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The bone morphogenetic protein system and the regulation of ovarian follicle development in mammals

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Summary

The bone morphogenetic protein (BMP) family consists of several growth factor proteins that belong to the transforming growth factor-β (TGF-β) superfamily. BMPs bind to type I and type II serine–threonine kinase receptors, and transduce signals through the Smad signalling pathway. BMPs have been identified in mammalian ovaries, and functional studies have shown that they are involved in the regulation of oogenesis and folliculogenesis. This review summarizes the role of the BMP system during formation, growth and maturation of ovarian follicles in mammals.

Keywords: Folliculogenesis, Oogenesis, Ovary, Transforming growth factor

Introduction

BMP active compounds have originally been identified in demineralized bone extracts that were capable to induce bone formation in ectopic sites (Urist, 1965). BMP family proteins have amino acid sequences similar to members of the TGF-β superfamily, which has over 35 members (Chang *et al.*, 2002). BMPs can be classified into several subgroups, including the BMP-2 and -4 group, the BMP-5, -6, -7 and -8 group and the BMP-9, -10 and -15 group (Ducy & Karsenty, 2000). The pattern of BMP expression has been described in various tissues and their proteins have become commercially available. It has been demonstrated in recent studies in vitro that BMP-2, -4 and -8 control primordial germ cell (PGC) formation (Dudley *et al.*, 2010), while BMP-6, -7 and -15 regulate primordial follicle activation and viability (Araújo *et al.*, 2010b; Celestino *et al.*, 2011), growth of secondary follicles (Frota *et al.*, 2011; Passos *et al.*, 2013) and maturation of antral follicles (Shimasaki *et al.*, 2004). Several BMPs have been described as autocrine and/or paracrine factors that regulate the development of ovarian follicle development.

Folliculogenesis is regulated by a delicate balance between endocrine and intra-ovarian factors (Artini *et al.*, 2007). Oocyte growth and maturation within pre-antral and antral follicle stages, respectively, are dependent on intra-ovarian factors, which in antral stages particularly are factors present in follicular fluid (Hsieh *et al.*, 2009; Padhy *et al.*, 2009). These follicular fluid factors are positively related to levels of these factors in blood serum (Qiao & Feng, 2011). Any imbalance or dysfunction among intra-ovarian factors can result in abnormal folliculogenesis and disorder in oocyte growth and maturation (Frank *et al.*, 2008). Altered balance of different factors, along with the intrafollicular fluid microenvironment may directly impair the potential development of the oocyte (Artini *et al.*, 2007; Padhy *et al.*, 2009), which would have a negative impact on fertilization, embryonic development and evolution of pregnancy using oocytes of antral follicles in *in vitro* fertilization systems (Qiao & Feng, 2011).

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In this report, we discuss the roles of the BMP system in regulating mammalian ovarian functions. We will focus on four main areas: (1) the structure of the BMP ligands and receptors; (2) their expression in the ovary; (3) BMP signal transduction and regulation of action; and (4) the role of BMPs on oogenesis and folliculogenesis.

BMP family members

According to Shimasaki *et al.* (2004), the BMP family comprises 18 members. As the discovery of the same or similar genes occurred simultaneously in different groups, different names were given for the same family of genes related to BMP as shown in Table 1. BMPs are regulatory in a series of physiological processes directly related to the functions of ovarian follicles at different stages of development.

Among members of the TGF-β superfamily, BMP stands out among the intra-ovarian factors. A structural feature of members of this superfamily is the presence of seven conserved cysteines, which are involved in the folding of the molecule into a single three-dimensional structure called a cystine knot (reviewed by Vitt *et al.*, 2001), the conserved cysteine residue that is not involved in the formation of cystine knot makes a disulfide bound between the two subunits. This results in the formation of covalently bound dimer, which allows the biological activity of these proteins (Wang *et al.*, 1990).

Members of the TGF-β superfamily are synthesized as precursor proteins that are composed of a signal sequence amino-terminal domain and a pro-mature domain (carboxy-terminal domain). The amino-terminal signal directs the precursor to a signalling pathway, while the pro-domain variable can facilitate dimerization. The signalling of the TGF-β superfamily is regulated at multiple levels, including connection and processing of extracellular ligand interactions and intracellular receptors (Kingsley, 1994; Bragdon *et al.*, 2011).

The BMP family members act through a family of transmembrane receptor, serine/threonine kinase receptors. Based on their structural and functional properties, the BMP receptors are divided into two subfamilies: receptors type I and type II. Receptors type I and II are glycoproteins of approximately 55 kDa and 70 kDa, respectively. The extracellular regions of these receptors contain about 150 amino acids with 10 or more cysteines that determine the folding area. A unique feature of type I receptors is an intracellular region of 30 amino acids immediately preceding the kinase domain. This stretch of 30 amino acids is called the GS (glycine- and serine-rich sequence) domain because of the sequence SGSGSG contained in this region (Wrana *et al.*, 1994).

The type I receptors have received different names, for example, similar to the activin receptor kinase 4 (ALK-4) (Attisano *et al.*, 1996). Due to the promiscuity of the type I receptor for different ligands of the TGF-β superfamily in most cases, the most suitable nomenclature is probably ALK. Receptors for various BMPs and their nomenclature are listed in Table 2.

BMP signal transduction

To exert their biological functions, BMPs bind to BMP receptor IA or IB types, inducing its activation. Then, BMPRII is recruited to promote phosphorylation of BMPRIA or BMPRIB. The binding induces phosphorylation of the GS domain in the type I receptor (Wieser *et al.*, 1995).

Although receptors type I and type II participate in the transduction of intracellular signalling of BMPs, the receptor binding and activity of certain ligands are regulated by co-receptors. The glycosylphosphatidylinositol (GPI) protein family members linked to repulsive molecular orientation (RGM), which includes the RGM-a, -b and -c, are co-receptors for BMP-2 and BMP-4 and reinforce the signalling of BMPs (Samad *et al.*, 2005). Xia *et al.* (2007) stated that RGM-b, also known as hemojuvelin and DRAGON, respectively, interacts with BMP receptors of which type I or type II, bind to BMP-2 and BMP-4, but not BMP-7 and TGF-β1. However, cells transfected with the RGM use both BMPRII and ActR-II for signalling BMP-2/4, suggesting that RGM facilitates the use of ActR-II by BMP-2/4.

