Transworld Research Network
37/661 (2), Fort P.O., Trivandrum-695 023, Kerala, India

Novel Concepts in Ovarian Endocrinology, 2007: 27-42 ISBN: 978-81-7895-309-0
Editor: Antonio González-Bulnes

2 Role of bone morphogenetic proteins in follicle development

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Abstract

The addition of the Bone Morphogenetic Proteins to the plethora of cytokines that affect the paracrine control of ovarian follicle development, although recent has been very fruitful to understand the physiology of the mammalian ovary. They proved to be involved key steps since the very beginning during the formation of the foetal ovary, passing to the entry of primordial follicles into the growing pool,

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granulosa and theca cell function and finally affecting the number of follicles capable of ovulating. The mammalian domestic species offer an excellent comparison to the rodent models used to investigate folliculogenesis. The sheep naturally occurring mutations in Bone Morphogenetic Proteins ligands and receptor are particularly interesting and have shed some light on very fundamental steps of folliculogenesis and on the regulation of ovulation rate.

Introduction

The Bone Morphogenetic Proteins (BMPs) are members of the Transforming Growth Factor beta (TGFbeta) family of growth factors and are deeply involved in the growth and differentiation of several cell lines including those of the female reproductive system and particularly the ovary [1].

The existence of several major genes (mutations) affecting ovulation rate in sheep has been known for more than 25 years [2] and some of them have been used extensively as models to understand the folliculogenesis in mammals [3, 4]. The genetic cause of some of these major genes were point mutations on BMP-15 and BMP receptor type 1 B (BMPRIB) genes, which brought the attention to the BMPs as regulators of ovarian follicle development and ultimately the ovulation rate in domestic species [5-8]. There are several recent reviews on the effect of members of the TGFbeta on folliculogenesis [9-11]. This review will focus on the effects of BMPs in the control of ovarian follicular growth in domestic animals but we will borrow information from rodent models whenever it becomes necessary.

BMPs signalling system

The BMPs comprises the largest family among the TGFbeta superfamily of growth factors. They are products of single genes that are translated as large preproteins composed of a signal peptide, predomain, and mature domain. After removal of the signal peptide, the proproteins undergo dimerization and are cleaved at a specific site (RXXR) by proteolytic enzymes, generating biologically active dimers of the mature protein. Recombinant protein expression systems revealed that when coexpressed, BMP-2, -4, -5, -6, and -7 can form heterodimers which often exhibit greater biological activity than their corresponding homodimers (for review see [9, 12]). There is also evidence that both BMP-15 and GDF-9 form non-covalent homodimers when expressed individually, while BMP-15/GDF-9 heterodimers are produced when both are co-expressed. When GDF-9 and BMP-15 are co-expressed the processing of both proproteins are reduced compared to that of individually expressed proproteins, suggesting that the proprotein heterodimer is less susceptible to proteolytic cleavage than the individual homodimers [13].

Table 1. Principal BMP ligands identified in the mammalian ovary, their preferred receptors and intracellular signalling pathway.

Ligand	Type II receptor	Type I receptor	Smad
BMP-2 BMP-4	BMPRII	BMPRIA (ALK 3) / BMPRIB (ALK6)	Smad1/5/8
BMP-6 BMP-7	BMPRII ActRII ActR2B	ActR1A (ALK 2) / BMPRIB (ALK6)	Smad1/5/8
BMP-15	BMPRII	BMPRIB (ALK6)	Smad1/5/8
GDF-9	BMPRII	TGFbetaR1 (ALK5)	Sma2/3

Adapted from [9] after [16]

The Table 1 summarises the ligands, receptors and signalling pathway of the BMPs most commonly present in the mammalian ovary. This family of peptides signals through a complex of single transmembrane domain receptors comprising of serine-threonine kinase receptors type I and II (Table 1). Upon ligation with the high affinity receptors, dimeric BMPs recruit the other receptors to form a heterotetrameric complex. The constitutively phosphorylated type II receptor transphosphorylate the type I receptor leading to the activation of their intracellular kinases. Once activated, the BMP type 1 receptor phosphorylates a receptor-regulated Smad (Smad 1, 5 or 8) which then heterodimerizes with a common Smad (Smad-4) and moves to the nucleus where the Smad complex associates with nuclear transcription factors and regulate the expression of target genes (for review see [14, 15]).

