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Review Article

The Role of Oocyte-Secreted Factors *GDF9* and *BMP15* in Follicular Development and Oogenesis

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Contents

Ovarian physiology is controlled by endocrine and paracrine signals, and the transforming growth factor β (TGFβ) superfamily has a pivotal role in this control. The *Bone morphogenetic protein 15* (*BMP15*) and *Growth differentiation factor 9* (*GDF9*) genes are relevant members of the TGFβ superfamily that encode proteins secreted by the oocytes into the ovarian follicles. Through a paracrine signalling pathway, these factors induce the follicular somatic cells to undergo mitosis and differentiation during follicular development. These events are controlled by a mutually dependent and coordinated fashion during the formation of the granulosa cell layers. Many studies have contributed to our knowledge concerning the paracrine factors acting within the follicular environment, especially regarding *GDF9* and *BMP15*. We aimed to review the relevant contributions of these two genes to animal reproductive physiology.

Introduction

The influence of endocrine and paracrine signalling over follicular somatic cell growth and differentiation during follicular development is not completely understood (Adashi and Rohan 1992; Galloway et al. 2000). Recent studies concerning intrafollicular communication between the oocyte and somatic cells reveal that the oocyte secretes growth factors and directly induces follicular development by a complex paracrine signalling process (Li et al. 2000; Su et al. 2008; McLaughlin and McIver 2009). Among these growth factors, two members of TGFβ superfamily are noteworthy: the *GDF9* and *BMP15* genes are both expressed by the oocyte during follicular development (McGrath et al. 1995; Dube et al. 1998; Bodensteiner et al. 1999; Galloway et al. 2000; Sendai et al. 2001; Juengel et al. 2002). These two factors are fundamental to the activation of primordial follicles and subsequently participate in all stages of follicular development (Bodensteiner et al. 1999; Eppig 2001; Juengel et al. 2002; Mandon-Pepin et al. 2003). They are also involved in the final events of maturation and ovulation, such as the expansion of *cumulus oophorus* cells (Lan et al. 2003; Su et al. 2004; Dragovic et al. 2005, 2007). Although there has been an increase in the number of growth factors characterized in the last few years, the understanding of the complex signalling network inside the follicle is still in process. In this review, we screened the most recent research regarding the roles of *GDF9* and *BMP15* in the genetic control of mammalian reproductive physiology, paying special attention to the livestock species.

Follicular Development and Crosstalk Between Oocyte, *cumulus oophorus* and Granulosa Cells

Follicles, which are the functional units of the ovary, are comprised of an oocyte surrounded by somatic cells. Follicles are classified as either non-cavitary pre-antral follicles (95% of the follicular population) or cavitary antral follicles (5% of the follicular population) (Figueiredo et al. 2007). Follicles are also classified into four developmental stages (primordial, primary, secondary and tertiary), according to their size and their responsiveness/dependency on gonadotropins (McGee and Hsueh 2000). In the antrum fluid of tertiary follicles, there is an intense paracrine signalling promoted by growth factors, many of which are members of the TGFβ superfamily and are directly involved in the proliferation of somatic cells and steroidogenesis control (Dong et al. 1996; Matzuk et al. 2002). In the third stage of development, follicles are dependent on gonadotropin to continue growing and 'surviving' (Vitt and Hsueh 2001). Follicles grow coordinately as a pool until the tertiary stage. At this point, another important event occurs: the follicle with the highest response to follicle stimulating hormone (FSH) in the growing pool becomes dominant. Concomitantly, granulosa cells start to express luteinizing hormone (LH) receptors in the mid to late follicular phase under the influence of FSH (Erickson et al. 1979). At this moment, the increasing production of estradiol (E_2) by antral follicles acts as an inhibitor of FSH release by the hypophysis gland, so FSH and LH act in synergy to support follicular development (Erickson et al. 1979). As a form of negative feedback, that decrease in FSH availability induces the subordinate follicles (those more dependent on FSH) to undergo atresia and degenerate. Therefore, only one dominant follicle in mono-ovulatory mammals, or a few dominants in poly-ovulatory species, continue to grow and will be able to undergo maturation and ovulation (McGee and Hsueh 2000).