SMAD proteins are components of intracellular signal transduction pathways of the TGF-β superfamily. The first member of this family is the protein Mothers Against Decapentaplegic (MAD) that has been identified in *Drosophila melanogaster* (Sekelsky *et al.*, 1995). Other members of this family have been identified based on their homology with MAD. Three MAD counterparts found in *Caenorhabditis elegans* are named as SMA-2, SMA-3, SMA-4 and mutation in their respective gene prevents development (Savage *et al.*, 1996). Homologues of SMA and MAD found in vertebrates are called SMADs, a combination of SMA and MAD (Derynck *et al.*, 1996). At least 10 vertebrate SMAD proteins have been identified so far (Massagué & Wotton, 2000).

The interaction between receptors induces phosphorylation of intracellular messengers (SMAD types 1, 5 and 8), which form complexes with SMAD co-regulator (SMAD-4), that accumulate in the nucleus and act as transcription factors by binding directly

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Table 1 Members of the BMP family and their alternative names

Ligand	Alternative names	References
BMP-1	mTLD	Martínez-Glez *et al*. (2012)
BMP-2	BMP-2A	Dathe *et al*. (2009)
BMP-3	Osteogenin	Hardy & Kramer (2000)
BMP-3B	GDF-10	Tandon *et al*. (2012)
BMP-4	BMP-2B	Nikaido *et al*. (1997)
BMP-5	–	Pierre *et al*. (2005)
BMP-6	Vgr-1	Gitelman *et al*. (1994)
BMP-7	OP-1	Macias *et al*. (1997)
BMP-8a	OP-2	Zhao and Hogan (1996)
BMP-8b	–	Cao *et al*. (2013)
BMP-11	GDF-11	Hannan *et al*. (2009)
BMP-12	GDF-7, CDMP-3	Yeh & Lee (2010)
BMP-13	GDF-6, CDMP-2	Williams *et al*. (2008)
BMP-14	GDF-5, CDMP-1	Chhabra *et al*. (2005)
BMP-15	GDF-9B	Bodin *et al*. (2007)
BMP-16	–	Luckenbach *et al*. (2011)
GDF-3	Vgr-2	Witthuhn & Bernlohr (2001)
GDF-15	PLAB, MIC-1, PDF, PTGF-β	Tanno *et al*. (2010)
MIS	AMH	Lee *et al*. (2003)

AMH: anti-Müllerian hormone; CDMP: cartilage-derived morphogenetic protein; GDF: growth differentiation factor; MIC: macrophage inhibiting cytokine; mTLD: mammalian Tolloid protein; MIS: Müllerian inhibiting substance; OP: osteogenic protein; PDF: prostate-derived fator; PLAB: placental bone morphogenetic protein; PTGF-β: placental TGF-β; Vgr: Vg-related protein;

Table 2 Ligands, receptors and SMADs of the BMP family

Ligand	Receptor type II	Receptor type I	References
BMP-2	BMPR-II	ALK-3 (BMPRIA)	Miyagi *et al.* (2012)
BMP-4	BMPR-II	ALK-6 (BMPRIB)	Miyagi *et al.* (2012)
BMP-6	ActR-II	ALK-2 (ActR-IA)	Goto *et al.* (2007)
BMP-7	ActR-IIB	ALK-6 (BMPRIB)	Glister *et al.* (2004)
BMP-15	BMPRII	ALK-6 (BMPRIB)	Glister *et al.* (2004)

to specific binding sites in the promoters of a target gene (Ohta *et al.*, 2008) or to other transcription factors (Lembong *et al.*, 2008), activating or inhibiting the expression of a gene (Massagué *et al.*, 2005). Figure 1 shows that BMP receptors I and II interaction induces phosphorylation of intracellular messengers (SMADs) that are moved to the nucleus to regulate the expression of specific genes.

BMP signalling can be monitored using antibodies that recognize the phosphorylated SMADs, because the signalling pathway is presented in a highly conserved manner across all species (Lembong *et al.*, 2008). The subcellular localization of phospho-SMAD1/5 passes from the nucleus to the cytoplasm during differentiation of PGCs in oogonia (Childs *et al.*, 2010). Mutations in SMAD-2, -3 and -4 were found in human tumors, suggesting that these genes function as tumor suppressors *in vivo* (Massagué, 1998). For example, human SMAD-4 is also known as DPC4 (deleted in pancreatic carcinoma locus 4), which is often deleted or mutated in pancreatic cancer (Hahn *et al.*, 1996).

Since the discovery of expression of BMP family members in ovarian cells (Israel *et al.*, 1992), many studies have been performed to demonstrate the importance of BMP system in regulating reproductive processes (Childs *et al.*, 2010; Otsuka *et al.*, 2011). At the extracellular level, BMP antagonists (e.g. noggin) interfere with ligand binding to the receptors of BMPs, as noggin binds to BMP receptors. The expression of noggin is potentially induced by the activity of BMPs and thus may contribute to the negative feedback mechanism controlling the action of BMPs (Song *et al.*, 2010), and inhibiting the growth of PGCs in culture systems (Ying & Zhao, 2001).