BMPs and the formation of the ovary in the foetus

The effect of BMPs on the folliculogenesis is first evidenced in the formation and early proliferation of the primordial germ cells (PGCs) in the foetus *in utero*. Mouse embryos homozygous null for BMP-4 contain no PGCs and also lack other extraembryonic mesoderm derived tissue such as the allantois, which along with the PGCs originate from the proximal epiblast. BMP- 4 null heterozygotes have fewer PGCs, due to a reduction in the size of the founding population [17].

Mouse embryos with BMP-8b gene knockout also have a reduction in the PGC numbers and the severity of the decrease varies according with the number of defective alleles and the genetic background of the mutants [18]. However, the effects of BMP-4 and BMP-8b on mouse PGC are not additive [18]. In epiblast cultures BMP-4 or BMP-8b alone cannot induce PGCs in vitro whereas they can when used in combination. Moreover, the PGC defects of BMP-8b mutants can be rescued by BMP-8b homodimers while BMP-4 homodimers cannot mitigate the PGC defects of BMP-4 null mutants,

suggesting that BMP-4 proteins are also required prior the synergistic action of BMP-4 and BMP-8b [19].

BMP-2 is expressed in the visceral endoderm at the time of PGC specification, and inactivation of BMP-2 results in a reduction in PGC number [20]. BMP-4 produced in the extraembryonic ectoderm signals through ALK2 expressed in the visceral endoderm to induce formation of PGCs from the epiblast. ALK2-deficient embryos are devoid of PGCs and the heterozygotes have reduced numbers, resembling BMP-4 null mutants [21].

Addition of BMP-4 to an *in vitro* organ culture induced formation and migration of PGC to the developing gonad, which was abolished by the addition of the BMP-antagonist noggin. Endogenous BMPs induced phosphorylation of Smad1/5/8 in the somatic cells of the urogenital ridges but not on migratory PGCs. suggesting that BMP signalling regulates PGC migration by controlling gene expression within the somatic cells along the migration route and within the genital ridges [22].

The expression of BMPs during foetal ovarian development at ages ranging from 25 to 120 days post coitum (dpc) has been studied in sheep by RT-PCR. Expression of GDF-9 mRNA started at 56 dpc and progressively increased reaching a maximum at 94 dpc and then decreasing at birth. BMP-15 showed a more restricted expression pattern, with high transcription from 94 dpc to birth. The pattern of BMPRIB expression was high from 56 dpc and remained high at all foetal stages [23]. Transcripts of BMP-4, BMPRII and BMPRIA were also detected in the sheep foetal ovary since 32 dpc [24].

The consistently high expression of BMPRIB in the foetus is consistent with an important role for this receptor in ovarian development. The growth of ovaries of foetuses carrying the Booroola mutation is retarded with lower germ cell numbers from 30 to 40 dpc, fewer oogonia entering meiosis at day 55, fewer developing primordial follicles at 75 and 90 dpc and growing follicles from 120 dpc [25]. However, the possible differences among the Booroola genotypes on the pattern of gene expression during the ovarian development have not been investigated.

Expression of BMP-4, -5, and 6 and GDF-9 mRNAs was also observed in the ovaries of neonatal pigs [26].

Ovarian BMP receptors

Receptors for BMPs are expressed at all stages of ovarian development. The expression of BMPRIA, BMPRIB and BMPRII investigated by immunohistochemistry in the sheep ovary showed strong immunostaining in the granulosa cell layer of follicles from the primary to late antral stages of development. Immunostaining for all three receptors was also present in the

oocyte, corpus luteum, ovarian surface epithelium and, to a lesser extent, the theca layer of antral follicles [27].

Bovine isolated theca, granulosa and oocytes studied by immunocytochemistry also expressed a range of BMP-responsive type-I (BMPRIB, ActRI) and type-II (BMPRII, ActRII, ActRIIB) receptors that after stimulation by BMP-4, 6 and 7 promoted cellular accumulation of phosphorylated Smad1 but not Smad2 [28].