Granulosa cells are also divided into anatomically and functionally distinct types during follicular growth and antrum formation: the cumulus cells, which have direct metabolic contact with the oocyte and the mural granulosa cells (the somatic lineage of the follicle's internal wall), which form a stratified epithelium alongside the basal lamina (Latham et al. 1999). In specialized cumulus cells, which are juxtaposed to the oocyte, there are cytoplasmic projections, such as desmosomes and gap junctions, which penetrate into the oocyte membrane through the *zona pellucida*. Through these

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cytoplasmic connections, the oocyte and cumulus cells share micronutrients and form a functional syncytium (Albertini et al. 2001; Makabe et al. 2006).

Historically, folliculogenesis control was attributed only to endocrine factors, acting directly in the ovary through the hypothalamic-pituitary-gonadal axis. Endocrine control is mediated by tissue-specific protein hormones: steroid hormones, cytokines and prostanoid hormones. The biology of the bovine oestrous cycle and the role of these hormones have been exhaustively described (for detail see Moore and Thatcher 2006). The events that occur during the oestrous cycle are regulated basically by a hormonal interaction of gonadotropin-releasing hormone (GnRH), FSH, LH, E_2, progesterone (P_4) and prostaglandins. However, new research has arisen focusing on intrafollicular signal-regulatory proteins that have a decisive role during early-to-late follicular development and coordinate the crosstalk between the oocyte and the follicular somatic cells (Webb et al. 2003). Therefore, oocyte-secreted factors can regulate folliculogenesis by modulating the growth and differentiation of granulosa cells. This regulation has an effect on FSH and LH through the expression of their receptors on target cells (Halvorson and Chin 1999; Findlay et al. 2002) and the expression of other modulators, such as, insulin growth factor 1 (IGF-1), inhibin, activin and androgens (Hickey et al. 2004). In general, the oocyte signalling factors induce the expression of genes associated with cumulus cell differentiation and mitosis. Moreover, these genes induce follicle growth and assist oocyte maturation through a positive feed-back mechanism. However, the precise role of each element in this intricate and complex signalling network is still under study.

TGFβ Superfamily Members *GDF9* and *BMP15*

The TGFβ superfamily is divided into two subgroups: BMP and TGFβ. This division was established according to the origin of the gene and its genetic structure (Chang et al. 2002). The involvement of this superfamily in ovarian physiology, cellular differentiation and fertility is propelling many research studies. The molecular structure of TGFβ superfamily proteins is quaternary, containing two β-strands and one α-helix. The α-helix is stabilized by three intermolecular disulphide bonds, which forms dimers between two identical protein monomers (homodimers) or between distinct TGFβ superfamily factors (heterodimers) (Vitt and Hsueh 2001).

The *GDF9* and *BMP15* genes contain two exons separated by a single intron that encode a rough endoplasmic reticulum (RER) signal peptide, a proregion and a mature peptide. The signal peptide region is encoded by the first exon, the proregion by segments of both exons and the mature peptide region by the second exon (McGrath et al. 1995). *GDF9* and *BMP15* are synthesized in the RER as pre-proproteins, constituted by a proregion and a mature carboxy-terminal domain (Chang et al. 2002). Post-translational processing is important for the secretion of biologically active *GDF9* and *BMP15* molecules (McMahon et al. 2008; Mottershead et al. 2008; Li et al. 2009). In this process, the signal peptide is removed and the proproteins undergo dimerization. As processing proceeds, specific proteolytic enzymes cleave the dimerized proproteins at the conserved furin cleavage sites (RHRR). The furin cleavage liberates the biologically active dimeric mature protein to be secreted by the cell (Liao et al. 2003). *GDF9* and *BMP15* are the closest paralogues of the TGFβ superfamily, and the mature regions of *GDF9* and *BMP15* can dimerize with themselves (homodimer), or with the mature regions of each other (heterodimer) when produced within the same cell. As they lack the seventh cysteine present in all other members of the TGFβ superfamily (Vitt and Hsueh 2001), they are unable to establish covalent interactions between their monomers. Therefore, *GDF9* and *BMP15* dimerize only by electrostatic and hydrophobic interactions, which presumably confer them to be more labile for their interactions (Chang et al. 2002).