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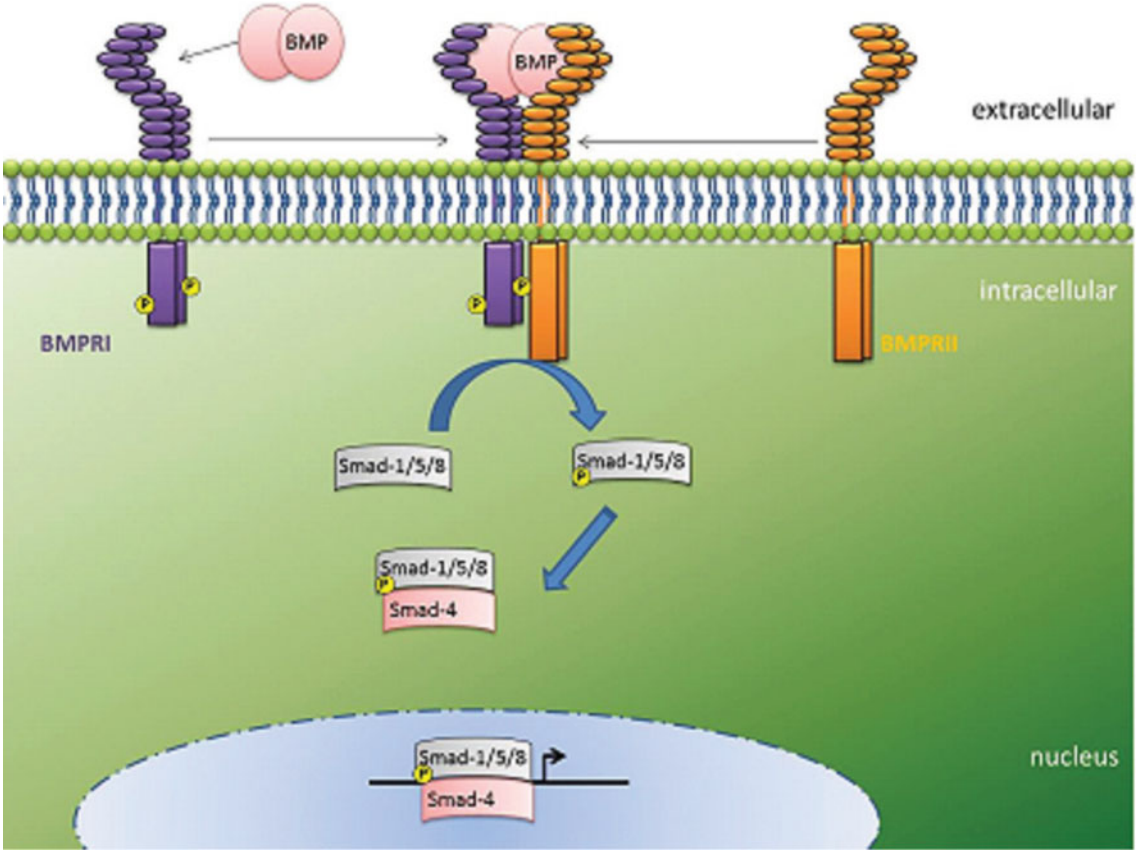

Figure 1 The interaction between BMP receptors I and II induces phosphorylation of intracellular messengers (SMADs) that are moved to the nucleus to regulate the expression of specific genes.

Expression of BMP receptors in the ovary

Several studies have reported the mRNA expression of BMP receptors in different follicular compartments of pre-antral and antral follicles of ruminants (bovine: Fatehi *et al.*, 2005; ovine: Wilson *et al.*, 2001, Souza *et al.*, 2002; caprine: Lima *et al.*, 2012). In the ovaries of murine (Erickson & Shimasaki, 2003) and caprine (Silva *et al.*, 2005), the expression of mRNA for BMPRII was demonstrated in oocytes and granulosa cells from primordial, primary, secondary and antral follicles. In goat antral follicles, the mRNA level of BMPRII showed a peak in 0.5 mm follicles, when compared with those of 0.2 and 1.0 mm (Costa *et al.*, 2012). Higher levels of BMPRII may potentiate the action of BMP-15 and GDF-9 in antral follicles, as these factors bind to BMPRII with high affinity (Edwards *et al.*, 2008). However, the expression of BMPRII and activin receptor-like kinase type 5 (ALK-5) in caprine follicles cultured *in vitro* is downregulated by follicle-stimulating hormone (FSH) (Frota *et al.*, 2011; Costa *et al.*, 2012).

The mRNA for BMPRIB was reported in ovaries of murine (Erickson & Shimasaki, 2003) and goats (Silva *et al.*, 2005), especially in oocytes, granulosa and theca cells from all classes of follicles. The mRNA level of BMPRIB was highest in goat ovarian follicles of 0.2 mm in relation to those with 1.0 mm (Costa *et al.*, 2012). Edwards *et al.* (2008) showed that BMP-4 binds to BMPRIB. With regard to BMP-15, it was found that BMP-15 could bind to multiple receptors in granulosa cells, i.e. BMPRII, activin type II receptor, ALK-2 and ALK-6. Among these receptors, ALK-6 is the most efficient in binding to BMP-15, while BMPRII is more effective in the bioactivity of BMP-15 (Wu *et al.*, 2012). Thus, BMP-15 signalling may be mediated by initial binding with ALK-6, which recruits BMPRII (Edwards *et al.*, 2008). In human, BMPRIA, BMPRIB, and BMPRII receptors were detectable in granulosa cells (Khalaf *et al.*, 2013). In addition, Kristensen *et al.* (2014) observed that, comparing transcript abundance in five size-matched populations of isolated pre-antral follicles, BMPRII is the most expressed receptors. Recently, a study in humans found that type 1 receptors ALK-3, ALK-4, ALK-5 and ALK-6 are expressed at moderate levels. In comparison, ALK-1, ALK-2, and ALK-7; are generally expressed at a low level in the human pre-antral

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Table 3 Locations of expression of BMP receptors in mammalian ovarian follicles

Type of follicle	Follicular compartment	BMP receptor	Species
Primordial	Oocyte	BMPRIA/IB/II	Ovine/Caprine
		BMPR-II	Murine/Bovine
	Granulosa cells	BMPR-II	Murine/Bovine
		BMPRIB/II	Caprine/Ovine
Primary	Oocyte	BMPRIA/IB/II	Ovine/Caprine
		BMPRII	Murine/Bovine
	Granulosa cells	BMPRII	Rat/Bovine
		BMPRIB/II	Caprine
		BMPRIA/IB/II	Ovine
Secondary	Oocyte	BMPRIA/IB/II	Ovine/Caprine
		BMPRII	Murine/Bovine
	Granulosa cells	BMPRII	Murine/Bovine
		BMPRIB/II	Caprine
		BMPRIA/IB/II	Ovine
	Theca cells	BMPRIB/II	Caprine
Antral	Oocyte	BMPRIA/IB/II	Ovine/Caprine
		BMPRII	Murine/Bovine
	Granulosa cells	BMPRII	Murine
		BMPRIA/II	Bovine
		BMPRIA/IB/II	Ovine/Caprine
	Theca cells	BMPRIB/II	Caprine
		BMPRIA/IB/II	Ovine

Murine (Erickson & Shimasaki, 2003; Edwards *et al.*, 2008); caprine (Silva *et al.*, 2005; Lima *et al.*, 2012); bovine (Fatehi *et al.*, 2005; Selvaraju *et al.*, 2013); ovine (Wilson *et al.*, 2001; Souza *et al.*, 2002).

follicles (Kristensen *et al.*, 2014). Table 3 shows the sites of expression of BMP receptors in ovarian follicles.

