HHS Public Access

Author manuscript

Vitam Horm. Author manuscript; available in PMC 2018 December 28.

Published in final edited form as:

Vitam Horm. 2018 ; 107: 317–348. doi:10.1016/bs.vh.2017.12.003.

Molecular Aspects and Clinical Relevance of GDF9 and BMP15 in Ovarian Function

Martina Belli and **Shunichi Shimasaki**[1]

University of California San Diego, School of Medicine, La Jolla, CA, United States

Abstract

Growth and differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) are oocyte-secreted factors with a leading role in the control of ovarian function in female reproduction, modulating both the cell fate of the somatic granulosa cells and the quality and developmental competence of the egg. This short review aims to consolidate the molecular aspects of GDF9 and BMP15 and their integral actions in female fertility to understand particularly their effects on oocyte quality and fetal growth. The significant consequences of mutations in the *GDF9* and *BMP15* genes in women with dizygotic twins as well as the clinical relevance of these oocyte factors in the pathogenesis of primary ovarian insufficiency and polycystic ovary syndrome are also addressed.

1. INTRODUCTION

The bidirectional communications between the oocyte and GCs in the ovary consist in the exchange of nutrients and signals that influence the growth and differentiation of both somatic cells and the egg by a complex regulatory network of autocrine, juxtacrine, and paracrine factors (Eppig, 2001; Gilchrist, Ritter, & Armstrong, 2004; Matzuk, Burns, Viveiros, & Eppig, 2002). Earlier observations on the importance of this communication throughout folliculogenesis provided evidence of the regulatory and nourishing roles of GCs and cumulus cells (CCs) on the oocyte (Anderson & Albertini, 1976; Gilula, Epstein, & Beers, 1978; Goodenough, Simon, & Paul, 1999), while more recent studies led to the conclusion that the oocyte exerts a key role in influencing somatic cells' proliferation, differentiation, and function through the production of soluble factors (Gilchrist, Lane, & Thompson, 2008). The oocyte is also able to influence its own developmental competence by inducing the production of positive regulatory factors in CCs that, in turn, act on it. For instance, factors produced by denuded oocytes, or exogenous recombinant GDF9 or BMP15, were able to increase the rates of blastocyst formation after in vitro maturation (IVM) and in vitro fertilization (IVF) of cumulus-oocyte complexes (COCs) (Dey et al., 2012; Gomez et al., 2012; Hussein, Sutton-McDowall, Gilchrist, & Thompson, 2011; Hussein, Thompson, & Gilchrist, 2006; Romaguera et al., 2010; Su, Hu, et al., 2014; Su, Wang, et al., 2014; Sudiman et al., 2014; Yeo, Gilchrist, Thompson, & Lane, 2008). Thus the oocyte, in addition to regulate the CC phenotype in its surrounding cells, can indirectly control and influence its own developmental competence.

[1]Corresponding author: e-mail address: sshimasaki@ucsd.edu.

2. DISCOVERY OF GDF9 AND BMP15

GDF9 and BMP15 were first described in 1993 (McPherron & Lee, 1993) and 1998 (Dube et al., 1998; Laitinen et al., 1998), respectively, as novel members of the TGF-β superfamily and oocyte-specific factors in the mouse ovary (Dube et al., 1998; Laitinen et al., 1998; McGrath, Esquela, & Lee, 1995; McPherron & Lee, 1993). The mRNAs of both proteins are expressed in oocytes of the postnatal mouse ovary throughout folliculogenesis with a very low or no expression in oocytes of primordial follicles and a strong expression in oocytes at all stages of developing follicles (Laitinen et al., 1998; McGrath et al., 1995). GDF9 mRNA is also detectable in ovulated oocytes (McGrath et al., 1995), and indeed, its EST cDNA was originally identified from a mouse two-cell embryo library (Laitinen et al., 1998), although its expression is rapidly lost thereafter (McGrath et al., 1995). These findings excited and rekindled many researchers who have been studying over a long period the role of OSFs in folliculogenesis and ovulation. It is now well recognized that these factors play pivotal roles in the normal proliferation and differentiation of GCs and for the acquisition of developmental competence by the oocyte.

3. GDF9 AND BMP15 STRUCTURES

All TGF-β superfamily members including GDF9 and BMP15 are synthetized as large preproproteins composed of a signal peptide, a prodomain that directs their dimerization and a mature domain (Shimasaki, Moore, Otsuka, & Erickson, 2004). After removal of the signal peptide, the dimeric proproteins are cleaved by furin-like proteases, and the dimeric mature proteins are secreted (Al-Musawi, Walton, Heath, Simpson, & Harrison, 2013; Shimasaki et al., 2004; Simpson et al., 2012). GDF9 and BMP15 are the most homologous proteins in amino acid sequence, and a peculiarity of these proteins is that the fourth cysteine residue is substituted by a serine. The fourth cysteine is the only cysteine that is involved in the formation of intersubunit disulfide bond and present in all TGF-β superfamily members except not only GDF9 and BMP15 but also GDF3, lefty-1, and lefty-2 (McPherron & Lee, 1993; Meno et al., 1997). Thus, a question was whether GDF9 and BMP15 proteins exist as monomers or noncovalently linked homodimers. They are now known to be noncovalently linked homodimers (Liao, Moore, Otsuka, & Shimasaki, 2003).