All members of the TGFβ superfamily have glycosylation target sites in their polypeptide sequences, and for most of them this post-translational modification is important for recognition by their receptors (Yoshino et al. 2006). The *GDF9* amino acid sequence has four putative glycosylation sites, three in the proregion and one in the mature peptide. The *BMP15* sequence has five putative glycosylation sites, three in the proregion and two in the mature peptide (Dube et al. 1998). *In vitro* studies show that, like any TGFβ member, glycosylations are required for the receptor to recognize the *GDF9* and *BMP15* factors, thus guaranteeing their bioactivity (McMahon et al. 2008). Therefore, the *GDF9* and *BMP15* expressed in bacterial-based systems do not retain full bioactivity (Eppig 2001).

Bone Morphogenetic Proteins (BMPs)

The term BMP was coined in 1965 to designate the active components in bone demineralization (Urist 1965). These proteins were classified as members of the TGFβ superfamily in 1986 (Wozney et al. 1988). Later, the presence of a functional BMP system in mammalian ovaries was described (Shimasaki et al. 1999). Currently, 12 different types of BMPs and eight GDFs are known in the BMP subfamily (Lehmann et al. 2003). Many proteins of this subfamily are expressed by oocytes, granulosa and theca cells. These factors act as intra-ovarian regulators of primordial follicle activation, somatic cell proliferation, steroidogenesis and oocyte maturation (Knight and Glister 2003). *BMP6*, *GDF9* and *BMP15* mRNAs were observed in the oocytes of mouse (McGrath et al. 1995; Dong et al. 1996), rat (Hayashi et al. 1999), and human (Fitzpatrick et al. 1998; Aaltonen et al. 1999), ovine and bovine (Bodensteiner et al. 1999; Galloway et al. 2000), brushtail possum (Eckery et al. 2002), and porcine (Brankin et al. 2005). Expression of *BMP2* and *BMP6* mRNA was observed in granulosa cells, and the expression of *BMP3b, BMP4, BMP6* and *BMP7* mRNA was detected in theca cells. This indicates that these factors participate in the bidirectional communication system between the follicular somatic cells and the oocyte (Gilchrist et al. 2006).

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GDF9

In ovine species, the *GDF9* gene is located on chromosome 5 and, within the ovary, is expressed exclusively by the oocyte (Sadighi et al. 2002). Although *GDF9* is occasionally referred to as an oocyte-specific factor, it is also expressed outside the ovary, most notably in the testis of mouse, rat, cow and human; in the pituitary gland of sheep and human; adrenal derived cell lines of mouse and human; and in foetal and neonatal mouse adrenal gland (Fitzpatrick et al. 1998; Pennetier et al. 2004; Faure et al. 2005; Farnworth et al. 2006; Nicholls et al. 2009; Wang et al. 2009). The *GDF9* sequence is conserved among mammals and is also very similar to *BMP15*; consequently, it was classified as a BMP subfamily member (Vitt et al. 2002). The knockout of *GDF9* can block the development of pre-antral follicles and cause infertility in female transgenic mice (Dong et al. 1996; Elvin et al. 1999). However, *GDF9* and *BMP15* knockout male mice are fertile with normal testis morphology and physiology and, besides *GDF9* and *BMP15* expression in extraovarian sites, no other effect of *GDF9* and *BMP15* knockout is observed (Dong et al. 1996; Elvin et al. 1999; Yan et al. 2001). The oocytes of knockout mice show absence and abnormal disposition of organelles, and the granulosa cells lack the capacity to undergo apoptosis. As apoptosis has been confirmed to be the mechanism of germ cell atresia in animals (Pesce and De Felici 1994; Ratts et al. 1995; Morita et al. 1999), this provides considerable insight into the role of *GDF9* in early folliculogenesis *in vivo* (Dong et al. 1996). The *GDF9* is necessary to optimize oocyte microenvironment, ovarian follicles growth and atresia, ovulation, fertilization and normal reproduction (Orisaka et al. 2009). Also, it induces the expression of the genes hyaluronic synthase 2 (Has2), cyclooxygenase 2 (COX2), pentraxin 3 (Ptx3), prostaglandin (Ptgs2) and gremlin (GREM1) in cumulus cells, which are essential for their expansion during oocyte maturation and before ovulation (Pangas and Matzuk 2005). Previous studies demonstrated the ability of recombinant *GDF9* to induce the expansion of cumulus cells in mice and secretion of an extracellular matrix. This matrix is composed mainly of hyaluronic acid, synthesized by Has2, which assists the cumulus-oocyte complex capture by the oviduct cells and enables proper fertilization (Elvin et al. 1999). Moreover, the *GDF9* knockdown by RNAi (RNA interference) injection into the oocyte eliminates the expansion of the cumulus cells, which corroborates the data supporting the essential role of this factor in cumulus cell expansion in mice (Gui and Joyce 2005).