Expression of BMP ligands and their role in oogenesis and folliculogenesis

The expression of BMPs in ovarian tissue has been reported in various studies. Table 4 shows the sites of expression of BMP family members in ovarian follicles. In human, it was demonstrated that BMP-2, BMP-4, BMP-5, BMP-6, BMP-7, and BMP-8A are expressed in ovarian granulosa cells from healthy ovulatory women (Khalaf *et al.*, 2013). Recently, in a study with human follicles that compared transcript abundance in five size-matched populations of isolated pre-antral follicles, BMP-15 and BMP-6 were the most expressed ligands. By comparison, BMP-1, BMP-2, BMP-3, BMP-4 and BMP-8B were generally expressed at a low level, whereas BMP-5, BMP-7, BMP-8A and BMP-10 were undetectable in the human pre-antral follicles (Kristensen *et al.*, 2014).

BMP-1, -2 and -4

In ovine ovaries, immunohistochemical localization demonstrated that BMP-1 was present in granulosa cells at all stages of follicular development, from primordial to large antral follicles, and that the levels of BMP-1 were not affected by the final follicle selection mechanism (Canty-Laird *et al.*, 2010). BMP-1 belongs to the family of metalloproteases and, through its procollagen C-proteinase activity, it can regulate the formation of extracellular matrix (ECM) during ovarian folliculogenesis (Canty-Laird *et al.*, 2010). The importance of the ECM for follicular development is exemplified by the observation that both the growth and atresia of ovine ovarian follicles are associated with dramatic changes in the composition of the collagenous ECM in which the theca and granulosa cells are embedded (Huet *et al.*, 1997, 1998; Berkholtz *et al.*, 2006).

Expression of mRNAs for BMP-2 was reported in granulosa cells of primary, secondary and antral follicles in murine ovaries (Erickson & Shimasaki, 2003), as well as in theca cells of bovine antral follicles (Fatehi *et al.*, 2005). According to Juengel *et al.* (2006), antral follicles in ovine ovaries express BMP-2 mRNA, present in granulosa cells. BMP-2 interacts with BMP-4 and has an additive effect on stimulating PGC generation in mice (Ying & Zhao, 2001). Studies that evaluate the effects of BMP-2 on early folliculogenesis are scarce, but *in vitro* studies with granulosa cells from ovine antral follicles have shown that BMP-2 increases the production of estrogen and inhibin-A after stimulation with FSH, thus promoting the *in*

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Table 4 Locations of expression of BMP family members in mammalian ovarian follicles

Type of follicle	Follicular compartment	BMP ligand	Species
Primordial	Oocyte	BMP-15	Murine
		BMP-6	Ovine
		BMP-6/15	Caprine
		BMP-4/7	Human
	Granulosa cells	BMP-1	Ovine
		BMP-4/7	Human
Primary	Oocyte	BMP-6	Ovine
		BMP-6/15	Caprine
		BMP-4/7	Human
		BMP-15	Murine
	Granulosa cells	BMP-1	Ovine
		BMP-2	Murine
		BMP-15	Caprine
		BMP-4/7	Human
	Theca cells	BMP-4	Murine
Secondary	Oocyte	BMP-6/15	Caprine/Murine
		BMP-6	Ovine
		BMP-4/7	Human
	Granulosa cells	BMP-1	Ovine
		BMP-2/6	Murine
		BMP-4/7	Human
		BMP-6/15	Caprine
	Theca cells	BMP-2/4/7	Murine
Antral	Oocyte	BMP-6/15	Caprine/Murine
		BMP-6	Ovine
		BMP-2/4/6	Bovine
	Granulosa cells	BMP-1	Ovine
		BMP-2/5/6	Murine
		BMP-4/6/7	Bovine
		BMP-6/7/15	Caprine
	Theca cells	BMP-2/3/3b/4/7	Murine
		BMP-2/4/6/7	Bovine
		BMP-7	Caprine
	Follicular fluid	BMP-2/15	Human

Rat (Erickson & Shimasaki, 2003; Pierre *et al.*, 2005; Juengel *et al.*, 2006); bovine (Glister *et al.*, 2004; Fatehi *et al.*, 2005); ovine (Juengel *et al.*, 2006; Canty-Laird *et al.*, 2010); caprine (Silva *et al.*, 2005; Frota *et al.*, 2013); human (Abir *et al.*, 2008; Sugiyama *et al.*, 2010; Wu *et al.*, 2012).

vitro differentiation of granulosa cells (Souza *et al.*, 2002). However, BMP-2 suppresses the synthesis of estradiol and androstenedione, while progesterone was shown to stimulate proliferation of porcine theca cells (Brankin *et al.*, 2005).

BMP-4 mRNA and protein are expressed in murine theca cells (Erickson & Shimasaki, 2003), as well as in granulosa, theca cells (Glister *et al.*, 2004) and oocytes from antral follicles in bovine species (Fatehi *et al.*, 2005). Several studies have demonstrated that BMP-4 is the main regulator of PGC formation in mammals (reviewed by Saitou & Yamaji, 2010). Analysis by real-time PCR showed that expression of BMP-4 increases during the transition from the PGC proliferation stage to the meiotic stage of differentiation (Lawson *et al.*, 1999; Ying *et al.*, 2000; Ross *et al.*, 2003). Additionally, BMP-4 causes a significant increase in expression of genes in PGCs and appears to be required for differentiation of human embryonic stem cells into PGCs (West *et al.*, 2010). BMP-4 furthermore promotes proliferation of PGCs in mouse (Ross *et al.*, 2003) and human (Childs *et al.*, 2010) gonads, and acts as a regulator of gametogenesis in multiple stages of development.