The proteolytic cleavage of the recombinant BMP15 and GDF9 proproteins expressed in vitro is not as efficient as expected, unless specific proteolytic enzymes are simultaneously overexpressed (Hayashi et al., 1999; Li, Rajanahally, Edson, & Matzuk, 2009; Liao et al., 2003; Liao, Moore, & Shimasaki, 2004; McNatty et al., 2005a; Mottershead et al., 2008; Otsuka, Moore, & Shimasaki, 2001; Pulkki et al., 2012; Reader et al., 2011; Saito, Yano, Sharma, McMahon, & Shimasaki, 2008). Similarly, in monkey follicular fluid, a strong and a faint band of GDF9 proprotein and mature protein, respectively, was observed, while BMP15 proprotein but not mature protein was detected (Duffy, 2003). Proproteins and mature proteins were also detected in human (Requena, Cruz, Agudo, Pacheco, & Garcia-Velasco, 2016; Wu et al., 2007) and dairy cow (Behrouzi, Colazo, & Ambrose, 2016) follicular fluid with different ratios. Thus, proproteins of GDF9 and BMP15 together with the respective mature proteins are secreted in vitro in cell culture and in vivo from the oocyte. Moreover, the secretion of mature and proproteins of these factors by the oocyte

depends on the species analyzed, and it can be different if produced in vivo or during in vitro culture (Gilchrist et al., 2004; Lin et al., 2012; McNatty et al., 2006). For instance, it was demonstrated that in vitro, but not in vivo, mouse COCs are deficient of the mature protein of BMP15 (Mester et al., 2015; Yoshino, McMahon, Sharma, & Shimasaki, 2006), or that sheep oocytes produce only unprocessed GDF9 and BMP15 in follicular fluid (McNatty et al., 2006), while during IVM they secrete mature proteins of the two factors (Lin et al., 2012). Further, in mouse, IVM oocytes produce a mixture of pro-GDF9 and mature GDF9 (Gilchrist et al., 2004), while in rat, IVM oocytes produce only mature GDF9 (Lin et al., 2012).

Some TGF-β superfamily members are secreted as a complex consisting of the mature dimer noncovalently bound to the prodomain (Israel, Nove, Kerns, Moutsatsos, & Kaufman, 1992; Jones et al., 1994; Thies et al., 2000; Wang et al., 1990). Saito et al. demonstrated that purified and bioactive recombinant human BMP15 is composed of the dimeric mature and proregion proteins (Saito et al., 2008). In contrast, Simpson et al. reported that recombinant human, but not mouse, GDF9 is inactive due to a latent complex with proregion proteins (Simpson et al., 2012). However, it is not always the case since purified bioactive recombinant human GDF9 produced from HEK293 (Liao et al., 2004) and HEK293T cells (Mottershead et al., 2008) was also reported. Further, both recombinant human BMP15 and GDF9 are phosphorylated and the phosphorylation is necessary for their bioactivity (McMahon, Sharma, & Shimasaki, 2008; Saito et al., 2008). The kinase for BMP15 was later identified to be Fam20C (Tagliabracci et al., 2012). Thus, the phosphorylation status of BMP15 and GDF9 should also be addressed with regard to their biological activity. The necessity of standardization in preparation and biochemical analysis for recombinant GDF9 was previously proposed by Pangas and Matzuk (2005), but unfortunately without substantial clues until now.

4. GDF9/BMP15 HETERODIMERS

As stated earlier, GDF9 and BMP15 share most homologous amino acid sequences, lack the fourth cysteine necessary for the covalent linkage between two subunits, and are expressed in the same cell (the oocyte) at the same time from the primary stage throughout folliculogenesis. Based on these information and our earlier experience in discovering activin (now called activin AB) that is composed of β_A and β_B subunits of inhibin A and B, respectively (Ling et al., 1986), we speculated that GDF9 and BMP15 may form heterodimers and demonstrated the first direct physical evidence of the heterodimeric GDF9/BMP15 molecule (Liao et al., 2003). Thereafter over a decade the integrated bioactivity of GDF9 and BMP15, observed in different species and functional studies, let many researchers approach on the formation of a functional heterodimer (Hanrahan et al., 2004; McIntosh et al., 2008; McNatty et al., 2005a, 2005b, 2004; Mottershead, Ritter, & Gilchrist, 2012; Peng et al., 2013). However, only recent studies demonstrated the functional evidence of the purified recombinant human GDF9/BMP15 heterodimer, which was named cumulin (Mottershead et al., 2015), able to exhibit highly potent bioactivity on GCs and to improve oocyte quality. Specifically, cumulin stimulates thymidine incorporation in primary mouse mural GCs (ED_{50}, 0.6ng/mL) and both SMAD2/3 and SMAD1/5/8 luciferase reporter activities in COV434 human GC line. Cumulin also regulates in vitro GC differentiation by

increasing mRNA expression of *Ptx3, Has2, Tnfaip6,* and *Ptgs2.* Covalent cumulin, in which the fourth cysteine is recovered, is more potent than noncovalent procumulin in these assays. Noncovalent procumulin, however, is more effective in the improvement of oocyte quality than the covalent cumulin, proving the important functional activity of the prodomain in these factors.