Recent studies with immunization against the mature region, or different peptide sequences of *GDF9* and *BMP15*, show an abnormal follicular development, perturbation of the oestrous cycle and altered ovulation rate in distinct ways in sheep and cattle (Juengel et al. 2002, 2004a, 2009; McNatty et al. 2007). The active long-term immunization against peptide sequences of mature regions of *GDF9* and *BMP15* provoke abnormal oestrous behaviour and arrest follicular development in ewes (Juengel et al. 2002). However, short-term immunization against different peptides (at the most N-terminal portion of the mature region of *GDF9* and *BMP15*), using DEAE dextran adjuvant, increases the ovulation rate in the immunized ewes (Juengel et al. 2004a). Moreover, distinct peptides based on the *GDF9* or *BMP15* mature region sequence can induce anovulation or increase the ovulation rate, depending on their position. The peptides against the most N-terminal portion of the mature region are more effective in inducing anovulation, while peptides representing the central portion of the mature region can increase ovulation in immunized ewes (McNatty et al. 2007). However, the same peptides are not effective in inducing anovulation or inducing a consistent increase in the ovulation rate in cows (Juengel et al. 2009). These results demonstrate the relevant role of *BMP15* and *GDF9* in the oestrous cycle control and normal follicular development in mammals, but their actions are distinct among different species.

BMP15

BMP15 (also known as *GDF9B*) was first described in 1998. It is highly homologous to *GDF9* and is expressed in the oocytes of primary follicles in sheep, human and rodent (Dube et al. 1998; Laitinen et al. 1998). Similar to *GDF9*, the mRNA and protein of *BMP15* are found in oocytes during all stages of folliculogenesis. Its expression is high between the primary to pre-ovulatory follicle stages in rodent species. However, in mice, *BMP15* protein expression does not occur until ovulation (Otsuka and Shimasaki 2002). Unlike *GDF9, BMP15* protein is found in the pituitary gland, testis and in several other tissues from many species, suggesting that *BMP15* activity is not exclusive to the ovary (Otsuka and Shimasaki 2002). *BMP15* mRNA was not detected in oocytes of primordial stage follicles and is only detected in growing follicles. However, similar to *GDF9*, it has a fundamental role in the regulation of follicular development in mammals (Galloway et al. 2000; Sendai et al. 2001).

The *BMP15* targets granulosa cells, which stimulates them to proliferate, and also modulates the expression of steroid hormones (Otsuka and Shimasaki 2002). There is a peak of *BMP15* expression during the moment of cumulus cells expansion, which occurs after oocyte maturation. This allows *BMP15* to interact with *GDF9* and coordinate the expression of the genes involved in cumulus cells expansion (Lan et al. 2003). *In vitro* experiments show that *GDF9* and *BMP15* have distinct effects on reproductive physiology in a specie-specific manner (Vitt et al. 2002). However, both genes are essential for proper follicular development, and natural mutations in these genes can provoke infertility in homozygous ewes (Hanrahan et al. 2004). Therefore, it seems that the maintenance of the precise expression level of *BMP15* and *GDF9* in oocytes is essential for efficient female fertility and proper follicular development (Liao et al. 2003). The presence of a regulatory feedback system between oocyte *BMP15/GDF9* and granulosa cell kit ligand could maintain the appropriate expression level of *BMP15* and *GDF9* in the oocyte, which is essential for their physiological functions (Otsuka and Shimasaki 2002).

To present full bioactivity, the *BMP15* protein undergoes three types of post-translational modifications in five distinct sites: *N*-glycosylation (Dube et al.