The earliest stages of folliculogenesis are also regulated by BMP-4 (Nilsson & Skinner, 2003). Ding *et al.* (2013) showed that BMP-4/SMAD signalling pathway initiates primordial follicle growth and prevents oocyte apoptosis. Moreover, BMP-4 was found to contribute in the reduction of the apoptosis levels of granulosa cells cultured *in vitro*. In this respect, BMP-4 stimulated the expression of survivin

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mRNA, but did not affect the expression of B-cell lymphoma-extra large (bcl-xL) or B-cell lymphoma-2-associated X (bax), which are pro-apoptotic genes (Kayamori *et al.*, 2009). BMP-4 also acts as an inhibitor of premature luteinization of granulosa cells from antral follicles enabling the required growth to become preovulatory follicles (Shimasaki *et al.*, 2004). Regarding the effects of BMP-4 in the final stages of oocyte maturation, Fatehi *et al.*, (2005) reported that addition of BMP-4 or BMP-2 during *in vitro* maturation of bovine COCs does not affect the process of cumulus cell expansion and oocyte maturation, as well as the formation and quality of blastocysts after *in vitro* fertilization. However, during culture of granulosa cells from antral follicles, BMP-4 potentiates the action of FSH by increasing the production of estradiol and inhibiting the synthesis of progesterone (rat: Shimasaki *et al.*, 1999; ovine: Mulsant *et al.*, 2001). In addition, BMP-2 and -4 reduced the mRNA expression of inhibitory SMAD-6 and -7 in cultured rat granulosa cells, leading to enhancement of BMP actions detected by DNA binding proteins of type 1 (Id-1) transcription (West *et al.*, 2010). These latter results revealed that ovarian steroidogenesis was modulated by specific somatostatin receptor (SSTR) activation, which occurs by upregulating endogenous BMP activity in growing follicles.

At different levels, the BMPs signalling pathway is controlled by positive and negative regulators. BMP-4 interacts with activin and GnRH to modulate the secretion of FSH in gonadotropic cells (Lee *et al.*, 2007; Nicol *et al.*, 2008). Ho & Bernard, (2009) demonstrated that BMP-2 induces phosphorylation of SMAD-1/-5 and promotes an increase in the expression of the β subunit of FSH in these cells. At different concentrations, BMP-4 increases FSH-induced estradiol production in rat granulosa cells and does not alter forskolin-induced estradiol production. Moreover, BMP-4 inhibits the FSH-induced progesterone production. However, the latter effects were not detected when rat granulosa cells were co-cultured with oocytes. In co-cultures of rat granulosa cells with oocytes both BMP-2 and BMP-4 inhibit synthesis of progesterone and estradiol, and stimulate synthesis of the p38-mitogen-activated protein kinase (p38MAPK) pathway by suppressing cAMP (Inagaki *et al.*, 2009).

In human granulosa cells there is an interrelationship between the BMP system and FSH. BMPs initially inhibit expression of the FSH receptors, while FSH stimulates endogenous BMP signalling. This balance is important for *in vitro* maintenance and development of granulosa cells (Miyoshi *et al.*, 2006). Table 5 shows the effects of BMP-1, -2 and -4 on oogenesis and folliculogenesis in the different mammalian species.

BMP-5, -6, -7 and -8

BMP-5 is expressed in porcine and murine ovaries (Shimizu *et al.*, 2004; Pierre *et al.*, 2005). Pierre *et al.* (2005) studied the expression of BMP-5 in rat ovaries by *in situ* hybridization, and found that mRNA was mainly expressed in granulosa and cumulus cells of early to large antral follicles from both healthy and atretic follicles; whereas low expression was detected in oocytes of small pre-antral follicles. In early follicles, BMP-5 is assumed to be involved in the development of secondary follicles but not primary (Shimizu *et al.*, 2004). In mice, BMP-5 enhanced the proliferation of granulosa cells from antral follicles, which was associated with an increase in cyclin D2 expression (Pierre *et al.*, 2005). Additionally, these authors also showed BMP-5 caused a marked dose-dependent inhibition of the FSH-induced progesterone production.

It has been demonstrated that BMP-6 is present in oocytes of all follicular categories in different species (murine: Erickson & Shimasaki, 2003; ovine: Juengel *et al.*, 2006; cattle: Glister *et al.*, 2004; goats: Frota *et al.*, 2013), as well as in granulosa and theca cells of several species (murine: Erickson & Shimasaki, 2003; ovine: Juengel *et al.*, 2006; cattle: Glister *et al.*, 2004). In caprine species, the levels of mRNA for BMP-6 in primary and secondary follicles are significantly higher than those observed in primordial follicles (Frota *et al.*, 2013). In this same species, addition of BMP-6 to culture medium promoted atresia in primordial follicles in organic culture (Araújo *et al.*, 2010b). Additionally, in *in vitro* cultured caprine secondary follicles, BMP-6, FSH and a combination of these compounds significantly increase follicular diameter and antrum formation (Frota *et al.*, 2013). In swine, in bigger follicles, Brankin *et al.* (2005) reported that BMP-6 maintained the theca cell viability during a culture period of 6 days. However, differences in cell types and animal species must be considered to understand the modes of BMP-6 action.

BMP-6 acts on bovine granulosa cells *in vitro* by stimulating their proliferation, promoting their viability and increasing their production of inhibin-A, activin-A and follistatin (Glister *et al.*, 2004). BMP-6 treatment reduces the secretion of forskolin-induced progesterone by cultured granulosa cells and luteinized theca cells *in vitro*, in a dose-dependent manner, without affecting the number of viable cells at the end of the growing period. Furthermore, treatment of cultured granulosa cells with BMP-6 decreased levels of forskolin-induced cytochrome P450 family, but had no significant effects on transcription levels of StAR and 3β-hydroxysteroid dehydrogenase/isomerase (HSD3B1) in the presence or absence of forskolin. BMP-6 also increased basal

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Table 5 Effects of BMP-1, -2 and -4 on oogenesis and folliculogenesis in the different mammalian species