Immunohistochemistry for BMPRIA, BMPRIB and BMPRII in pig ovaries demonstrated the presence of all three receptors in the foetal egg nests and in the granulosa cell layer of follicles ranging from primordial to late antral stages. Immunostaining was also observed in oocytes, theca layer, corpus luteum and ovarian surface epithelium [29].

The mRNAs of BMPRII, BMPRIA, and BMPRIB were also detected in the ovary of cycling goats by RT-PCR. Transcripts for all BMP receptors were detected in primordial, primary, and secondary follicles as well as in oocyte and granulosa cells of antral follicles and in the corpora lutea [30].

Ovarian BMP expression

Ovine and bovine ovaries express GDF-9 mRNA in the oocyte of follicles from the primordial stage onwards, in a pattern consistent with a role in the initiation and maintenance of folliculogenesis in these species [31]. The sheep ovary also expresses BMP-15 with transcripts being detected in primary follicles after the granulosa cells have undergone two doublings [5].

BMP expression in the sheep ovary investigated by northern blot of ovarian tissues comprising large and small antral follicles, isolated granulosa and theca cells from large antral follicles showed that BMP-4 and -7 are highly expressed in all tissues investigated. BMP-2 is expressed on granulosa cells at a lesser intensity but nevertheless present in the sheep ovary, and BMP-6 is expressed in the oocyte since RNA expression was observed in intact follicles but not in granulosa or theca cells [3]. The expression of BMP-6 in the sheep ovary was also observed by immunohistochemistry in the oocyte, granulosa, and thecal layers of antral follicles [32]. A recent study using in situ hybridization confirmed the BMP- 6 expression restricted to sheep oocytes but did not detected BMP-2, 4 and 7 in any cells of non-atretic ovarian follicles. However, expression of all BMPs was evident by RT-PCR using RNA from isolated granulosa cells [33].

In the cattle ovary, immunohistochemistry revealed expression of BMP-4 and 7 in isolated theca cells while BMP-6 was expressed selectively in granulosa cells and oocytes [28]. Another study corroborated the protein expression pattern of BMP-4 and found that expression by immunohistochemistry of BMP-2 was limited to theca interna and approximately 25% of oocytes of antral follicles [34]. However the same study found that BMP-4 mRNA

expression by PCR was present also in the granulosa layer and oocytes of antral follicles but BMP-2 mRNA expression remained confined to the theca cells [34].

Investigation of BMPs in pig ovaries by Western blotting, showed that oocytes and follicular fluid contained BMP- 2 which was also observed in granulosa and theca cells. BMP-6 protein was evident on oocytes, follicular fluid and in granulosa cells but lacking in theca cells [35]. The pig ovary also shows expression of BMP-4, -5, -6, -15 and GDF-9 mRNAs [26, 36].

Investigation of mRNA expression of GDF-9, BMP-15 in the goat ovary using RT-PCR detected transcripts of both genes in primordial, primary, and secondary follicles as well as in oocyte and granulosa cells of antral follicles. GDF-9 and BMP-15 protein localization investigated by immunohistochemistry were observed in oocytes of all types of follicles and granulosa cells of primary, secondary, and antral but not primordial follicles [30].

BMPs action on granulosa cells

BMP-4 reduces progesterone secretion by ovine granulosa cells cultured *in vitro* [6, 37]. This inhibitory action was associated with a decrease at mRNA and protein levels of steroidogenic acute regulatory protein (StAR) and P450 side-chain cleavage (P450scc) [38]. Addition of BMP-2, -4, -6 or -7 inhibited progesterone production from cultured ovine granulosa cells without affecting cellular proliferation [33]. Another study using BMP-2, -4, -6, -15 or GDF-9 also reported reduction of progesterone secretion by sheep granulosa cells in culture and no effect on cell proliferation by any of the tested growth factors apart from BMP-4 [24]. However the physiological significance of these findings is unknown because the follicle mainly secretes oestradiol rather than progesterone prior to luteinization. The unequivocal marker of granulosa cell differentiation is its ability to secrete oestradiol. Culture of sheep granulosa cells differentiated *in vitro* in a serum-free medium, showed that BMP-2 enhanced oestradiol and inhibin-A production but had no effect on cell proliferation [27]. A recent study using the same culture system that maintain granulosa cell phenotype confirmed the action of BMP-2 and showed that BMP-4 and -6 also stimulated oestradiol without causing any change on cell number [32].