Mottershead et al. also demonstrated interesting data, suggesting that mature GDF9 and BMP15 as well as pro-GDF9 and pro-BMP15 form heterodimers extracellularly. They further proposed that the latent GDF9 is activated following the heterodimerization with BMP15 to form cumulin, either within the oocyte or in the extracellular matrix (Mottershead et al., 2015). This could explain the potent synergistic action observed between BMP15 and GDF9 in terms of mitogenic and differentiating activity on GCs as well as on oocyte quality, raising cumulin to the position of main regulator of fertility in monoovulatory mammals. Therefore, the differential or synergistic effect of GDF9 and BMP15 may occur depending on whether they exert their bioactivity separately or cooperatively by forming cumulin, respectively.

BMP15 binds to the complex of ALK6 and BMPRII on GC surface and activates SMAD1/5/8 intracellular pathway (Moore, Otsuka, & Shimasaki, 2003; Pulkki et al., 2012; Shimasaki et al., 2004), whereas GDF9 activates ALK5 and BMPRII receptors and triggers SMAD2/3 pathway downstream (Kaivo-Oja et al., 2003; Mazerbourg et al., 2004; Roh et al., 2003; Vitt, Mazerbourg, Klein, & Hsueh, 2002), although it is controversial whether ALK5 is the only type I receptor for GDF9 as *Alk5* conditional knockout mice have no defects in follicular development and cumulus expansion (Li et al., 2011), which is a distinct phenotype from *Gd/9*-null mice (Dong et al., 1996). Interestingly, it is suggested that cumulin binds to a receptor complex formed by two BMPRII, one ALK6, and one ALK5/4 molecule, which can activate the two distinct SMAD pathways (i.e., SMAD1/5/8 and SMAD2/3). This last evidence is in contrast to the work of Peng et al., which showed that the GDF9/BMP15 heterodimer signals exclusively through SMAD2/3 pathway, since it can bind to but not activate the ALK6 receptor (Peng et al., 2013).

5. SPECIES-SPECIFIC ROLES OF GDF9 AND BMP15

The significance of GDF9 and BMP15 factors in the regulation of ovarian function emerged with the studies of deficient mouse models of GDF9 (Dong et al., 1996) and BMP15 (Yan et al., 2001). *Gd/9*-deficient female mice are infertile and show a block of folliculogenesis at the stage of primary follicles, when one layer of cuboidal GCs surrounds the oocyte (Dong et al., 1996). Ovaries from *Gdf9*-deficient female mice appear smaller in size, the number of primordial and primary follicles is higher compared with control, no corpora lutea are observed, and atretic follicles are missing. Without the support of highly differentiated GCs, oocytes are prevented to acquire meiotic competence, thus altering the reproductive function. Moreover, the hypogonadal phenotype related to the absence of GDF9 induces higher serum levels of FSH and LH as compared with control. Deficient males are not affected in the reproductive phenotype as they proved to be fertile (Carabatsos, Elvin, Matzuk, & Albertini, 1998; Dong et al., 1996).

In contrast, *Bmp15*-deficient female mice are subfertile, with a lower litter size and a lower number of litters per month (Pangas & Matzuk, 2004; Yan et al., 2001), demonstrating a less essential role for BMP15 in mouse reproductive function compared with GDF9. *Bmp15*-null male mice are fertile with normal testis size (Yan et al., 2001), highlighting the importance of this factor restricted to the female reproductive function. Also, mice lacking BMP15 and GDF9 showed lower expression of epidermal growth factor receptor (EGFR) compared with wild-type mice, and the activation of SMAD2/3 and extracellular signal-regulated kinases 1 and 2 signaling in CCs triggered by the oocyte was poor (Su et al., 2010). Thus, the oocyte, via paracrine signaling of OSFs, guides the acquisition of responsiveness of CCs to EGF signals, promotes a correct cumulus expansion, and improves its own meiotic maturation and developmental competence in the follicle (Ritter, Sugimura, & Gilchrist, 2015; Su et al., 2010; Sugimura et al., 2015). Moreover, even though BMP15 was shown to have a role in the preservation of gap junction communication between the oocyte and CCs (Sugimura et al., 2014), the action of OSFs on cumulus expansion is species specific, as for some species (e.g., in the mouse) the activation of SMAD2/3 and/or SMAD1/5/8 is required (Buccione, Schroeder, & Eppig, 1990; Dragovic et al., 2007), whereas in the cow the presence of OSFs is not critical (Sugimura et al., 2014).

Of interest in species-specific differences in the role of GDF9 and BMP15 is the dissimilarity between mono- vs polyovulatory species (Dong et al., 1996; Galloway et al., 2000; Moore, Erickson, & Shimasaki, 2004). For instance, BMP15 action is more important in sheep (mono-ovulatory) rather than mice (polyovulatory) in the first stage of follicle development (Eppig, 2001). In fact, while *Bmp15*-null female mice show impaired fertility but an overall normal follicular morphology (Yan et al., 2001), sheep with spontaneous homozygous mutations in the *Bmp15* gene show no further follicle development beyond the primary stage, which lead to sterility (Eppig, 2001; Galloway et al., 2000; Hanrahan et al., 2004).

