Involvement of Bone Morphogenetic Proteins (BMPs) in Ovarian Function and Infertility

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Abstract: Advances in assisted reproduction techniques and the treatment of diseases known to be correlated with infertility such as polycystic ovary syndrome and premature ovarian failure require a better understanding of ovarian physiology. Despite the enormous quantity of information produced over the last two decades, the mechanisms controlling follicular development are not fully understood. Ovarian function is regulated by interactions between gonadotropins, follicle stimulating hormone, luteinizing hormone and local ovarian factors such as inhibins, activins, bone morphogenetic protein-15 (BMP-15) and growth differentiation factor-9 (GDF-9), all members of the transformation and growth factor-β (TGF-β) superfamily. There is evidence of a functional ovarian BMP system with countless genes involved in normal follicular development and in fertility. The present review summarizes the ligands of the TGF-β superfamily, their receptors and signaling pathways and discusses the ovarian functions of the BMPs secreted by the oocytes as critical regulators of fertility.

INTRODUCTION

The progress of the ovarian follicle through the successive stages of folliculogenesis depends on an effective bidirectional communication pathway between the oocyte and the somatic follicular cells and also requires endocrine signals, mainly gonadotropins and steroids which act on the receptors on the two cell types and interact with local autocrine/paracrine signaling pathways. Several growth factors, including the members of the TGF-β superfamily, are expressed by the oocyte and by follicular cells in specific stages of development and function as intraovarian regulatory molecules involved in follicular recruitment, proliferation/atresia of granulosa and theca cells, steroidogenesis, oocyte maturation, ovulation, and luteinization [1, 2]. Normal follicular development depends on the coordination between the processes of proliferation/differentiation of somatic cells and of growth/maturation of the oocyte, and the oocyte-granulosa cell signaling is of critical importance for insuring this coordination, promoting integrated cell functions [3, 4]. Intercellular communication occurs *via* transzonal cytoplasmic processes and paracrine mediators. Particularly important among them are the stem cell factor, also known as kit-ligand (KL), and bone morphogenetic proteins (BMPs), the GDF-9 and BMP-15 or GDF9B [4]. Mutations that inactivate the GDF-9 and BMP-15 genes are associated with premature ovarian failure and infertility in sheep and in women, indicating the fundamental participation of these growth factors in the regulation of folliculogenesis and ovulation [5, 6]. In addition, a GDF-9/BMP-15-KL feedback loop has been described as an important signaling mechanism on the oocyte-granulosa cell interface, as well as a mechanism of coordination of follicular development [1, 7, 8].

THE TGF-β SUPERFAMILY

The extracellular signaling molecules of the TGF-β superfamily comprise approximately 30 structurally related and functionally distinct proteins which in mammals include three TGF-β isoforms (TGF-β1,2,3), anti-müllerian hormone (AMH), two inhibins (A and B), three activins (A, B, AB), 20 BMPs (BMP-1-BMP-20), and nine growth and differentiation factors (GDF-1-GDF-9) [9]. Collectively, the ligands of the TGF-β superfamily have regulatory functions in a wide variety of tissues, with a well-documented participation in processes such as the induction of the embryonic mesoderm, differentiation of the genital duct, ovarian folliculogenesis, and other physiological processes [10, 11]. Among the factors expressed in the ovary are GDF-9, GDF-9B (BMP-15), inhibins, activins, TGF-β, BMPs 2, 4, 6, 7, and AMH [2].

THE OOCYTE GROWTH FACTORS: THE BMPs

BMPs are multifunctional cytokines that can regulate cell proliferation/differentiation, deposition of the extracellular matrix, and apoptosis [12]. Among the 20 known BMPs, eight are implicated in the formation and/or function of mammalian germ cells and in ovarian formation/function (BMP-2, BMP-4, BMP-6, BMP-7, BMP-15 and GDF-9) [13-16], with GDF-9, BMP-15 and BMP-6 being selectively expressed by the oocyte in different species in the early stages of folliculogenesis [2, 17-19]. BMPs are synthesized and secreted as prepropeptides and are proteolytically cleaved to form activated disulfide-linked dimers, most of them being homodimers. However, the gene products of BMP-15 and GDF-9 can form heterodimers [20].