Experiments with bovine granulosa cells cultured under the same conditions as ovine, demonstrated that BMP-4, -6 and -7 enhanced secretion of oestradiol, inhibin-A, activin-A and follistatin and increased viable cell number but suppressed progesterone secretion, consistent with an action to prevent or delay atresia and/or luteinization [28]. Granulosa cells obtained from cattle large follicles, treated with GDF-9 showed a dose-dependent decrease in progesterone and oestradiol production, but promoted granulosa cell proliferation. In the same culture system, treatment with BMP-4 also

inhibited steroidogenesis but had no effect on cell numbers [39]. Suggesting that the action of BMPs in the cow granulosa cells can be altered by the culture system employed.

Porcine granulosa cells cultured in a serum-free system with increasing concentrations (0 to 100 ng/ml) of BMP-2 or BMP-6, showed that both BMPs suppressed progesterone production in a dose-dependent manner, but had no effect on cell proliferation apart from BMP-6 at the highest dose. BMP-2 and -6 decreased cyclic adenosine monophosphate (cAMP) and protein expression of 3beta-hydroxy- steroid dehydrogenase (3beta-HSD), while StAR expression was only affected by BMP-6 treatment. Supporting the hypothesis that BMP-2 and -6 act as luteinization inhibitors in the pig granulosa [35].

BMPs action on theca cells

The effects of BMPs on theca cell function are variable depending on dose, culture system and species. In cultured bovine theca cells BMP-4, -6, and -7 suppressed both basal and LH-induced androgen production while had only a moderate effect on progesterone production and cell number [40]. Semiquantitative RT-PCR showed that all three BMPs induced a marked reduction of mRNA for P450 17alpha-hydroxylase (P450c17). While abundance of mRNA encoding StAR, P450ssc and 3beta-HSD were also reduced but to a much lesser extent. The marked reduction in cellular content of P450c17 protein after BMP treatment was confirmed by immunocytochemistry. Treatment of bovine theca cells with BMPs led to accumulation of phosphorylated Smad1, but not Smad2, confirming the signalling through the Smad1 pathway [40].These findings suggest that in this species BMPs suppress androgen production by theca cells due direct inhibition of P450c17.

In the pig BMP-2 and -6 also suppress progesterone and androstenedione synthesis by cultured theca cells. Oestradiol synthesis is suppressed by BMP-2, but not BMP-6, and theca cell proliferation is stimulated by BMP-6, while BMP-2 show no action. Both BMP-6 and -2 inhibited cAMP release theca cells. Addition of BMP-2 and 6 to co-cultured theca and granulosa cells suppresses progesterone and androstenedione synthesis while stimulating cell proliferation [41].

The effect of BMP-2, -4, and -6 was also examined in sheep theca cells in culture. High doses of BMPs inhibited LH-stimulated androstenedione production, whereas lower doses stimulated cellular proliferation [32].

In summary these observations suggest that BMPs may stimulate aromatase activity in granulosa cells but inhibit androgen production by theca cells. In this way premature luteinization of the developing follicle is prevented.

BMPs antagonists

The effect of BMPs can also be regulated in the extracellular milieu by antagonists such as noggin, follistatin, chordin and cerberus, which bind to ligands with high affinity interfering in receptor coupling. The action of BMPs is also regulated at the membrane site by pseudo receptors like BAMBI and soluble forms of type I receptors (for review see [42]).

A bioassay based on progesterone production by ovine granulosa cells in culture, showed that follistatin was a strong antagonist of activin A, but not BMP-2 or BMP-4 actions. In contrast, noggin, a known specific BMP antagonist, had no effect on activin-A but strongly neutralized BMP-2 and BMP-4 actions. Both follistatin and noggin showed a lesser reduction on BMP-6 action [43]. The relative binding affinity of follistatin for BMP-4, -6 and -7 were 10, 5 and 1% respectively when compared to activin [28]. Moreover, studies on bovine granulosa cells showed that preincubation of BMP-4, -6 or -7 with excess follistatin abolished BMP-4-induced phosphorylation of Smad-1, partially reversed Smad-1 phosphorylation induced by BMP-6 while it had no inhibitory effect on BMP-7-induced Smad-1 phosphorylation [28].

