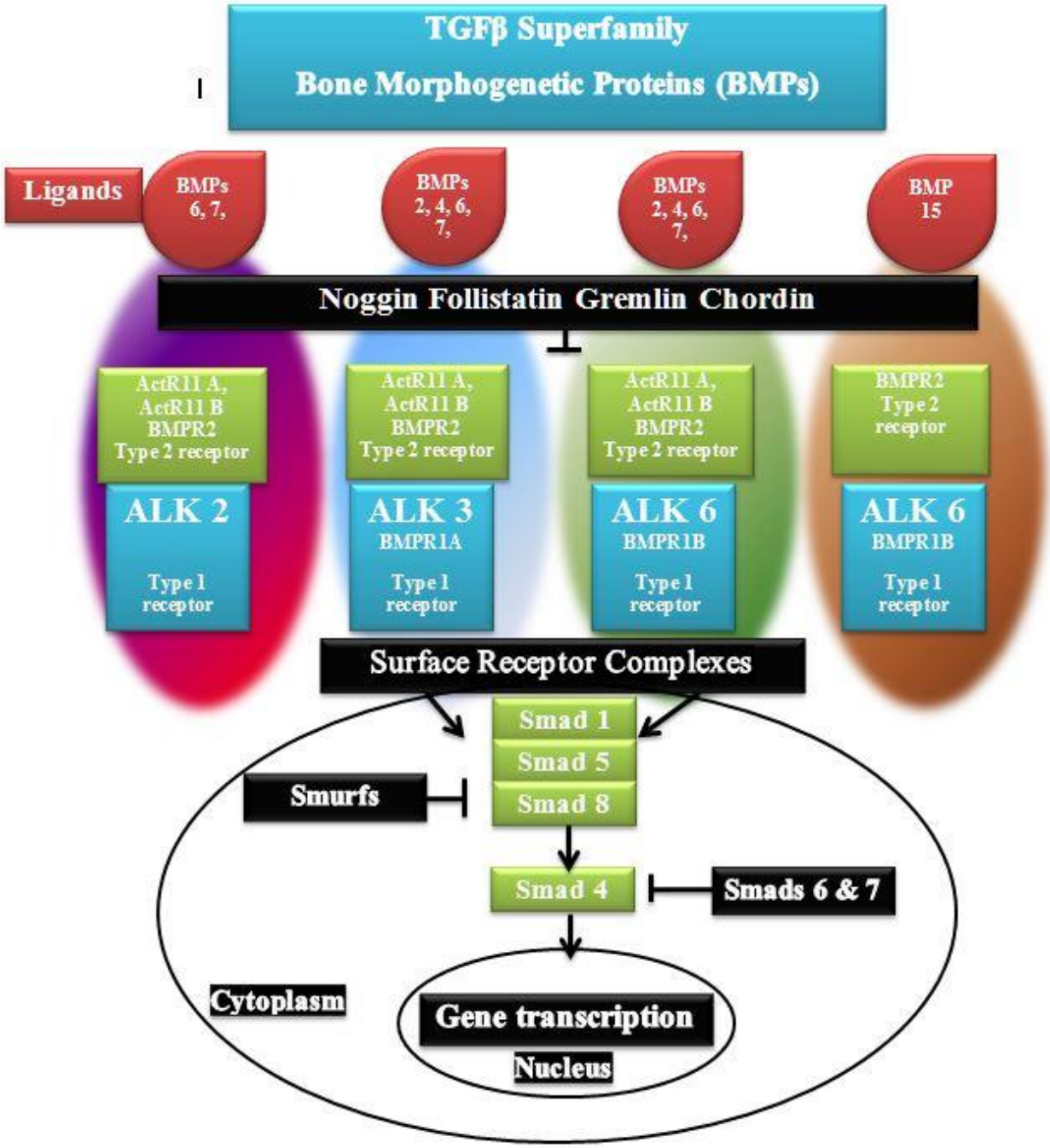

Figure 4 BMP signalling pathway

BMP ligand signalling and the formation of hetero-tetrameric receptor complex with a type 1 and type 2 TGFβ receptor (4 oval shapes). Phosphorylation of downstream signalling 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.

In addition to activation of canonical Smad-mediated signalling 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 signalling adds another dimension to the complex signalling that regulates folliculogenesis (Tajima, et al. 2003).

Observations on intraovarian roles of different BMPs

BMP2 Evidence suggests BMP2 is involved in oocyte endowment, primordial pool assembly, and activation of primordial follicles (Lawson, et al. 1999, Ying and 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 supressed synthesis (Lee, et al. 2007). BMP2 also increased granulosa cell oestrogen and inhibin B production and FSHR expression in culture; however it had no effect on proliferation (Shi, et al. 2011, Souza, et al. 2002). BMP2 has a relatively low affinity to bind with BMPR1B, and binds preferentially with another type 1 TGFβ receptor, BMPR1A (ALK3) however species difference exist (Miyazono, et al. 2010).

BMP4 BMP4 is produced by both theca and granulosa cells in the bovine and human model (Glister, et al. 2004b, Khalaf, et al. 2013). BMP4 has been shown to bind precociously with BMPR1A and BMPR1B in sheep (Fabre, et al. 2006), signalling 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 StAR and 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 signalling pathways to the TGFβ which involves the MAPK family, in particular ERK1/2 (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, steroidogenic acute regulatory protein (StAR), and P450 Side Chain Cleavage (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 and McFarlane 2011). Whilst FSH-induced progesterone biosynthesis was inhibited by BMP4, it was shown to enhance FSH-induced oestrogen 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 oestrogen, inhibin and activin production (Glister, et al. 2004b).

BMP4 is also involved in oocyte endowment, primordial follicle assembly and primordial follicle activation (Lawson, et al. 1999, Lee, et al. 2004, Lee, et al. 2001, Nilsson and Skinner 2003, Ying and 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, et al. 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).

BMP7 At the ovarian level, BMP7 is mostly expressed by theca cells, but with some expression by granulosa cells (Glister, et al. 2004a, 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 mono-ovulatory 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 2013). Differences in response are likely due to granulosa cells either being luteinised (caused by the addition of serum during culture) or attributed to being collected from gonadotrophin-stimulated ovaries in humans.

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. 2004b, Khalaf, et al. 2013, Wu, et al. 2007)(add more references). BMP6 is involved in proliferation, steroidogenesis, and cyto-differentiation of granulosa cells in a species-specific manner. In the goat, BMP6 did not increase granulosal FSHR, yet it increased LHR expression (Zhu 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, et al. 2001a). 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, et al. 2001a). 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).

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 and Shimasaki 2010, Otsuka and Shimasaki 2002, Pulkki, et al. 2012). From the primary follicle stage onwards, 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, et al. 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 cyto-differentiation, independently of FSH, yet BMP15 supresses FSH regulated progesterone synthesis by reducing StAR and CYP11A1 in the rat (Otsuka, et al. 2001b).

It has also been shown that BMP15 decreased FSH-induced progesterone biosynthesis by decreasing FSHR mRNA, ultimately inhibiting luteinisation. (Moore, et al. 2003, Otsuka and 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, et al. 2001c, Shimasaki, et al. 1999). Moreover, in the pituitary, BMP15 has been associated with increasing FSHβ expression (Otsuka and Shimasaki 2002).