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1998; Hashimoto et al. 2005; Li et al. 2009), *O*-glycosylation (Saito et al. 2008) and *C*-phosphorylation (McMahon et al. 2008). The amino acid sequence of mouse *GDF9* contains four putative N-linked glycosylation sites, one of which is located in the mature region (McPherron and Lee 1993; Gilchrist et al. 2004a). Inadequate post-translational modifications, also known as inadequate protein maturation, can create aberrations with direct consequences on female reproductive physiology (Saito et al. 2008). These abnormalities provide molecular evidence of intracellular interactions between *BMP15* and *GDF9*.

As opposed to *GDF9*, *BMP15* knockout female mice present only a slight decrease in fertility, characterizing a subfertility phenotype (Yan et al. 2001). However, when the *BMP15*$^{-/-}$ genotype is introduced into a *GDF9*$^{+/-}$ background, the females (*BMP15*$^{-/-}$ *GDF9*$^{+/-}$) show severe defects in ovary morphology and are sterile (Yan et al. 2001). These results show a synergic effect between *GDF9* and *BMP15*, corroborating the idea of a functional interaction between these two genes *in vivo*. However, the absence of *GDF9* and *BMP15* has distinct phenotypes among different species, because these naturally occurring mutations that nullify *BMP15* activity lead to infertility in homozygous ewes (Galloway et al. 2000).

As the *GDF9* and *BMP15* are the only members of the TGFβ superfamily that do not contain the fourth cysteine of the cysteine knot structure, their subunits are not covalently linked and can form homo and heterodimers *in vivo* (McIntosh et al. 2008). It is possible that the secreted *GDF9/BMP15* heterodimer is important for follicle somatic cell mitosis promoted by the oocyte through the use of the same receptors used by the *GDF9* and *BMP15* homodimers (Gilchrist et al. 2004b). The post-translational processing of *BMP15* and *GDF9* present differences in distinct species; recombinant mouse *BMP15* is less efficiently processed in mouse cells than in human and sheep counterparts. However, human, mouse and sheep recombinant *GDF9* are easily processed (Liao et al. 2003). Consequently, differences between the structures of the mouse and human genes could be expected and have important consequences on the function of *BMP15*.

TGFβ Superfamily *GDF9* and *BMP15* Signalling Pathways

The signalling pathway of TGFβ superfamily members begins when the ligands are recognized by their specific heterotetrameric receptor complex. This complex is formed by one homodimer of serine-threonine kinase type I and one homodimer of serine-threonine kinase type II (Souza et al. 2007). In the presence of the ligands, the type I dimer recruits the type II dimer, forming the heterotetrameric complex. This union induces the transphosphorylation of serine residues of the type I receptor by the kinase activity of the type II receptor. This phosphorylation activates the intracellular kinase of the type I homodimer, which in turn phosphorylates its intracellular signallizing substrates named responsive Smads (for details see Moustakas and Heldin 2009). Responsive Smad proteins (R-Smads) are a family of transcription factors found in all vertebrates, insects and nematodes and are the only substrate for the TGFβ superfamily receptors (Massagué 1998). However, there are approximately 27 TGFβ superfamily ligands for five type I receptors, seven for type II and a repertoire of five different intracellular target R-Smads, making this one of the most complex signalling networks in mammals (Kang et al. 2009; Moustakas and Heldin 2009).

Once activated by phosphorylation, the R-Smad molecules interact with a common Smad (Co-Smad), also named Smad 4, which binds to all phosphorylated R-Smads. Subsequently, the R-Smad/Co-Smad complex translocates to the nucleus, where it interacts with specific transcription factors that regulate the expression of several target genes (Massagué 1998). The TGFβ-activin receptors remain active for at least 3–4 h after ligand binding. During this time, the R-Smad/Co-Smad complex was maintained in the nucleus either activating or repressing transcription, which depends on their association and context with others transcription factors (Derynck and Zhang 2003). Aside from R-Smads and Co-Smad, there are the inhibitory Smads (I-Smads 6 and 7), which are a distinct subclass of Smads that antagonize TGFβ signalling transduction. I-Smad 7 interacts with all activated type I receptors to inhibit R-Smad phosphorylation and transcriptional regulation. I-Smad 6 competes with activated R-Smads to form a complex with Smad4, inhibiting the normal path of signalling in a competitive way (Souchelnytskyi et al. 1998). The classic BMP and TGFβ-activin pathways can also be inhibited by a dominant-negative antagonist named *BAMBI*. This antagonist has structural features that resemble type I receptors, except that it lacks the intracellular serine-threonine kinase domain. Therefore, *BAMBI* can compete with receptors type I to bind the ligands and inhibit the signalling to the TGFβ-activins and BMPs (Grotewold et al. 2001).