Bovine theca cultures treated with BMP-4, -6, and -7 and coincubated with BMP antagonists, chordin, gremlin, and follistatin, demonstrated that gremlin and chordin were effective antagonists of BMP-4 and -7 induced suppression of androstenedione (A4) secretion but had no effect on BMP-6 action. Follistatin failed to reverse the effects of BMPs on androgen production [40].

BMP effects on cumulus expansion

Recent data has suggested that BMPs may be important regulators of the dialogue between the oocyte and its surrounding cumulus granulosa cells. When the oocyte is removed from the cow follicle cultured in vitro the surrounding granulosa cells undergo apoptosis which can be prevented by the addition of BMP-15 [44]. In the same work effects of other BMPs were much less (BMP-6) or absent (GDF-9).

In contrast several studies in the mouse and rat suggest that GDF-9 may play a crucial role in cumulus expansion. When GDF-9 antisense RNA was microinjected into cultured rat follicles, there was a striking increase of GC apoptosis and inhibition of follicle growth [45]. A study demonstrated the induction of cumulus expansion of oocytectomized mouse COCs in presence of GDF-9, FSH and foetal calf serum (FCS), setting the GDF-9 as candidate to be the cumulus-expansion enabling factor (CEEF) [46]. More recently, two other studies provided additional evidence that GDF-9 may be the elusive CEEF. The first one, using a RNA interference strategy to knockdown the expression of GDF-9 and BMP-15 proteins in mouse oocytes microinjected with dsRNAs, showed that GDF-9 knockdown oocytes, but not BMP-15, were unable to induce cumulus expansion in oocytectomized mouse COCs in presence of FSH [47]. A distinct result was obtained in the second study

employing GDF-9, TGFbeta, and their respective antagonists, mAb-GDF-9 (monoclonal anti-human GDF-9) and TGFbRII ECD (extra cellular domain of TGFbeta type II receptor). This work showed that neither GDF-9 nor TGFbeta alone could account for the oocyte-secreted factors with CEEF proprieties [48]. However, the evidence that the CEEF is composed by molecules of the TGFbeta superfamily is further confirmed by the fact that disruption of SMAD 2/3 signalling completely ablated the initiation of mouse cumulus expansion [49].

In summary these studies support the concept that BMPs including oocyte derived factors such as GDF-9 and BMP15 play an important part in regulating the activity of the somatic (granulosa) cell component of the follicle. Whether the contrasting studies with different ligands are due to species differences or in the test system used is unknown.

BMPs and ovulation rate

A number of strains of sheep have been observed which have a lambing and ovulation rate well above that observed in the parent breed (usually 1-2). It has now been shown to be associated with single point mutations in genes of the BMPs or their receptors (Table 2).

The first naturally occurring mutations (Table 2) in a BMP ligand was identified in Romney ewes selected for their increased ovulation rate [5]. Heterozygous ewes of the Hanna and Inverdale point mutations on the BMP-15 gene have an extra ovulation above wild type, while homozygous ewes are infertile and have streak ovaries devoid of follicles with more than one layer of granulosa cells [50]. The BMP-15 gene has proved to be a mutation “hot spot” in prolific sheep with identification of the Galway and Belclare mutations in Cambridge and Belclare ewes [51] and more recently the Lacaune mutation identified in the Lacaune sheep breed [52]. All mutations found so far in the BMP-15 gene are associated with an increased ovulation rate and sterility in heterozygous and homozygous ewes, respectively [5, 51, 52]. Ewes heterozygous simultaneously for both Belclare and Galway or Inverdale and Hanna mutations were also infertile showing a phenotype similar to the observed in homozygous ewes for each mutation [50, 51]. Recently, the Galway mutation was also found in the Small Tailed Han breed with heterozygous mutant ewes producing 0.55 more lambs than the wild-type [53].

The High Fertility mutation (Table 2) was identified in the mature domain of another oocyte secreted cytokine, GDF-9. Similar to the BMP-15 mutations it increases ovulation rate in heterozygous ewes while arresting folliculogenesis at primary stage in homozygous females [51].

Table 2. Major genes affecting ovulation rate in sheep and in which the causing mutation has been identified.