Other TGFβ superfamily members (GDF9, GDF3, AMH)

GDF9 and GDF3 are TGFβ superfamily members with close homology to the BMPs, and signal via ALK4 and 5, and via Smad2 and 3. GDF9 is also produced by the oocyte from primordial follicles onwards, and is known to supress 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).

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 decrease and GDF3 expression increases.

AMH Another TGFβ superfamily member that has attracted considerable interest with regard to its intraovarian role is AMH. Whilst several BMPs have been shown to promote primordial follicle activation and increase the pool of growing follicles in cultured neonatal mouse ovaries (Skinner references), AMH exerts an inhibitory effect (Josso, et al. 2001. In addition AMH inhibits FSH-dependent follicle growth and oestrogen production at later follicle stages whilst several BMPs have the opposite effect {Gouédard, 2000 #1060). AMH signals via its own type 2 receptor (AMHR2) forming a signalling complex with either BMPR1B (ALK6) or BMPR1A (ALK3) (Josso and 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, et al. 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.

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 showing that the mesenchymal pericyte migrates towards 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 signalling 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, et al. 2001).

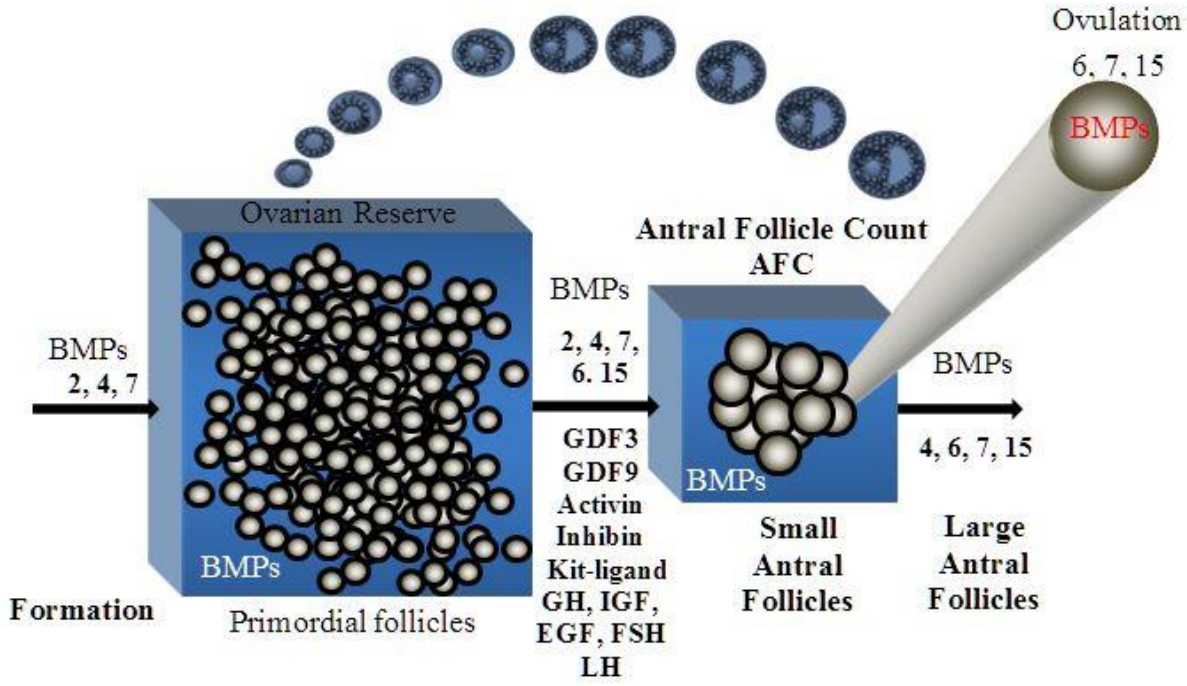

Figure 5 BMP signalling and follicle development

The involvement of BMP signalling in embryonic ovarian formation of primordial follicles; activation of the primordial to primary follicle; antral follicle formation and recruitment into cyclic folliculogenesis to ovulation.

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, et al. 2007). The TGFβ super family is involved with the proliferation of pericytes (Sweeney, et al. 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.

BMP 15 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, et al. 2000). Whereas, GDF9 knockout mice are infertile due to arrested primordial to primary growth transition (Dong, et al. 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 signalling complex with BMPR1B. However, in humans, AMHR2 is evidently not expressed in 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 leukaemia inhibitory factor (LIF), forkhead box O3 (Foxo 3), growth hormone, and the phosphatidylinositol 3-kinase (PI3K)-AKT signalling axis pathway (Albamonte, et al. 2013, Castrillon, et al. 2003, John, et al. 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.

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). Immunisation to inhibit BMP4 and BMPR1B signalling 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) (also skinner

lab) Similarly, Booroola sheep, with a partially attenuating mutation to the BMPR1B, retained more primordial follicles over time compared to the wild type sheep (Ruoss, et al. 2009).

BMP receptor activity in the ovary

The BMP ligands, that 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, Shi, et al. 2011, Shi, et al. 2010, Zhu 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 o f FSHR on the granulosa cell (Fig. 6) (Erickson, et al. 1979, Nielsen, et al. 2011, Rice, et al. 2007, Sen and Hammes 2010, Sen, et al. 2014).

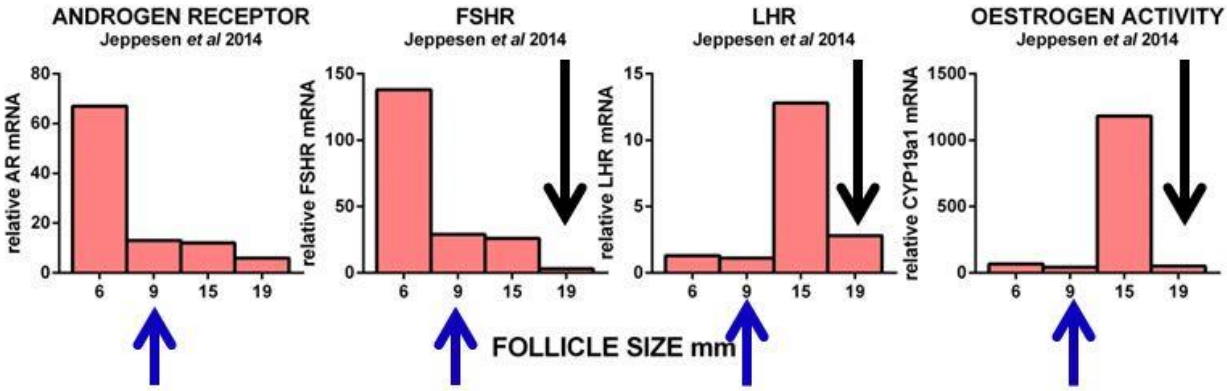

Figure 6 The stage-specific relationship between granulosal receptor expression and oestrogen activity during folliculogenesis in a natural cycle.

Dominant follicle selection took place when the androgen receptor and FSHR expression decreased, and LHR expression increased (indicated by the blue upwards-arrow). Down-regulation of FSHRs, LHRs and the cessation of proliferation occurs in the pre-ovulatory follicles in humans and animals, (indicated by the black downwards-arrow). *CYP19a1* is the gene that encodes aromatase, essential for the production of oestrogen. Based on (Gasperin, et al. 2014, Jeppesen, et al. 2012).