BMP15 uses a classic path of BMP signal transduction, binding to type II receptor BMPRIIB and to a specific type I receptor *ALK6* (BMPRIB) that results in the activation of R-Smads 1, 5 and 8 (ten Dijke et al. 2002). *GDF9* uses the TGFβ-activin signalling pathway, binding to the same type II receptor, BMPRIIB and a specific type I receptor, *ALK5* (TβRI), which results in the activation of R-Smads 2 and 3 (Vitt et al. 2002; Mazerbourg et al. 2004). The mRNA of *ALK5* was detected in oocytes at all follicle stages of humans, sheep and mice. In granulosa cells, *ALK5* expression was detected in follicles of the primordial stage in mice, in the primordial to primary stage in humans and in the pre-antral follicles of all species (Juengel et al. 2004b). *ALK6* is expressed in granulosa cells from the primary through late pre-antral follicle stages in a lower extension by the theca cells in ovine species and in antral follicles in bovines (Glister et al. 2004). The *BMPRIIB* receptor is expressed in the granulosa cells of primordial follicles in ruminants and in pre-antral follicles in rodents, and it continues to be expressed in all subsequent stages of folliculogenesis (Edwards et al. 2008). *BMPRII* receptor is essential for *GDF9* signalling in granulosa cells (Mazerbourg et al. 2004) and the cooperative effects of *GDF9* and *BMP15* on granulosa cell proliferation are blocked by the extracellular domain of *BMPRII* in sheep (Edwards et al. 2008).

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Natural Mutations in *GDF9* and *BMP15* Genes and their Effect on Reproduction

Research studies of natural prolific sheep lineages have shown that characteristics such as smaller body size and high ovulation rates are frequently determined by major genes. When these genes are able to determine reproductive phenotypes, they are called fecundity genes (*Fec*) (Davis 2005). The first description of mutations in a major gene that increase prolificacy in two lineages of Romney sheep (Hanna and Inverdale) were mapped in the centre of the X chromosome in a locus named *FecX* (Davis et al. 1991). This region is orthologous to the human Xp11.2–11.4 chromosome region, and DNA sequencing analysis identified two distinct single nucleotide polymorphisms (SNPs) in the *BMP15* gene named $FecX^H$ (*Hanna* allele) and $FecX^I$ (*Inverdale* allele) (Galloway et al. 2000). Subsequently, a third polymorphism was identified in a distinct locus, and this new mutation was described independently by three groups in the Booroola lineage of Merino sheep. The new SNP was found in the coding sequence of the *ALK6* (*BMPRIB*) receptor gene located on chromosome 6, and this locus/allele was named $FecB^B$ (Mulsant et al. 2001; Souza et al. 2001; Wilson et al. 2001). The last gene to be associated with prolific phenotypes in sheep was *GDF9*, where a SNP was identified in the Belclare and Cambridge breeds, and this new allele was named $FecG^H$ (Hight Fertility) (Hanrahan et al. 2004). In the same work, two new SNPs in the *BMP15* sequence were identified: $FexX^G$ (Galway) and $FecX^B$ (Belclare). Since then, three additional alleles associated with prolificacy were identified in the *BMP15* gene: $FecX^L$, which was found in Lacaune sheep (Bodin et al. 2007), and one 17bp deletion ($FecX^R$), which was found in Aragonesa sheep (Martinez-Royo et al. 2008; Monteagudo et al. 2009). A novel mutation in the mature region of the *GDF9* protein was also reported in Thoka-Cheviot sheep (*FecT* allele). Heterozygous ewes have increased fertility, but homozygous ewes are infertile because of a complete absence of follicular development, in spite of apparently normal activation of the oocyte and expression of a number of oocyte-specific genes (Nicol et al. 2009). All of the *BMP15* and *GDF9* variants that have been described have the same phenotype: the heterozygote animals are prolific, with an increase in the ovulation rate ranging from 35% to 95% (McNatty et al. 2005), whereas the homozygote animals are sterile because of a failure of ovarian follicles to progress beyond the primary stage of development (Davis et al. 1992; Galloway et al. 2000; Hanrahan et al. 2004; Bodin et al. 2007; Martinez-Royo et al. 2008; Monteagudo et al. 2009; Nicol et al. 2009). The data suggest that all mutations described in the pre-propeptide of *BMP15* ($FecX^R$, $FecX^G$) or in the mature peptide of *GDF9* ($FecG^H$, *FecT*) and *BMP15* ($FecX^H$, $FecX^I$, $FecX^L$, $FecX^B$) are associated with a loss of function in gene activity. In this case, the *BMP15* and *GDF9* mutations result in either the reduction of mature protein levels or an altered bond between ligands and receptors found in the granulosa and cumulus cells surface (Liao et al. 2003). Recently, our group has identified a new polymorphism in the mature peptide of *GDF9* gene in a prolific flock of Brazilian Santa Inês sheep. This allele was named $FecG^E$ (Embrapa) and presents a distinct phenotype related to the other *FecX* and *FecG* alleles (here we suggest that the Thoka polymorphism should be renamed to $FecG^T$ allele to be in conformity with the former nomenclature of the *FecG locus*; Hanrahan et al. 2004). The $FecG^E$ heterozygote ewes are 27% more prolific than the wild-type ewes; the homozygote ewes show an even greater increase in prolificacy (58%), and there are no records of sterility among the $FecG^{E/E}$ animals (Silva et al. 2010). This alternative phenotype brings a new perspective for the study and better understanding of the paracrine control of ovulation quota in mammals.