Why is the role of BMP15 in female mice during folliculogenesis less critical than that in ewes? One of the relevant findings is that the functional mature BMP15 in the mouse ovary is not detectable before the ovulatory stage (Yoshino et al., 2006). Three days before ovulation, however, the mature BMP15 is produced and induces cumulus expansion in mouse COCs along with increased expression of essential EGF-like growth factors (Park et al., 2004; Yoshino et al., 2006). These findings are consistent with the phenotype of *Bmp15*-null mice, which exhibit normal folliculogenesis but have defects in the ovulation process (Yan et al., 2001). The species-specific differences in the phenotypes caused by *Bmp15* mutations may thus be attributed to the temporal variations in the production of the mature form of BMP15. Additionally, Crawford et al. reported that mean expression levels of BMP15 mRNA are lower than GDF9 mRNA in denuded mouse oocytes (Crawford & McNatty, 2012) in contrast to the similar ratio of BMP15 and GDF9 produced by the oocyte in sheep (Crawford & McNatty, 2012). Indeed, ewes with spontaneous homozygous mutations in the *Bmp15* or *Gdf9* gene have high similarity in their infertile phenotype (McNatty et al., 2005). Ewes with spontaneous heterozygous mutations in *Bmp15* or *Gdf9* also share the increased fecundity with an augmented ovulation rate and increased litters of twins and triplets, due to an amplified sensitivity to LH and development of secondary follicles followed by an increased number of antral follicles (Galloway et al., 2000;

Hanrahan et al., 2004; Juengel, Hudson, Whiting, & McNatty, 2004), showing that the dosage of BMP15 or GDF9 in the oocyte can modulate fertility (Galloway et al., 2000; Moore et al., 2004).

6. OOCYTE ACTIONS ON GCs

The evidence of a critical function of the oocyte in promoting proliferation of GCs in preantral and antral follicles dates back to the early 1990s with the studies of Vanderhyden, Cohen, and Morley (1993) and Vanderhyden, Telfer, and Eppig (1992). The oocyte was proved to induce GC proliferation through the production of a soluble factor that was acting in a paracrine fashion on GCs, most likely via follicular fluid. Experiments with mouse CCs from COCs or GCs from preantral follicles cultured with oocyte-conditioned medium suggested that no direct contact between the oocyte and somatic cells was required to proliferate (Vanderhyden et al., 1992). However, the ability of the oocyte to promote GC proliferation and differentiation is highly influenced by the developmental stage of the oocyte, besides the developmental state of GC itself (Gilchrist, Ritter, & Armstrong, 2001). In particular, the meiotic status of the oocyte determines the mitogenic influence on GC proliferation. During meiotic competence acquisition the oocyte highly promotes GC proliferation and starts losing this capability at the metaphase I of the meiosis. Coculturing experiments with meiotically arrested GV oocytes and mural GCs gave the highest GC proliferation rate that was gradually decreased when GCs were cultured with maturing oocytes, ovulated oocytes, or zygotes (Gilchrist et al., 2001). In fact, meiotically growing incompetent oocytes, even if they were proved to express BMP15 and GDF9 (Dube et al., 1998; McGrath et al., 1995), are less effective in regulating follicular cell function than fully grown GV-stage oocytes (Gilchrist et al., 2001). When oocytes from secondary follicles were paired with somatic cells from newborn mouse ovaries and implanted beneath the renal capsules of ovariectomized female mice, a double rate of follicle growth along with GC differentiation was observed after only 9 days from implantation. In this study, the oocytes in the newly formed and developed follicles were able to resume meiosis, be fertilized, and complete the preimplantation stages of development in vitro (Eppig, Wigglesworth, & Pendola, 2002).

OSFs modulate the production of factors that control ovarian functions in promoting follicular growth, including inhibin (Lanuza, Groome, Baranao, & Campo, 1999), luteinizing hormone receptor (Eppig, Pendola, & Wigglesworth, 1998; Eppig, Wigglesworth, Pendola, & Hirao, 1997), and kit ligand (Joyce, Pendola, Wigglesworth, & Eppig, 1999), and contribute to the regulation of gene expression (Paradis et al., 2010; Regassa et al., 2011), metabolism (Sugiura et al., 2007), and apoptosis (Hussein, 2005). As for apoptosis, the level of CC apoptosis was increased when the oocytes were removed from the COCs but reversed when the oocytes were added back to the CC culture (Hussein, 2005), suggesting that OSFs play a role to prevent CC apoptosis. Oocyte-secreted BMP15 and BMP6 as well as theca cell-secreted BMP7 (Erickson & Shimasaki, 2003) were shown to protect spontaneous CC apoptosis, but GDF9 has no significant effect (Hussein, 2005). This is likely to be due to the different signaling pathways, namely SMAD1/5/8 for BMP15, BMP6, and BMP7, and SMAD2/3 for GDF9.