Dominant follicle selection takes place when the androgen receptor expression reduces and oestrogen 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, Regan, et al. 2017). At the time of maturation of the follicle, down-regulation of BMPR1B, FSHR, and LHR expression is associated with reduced proliferation and a shift from oestrogen synthesis to progesterone synthesis in the ovulatory follicles in humans and animals expression (Fig. 6, 7) (Regan, et al. 2016, Regan, et al. 2017, Regan, et al. 2015b, Rice, et al. 2007). This shift in steroidogenesis requires the progesterone-suppressive BMP signalling to be down-regulated in the largest follicles (Regan, et al. 2016, Regan, et al. 2017). In addition, medium to large antral follicles require substantial androgen substrate to generate oestrogen, and a reduction in BMP signalling reflects the ability of BMPs to regulate thecal androgen production (Glister, et al. 2005).

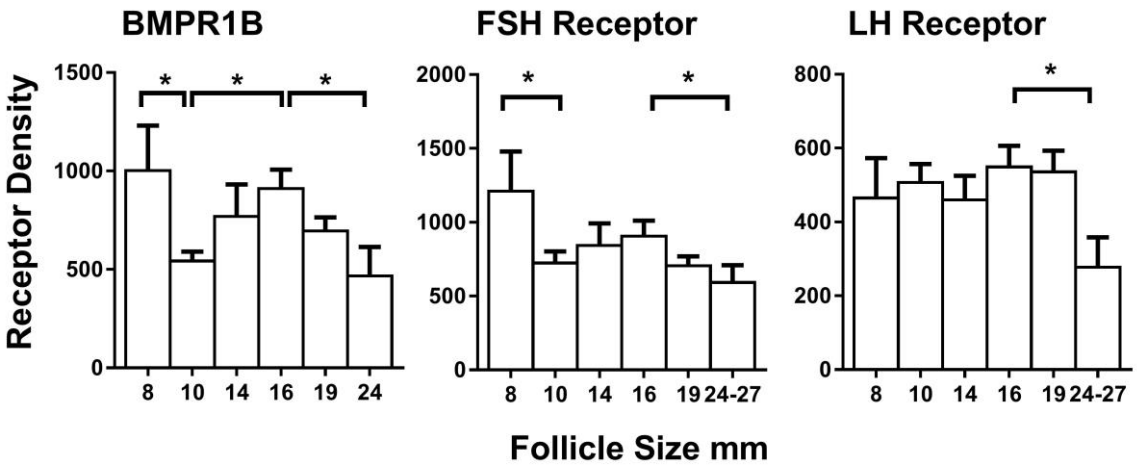

Figure 7 The stage-specific relationship between granulosal receptor expression during folliculogenesis in human IVF cycles.
Granulosal BMPR1B, FSHR, and LHR protein density and follicle size profile of young patients with a typical ovarian reserve for the age group. The patients were 23-30 years old and stimulated with gonadotrophins during an IVF cycle. Values in graphs are means ± S.E.M., and differences were considered significant if *p<0.05 and **p<0.01.

(Regan, et al. 2016, Regan, et al. 2017) copy write Elsevier

Granulosa cells are unique in the ovary because they express FSHRs, which are required for the synthesis of oestrogen expression (Fig. 8) (Miller 2011). Theca cells express LHRs and synthesise androgens, which are used by the granulosa cells as substrate for oestrogen synthesis. The receptor density of BMPRIB on granulosal cells fluctuates bi-phasically during menstrual cycle in unison with the FSHR in young women and Merino sheep (Regan, et al. 2016, Regan, et al. 2015b).

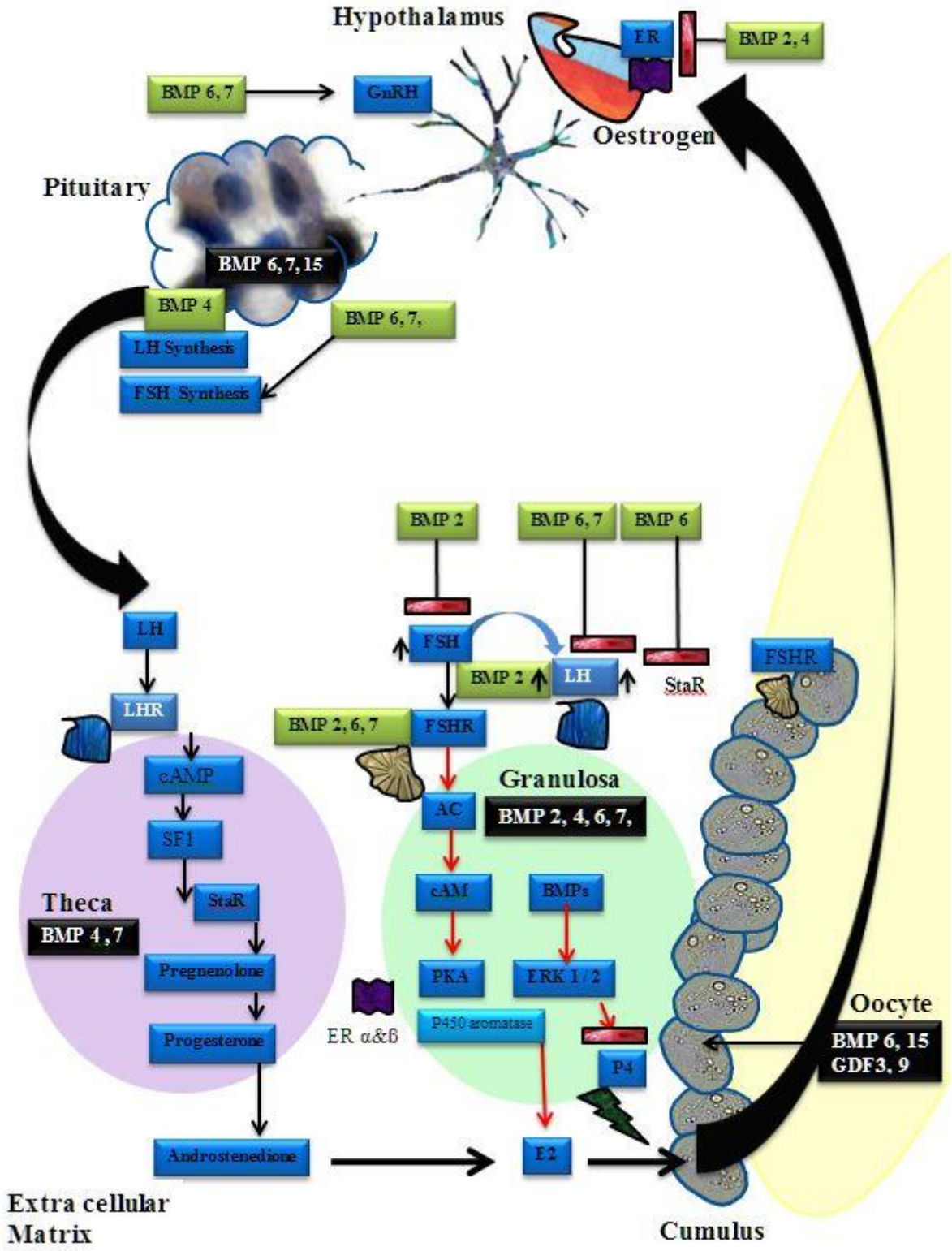

Figure 8 Hypothalamic-pituitary ovarian axis of regulation and the granulosa and theca cell interaction.