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OVARIAN FUNCTIONS OF BMPs SECRETED BY OOCYTE

The expression and bioactivity of oocyte growth factors have been investigated in studies mainly focusing on murine culture systems and have demonstrated that the oocyte regulates various parameters of follicular cell function *in vitro*, particularly by stimulating mitotic activity and estradiol secretion and inhibiting progesterone synthesis [21, 22]. These data have also been reported for swine, sheep, bovine, non-human primate and human ovaries [13, 23-28]. Deletion of the *gdf9* gene causes infertility with blockade of folliculogenesis in the primary stage in mice [14] and results in down-regulation of mRNA expression for activin Bβ, follistatin and cyclooxygenase 2, as well as a significant up-regulation of KL expression [3], with the development of follicles with atypical granulosa cells (GC) and without the theca cell (TC) layer. Thus, GDF-9 can bind to the receptors of granulosa cells and regulate the expression of various gene products [29]. During terminal follicle maturation, GDF-9 performs important functions in order to trigger the mechanisms associated with ovulation, such as: 1) maintaining the structural integrity of the preovulatory follicle, inducing hyaluronan synthetase 2 and pentraxin 3 and suppressing the plasminogen activator urokinase; 2) stimulating prostaglandin and progesterone synthesis; 3) suppressing early luteinization of cumulus GC, thus inhibiting the synthesis of LHR mRNA and the expansion of the cumulus [30]. BMP-15 stimulates cell proliferation with an FSH-independent mitogenic effect in primary rat GC cultures [18, 31]. It also suppresses the expression of FSH receptors and stimulates KL expression [32]. The biological effects of BMP-6 on GC differ from those of other BMPs. BMP-6 has no mitogenic effect on GC [33] and inhibits the production of P4 induced by forskolin, and the expression of StAR and P450scc mRNA [34]. The mechanism by which BMP-6 inhibits the action of FSH probably involves down-regulation of adenylate cyclase activity, in contrast to BMP-15, which inhibits the basal expression of FSH receptors.

RECEPTOR AND INTRACELLULAR SIGNALING OF BMPs

The members of the TGF-β superfamily bind to the serine/threonine kinase membrane receptors and activate the Smad transcription factors [35, 36]. In the human genome, the serine/threonine kinase receptor family comprises 12 members, five of them being type II receptors: type II BMP receptor (BMPRII), type II anti-müllerian hormone receptor (AMHRII), type II TGF-β receptor, and type II activin receptors (ATCRIIA and ATCRIIB). There are also seven type I receptors referred to as activin kinase-like (ALK) receptors [37]. Both receptor types consist of approximately 500 amino acids and present an aminoterminal extracellular domain with 10 or more cysteines, a transmembrane region, and a carboxy terminal serine/threonine kinase domain. The ligands activate two main intracellular signaling pathways characterized by the activation of two different Smad protein groups: Smad 1/5/8 and Smad 2/3 (Fig. **1**) [38, 39]. BMPs, like other ligands of the TGF-β family, act on serine/threonine kinase receptors [12], and signal transduction requires the formation of a hetero-oligomeric complex with BMP receptors (BMPRs) of types II and IA or IB (ALK)