7. OSF EFFECTS ON THE OOCYTE DEVELOPMENTAL COMPETENCE

GDF9 and BMP15 have critical roles in the ovary for the transition after primary stage of follicle development, but they also control later events in follicle development. The absence of GDF9 or BMP15 in mice, as previously described, brings to a phenotype of infertility or reduced fertility, respectively, due to misregulation of GCs and low developmental competence of the oocyte (Su et al., 2004). However, the treatment of COCs with recombinant GDF9, BMP15, or denuded oocytes during IVM led to higher rates of blastocyst formation and fetal yield after IVM and IVF of COCs in cattle (Dey et al., 2012; Hussein et al., 2011, 2006; Su, Hu, et al., 2014; Su, Wang, et al., 2014), in mice (Glister, Groome, & Knight, 2003; Sudiman et al., 2014; Yeo et al., 2008), in goats (Romaguera et al., 2010), and in pigs (Gomez et al., 2012). BMP15 in particular seems to interact with amphiregulin (one of EGF-like growth factors) to enhance oocyte developmental competence, via a prolonged gap-junctional coupling between the oocyte and CCs that would allow the passage of metabolites to the oocyte (Sugimura et al., 2014). The implication of these results resides in the improvement of IVM techniques, in which compromised oocytes from IVM cycles might benefit from treatment with OSFs to restore a good developmental competence. In fact, IVM oocytes might not be able to secrete oocyte factors that are able to sustain oocyte meiotic maturation, as demonstrated by the evidence that murine IVM COCs lack a processed form of BMP15 compared with in vivo matured COCs (Sugimura et al., 2014). The synergistic effects of BMP15 and GDF9 in the improvement of oocyte meiotic competence might be related to a better nuclear and cytoplasmic maturation, the prevention of zona pellucida hardening, a correct fertilization pattern, and the higher expression of glutathione peroxidase 1 in the oocyte (Dey et al., 2012).

8. GDF9 AND BMP15 IN DIZYGOTIC TWINNING, POI, AND PCOS

As stated earlier, GDF9 and BMP15 can influence female reproduction. Published data that have been accumulated so far indicate that non-synonymous mutations in the *GDF9* and *BMP15* genes are correlated with dizygotic (DZ) twinning (Montgomery et al., 2004; Palmer et al., 2006; Zhao et al., 2008) (Table 1) as well as gynecological diseases such as primary ovarian insufficiency (POI) (Bouilly et al., 2016; Di Pasquale, Beck-Peccoz, & Persani, 2004; Di Pasquale et al., 2006; Dixit et al., 2005, 2006; Ferrarini et al., 2013; Kovanci et al., 2007; Laissue et al., 2006; Ledig, Ropke, Haeusler, Hinney, & Wieacker, 2008; Mayer, Fouquet, Pugeat, & Misrahi, 2017; Rossetti et al., 2009; Tiotiu et al., 2010; Wang et al., 2013, 2010; Zhao et al., 2007) (Table 2) and polycystic ovary syndrome (PCOS) (Liu et al., 2011; Mehdizadeh, Sheikhha, Kalantar, Aali, & Ghanei, 2016; Wang et al., 2010) (Table 3).

8.1 DZ Twinning

The spontaneous conception of DZ twinning rate is influenced by a combination of male and female reproductive factors as it reflects the frequency of double ovulation, the successful fertilization, and the survival of the zygote (Tong & Short, 1998). Of GDF9 and BMP15 as potentially associated genes with DZ twinning is the genetic influence in the *GDF9* gene

more frequent than that in the *BMP15* gene (Hoekstra et al., 2008). The first evidence of association between DZ twinning and GDF9 mutation was the finding of a loss-of-function mutation (c.207_210delATCT, p. L69LfsTer88) in two sisters with spontaneous DZ twins (Montgomery et al., 2004) (Table 1). This mutation alters the reading frame for the protein introducing a stop codon at codon position 88 of exon 1. Other mutations in the *GDF9* gene with insertion/deletions (c.392–393insT and c.1268–1269delAA) or missense codons (P103S and T121L in the proregion; P374L and R454C in the mature region) were found (Palmer et al., 2006). Variants P103S and R454C were also reported in POI women (see below), supporting the hypothesis that mutations causative of DZ twinning may be candidates for the development of POI as well and vice versa (Inagaki & Shimasaki, 2010). In biochemical and functional studies for in vitro GC proliferation, it was demonstrated that mutations P103S and P374L abrogated mature GDF9 expression, suggesting a 50% decrease in GDF9 levels in heterozygous women carrying these mutations (Inagaki & Shimasaki, 2010; Simpson et al., 2014). Pooling the prevalence data of GDF9 variants in Table 1 provides a significant difference ($P = 0.0006$) between DZ twinning and controls by Fisher's exact test (https://www.graphpad.com/quickcalcs/contingency1.cfm). In this regard, Palmer et al. previously predicted the frequency differences of individual variants in the *GDF9* gene found in mothers of DZ twins and normal controls by contingency χ^2 testing with χ^2 and asymptotic P values estimated, allowing for the family nature of the data as implemented in MENDEL binomial link measured genotype model (Palmer et al., 2006). They found that the frequencies of the variants P103S, P374L, and c.1268_1269delAA (frame shift) were significantly higher ($P < 0.05$, denoted by asterisks in Table 1) in mothers of DZ twins than the control (Palmer et al., 2006), supporting the results by Fisher's exact test.