Protein	Species	Cell type	Action of BMP
BMP-1	Ovine	Theca cells	Regulate the formation of ECM during folliculogenesis
BMP-2	Ovine	Granulosa cells	Increases the production of estrogen and inhibin-A, promoting the differentiation
	Human	Granulosa cells	Enhances the secretion of inhibin-B, reduces the expression of PCSK6
	Porcine	Theca cells	Suppresses the synthesis of estradiol and androstenedione
	Murine	Granulosa cells	Promotes expression of FSH-induced P450- aromatase, decreases the mRNA levels for FSH-induced StAR protein
BMP-4	Human	Embryonic stem cells	Promotes differentiation of *stem cells* into PGCs
		Primordial germ cells	Enhances genes expression and proliferation
	Murine	PGCs	Proliferation
		Granulosa cells	Increases FSH-induced estradiol production, inhibits premature luteinization
		Theca cells	Inhibits the production of FSH-induced progesterone
	Bovine	Granulosa cells	Reduces apoptosis levels, stimulates the expression of survivin mRNA, enhances the production of estradiol, inhibin and follistatin-A, inhibits progesterone production
	Ovine	Granulosa cells	Increases the production of estradiol and inhibit the synthesis of progesterone, decrease the expression of cAMP, StAR and P450scc

Murine (Shimasaki *et al.*, 1999; Lawson *et al.*, 1999; Ying *et al.*, 2000; Ross *et al.*, 2003; Ho & Bernard, 2009; Inagaki *et al.*, 2009; West *et al.*, 2010); bovine (Fatehi *et al.*, 2005); ovine (Mulsant *et al.*, 2001; Souza *et al.*, 2002); human (Miyoshi *et al.*, 2006; Childs *et al.*, 2010); porcine (Brankin *et al.*, 2005).

expression of cytochrome P450 mRNA. Follistatin partially reverses the suppressive effect of BMP-6 on the secretion of progesterone in granulosa cells and theca cells. Additionally, BMP-6 decreases the secretion of activin-A in presence of forskolin (Kayani *et al.*, 2009).

In mouse granulosa cells, BMP-6 inhibits the expression of LH receptors and progesterone synthesis, through inhibition of steroidogenic enzymes (Otsuka *et al.*, 2001). BMP-6 acts by retarding the follicular differentiation process, providing the rapid growth of follicles through the multiplication of granulosa cells. This growth factor decreases drastically during the selection of the dominant follicle, the reduction possibly being related to the mechanism by which dominant follicles are selected (Shimasaki *et al.*, 2004). Administration of BMP-6 to aged female mice during superovulation significantly stimulates ovarian mRNA and protein expression of vascular endothelial growth factor (VEGF), which is involved in angiogenesis (Park *et al.*, 2012). Developing follicles require a higher nutrient availability carried by newly vessels, formed from the previous stimulation of angiogenic factors, such as VEGF. Thus, during ovulation induction, BMP-6 plays an important role in the improvement of oocyte quality and ovarian response of aged female, possibly by regulating of ovarian VEGF expression. Moreover, BMP-6 might play a role in ovulation by increasing the accumulation of neutrophils in the ovulatory follicle and suppressing the effect of protease inhibitors (Akiyama *et al.*, 2014).

BMP-7 is produced by theca cells of secondary and antral follicles (Shimasaki *et al.*, 2004). Human fetal follicles also express the mRNA for BMP-7 in granulosa cells, oocytes and oogonia (Abir *et al.*, 2008). In goat antral follicles, the levels of BMP-7 mRNA are significantly higher in mural granulosa/theca cells from large antral follicles (>3 mm) than those observed in small antral follicles (<3 mm) (Frota *et al.*, 2013). In follicles of murine species, however, the mRNA expression of BMP-7 was restricted to granulosa cells (Lee *et al.*, 2004), but in ovine follicles, BMP-7 was detected in all follicular compartments (Juengel *et al.*, 2006).

In vitro studies demonstrated that BMP-7 promotes growth and activation of primordial follicles, and stimulates the expression of receptors for FSH in murine ovaries (Lee *et al.*, 2004). BMP-7 also contributed to maintain the survival and ultrastructure of primordial follicles after caprine ovarian tissue

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culture (Araújo *et al.*, 2010a). After injection of BMP-7 in the rat ovarian bursa increased the number of primary, secondary and antral follicles, indicating that, in murine, the BMP-7 is able to promote the growth and activation of primordial follicles (Lee *et al.*, 2001). Additionally, BMP-7 alone or together with FSH significantly increased growth and antrum formation in cultured caprine follicles (Frota *et al.*, 2011).

Human granulosa cells from antral follicles cultured in presence of BMP-6 or -7 showed a significant increase in expression of GDF-3 mRNA (Shi *et al.*, 2012). The authors furthermore found that growth of cultured human granulosa cells was linked to the presence of GDF-3, BMP-6 or BMP-7. In a previous study with *in vitro* human granulosa cells, Shi *et al.* (2010) reported a BMP-7 induced reduction in LH receptor gene expression. In addition, cultured human granulosa cells show a reduction in the order of 50–60% in the expression of PCSK6, when cultured in the presence of BMP-2, -6, -7 or -15 (Akiyama *et al.*, 2012). PCSK is a protease that cleaves NODAL (protein belonging to the TGF-β superfamily; see Table 1) into an active form (Constam & Robertson, 2000). In addition, BMP-7 increases the expression of cytochrome P450 enzyme aromatase favoring the production of estradiol *in vivo* (Lee *et al.*, 2001) and *in vitro* (Miyoshi *et al.*, 2007). Granulosa cells showed a reduction in the level of carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD) protein after their culture in medium supplemented with BMP-7. BMP-7 significantly increased mRNA expression of X-linked inhibitor of apoptosis protein (XIAP), and BMP-7 furthermore significantly decreased the expression of caspase-3 and caspase-9 (Kayamori *et al.*, 2009). Because progesterone is important for the process of ovulation in mammals (Yoshimura & Wallach, 1987), inhibition of progesterone production by BMP-7 can be related to the mechanisms of ovulation inhibition (Lee *et al.*, 2001).

Expression of BMP-8 in the ovary was still not reported, but its effect on the formation of PGCs was demonstrated by Ying *et al.*, (2000). These authors showed that BMP-8 is expressed in the extra-embryonic ectoderm of mouse embryos, and that a mutation in the gene which produces this protein results in a decrease in the population of PGCs. Such a decrease may lead to a reduction in the follicle population in adult animals. Table 6 shows the effects of BMP-5, -6, -7 and -8 on oogenesis and folliculogenesis in the different mammalian species.