[12, 40]. BMPR-II has autophosphorylation activity and is responsible for the activation of the type I receptor after the complex is formed. After receptor activation, a signaling cascade is started, involving the phosphorylation, heterodimerization and migration of Smad proteins to the nucleus [40]. The activated Smad complexes translocated to the nucleus, together with other nuclear co-factors, regulate the transcription of target genes [11] (Fig. **1**). The transcription factor-Smad interaction determines the precise response to the ligand in different cell types and in cooperation with other signaling pathways [41]. GDF-9 interacts with the BMPRII and ALK5 receptors and activates the Smad 2/3 pathway, while BMP-15 activates the Smad 1/5/8 pathway by interacting with the BMPRII and ALK6 receptors, as observed in rat GC [31]. The activation of distinct signaling pathways by homodimers of GDF-9 and BMP-15 may be associated with specific functions of these BMPs in follicular development. However, GDF-9 and BMP-15 can form homo- or heterodimers [20, 42] and the latter can activate, like GDF-9, a hybrid of the two signaling pathways [33]. The three types of BMP receptors are expressed in the different types of ovarian cells, including the oocyte, GC, TC, and epithelial and endothelial ovarian cells of rats, sows, sheep and women [15, 28, 43-45]. BMPRs have also been observed in the fetal swine ovary, indicating their involvement in the formation of ovarian follicles [44]. The presence of BMP receptors and the ability of these factors to modulate steroidogenesis are indicative of a functional intraovarian BMP system.

REGULATION OF BMP EXPRESSION IN THE OVARY

It is still unclear which factor is responsible for the activation or regulation of BMP expression in the ovary. Two factors pointed out as regulatory of BMP activity are follistatin and KL. Follistatin can specifically bind to BMPs and attenuate changes induced by these proteins in bovine and rat GC [16, 46]. BMP-15 and GDF-9 respectively stimulate and inhibit the expression of KL and KL inhibits the expression of BMP-15 [7, 8]. Thus, follistatin and KL may represent two potential regulatory mechanisms for the control of BMP activity in the ovary. However, these interactions and others still need to be clarified.