Hypothalamic, pituitary control of ovarian growth and differentiation of the ovarian follicle comprised of the Theca (circle on left), granulosa (circle 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 signalling activity (green) and inhibition (red bar). Delayed

expression of LHR (white text) on granulosa compared to the theca cells; oestroen receptor (ER). Based on (Bao, et al. 1997, Chen, et al. 2008, Dijke, et al. 2003, Feary, et al. 2007, Fitzpatrick, et al. 1997, Hillier, et al. 1994, Hussein, et al. 2005, Kayani, et al. 2009, Miller and Bose 2011, Miyazono, et al. 2005, Miyoshi, et al. 2007, Moore, et al. 2001, Nicol, et al. 2008, Pierre, et al. 2004, Rice, et al. 2007, Seger, et al. 2001, Sugawara, et al. 2000, Sullivan, et al. 1999, Takeda, et al. 2012, ten Dijke, et al. 2003, Yamamoto, et al. 2002, Yuan 1998)

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

Proliferation of the granulosa and theca cells continues as the rise in oestrogen 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 oestrogen occurs, androgen levels rise, creating an androgen dominant follicle. Greater androstenedione to oestrogen ratios have been shown to result in an elevated level of granulosa cell apoptosis and follicle demise (Yuan and Giudice 1997).

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 follicle(s) with a higher density of gonadotrophin receptors are presumed to be more responsive to the gonadotrophins, and continue to increase in size (Bächler, et al. 2014, Gougeon 1986, LaPolt, et al. 1992).

FSHR are expressed exclusively by granulosa cells that respond to pituitary-derived FSH by proliferating and increasing oestrogen 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).

Down-regulation of granulosal BMPR1B and FSHR expression has been observed at the stage of cyclic dominant follicle selection, occurring between ~8 to10 mm in the human and ~1 to 1.7 mm in the Merino sheep (Regan, et al. 2016, Regan, et al. 2015b). As mentioned above, follicles are selected as a consequence of the decline in pituitary FSH and only follicles with the newly acquired LHR can sustain oestrogen production during the preovulatory phase. Suppression of granulosal progesterone synthesis in favour of FSH-dependent oestrogen production, appears to be governed by the action of the BMPs (Knight and 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-oestrogen 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, et al. 1999, Ginther, et al. 2012, Luo, et al. 2011, Picton and McNeilly 1991).

BMPs and ovulation rate

The number of pre-ovulatory 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).

Figure 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 signalling 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 localised on sheep granulosa cells from the primordial follicle stage onwards (Al-Samerria and Almahbobi 2014 , Anthony, et al. 2015, Chen, et al. 2008, Erickson and 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. 2004a). 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. 2004a, Regan, et al. 2016, Regan, et al. 2017, Regan, et al. 2015a).

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 and 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 LH or FSH, they produced more cAMP, oestrogen, and androstenedione from the same number of cells from the large antral follicle (Campbell, et al. 2006). An increased cellular capacity to produce oestrogen 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. 2015b).

Recently, compelling data show that the expression of mature surface granulosa receptors for FSHR, LHR, and BMPR1B are significantly elevated in the Booroola compared to the young wild type Merino sheep (Regan, et al. 2015b). 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 up-regulation of FSHR, earlier acquisition of LHR, and an increase of BMPR1B itself in the Booroola sheep. The increased signalling resulted in multiple ovulations and an increased birth rate of ~5 (Otsuka, et al. 2001c, Regan, et al. 2015b). 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 immunisation 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 down-regulation 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). Immunisation against AMH alone would, therefore, increase ovulation rate but not granulosa cell proliferation governed by other BMPs. Whereas, immunisation 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 immunisation against BMP15 increased the ovulation rate from 1-2 to ≥3 without affecting plasma progesterone concentration (Juengel, et al. 2004, Juengel, et al. 2011).

In another study, complete neutralisation 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 pre-ovulatory follicles (Campbell, et al. 2009). Although the effect of the infusion was short-lived, the oestrogen 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 pre-antral follicle development.

BMPs and the terminal stage of folliculogenesis

Granulosa cell proliferation continues and the ovarian follicle increases in size from 10 mm to 20+ mm in the human, producing large quantities of oestrogen 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 reorganisation, 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 synthesis large amounts of progesterone (Westergaard, et al. 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 and Menon 2014, Regan, et al. 2016, Regan, et al. 2015b, Zhang and Roy 2004).

This process collectively referred to as luteinisation appears to be associated with BMPR1B, FSHR, and LHR down-regulation in the leading dominant follicles (Fan, et al. 2009, Izadyar, et al. 1998, Regan, et al. 2017, Regan, et al. 2015a). Furthermore, LHR density peaks in the pre-ovulatory 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 oestrogen levels (Jeppesen, et al. 2012, LaPolt, et al. 1992, Ophir, et al. 2014, Regan, et al. 2017, Regan, et al. 2015a).

In women reaching the end of their reproductive lifespan, dysregulation of granulosal BMPR1B (Fig. 10) and FSHR occurs (Regan, et al. 2016, Regan, et al. 2017).

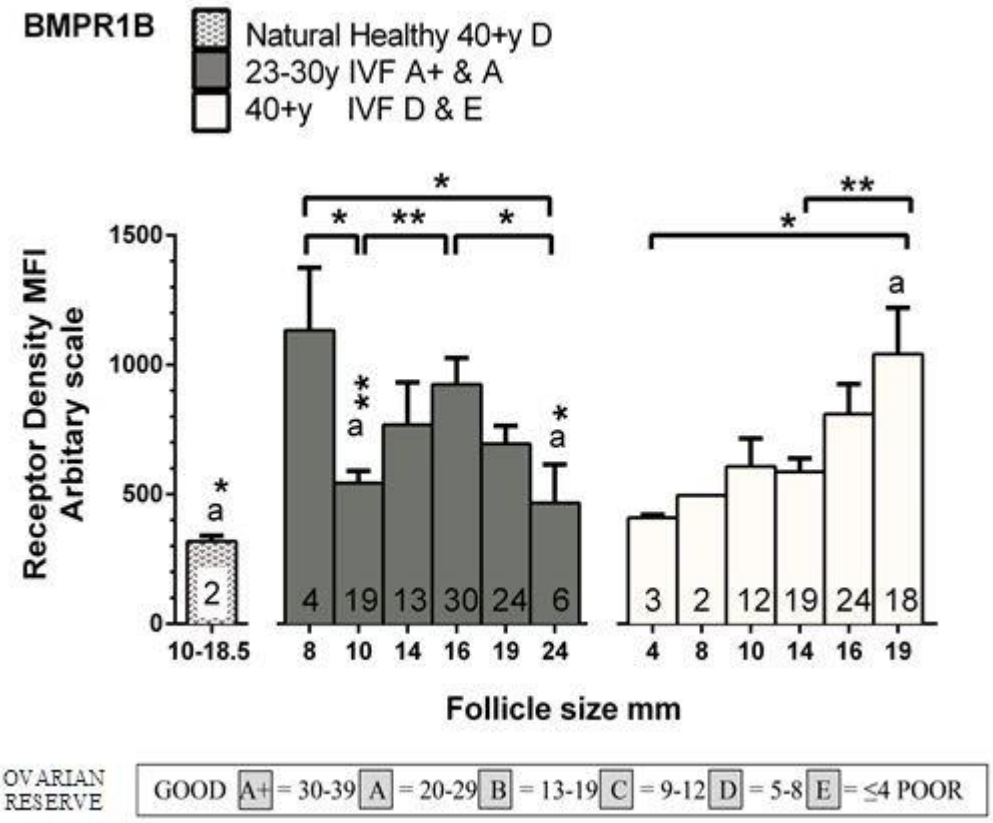

Fig. 10 Granulosal BMPR1B density from follicles of different sizes collected from young and older IVF patients compared to an unstimulated natural healthy cycle.