Mutation	Gene	Base	Aminoacid	Location
Galway	BMP-15	C718T	Gln239STOP	Prepeptide
Hanna	BMP-15	C871T	Glu291STOP	Mature peptide
Inverdale	BMP-15	T896A	Val299Asp	Mature peptide
Lacaune	BMP-15	G963A	Cys321Tyr	Mature peptide
Belclare	BMP-15	C1100T	Ser367Ile	Mature peptide
High Fertility	GDF-9	C1184T	Ser395Phe	Mature peptide
Booroola	BMPR1B	A746G	Gln249Arg	Intracellular domain

The Booroola mutation is the only one so far identified in a receptor instead of a BMP ligand (Table 2). It is caused by a single point mutation in the BMP receptor type IB, which is located in the subdomain 3 of the receptor kinase domain [6-8]. It has an additive effect on ovulation with wild type females (++) presenting 1-2 ovulations, heterozygous (B+) showing three or four ovulations and homozygous (BB) ewes having five or more ovulations per cycle [54]. The Booroola mutation was initially described in flocks with a Merino origin, but since then has been found in the Garole, Javanese, Hu and Han sheep breeds [55, 56].

Ewes carrying both the Inverdale and the Booroola mutations had an ovulation rate that was greater than expected for an additive effect alone [57]. Small Tailed Han ewes carrying mutations in both BMPRIB and BMP-15 genes (Booroola and Galway) also had greater litter size than those with either mutation alone [53]. The BMP-15 signalling pathway [58] through BMPRII and BMPRIB activating smad 1/5/8 phosphorylation can offer support to these observations.

Ewes bearing one mutant allele of the BMP-15 gene (Galway or Belclare mutations) and a mutant allele of the GDF-9 gene (High Fertility mutation) have an additive effect on the ovulation rate supporting the view that GDF-9 and BMP-15 have distinct biological effects [51]. This view is also supported by evidence derived from immunization studies, which demonstrated that BMP-15 and GDF-9 are both essential for normal follicular development and control of ovulation rate [59].

The mechanism of action of the mutations affecting ovulation rate in sheep are not fully elucidated but nevertheless several hypothesis have been put forward. However the characteristic that is consistent among these animals is that prolific sheep ovulate more follicles, which mature at smaller diameter producing a similar endocrine milieu [60, 61]. It is likely that the major effect of the mutated gene is evident in the ovary. This local effect has been demonstrated in ewes Booroola rendered hypogonadotrofic by GnRH antagonist and infused with a standard gonadotrophin regime, which maintained the difference in ovulation rate characteristic of each genotype

[62]. This increased ovulation rate in sheep carrying Booroola mutation is related to a reduced rate of atresia, since animals of an old age carrying or not the mutation show no differences on the estimated remaining follicular pool [63].

The cause of increased ovulation in the ewes carrying mutations in the BMP-15 gene could be explained by a simple reduction in the protein production, since the Hanna and Galway mutation induce a premature stop codon leading to a truncation in the protein [5, 51]. In vitro protein expression systems show that the BMP-15 with the Inverdale mutation can form non-covalent dimers but the processing efficiency of BMP-15 mutant proprotein is significantly lower than wild-type BMP-15. When GDF-9 is co-expressed, the processing and secretion of mutant BMP-15 is abolished, and the processing of GDF-9 is also marked reduced, suggesting that the heterodimers of mutant BMP-15/GDF-9 proproteins are not susceptible to proteolytic cleavage [13]. Expanding the use of expression systems to BMP-15 with the Belclare or Inverdale mutations and GDF-9 with the High Fertility mutation showed that when individually expressed, both BMP-15 mutations did not disturb the processing, secretion, and dimerization of the mature proteins or had any effect on the biological activity of the molecules. However, when mutant BMP-15 is co-expressed with wild-type GDF-9, the secretion of mutant BMP-15 and GDF-9 is marked reduced. The expression of mutant GDF-9 with wild-type BMP-15 has no effect on BMP-15 and mutant GDF-9 secretion. In addition, when mutant GDF-9 is co-expressed with mutant BMP-15, the secretion levels of both proteins were significantly lower than those of cells co-expressing wild type GDF-9 and mutant BMP-15 [64]. Protein expression in vitro of BMP-15 with the Lacaune mutation resulted in an impairment of the maturation process of the BMP15 protein, resulting in a defective secretion of both the precursor and mature peptide, which was not due to any alteration in the BMP-15 transcription levels [52]. Interestingly, the main effect of the mutations seems to be at post-transcriptional level because mRNA abundance of BMP-15 or GDF-9 are also not altered by the Inverdale Mutation [5, 65]. Since BMP-15 and GDF-9 are both product of the oocyte in sheep, and expressed in a very similar pattern, the common mechanism among all mutations would be a reduction on the processing and hence biologically active ligands.