BMPs AND INFERTILITY

The recent interest in BMPs derives from observations that correlate induced or spontaneous genetic mutations in members of the BMP family with impairment of ovarian development and ovulation [6, 14, 47, 48]. Spontaneous mutations in the ovine and murine BMP-15 (*FecX*) genes and in the ovine GDF-9 (*FeGC*) gene may drastically alter fertility and ovulatory rate in this mammalian species. Deletion of the *bmp15* or *gdf9* genes (sheep, mice) or immunization against BMP-15 (sheep) results in infertile animals [49, 50]. Infertility is due to delayed follicular development and histological examination reveals large oocytes, absent or abnormal granulosa proliferation, and faulty thecal recruitment (GDF-9-knockout mice) [14, 51]. Inverdale and Hanna sheep, by presenting BMP-15-inactivating mutations, represent an interesting model for the understanding of the functions of this protein in the control of folliculogenesis.
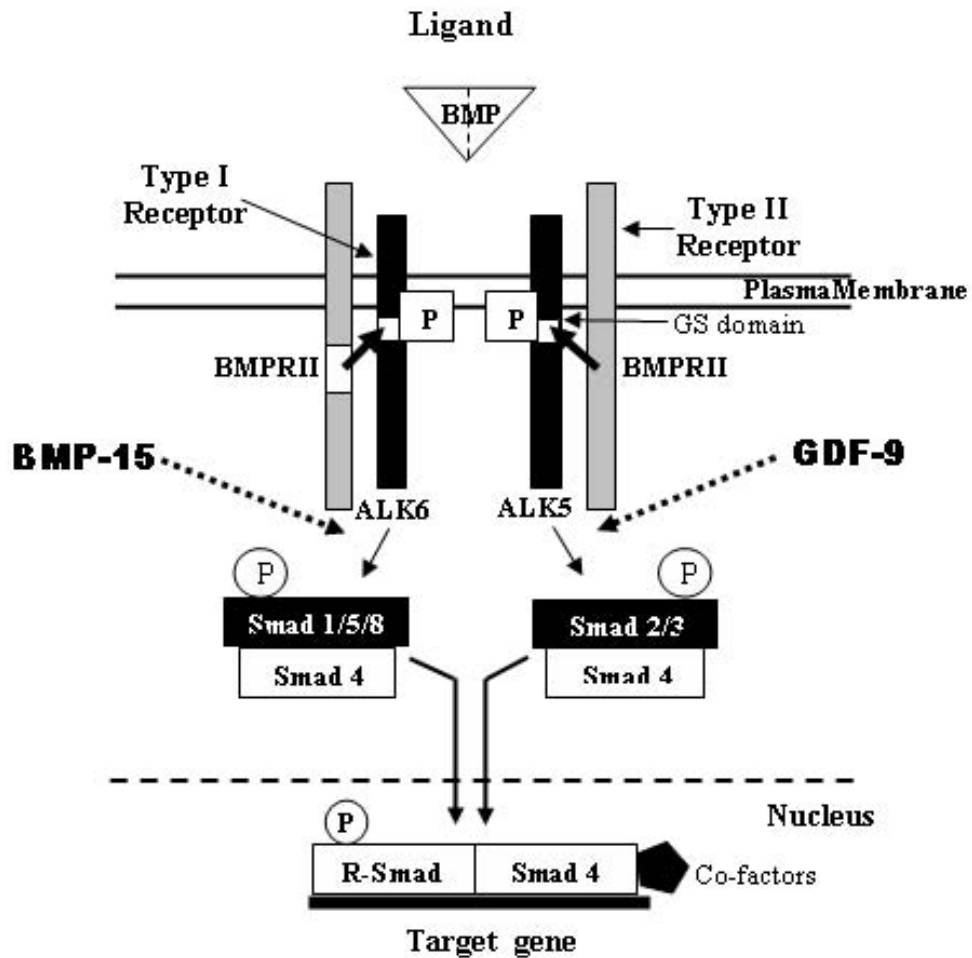
Fig. (1). Receptors and signaling pathways of BMP-15 and GDF-9. The GDF-9 or BMP-15 dimers bind to specific serine-threonine kinase type I and type II receptors and the formation of the tetramer (2 type 1 and 2 type II receptors for each ligand) permits the phosphorylation of the type I receptor by the type II receptor in the glycine-serine (GS) domain, resulting in the formation of the signal transduction complex. Type I ALK5 and ALK6 receptors respectively recognize and phosphorylate R-Smads 2/3 and 1/5/8. The phosphorylated R-Smads form complexes with Smad-4 (Co-Smads) and are translocated to the nucleus, where they interact with specific DNA motifs. The effective binding to the target gene is modulated by various nuclear cofactors that co-activate or co-repress transcription.

Mutation in homozygosis ($FecX^I$/$FecX^I$) causes infertility with blockade of folliculogenesis in the primary stage [51], while mutation in heterozygosis ($FecX^I$/$FecX^+$) causes an increase in ovulatory rate and multiple births [6, 52]. Similarly, spontaneous mutations in the *gdf9* gene in Belclare and Cambridge sheep cause a dose-dependent increase in ovulatory rates [49]. In the Booroola race, spontaneous mutations that inactivate the genes coding for BMP receptors, such as BMPR-IB (*FecB*), also cause similar changes in ovulatory rate [45, 53]. It has been suggested that the reduced availability of BMP-15 in heterozygous sheep may stimulate follicular development by increasing sensitivity to FSH, since *in vitro* studies indicate that BMP-15 inhibits the expression of FSH receptors [19]. In fact, the characteristics of early follicular development are larger oocytes and granulosa cells more responsive to FSH and with a lower proliferative activity than normal GC [52, 53]. In this case, the ovary produces a large number of smaller and more estrogenic follicles that escape endocrine control, most of them progressing to ovulation. Some authors have investigated in human ovaries the