Granulosal BMPR1B protein density and follicle size profile of a natural healthy unstimulated patient of 41y with an AFC of D, before the LH surge, (patterned bar). Patients, 23-30 y stimulated, IVF cycle with an AFC of A+ & A, (grey bar). Patients, 40+ y stimulated IVF cycle with an AFC of D & E, (white bar). IVF patients were grouped according to ovarian reserve measured indirectly by the antral follicle count (AFC). 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 ± S.E.M., 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 analysed for that group. Based on figure with **copy write Elsevier**

As the ovarian pool of primordial follicles depletes in women, receptor density continue to increase with follicle growth. It is proposed that the lack of receptor down-regulation 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, Regan, et al. 2017).

References

Abir, R, A Ben-Haroush, N Melamed, C Felz, H Krissi, and B Fisch 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, BK Tsang, BR Downey, GJ Marcus, and DT Armstrong 1980 Interrelationships Between Follicular Fluid Steroid Levels, GonadotropiC Stimuli, and Oocyte Maturation During Preovulatory Development of Porcine Follicles. *Biology of Reproduction* **23** 621-627.

Al-Samerria, S, I Al-Ali, JR McFarlane, and G Almahbobi 2015 The impact of passive immunization against BMPR1B and BMP4 on follicle development and ovulation. *Reproduction*.

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

Albamonte, MI, MS Albamonte, I Stella, L Zuccardi, and AD Vitullo 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, F Shehata, E Shalom-Paz, SL Tan, and T Tulandi 2011 Age-related normogram for antral follicle count: McGill reference guide. *Fertility and Sterility* **95** 663-666.

Amsterdam, A, R Sasson, I Keren Tal, D Aharoni, A Dantes, E Rimon, A Land, T Cohen, Y Dor, and L Hirsh 2003 Alternative pathways of ovarian apoptosis: death for life. *Biochemical pharmacology* **66** 1355-1362.

Anthony, E, A Pierre, Nd Clemente, J-Y Picard, P Jarrier, C Mansanet, D Monniaux, and S Fabre 2015 Anti-Müllerian Hormone Regulation by the Bone Morphogenetic Proteins in the Sheep Ovary: Deciphering a Direct Regulatory Pathway. *Endocrinology* **156** 301-313.

Anzalone, CR, L-S Hong, JKH Lu, and PS LaPolt 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, VR, Silva C.M.G., Magalhães D.M., Silva G.M., Báo S.N., Silva J.R.V., Figueiredo J.R. & Rodrigues A.P.R. 2010 Effect of Bone Morphogenetic Protein-7 (BMP-7) on in vitro survival of caprine preantral follicles. *Pesq. Vet. Bras.* **30** 305-310.

Austin, EJ, M Mihm, ACO Evans, PG Knight, JLH Ireland, JJ Ireland, and JF Roche 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, D Menshykau, C De Geyter, and D Iber 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, G Adams, and R Pierson 2012 Ovarian antral folliculogenesis during the human menstrual cycle: a review. *Human Reproduction Update* **18** 73-91.

Baird, D, and B Campbell 1998 Follicle selection in sheep with breed differences in ovulation rate. *Mol Cell Endocrinol* **145** 89 - 95.

Bao, B, HA Garverick, GW Smith, MF Smith, BE Salfen, and RS Youngquist 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.

Bomsel Helmreich, O, A Gougeon, A Thebault, D Saltarelli, E Milgrom, R Frydman, and E Papiernik 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, RL Quinn, R Webb, and MG Hunter 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, K McNatty, P Smith, D Heath, N Hudson, D Phillips, B McLeod, and G Davis 1993 Ovaries of ewes homozygous for the X-linked Inverdale gene (FecXI) are devoid of secondary and tertiary follicles but contain many abnormal structures. *Biol Reprod* **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, M Clinton, and R Webb 2012 The role of anti-mullerian hormone (AMH) during follicle development in a monovulatory species (sheep). *Endocrinology* **153** 4533-4543.

Campbell, BK, H Dobson, DT Baird, and RJ Scaramuzzi 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, BK, NR Kendall, and DT Baird 2009 Effect of Direct Ovarian Infusion of Bone Morphogenetic Protein 6 (BMP6) on Ovarian Function in Sheep. *Biology of Reproduction* **81** 1016-1023.

Campbell, BK, CJH Souza, AJ Skinner, R Webb, and DT Baird 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, DH, L Miao, R Kollipara, JW Horner, and RA DePinho 2003 Suppression of Ovarian Follicle Activation in Mice by the Transcription Factor Foxo3a. *Science* **301** 215-218.

Chen, AQ, S Yu, Z Wang, Z Xu, and Z Yang 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, AJ, RAL Bayne, AA Murray, SJMD Silva, CS Collins, N Spears, and RA Anderson 2010 Differential expression and regulation by activin of the neurotrophins BDNF and NT4 during human and mouse ovarian development. *Developmental Dynamics* **9999** NA.

Di Pasquale, E, R Rossetti, A Marozzi, B Bodega, S Borgato, L Cavallo, S Einaudi, G Radetti, G Russo, M Sacco, M Wasniewska, T Cole, P Beck-Peccoz, L Nelson, and L Persani 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.

Dijke, P, O Korchynskyi, G Valdimarsdottir, and M-J Goumans 2003 Controlling cell fate by bone morphogenetic protein receptors. *Molecular and Cellular Endocrinology* **211** 105-113.

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

Durlinger, ALL, MJG Gruijters, P Kramer, B Karels, HA Ingraham, MW Nachtigal, JTJ Uilenbroek, JA Grootegoed, and APN Themmen 2002 Anti-Mullerian Hormone Inhibits Initiation of Primordial Follicle Growth in the Mouse Ovary. *Endocrinology* **143** 1076-1084.