GDF9 and *BMP15* mutations have been recently associated with various human reproductive abnormalities. Heterozygous, non-conservative substitutions in *BMP15* were associated with premature ovarian failure because of ovarian dysgenesis in women (Di Pasquale et al. 2004, 2006; Laissue et al. 2008). The altered protein lacks the C-terminal region, which contains the mature region of *BMP15* (Dixit et al. 2006). Moreover, non-conservative substitutions and abnormal expression of *GDF9* were also associated with polycystic ovarian syndrome and premature ovarian failure (Dixit et al. 2005; Laissue et al. 2006). Normal ovulation in human species requires two functional *BMP15* copies, considering that the presence of one heterozygous mutation is sufficient to cause premature ovarian failure, hypergonadotropic ovarian failure and dizygotic twins (Di Pasquale et al. 2004; Laissue et al. 2006; Zhao et al. 2008). It is possible that the misfolding of *BMP15* and *GDF9* proregions in the muted variants could affect the normal proteolytic processing and consequently inhibit the release of the mature region. This may lead to the production of abnormal dimers or inhibition of the dimerization process (Laissue et al. 2006). In addition, three markers linked to the *BMP15* gene were associated with high follicle production in women submitted to recombinant FSH stimulation (González et al. 2008).

Therefore, the relative importance of *BMP15* and *GDF9* during folliculogenesis regulation can be different between sheep, mice and humans. These differences may be attributed to the mono *versus* polyovulatory behaviour of these animals (Galloway et al. 2000; Moore et al. 2004). It is also possible that the observable differences are attributable to the nature of the mutations in the *BMP15* gene; a single point mutation in sheep *versus* a deletion of the entire second exon in mice (Liao et al. 2003). However, based on the present data, the comprehension of molecular events that modulate reproductive physiology is still insufficient to explain why different mammal species show a broad variety in allelic interactions of *BMP15* and *GDF9* variants.

Conclusion

In the last few years, the comprehension of reproductive physiology in mammals is showing an impressive advance. This increased understanding is propelled by research studies focused on growth and differentiation

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factors, mainly the members of TGFβ superfamily, which are expressed by follicular somatic cells and oocytes. These factors establish a complex intrafollicular gradient that activates an intricate signalling network, which mediates the cellular growth and differentiation of follicle cells. The paracrine factors produced inside the follicle also modulate the effects of the pituitary hormones (LH and FSH) on estradiol and progesterone production. Therefore, these factors mediate the connections between the hypothalamic-gonadal axis and the ovaries and determine the ovulation quota in different mammalian species.

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Conflicts of interest

None of the authors have any conflicts of interest to declare.

Author contributions

Fernanda Paulini and Eduardo O Melo drafted the paper.

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