The hypothesis of increased ovulation rate in animals with reduced biologically available BMP-15 and GDF-9 is supported by studies in rodents that indicate that BMP-15 reduced the expression of FSH receptor mRNA in granulosa cells and consequently the levels of FSH induced expression of mRNAs encoding the steroidogenic enzymes StAR, P450scc, P450 aromatase and 3beta-HSD. There was also a reduction in the expression of LH receptor, and inhibin/activin subunits [66]. Granulosa cells cultured with GDF-9 reduce

FSH-stimulated progesterone and oestradiol production and also In FSH-induced LH receptor expression. This inhibitory on granulosa cell differentiation were accompanied by decreases in the FSH-induced cAMP production [67]. However, in rodents both GDF-9 and BMP-15 induce marked increase in granulosa cells numbers in vitro [12] while in sheep they have no effect on granulosa proliferation [24].

In vitro, ovarian granulosa cells from BB ewes are less responsive than granulosa cells ++ ewes to the inhibitory effect of BMP-4 on progesterone secretion in vitro [6]. The presence of the Booroola mutation is associated with a decreased responsiveness of granulosa cells to the action of BMP-4 but not TGFbeta-1 and activin-A, which show similar inhibitory effects on progesterone secretion by granulosa cells disregarding the genotype. Transfection experiments with co-expression of Booroola mutant BMPR1-B and BMPR2 failed show activation of BMPRIB by BMP-4, suggesting that the mutant receptor is associated with a reduction in signalling activity [37].

A recent study that compared the behaviour of granulosa and theca cells from BB and ++ ewes *in vitro*. Showed that when exposed to the same gonadotrophin environment granulosa cells from mutant ewes produce more oestradiol and inhibin-a with the similar action being observed in the androstenedione secretion by theca cells. Cells from mutant ewes are also more sensitive to the action of BMP-2, -4, and -6 with the granulosa cells producing more oestradiol and inhibin and theca cells higher androstenedione secretion when treated with same concentration of BMP. These results provide evidence to support the hypothesis that the Booroola mutation increases the BMP response of somatic cells when stimulated to differentiate by gonadotrophin [32].

Taking in account these observations, we propose that the Booroola mutation could act on a novel pathway involving BMPs that inhibits granulosa cell differentiation. This inhibitory pathway involves the BMPR1B and in the wild type normally acts to prevent the development of more than one or two ovulatory follicles. The mutated form of the receptor is partially disabled in the Booroola [37] and hence fewer developing follicles become atretic and more are available at the point of selection of the ovulatory follicles. Alternatively the mutant form of the receptor could be stimulatory since the cells from mutant animals show earlier differentiation in vitro even without addition of exogenous BMPs [32]. Further experiments investigating the relevant components of the BMP pathways in the ovary should help clarify the mechanisms involved in the increase in ovulation rate in high fecundity breeds of sheep.

Conclusions

The expression pattern of BMPs in the mammalian ovary with ligands in the oocyte, granulosa and theca cells, along with the widespread distribution of their receptors and antagonists, give the opportunity for the establishment of

several levels of local regulation of folliculogenesis and ovulation rate. The BMPs are implicated in the regulation of several key points of ovarian and follicle development spreading from the very beginning during the formation of the foetal ovary, regulation of primordial follicles entry into the growing pool, granulosa and theca cell function and finally affecting the number of follicles capable of ovulating. The mammalian domestic species can offer an excellent counterpoint to the rodent models used to investigate folliculogenesis. The sheep naturally occurring mutations in BMP ligands and receptor are of particular interest and have shed some light on very fundamental steps of folliculogenesis and on the regulation of ovulation rate.

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