BMP-15

Several studies have shown that BMP-15 is involved in the normal fertility in mammals, playing a role in the regulation of follicular (Kedem *et al.*, 2011; Passos *et al.*, 2013) and cumulus cell functions (Erickson & Shimasaki, 2001; Teixeira Filho *et al.*, 2002; Gilchrist *et al.*, 2008; Chen *et al.*, 2009). The expression of mRNAs for BMP-15 was detected in primordial, primary and secondary follicles, as well as in oocytes and granulosa cells from goat antral follicles (Silva *et al.*, 2005). BMP-15 protein was detected in oocytes of all types of follicles and in granulosa cells from primary follicles onward (Silva *et al.*, 2006). BMP-15 was shown to be expressed in primary oocytes from follicles of rodents, ovine and humans (Juengel & McNatty, 2005). BMP-15 was also detected in follicular fluid of human follicles (Wu *et al.*, 2007). In addition, data from real-time PCR showed a significant reduction in the levels of BMP-15 transcripts in cultured follicles, with a decrease of two times in its levels during follicular development (Sánchez *et al.*, 2009).

Mutations in the BMP-15 are related with the occurrence of diseases directly related to reproductive activity, such as polycystic ovary syndrome and premature ovarian failure (Takebayashi *et al.*, 2000). Immunization studies showed that BMP-15 proteins are involved in the normal ovine follicular development, including both the early and later stages of growth (Juengel *et al.*, 2002; Wu *et al.*, 2007). During transition from primordial to primary follicles, BMP-15 is involved in the ovine granulosa cell proliferation or for the prevention of their differentiation (Galloway *et al.*, 2000). Lima *et al.* (2012) showed that BMP-15 is effective in promoting antral cavity formation and maintenance of follicular growth during culture of goat pre-antral follicles. A recent study demonstrated that BMP15 down-regulates connexin 43 (Cx43) in human granulosa cells. In this study, the reduction of Cx43 contributes to a decrease in the activity of intercellular communication, which indicates that the Smad1/5/8 signalling pathway is required for the BMP15-induced down-regulation of Cx43 (Chang *et al.*, 2014). However, Sugimura *et al.* (2014) showed that BMP15 was equally effective in maintaining gap-junctional communication (GJC) even in the presence of amphiregulin. They also reported that amphiregulin stimulation of COC glycolysis and BMP15 preservation of GJC may facilitate efficient transfer of metabolites from cumulus cells to the oocyte thereby enhancing oocyte developmental competence. In bovine species, BMP15 stimulates expansion of *in vitro*-matured bovine COCs by driving glucose metabolism toward hyaluronic acid production and controlling the expression of genes in the ovulatory cascade (Caixeta *et al.*, 2013).

BMP-15 is capable of maintaining a low incidence of apoptosis in cumulus cells (Wu *et al.*, 2007). Studies on rat granulosa cells demonstrated that recombinant BMP-15 stimulates the proliferation of

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Table 6 Effects of BMP-5, -6, -7 and -8 on oogenesis and folliculogenesis in the different mammalian species

Protein	Species	Follicle and/or cell type	Action of BMP
BMP-5	Porcine	Granulosa cells	Proliferation
BMP-6	Bovine	Granulosa cells	Proliferation, viability and production of inhibin-A, activin-A and follistatin
	Caprine	Primordial follicles	Negatively affects the survival and ultrastructure
		Secondary follicles	Increases follicular diameter and antrum formation
	Human	Granulosa cells	Increases expression of GDF-3 mRNA, reduction expression of PCSK6
	Murine	Granulosa cells	Inhibits the expression of LH receptors and progesterone synthesis, stimulates expression of Id-1 and VEGF
	Bovine	Granulosa cells	Reduces the secretion of forskolin-induced progesterone and activin-A, CYP11A1, increased basal expression of CYP17A1
		Theca cells	Promotes luteinization
BMP-7	Human	Granulosa cells	Increases expression of GDF-3, reduces LH receptor and expression of PCSK6
	Caprine	Primordial follicles	Improve the survival and growth
		Secondary follicles	Increases growth and antrum formation
		Primordial follicles	Promotes growth and activation
	Murine	Granulosa cells	Stimulates the expression of FSHR and mitosis, inhibits the production of progesterone
		Granulosa cells	Increases estradiol production and inhibits progesterone synthesis
	Bovine	Granulosa cells	Reduces CAD protein, expression of caspase-3 and caspase-activated 9, stimulates expression of surviving mRNA and XIAP mRNA
BMP-8	Murine	PGCs	Proliferation

Murine (Shimasaki *et al.*, 1999; Ying *et al.*, 2000; Lee *et al.*, 2001; Otsuka *et al.*, 2001; Lee *et al.*, 2004; Park *et al.*, 2012); bovine (Fatehi *et al.*, 2005; Kayani *et al.*, 2009; Kayamori *et al.*, 2009); caprine (Araújo *et al.*, 2010a,b; Frota *et al.*, 2011, 2013); human (Seidah & Chretien, 1999; Shi *et al.*, 2010, 2012); porcine (Shimizu *et al.*, 2004). FSHR, follicle stimulating hormone receptor.

these cells independent of FSH, but decreased the effects of FSH on progesterone production without affecting that of estradiol (Otsuka *et al.*, 2000). BMP-15 furthermore stimulates expression of kit ligand (KL) in rat granulosa cells (Otsuka & Shimasaki, 2002), and it also stimulates the expression of epidermal growth factor (EGF) in cumulus cells of mice (Yoshino *et al.*, 2006).

Oocytes recovered from follicles with high levels of BMP-15 in the follicular fluid had a higher rate of fertilization, cleavage and better quality in the developing embryo. BMP-15 showed a positive correlation with the levels of estradiol and a negative correlation with those of FSH in the follicular fluid. In addition, BMP-15 can serve as an indicator of mature oocyte cytoplasm. BMP-15 can inhibit the action of stimulating FSH production of pregnancy-associated plasma protein-A (PAPP-A) in a dose-dependent manner. In addition, by controlling the expression of PAPP-A in granulosa cells, BMP-15 and FSH play a role in the selection of the dominant follicle and oocyte maturation (Wu *et al.*, 2012). Table 7 summarizes the effects of BMP-15 on oogenesis and folliculogenesis in the different mammalian species.