In contrast, Zhao et al. identified several mutations and deletions/insertions in the *BMP15* gene in mothers of DZ twins, three of which (P174S, A311T, and R392T) were not detected in 1512 controls (Zhao et al., 2008). However, no significant evidence has been provided in these variations in DZ twinning (Zhao et al., 2008). Fisher's exact test ($P = 0.8328$) also supports their conclusion (Table 1).

It was shown that mothers of DZ twins reach menopause significantly earlier than those of monozygotic twins, with a higher number of diagnoses for POI (Gosden et al., 2007; Martin, Healey, Pangan, Heath, & Turner, 1997), highlighting the correlation between the faster depletion of follicle reserve with multiple ovulation and the early ovarian insufficiency (Moore et al., 2004). Given that reduced levels of BMP15 and/or GDF9 in ewes are associated with increased ovulation rate and litter size, women with GDF9 (and possibly BMP15) mutations may have an increased number of dominant follicles, resulting in an increased likelihood of bearing DZ twins.

8.2 Primary Ovarian Insufficiency

POI is a dysfunction of the ovary caused by heterogeneous factors (viral infections, autoimmune disorders, genetic disorders, iatrogenic causes), among which the genetic component plays a substantial role, as the synergistic action of different mutations may underlie the development of this phenotype (Rossetti, Ferrari, Bonomi, & Persani, 2017; Tucker, Grover, Bachelot, Touraine, & Sinclair, 2016). Relatively a few genes are considered

involved in the development of syndromic and nonsyndromic POI (Cox & Liu, 2014; Fortuno & Labarta, 2014). Approximately 1% of women are affected by POI presenting prolonged amenorrhea (4–6 months) before the age of 40, which is a major cause of infertility, and they show elevated plasma gonadotropin levels. *BMP15* and *GDF9* gene variants were found to have a high incidence on the POI phenotype, namely 0%–12% prevalence ofBMP15 variants (Dixit et al., 2006; Ma et al., 2015; Persani, Rossetti, Di Pasquale, Cacciatore, & Fabre, 2014) and 1%–4% prevalence of GDF9 variants (Dixit et al., 2006; Laissue et al., 2006; Ma et al., 2015; Qin, Jiao, Simpson, & Chen, 2015) in certain ethnicities.

The first identified human BMP-15 mutation associated with hypergonadotropic ovarian failure due to ovarian dysgenesis was a heterozygous Y235C missense mutation (Di Pasquale et al., 2004). Thereafter, a number of nonsynonymous mutations in the *BMP15* and *GDF9* genes were identified in women with POI as follows: (i) *BMP15,* S5R, E51IfsTer27, R61W, R61E, E64AfsTer12, R68W, R76C, R76H, H81R, N103S, R138H, L148P, A180T, A180F/S+V, F194S, N196K, G199R, H200Y, R206H, E211X, W221R, Y235C, I243G, Ins263L, and R329C; and (ii) *GDF9,* D57Y, K67E, P103S, R146C, T148A, S186Y, V216M, T238A, S428T, and R454C (Table 2).

The biochemical and biological properties of these variants have been extensively studied. For example, BMP15 variants of R68W, R138H, L148P (Rossetti et al., 2009), R76C, and R206H (Inagaki & Shimasaki, 2010) located in the proregion lead to a reduced production of mature BMP15 in an in vitro assay, showing that reduced biological effects may be due to an impaired processing or a decreased precursor stability (Inagaki & Shimasaki, 2010; Rossetti et al., 2009). Ins263L might not have a functional abnormality since it is present also in control women and might represent, together with N103S, a polymorphism with no functional significance (Bouilly et al., 2016; Di Pasquale et al., 2006; Dixit et al., 2006; Laissue et al., 2006; Rossetti et al., 2009; Tiotiu et al., 2010; Wang et al., 2010).

A study from Dixit and coworkers in 2006 found a homozygous mutation, E211X, in the proregion of BMP15 associated with POI (Dixit et al., 2006). This variant is likely to represent a knockout of the *BMP15* gene, with the production of a prematurely truncated protein that leads to the lack of the mature protein. The patient carrying the mutation was infertile and presented ovarian hypoplasia, together with other developmental defects. A study from 2010 on Chinese POI patients showed the identification of a missense mutation in BMP15 in the mature region (R329C) that might impair the correct folding and final three-dimensional structure of BMP15 subunit as well as the formation of homo- or heterodimers with GDF9 (Wang et al., 2010). A more recent study was published on a family with two sisters who exhibited to be compound heterozygous with E51IfsTer27 inherited from their father and E64AfsTer12 from their mother (Mayer et al., 2017). Due to frame-shift mutations, both of these mutant BMP15 proteins are prematurely truncated in the proregion, precluding mature BMP15 production. Thus, a deletion in the two alleles of the *BMP15* gene in two sisters revealed a human “knockout-like” effect, which resulted in primary amenorrhea in one sister and primo-secondary amenorrhea in the other. Moreover, hormonal analysis showed high FSH and LH, very low estradiol, and low but detectable AMH. Five years later, FSH and LH levels were elevated, estradiol level lowered, and AMH

became undetectable, indicating a complete loss of growing follicles. Interestingly, their brother, hemizygous for BMP15, showed asthenozoospermia but refused further investigations.