Edson, MA 2009 The mammalian ovary from genesis to revelation. *Endocrine reviews* **30** 624-712.
Edson, MA, RL Nalam, C Clementi, HL Franco, FJ DeMayo, KM Lyons, SA Pangas, and MM Matzuk 2010 Granulosa Cell-Expressed BMPR1A and BMPR1B Have Unique Functions in Regulating Fertility but Act Redundantly to Suppress Ovarian Tumor Development. *Mol Endocrinol* **24** 1251-1266.
Edwards, RG, L R, and B P 1996 Time to revolutionize ovarian stimultion. *Human Reproduction* **11** 917-919.
Edwards, RG, and PC Steptoe 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, and S Shimasaki 2001 The physiology of folliculogenesis: the role of novel growth factors. *Fertility and Sterility* **76** 943 - 949.
Erickson, G, and S Shimasaki 2003 The spatiotemporal expression pattern of the bone morphogenetic protein family in rat ovary cell types during the estrous cycle. *Reprod Biol Endocrinol* **1(9)** 1-20.
Erickson, GF, C Wang, and AJW Hsueh 1979 FSH induction of functional LH receptors in granulosa cells cultured in a chemically defined medium. *Nature* **279** 336-338.
Fabre, S, A Pierre, P Mulsant, L Bodin, E Di Pasquale, L Persani, P Monget, and D Monniaux 2006 Regulation of ovulation rate in mammals: contribution of sheep genetic models. *Reproductive Biology and Endocrinology* **4** 20.
Fabre, S, A Pierre, C Pisselet, P Mulsant, F Lecerf, J Pohl, P Monget, and D Monniaux 2003 The Booroola mutation in sheep is associated with an alteration of the bone morphogenetic protein receptor-IB functionality. *J Endocrinol* **177** 435 - 444.
Fan, H, Z Liu, M Shimada, E Sterneck, PF Johnson, SM Hedrick, and JS Richards 2009 MAPK3/1 (ERK1/2) in ovarian granulosa cells are essential for female fertility. *Science,* **324** 938-941.
Faure, MO, L Nicol, S Fabre, J Fontaine, N Mohoric, A McNeilly, and C Taragnat 2005 BMP-4 inhibits follicle-stimulating hormone secretion in ewe pituitary. *The Journal of endocrinology* **186** 109-121.
Feary, E, J Juengel, P Smith, M French, A O'Connell, S Lawrence, S Galloway, G Davis, and K McNatty 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, SL, DL Carlone, RL Robker, and JS Richards 1997 Expression of aromatase in the ovary: Down-regulation of mRNA by the ovulatory luteinizing hormone surge. *Steroids* **62** 197-206.
Fowler, PA, T Sorsa, WJ Harris, PG Knight, and HD Mason 2001 Relationship between follicle size and gonadotrophin surge attenuating factor (GnSAF) bioactivity during spontaneous cycles in women. *Human Reproduction* **16** 1353-1358.
Galloway, S, S Gregan, T Wilson, K McNatty, J Juengel, O Ritvos, and G Davis 2002 Bmp15 mutations and ovarian function. *Molecular and Cellular Endocrinology* **191** 15 - 18.
Garcia, J, GS Jones, AA Acosta, and GL Wright 1981 Corpus luteum function after follicle aspiration for oocyte retrieval. *Fertility and Sterility* **36** 565-572.
Gasperin, BG, R Ferreira, MT Rovani, V Bordignon, R Duggavathi, J Buratini, JFC Oliveira, and PBD Gonçalves 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, L Ritter, and D Armstrong 2004 Oocyte-somatic cell interactions during follicle development in mammals. *Anim Reprod Sci* **82-83** 431 - 446.
Ginther, OJ, MA Beg, EL Gastal, MO Gastal, AR Baerwald, and RA Pierson 2005 Systemic concentrations of hormones during the development of follicular waves in mares and women: a comparative study. *Reproduction* **130** 379-388.

Ginther, OJ, FA Khan, MA Hannan, MB Rodriguez, G Pugliesi, and MA Beg 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, C Kemp, and P Knight 2004a 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, CF Kemp, and PG Knight 2004b 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, S Richards, and P Knight 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.

Gougeon, A 1986 Dynamics of follicular growth in the human: a model from preliminary results. *Hum. Reprod.* **1** 81-87.

Hanrahan, J, S Gregan, P Mulsant, M Mullen, G Davis, R Powell, and S Galloway 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). *Biol Reprod* **70** 900 - 909.

Hansen, KR, GM Hodnett, N Knowlton, and LB Craig 2011 Correlation of ovarian reserve tests with histologically determined primordial follicle number. *Fertility and Sterility* **95** 170-175.

Hillier, S, P Whitelaw, and C Smyth 1994 Follicular oestrogen synthesis: the 'two-cell, two-gonadotrophin' model revisited. *Mol Cell Endocrinol* **100** 51 - 54.

Hussein, T, D Froiland, F Amato, J Thompson, and R Gilchrist 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, F Otsuka, T Miyoshi, M Yamashita, M Takahashi, J Goto, J Suzuki, and H Makino 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, and S Shimasaki 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, HA, DS Lala, Y Ikeda, X Luo, WH Shen, MW Nachtigal, R Abbud, JH Nilson, and KL Parker 1994 The nuclear receptor steroidogenic factor 1 acts at multiple levels of the reproductive axis. *Genes & development* **8** 2302-2312.

Izadyar, F, E Zeinstra, and MM Bevers 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, JV, SG Kristensen, ME Nielsen, P Humaidan, M Dal Canto, R Fadini, KT Schmidt, E Ernst, and C Yding Andersen 2012 Lh-receptor gene expression in human granulosa and cumulus cells from antral and preovulatory follicles. *The Journal of Clinical Endocrinology & Metabolism* **97** E1524-E1531.

John, GB, LJ Shirley, TD Gallardo, and DH Castrillon 2007 Specificity of the requirement for Foxo3 in primordial follicle activation. *Reproduction* **133** 855-863.

Josso, N, and Nd Clemente 2003 Transduction pathway of anti-Müllerian hormone, a sex-specific member of the TGF-β family. *Trends in Endocrinology & Metabolism* **14** 91-97.

Josso, N, N di Clemente, and L Gouédard 2001 Anti-Müllerian hormone and its receptors. *Molecular and Cellular Endocrinology* **179** 25-32.

Juengel, J, N Hudson, L Whiting, and K McNatty 2004 Effects of immunization against bone morphogenetic protein 15 and growth differentiation factor 9 on ovulation rate, fertilization, and pregnancy in ewes. *Biol Reprod* **70** 557 - 561.

Juengel, J, L Quirke, S Lun, D Heath, P Johnstone, and K McNatty 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, DG Mottershead, S Mazerbourg, S Myllymaa, S Duprat, RB Gilchrist, NP Groome, AJ Hsueh, and O Ritvos 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. *Journal of Clinical Endocrinology & Metabolism* **90** 271-278.

Kayani, AR, C Glister, and PG Knight 2009 Evidence for an inhibitory role of bone morphogenetic protein(s) in the follicular–luteal transition in cattle. *Reproduction* **137** 67-78.

Khalaf, M, J Morera, A Bourret, Y Reznik, C Denoual, M Herlicoviez, H Mittre, and A Benhaim 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, NA, DE Battaglia, TK Woodruff, V Padmanabhan, LC Giudice, WJ Bremner, and MR Soules 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 & Metabolism* **85** 4520-4525.

Knight, PG, and C Glister 2006 TGF-β superfamily members and ovarian follicle development. *Reproduction* **132** 191-206.

Knight, PG, L Satchell, and C Glister 2012 Intra-ovarian roles of activins and inhibins. *Molecular and Cellular Endocrinology* **359** 53-65.