correlation between mutations in the *gdf9* and *bmp15* genes and diseases such as premature ovarian failure (POF) and polycystic ovary syndrome (POS) [47, 48, 54]. The detection of GDF-9 and BMP-15 mRNA in normal human oocytes indicates that these factors may play a fundamental role in folliculogenesis and fertility in women [55]. POF occurring before 40 years of age is a heterogeneous condition caused by autoimmune or genetic changes and in most cases its etiology is unknown [56]. This condition may mean that the follicular cohort suffers atresia at accelerated rates or that the follicles do not respond to gonadotropin stimulation [54]. Evaluation of *bmp15* and *gdf9* in different populations of women with POF has suggested that these changes rarely occur in this disease [54]. Mutational analyses of these genes in a group of women with a clinical diagnosis of POF have not reached conclusive results but indicate that this is a plausible explanation for POF. However, discordant results have been obtained for different populations and ethnic groups and some authors have suggested that mutations in the *gdf9* gene associated with POF present a spectrum that varies ac-

cording to the population, rather than representing a single mutation shared by all populations [57]. Polycystic ovary syndrome (POS) is one of the most common causes of anovulation, infertility and menstrual irregularity in women, affecting 5 to 10% of women of reproductive age. POS has been defined as a syndrome involving polycystic ovaries, hyperandrogenism, and chronic anovulation, with the exclusion of specific diseases of the ovaries, adrenal glands and pituitary [58]. There is evidence indicating that the mechanism of POS involves ovarian dysfunction [59]. In POS, the ovaries contain double the normal number of growing follicles, supporting this hypothesis. This finding is particularly significant by suggesting that all steps in the folliculogenesis process can be aberrant in this syndrome. In addition, there is evidence that, in POS, follicular growth and development stops when the follicles reach 4 to 7 mm, causing an accumulation of large numbers of small follicles in the tunica albuginea. The interstitial theca cells associated with follicle development exhibit high levels of androgen biosynthesis and also support the role of the ovaries in POS [60]. This evidence raises the hypothesis that abnormal folliculogenesis occurs in POS [55]. Due to altered folliculogenesis and cyst formation in this syndrome, the expression of GDF-9 and BMP-15 was assessed in women with POS and in women with polycystic ovaries during follicular development. The mRNA levels of GDF-9, but not of BMP-15, were found to be reduced in this group of women during the phase of differentiation and growth [55]. In assisted reproduction techniques the quality of the oocytes obtained with controlled ovarian hyperstimulation varies widely. Only half of these fertilized oocytes complete preimplantation development and few of them do implant. Some studies have demonstrated that changes in the expression of genes such as *gdf9* and *bmp15* in the oocytes or in pentraxin 3 in cumulus cells can be monitored for an appropriate selection of the oocyte to be fertilized [61]. These observations illustrate the importance of BMP-15 as a regulator of fertility and indicate that the BMPs secreted by the oocyte are the apex of the regulatory mechanisms associated with folliculogenesis. However, fertility and the development of healthy follicles and oocytes depend on various paracrine and endocrine factors in addition to BMPs. The IGF/IGFBP system, the fibroblast growth factors, epidermal growth factor/TGF-α, the endothelial growth factors, interleukins and members of the TGF-β superfamily such as activins, follistatins, inhibins and anti-müllerian hormone are among several other intraovarian peptides involved in the control of folliculogenesis.

CONCLUSIONS

The severe consequences of mutations and deletions in the *gdf9* and *bmp15* genes for the number of dominant follicles and the ovulatory rate of mice and sheep demonstrate the fundamental importance for ovarian function of these proteins secreted by the oocyte. However, little information is available about the actions of these factors on human infertility. In view of the impact of GDF-9 and BMP-15 on reproduction in animal models, it is clear that the GDF-9/BMP-15 system represents an important focal approach to the treatment of infertile women. Studies defining the precise involvement of these factors in human ovarian physiology/physiopathology are needed for the advancement of basic science and for the potential application of the knowledge obtained to clinical biotechnology involving the ovarian BMP system.

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