As described earlier, an increasing number of BMP15 variants have been identified in women with POI in different ethnicities. However, the statistical significance of the mutations in POI has not been provided in each study due to low numbers of patients and controls examined. In this regard, Persani et al. performed an excellent work in which they put together all BMP15 variants identified by January 2014 (the date in which the manuscript was submitted) by different laboratories in different ethnicities and found that the prevalence of nonsynonymous mutations in the POI cohorts is 10-fold higher than in the control populations ($P < 0.0001$ by Fisher's exact test), thus supporting the pathogenic role of these rare variants (Persani et al., 2014). We repeated this calculation with all nonsynonymous BMP15 variants identified by March 2017. As a result, the P value of the difference between the POI and normal cohorts in the prevalence of non-synonymous mutations of BMP15 is 0.0170 (Table 2). However, if the relatively common variants, N103S and Ins263L, are excluded from the calculation, the P value would be <0.0001, which is consistent with the previous report by Persani et al. (2014). Collectively, the mutation rate in the *BMP15* gene in women with POI is significantly higher than the normal control.

Variants K67E, P103S, and S428T rose to misfolded GDF9 proproteins when they were expressed in vitro, resulting in significantly reduced or nearly abrogated mature proteins (Inagaki & Shimasaki, 2010; Simpson et al., 2014). It is of interest that variants S186Y, V216M, and T238A, located in the proregion of GDF9, have reduced association with the mature domain as compared with the wild-type control, thus the availability of the mature protein dimers is increased (Simpson et al., 2014). The variants P103S and R454C are common in mothers of DZ twins as described earlier (Palmer et al., 2006). Another GDF9 variant, R146C, was found in 3 out of 139 Chinese women with diminished ovarian reserve but absent in the control population ($n = 152$) (Wang et al., 2013). This mutation reduced the levels of mature GDF9 protein in transfected cells presumably through disruption of its three-dimensional structure. Moreover, variant R146C reduced the ability of the wild-type GDF9 to stimulate GC proliferation and to activate SMAD2 signaling, supporting the hypothesis that the R146C variant is deleterious and thus could have pathogenic effects (Wang et al., 2013). Putting all GDF9 variants detected in women with POI together, the GDF9 mutation rate in POI appeared to be significantly higher than control women ($P = 0.0484$) (Table 2).

Women with nonsyndromic POI showed heterozygous mutations not only in *BMP15* or *GDF9* but also in other key genes of ovarian function, such as *NOBOX, FOXL2, SOHLH1, FIGLA, GALT, STAG3, HFM1, SYCE1, MCM8, MCM9, SCM1β, REC8, LHX8,* and many more, involved in cell functions such as regulation of transcription, meiosis, and DNA repair (Bouali et al., 2016; Bouilly et al., 2016; Qin et al., 2015). A recent study on whole-exome sequencing of Chinese POI patients showed a novel homozygous truncating variant in the *NOBOX* gene (chr7:144098161delC) that impaired severely the transcriptional activation of

the *GDF9* gene in functional analyses, suggesting that a loss-of-function effect on NOBOX transcriptional activity could be associated with POI via reduced GDF9 (Li et al., 2017).

8.3 Polycystic Ovary Syndrome

PCOS affects 5%–10% of women in their reproductive age and is considered the most common endocrinological disorder characterized by anovulation, ovarian cysts, elevated plasma level of androgens, hirsutism, insulin resistance, obesity, and menstrual irregularities that lead to infertility (Dunaif, 1997; Fauser et al., 2012; Franks, 1995; Legro et al., 1998). Genetic factors have been shown to influence the impaired follicular development that contribute to PCOS pathogenesis, and the dysregulation of oocyte BMP15 and GDF9 expression in women with PCOS has been reported; however, a consistent conclusion with regard to the dysregulated levels of BMP15 and GDF9 in PCOS remains to be lead (de Resende et al., 2012; Teixeira Filho et al., 2002; Wei et al., 2014; Wei, Liang, Fang, & Zhang, 2011; Zhao et al., 2010).

Nonsynonymous mutations in the *BMP15* gene were also identified in PCOS patients (L12V, A37P, P57A, R96S, N103S, H200Y, and Ins263L) (Liu et al., 2011; Mehdizadeh et al., 2016) (Table 3). Several of these mutations (L12V, A37P, P57A, R96S, and N103S) in the *BMP15* gene were found in PCOS, but not normal, women (Liu et al., 2011). However, the prevalence of all these BMP15 variants in women with PCOS is not significantly different from that in normal controls (Table 3). Nonsynonymous mutations were also found in the *GDF9* gene in PCOS patients (N5L, L40V, M45V, D57Y, R146C, Y342F, S425R, and S428T) (Wang et al., 2010) with no significant difference in mutation rates between PCOS and normal women (Table 3). Despite the presence of a variety of studies indicating a correlation between genetic variations in key genes of ovarian function and PCOS development, other evidence exists in support of the absence of such associations between BMP15 and GDF9 polymorphisms and PCOS susceptibility (Sproul, Jones, Mathur, Azziz, & Goodarzi, 2010; Takebayashi et al., 2000).