LaPolt, PS, JL Tilly, T Aihara, K Nishimori, and AJ Hsueh 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, KA, NR Dunn, BA Roelen, LM Zeinstra, AM Davis, CV Wright, JP Korving, and BL Hogan 1999 Bmp4 is required for the generation of primordial germ cells in the mouse embryo. *Genes & development* **13** 424-436.

Lee, K, V Khivansara, M Santos, P Lamba, T Yuen, S Sealfon, and D Bernard 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-S, S-J Yoon, T-K Yoon, K-Y Cha, S-H Lee, S Shimasaki, S Lee, and K-A Lee 2004 Effects of bone morphogenetic protein-7 (BMP-7) on primordial follicular growth in the mouse ovary. *Molecular reproduction and development* **69** 159-163.

Lee, W, F Otsuka, R Moore, and S Shimasaki 2001 Effect of bone morphogenetic protein-7 on folliculogenesis and ovulation in the rat. *Biol Reprod* **65** 994 - 999.

Li, Y, R-Q Li, S-B Ou, N-F Zhang, L Reng, L-N Wei, Q-X Zhang, and D-Z Yang 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, K Kobayashi, M van Dinther, SH van Heiningen, G Valdimarsdottir, T van Laar, M Scharpfenecker, CWGM Lowik, M-J Goumans, Pt Dijke, and E Pardali 2009 VEGF and inhibitors of TGF{beta} type-I receptor kinase synergistically promote blood-vessel formation by inducing {alpha}5-integrin expression. *J Cell Sci* **122** 3294-3302.

Luo, W, A Gumen, J Haughian, and M Wiltbank 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, M Banah, P McCloud, J Hee, and H Burger 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, M Dyson, D Eubank, B Clark, E Lalli, P Sassone Corsi, A Zeleznik, and D Stocco 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, N Inoue, N Manabe, and S Ohkura 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, B Barboni, M Turriani, G Galeati, A Zannoni, G Castellani, P Berardinelli, and PA Scapolo 2001 Follicle Activation Involves Vascular Endothelial Growth Factor Production and Increased Blood Vessel Extension. *Biology of Reproduction* **65** 1014-1019.

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

McNatty, K, L Moore, N Hudson, L Quirke, S Lawrence, K Reader, J Hanrahan, P Smith, N Groome, M Laitinen, O Ritvos, and J Juengel 2004 The oocyte and its role in regulating ovulation rate: a new paradigm in reproductive biology. *Reproduction* **128** 379 - 386.

McNatty, KP, KM Henderson, S Lun, DA Heath, K Ball, NL Hudson, J Fannin, M Gibb, LE Kieboom, and P Smith 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, KP, NL Hudson, L Whiting, KL Reader, S Lun, A Western, DA Heath, P Smith, LG Moore, and JL Juengel 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.

Menon, KMJ, and B Menon 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, and H Bose 2011 Early steps in steroidogenesis: intracellular cholesterol trafficking. *Journal of lipid research* **52** 2111-2135.

Miller, WLaA, Richard J 2011 The Molecular Biology, Biochemistry, and Physiology of Human Steroidogenesis and Its Disorders. *Endocrine Reviews,* **32(1):** 81–151.

Mitsui, Y, H Hirata, N Arichi, M Hiraki, H Yasumoto, I Chang, S Fukuhara, S Yamamura, V Shahryari, G Deng, S Saini, S Majid, R Dahiya, Y Tanaka, and H Shiina 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, Y Kamiya, and M Morikawa 2010 Bone morphogenetic protein receptors and signal transduction. *Journal of Biochemistry* **147** 35-51.

Miyazono, K, S Maeda, and T Imamura 2005 BMP receptor signaling: transcriptional targets, regulation of signals, and signaling cross-talk. *Cytokine Growth Factor Rev* **6** 251 - 263.

Miyoshi, T, F Otsuka, K Inagaki, H Otani, M Takeda, J Suzuki, J Goto, T Ogura, and H Makino 2007 Differential regulation of steroidogenesis by bone morphogenetic proteins in granulosa cells: Involvement of extracellularly regulated kinase signaling and oocyte actions in follicle-stimulating hormone-induced estrogen production. *Endocrinology* **148** 337-345.

Monniaux, D, F Clément, R Dalbiès-Tran, A Estienne, S Fabre, C Mansanet, and P Monget 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, RK, F Otsuka, and S Shimasaki 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, RK, F Otsuka, and S Shimasaki 2003 Molecular basis of bone morphogenetic protein-15 signaling in granulosa cells. *Journal of Biological Chemistry* **278** 304-310.

Mulsant, P, F Lecerf, S Fabre, L Schibler, P Monget, I Lanneluc, C Pisselet, J Riquet, D Monniaux, I Callebaut, E Cribiu, J Thimonier, J Teyssier, L Bodin, Y Cognié, N Chitour, and J-M Elsen 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, MO Faure, JR McNeilly, J Fontaine, C Taragnat, and AS McNeilly 2008 Bone morphogenetic protein-4 interacts with activin and GnRH to modulate gonadotrophin secretion in LbetaT2 gonadotrophs. *Journal of Endocrinology* **196** 497-507.

Nielsen, ME, IA Rasmussen, SG Kristensen, ST Christensen, K Møllgård, E Wreford Andersen, AG Byskov, and C Yding Andersen 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, EE, and MK Skinner 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, O Yoshino, Y Osuga, J Shi, H Hiroi, T Yano, and Y Taketani 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, Y Yung, E Maman, N Rubinstein, GM Yerushalmi, J Haas, E Barzilay, and A Hourvitz 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, RK Moore, and S Shimasaki 2001a Biological Function and Cellular Mechanism of Bone Morphogenetic Protein-6 in the Ovary. *Journal of Biological Chemistry* **276** 32889-32895.

Otsuka, F, RK Moore, and S Shimasaki 2001b Biological function and cellular mechanism of bone morphogenetic protein-6 in the ovary. *J Biol Chem* **276**.

Otsuka, F, and S Shimasaki 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, S Yamamoto, G Erickson, and S Shimasaki 2001c Bone morphogenetic protein-15 inhibits follicle-stimulating hormone (FSH) action by suppressing FSH receptor expression. *J Biol Chem* **276** 11387 - 11392.

Pangas, SA 2012 Regulation of the ovarian reserve by members of the transforming growth factor beta family. *Molecular reproduction and development* **79** 666-679.

Picton, HM, and AS McNeilly 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, C Pisselet, J Dupont, B Mandon-Pepin, D Monniaux, P Monget, and S Fabre 2004 Molecular basis of bone morphogenetic protein-4 inhibitory action on progesterone secretion by ovine granulosa cells. *J Mol Endocrinol* **33** 805 - 814.

Pulkki, M, D Mottershead, A Pasternack, P Muggalla, H Ludlow, M van Dinther, S Myllymaa, K Koli, P ten Dijke, M Laitinen, and O Ritvos 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 Rev*. *Genet*. **4** 690-700.

Reddy, P, L Liu, D Adhikari, K Jagarlamudi, S Rajareddy, Y Shen, C Du, W Tang, xe, xe, xe, T inen, SL Peng, Z-J Lan, AJ Cooney, I Huhtaniemi, and K Liu 2008 Oocyte-Specific Deletion of Pten Causes Premature Activation of the Primordial Follicle Pool. *Science* **319** 611-613.