Recent studies have demonstrated in different animals (i.e. mouse, rat, pig, ovine, cow and red deer) that the expression of BMP-15 and GDF-9 is tightly co-regulated (Crawford & Mcnatty, 2012). In fact, BMP-15 and GDF-9 play roles in regulating cumulus cell functions through the processes of mitosis, proliferation, apoptosis, luteinization, metabolism and expansion through mitogenic signalling transduction mechanisms (Qiao & Feng, 2011). After immunizing mice against selected proregion peptide sequences of BMP-15 and GDF-9 or a recombinant full-length mouse proregion GDF-9 protein, McIntosh *et al.* (2012) showed that both factors have physiologically important roles in regulating ovulation rate and litter size in mice. Peng *et al.* (2013) demonstrated that GDF-9:BMP-15 heterodimers are the most bioactive ligands in the regulation of cumulus expansion genes in mice and human. GDF-9 and BMP-15 play crucial roles in

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Table 7 Effects of BMP-15 on oogenesis and folliculogenesis in the different mammalian species

Species	Follicular compartment	Action of BMP-15
Ovine	Granulosa cells	Proliferation
Human	Primordial follicles	*In vitro a*ctivation
	Cumulus cells	Proliferation and modulate FSH-dependent cytodifferentiation
	Follicular fluid	Predicting oocyte quality and subsequent embryo development, is correlated with estradiol level
Bovine	Secondary follicles	*In vitro* growth
Caprine	Primordial follicles	*In vitro* activation
	Secondary follicles	*In vitro* growth
Murine	Granulosa cells	Proliferation, decrease progesterone production and stimulates expression of KL
	Cumulus cells	Proliferation and stimulation of EGF expression

Murine (Otsuka *et al.*, 2000, 2002; Yoshino *et al.*, 2006; McIntosh *et al.*, 2012; Peng *et al.*, 2013); bovine (Passos *et al.*, 2013); caprine (Celestino *et al.*, 2011; Lima *et al.*, 2012); human (Teixeira Filho *et al.*, 2002; Gilchrist *et al.*, 2008; Chen *et al.*, 2009; Qiao & Feng, 2011; Kedem *et al.*, 2011); ovine (Galloway *et al.*, 2000; Juengel *et al.*, 2002).

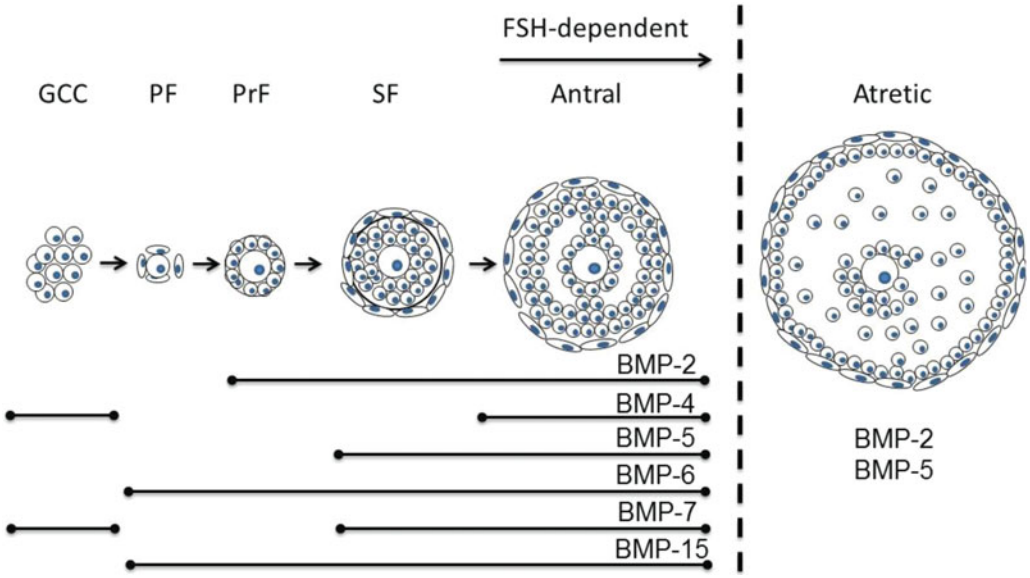

Figure 2 Action of BMPs in the different stages of follicle development in mammalian species. The BMP system acts to promote follicular development and cellular growth and regulates the functions of different components of the ovarian follicles. Legend: GCC, germ cell cohorts; PF, primordial follicle; PrF, primary follicle; SF, secondary follicle.

follicular development, ovulation, oocyte maturation and embryo development (Juengel & McNatty, 2005; Knight & Glister, 2006; Hutt & Albertini, 2007; Gilchrist *et al.*, 2008). Under *in vitro* conditions, the positive role of BMP-15 and GDF-9 in the oocyte maturation and rate of blastocyst production was observed in a co-incubation of cumulus–oocyte complexes in the presence of these factors that promotes oocyte maturation and enhances blastocyst production (Hussein *et al.*, 2006). Interestingly, both GDF-9 and BMP-15 are involved in the folliculogenesis in humans and their abnormal expression may be related to female infertility (Gilchrist *et al.*, 2008). Results suggested that BMP-15 may be a good indicator of oocyte maturity and fertilization ability (Wu *et al.*, 2007). As GDF-9 and BMP-15 are both present in follicles throughout most stages of follicular growth, it is important to consider whether these growth factors are synergistic or redundant in processes where they have similar activity alone (McNatty *et al.*, 2005). There are clinical implications for BMP-15 and GDF-9, specifically as a potential tool to help overcome female infertility. Figure 2 summarizes the stages of follicular development in which each BMP acts.

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Conclusions and final considerations

In summary, this review shows the importance of BMPs for the control of oogenesis and folliculogenesis in mammalian species. BMP-2 and -4 modulate hormone production of granulosa and theca cells contributing to follicular growth. BMP-5, -6, -7 and -8 act in follicular development and BMP-15 contributes to the oocyte and follicular maturation. These data show that a complex combination of BMPs is involved in the control of primordial germ cell formation, oocyte growth and maturation, as well as on follicle development and ovulation.

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