Regan, SLP, PG Knight, J Yovich, J Stanger, Y Leung, F Arfuso, A Dharmarajan, and G Almahbobi 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, SLP, PG Knight, JL Yovich, JD Stanger, Y Leung, F Arfuso, A Dharmarajan, and G Almahbobi 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, SLP, JR McFarlane, T O'Shea, N Andronicos, F Arfuso, A Dharmarajan, and G Almahbobi 2015a Flow cytometric analysis of FSHR, BMPR1B, LHR and apoptosis in granulosa cells and ovulation rate in merino sheep. *Reproduction* **150** 151-163.

Regan, SLP, JR McFarlane, T O'Shea, N Andronicos, F Arfuso, A Dharmarajan, and G Almahbobi 2015b Flow cytometric analysis of FSHR, BMRR1B, LHR and apoptosis in granulosa cells and ovulation rate in merino sheep. *Reproduction* **150** 151-163.

Rice, S, K Ojha, S Whitehead, and H Mason 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 & Metabolism* **92** 1034-1040.

Richards, JS 1994 Hormonal Control of Gene Expression in the OVary. *Endocrine reviews* **15** 725-751.

Robinson, RS, KJ Woad, AJ Hammond, M Laird, MG Hunter, and GE Mann 2009 Angiogenesis and vascular function in the ovary. *Reproduction* **138** 869-881.

Rodgers, RJ, and HF Irving-Rodgers 2010 Formation of the Ovarian Follicular Antrum and Follicular Fluid. *Biology of Reproduction* **82** 1021-1029.

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

Seger, R, T Hanoch, R Rosenberg, A Dantes, WE Merz, JF Strauss, and A Amsterdam 2001 The ERK Signaling Cascade Inhibits Gonadotropin-stimulated Steroidogenesis. *Journal of Biological Chemistry* **276** 13957-13964.

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

Sen, A, H Prizant, A Light, A Biswas, E Hayes, H-J Lee, D Barad, N Gleicher, and SR Hammes 2014 Androgens regulate ovarian follicular development by increasing follicle stimulating hormone receptor and microRNA-125b expression. *Proceedings of the National Academy of Sciences* **111(8)** 3008-3013.

Shi, J, O Yoshino, Y Osuga, I Akiyama, M Harada, K Koga, A Fujimoto, T Yano, and Y Taketani 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, O Yoshino, Y Osuga, K Koga, Y Hirota, T Hirata, T Yano, O Nishii, and Y Taketani 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, O Yoshino, Y Osuga, K Koga, Y Hirota, E Nose, O Nishii, T Yano, and Y Taketani 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, O Yoshino, Y Osuga, O Nishii, T Yano, and Y Taketani 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, R Moore, F Otsuka, and G Erickson 2004 The bone morphogenetic protein system in mammalian reproduction. *Endocr Rev* **25** 72 - 101.

Shimasaki, S, RJ Zachow, D Li, H Kim, S-i Iemura, N Ueno, K Sampath, RJ Chang, and GF Erickson 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, MK 2005 Regulation of primordial follicle assembly and development. *Hum Reprod Update* **11** 461-471.

Slot, KA, J Kastelijn, A Bachelot, PA Kelly, N Binart, and KJ Teerds 2006 Reduced recruitment and survival of primordial and growing follicles in GH receptor-deficient mice. *Reproduction* **131** 525-532.

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

Sugawara, T, M Saito, and S Fujimoto 2000 Sp1 and SF-1 Interact and Cooperate in the Regulation of Human Steroidogenic Acute Regulatory Protein Gene Expression. *Endocrinology* **141** 2895-2903.

Sullivan, MW, A Stewart Akers, JS Krasnow, SL Berga, and AJ Zeleznik 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.

Suzuki, N, N Yoshioka, S Takae, Y Sugishita, M Tamura, S Hashimoto, Y Morimoto, and K Kawamura 2015 Successful fertility preservation following ovarian tissue vitrification in patients with primary ovarian insufficiency. *Human Reproduction* **30** 608-615.

Sweeney, MD, S Ayyadurai, and BV Zlokovic 2016 Pericytes of the neurovascular unit: key functions and signaling pathways. *Nat Neurosci* **19** 771-783.

Tajima, K, A Dantes, Z Yao, K Sorokina, F Kotsuji, R Seger, and A Amsterdam 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.

Takeda, M, F Otsuka, H Takahashi, K Inagaki, T Miyoshi, N Tsukamoto, H Makino, and M Lawson 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.

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

Taylor, PD, H Wilson, SG Hillier, SJ Wiegand, and HM Fraser 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.

ten Dijke, P, O Korchynskyi, G Valdimarsdottir, and M Goumans 2003 Controlling cell fate by bone morphogenetic protein receptors. *Mol Cell Endocrinol* **211** 105 - 113.

Val, P, A-M LefranÃƒ ois-Martinez, G VeyssiÃƒ re, and A Martinez 2003 SF-1 a key player in the development and differentiation of steroidogenic tissues. *Nuclear receptor* **1** 8-8.

van Rooij, IAJ, FJM Broekmans, GJ Scheffer, CWN Looman, JDF Habbema, FH de Jong, BJCM Fauser, APN Themmen, and ER te Velde 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, M Hayashi, C Klein, and A Hsueh 2000 Growth differentiation factor-9 stimulates proliferation but suppresses the follicle-stimulating hormone-induced differentiation of cultured granulosa cells from small antral and preovulatory rat follicles. *Biol Reprod* **62** 370 - 377.

Webb, R, Campbell BK. 2007 Development of the dominant follicle: mechanisms of selection and maintenance of oocyte quality. *Society of Reproduction and Fertility supplement* **vol:64** 141 -163

Weenen, C, JSE Laven, ARM von Bergh, M Cranfield, NP Groome, JA Visser, P Kramer, BCJM Fauser, and APN Themmen 2004 Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. *Mol. Hum. Reprod.* **10** 77-83.

Westergaard, L, IJ Christensen, and KP McNatty 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, X-Y Wu, JL Juengel, IK Ross, JM Lumsden, EA Lord, KG Dodds, GA Walling, JC McEwan, AR O'Connell, KP McNatty, and GW Montgomery 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, L Tang, J Cai, X-E Lu, J Xu, X-M Zhu, Q Luo, and H-F Huang 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, HA Garverick, GW Smith, MF Smith, SA Hamilton, and RS Youngquist 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.

Yamamoto, T, F Saatcioglu, and T Matsuda 2002 Cross-talk between bone morphogenic proteins and estrogen receptor signaling. *Endocrinology* **143** 2635-2642.

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, and GQ Zhao 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, Bao, B, Garverick, HA, Youngquist, RS, Lucy, MC 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. *Domest Anim Endocrinol*. **15(1) 55-63**

Yuan, W, and L Giudice 1997 Programmed Cell Death in Human Ovary Is a Function of Follicle and Corpus Luteum Status. *The Journal of Clinical Endocrinology & Metabolism* **82** 3148-3155.

Zeleznik, AJ 2001 Follicle selection in primates: "many are called but few are chosen". *Biology of Reproduction* **65** 655-659.

Zhang, Y-M, and SK Roy 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, Song Y, Cao B 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.