9. PERSPECTIVES

Most of the mutations in the *GDF9* and *BMP15* genes identified in mothers of DZ twins and women with POI and PCOS are located in the proregion of the proprotein. As the proregion is necessary for dimerization of the mature protein (Hogan, 1996), mutations in the proregion may cause the impaired processing of the proproteins by forming misfolded proprotein dimers and thereby negatively impact the production of functional mature protein dimers. This impairment could occur in various combinations of GDF9 or BMP15 subunits (i.e., homodimers of the mutant GDF9, heterodimers of the mutant GDF9 and the wild-type GDF9, heterodimers of the mutant GDF9 and the wild-type BMP15, homodimers of the mutant BMP15, heterodimers of the mutant BMP15 and the wild-type BMP15, and heterodimers of the mutant BMP15 and the wild-type GDF9).

Misfolded proteins are degraded in the endoplasmic reticulum (ER) by the process termed ER-associated degradation (McCracken & Brodsky, 1996), which is necessary for protein quality control in the eukaryotic secretory pathway to ensure that only properly folded proteins transit through cellular organelles (Ellgaard & Helenius, 2001). These pathological

events caused by adoption of nonnative protein conformations lead to a large range of conditions recognized as conformational diseases (Convertino, Das, & Dokholyan, 2016; Kopito & Ron, 2000). In the control process of misfolded proteins, chaperones control the folding status of proteins by preventing and reversing protein aggregation together with ATP-dependent proteases (Weibezahn, Bukau, & Mogk, 2004). As a therapeutic strategy for conformational diseases, cell-permeable pharmacological chaperones are expected to selectively provide a molecular scaffold for misfolded GDF9 and BMP15 proproteins to recover their native folding, leading to the secretion of functional mature proteins.

ACKNOWLEDGMENTS

We apologize to researchers whose work could not be cited in this review due to space constrains. This work was supported by the NIH Grant 2P50HD012303–34A1 awarded in part to S.S. and by the UC San Diego Department of Reproductive Medicine Fund 60121B awarded to S.S.

REFERENCES

Al-Musawi SL, Walton KL, Heath D, Simpson CM, & Harrison CA (2013). Species differences in the expression and activity of bone morphogenetic protein 15. Endocrinology, 154, 888–899. [PubMed: 23284103]

Anderson E, & Albertini DF (1976). Gap junctions between the oocyte and companion follicle cells in the mammalian ovary. Journal of Cell Biology, 71, 680–686. [PubMed: 825522]

Behrouzi A, Colazo MG, & Ambrose DJ (2016). Alterations in bone morphogenetic protein 15, growth differentiation factor 9, and gene expression in granulosa cells in preovulatory follicles of dairy cows given porcine LH. Theriogenology, 85, 1249–1257. [PubMed: 26794084]

Bouali N, Francou B, Bouligand J, Lakhal B, Malek I, Kammoun M, et al. (2016). NOBOX is a strong autosomal candidate gene in Tunisian patients with primary ovarian insufficiency. Clinical Genetics, 89, 608–613. [PubMed: 26848058]

Bouilly J, Beau I, Barraud S, Bernard V, Azibi K, Fagart J, et al. (2016). Identification of multiple gene mutations accounts for a new genetic architecture of primary ovarian insufficiency. The Journal of Clinical Endocrinology and Metabolism, 101, 4541–4550. [PubMed: 27603904]

Buccione R, Schroeder AC, & Eppig JJ (1990). Interactions between somatic cells and germ cells throughout mammalian oogenesis. Biology of Reproduction, 43, 543–547. [PubMed: 2289008]

Carabatsos MJ, Elvin J, Matzuk MM, & Albertini DF (1998). Characterization of oocyte and follicle development in growth differentiation factor-9-deficient mice. Developmental Biology, 204, 373–384. [PubMed: 9882477]

Chand AL, Ponnampalam AP, Harris SE, Winship IM, & Shelling AN (2006). Mutational analysis of BMP15 and GDF9 as candidate genes for premature ovarian failure. Fertility and Sterility, 86, 1009–1012. [PubMed: 17027369]

Convertino M, Das J, & Dokholyan NV (2016). Pharmacological chaperones: Design and development of new therapeutic strategies for the treatment of conformational diseases. ACS Chemical Biology, 11, 1471–1489. [PubMed: 27097127]

Cox L, & Liu JH (2014). Primary ovarian insufficiency: An update. International Journal of Women's Health, 6, 235–243.

Crawford JL, & McNatty KP (2012). The ratio of growth differentiation factor 9: Bone morphogenetic protein 15 mRNA expression is tightly co-regulated and differs between species over a wide range of ovulation rates. Molecular and Cellular Endocrinology, 348, 339–343. [PubMed: 21970812]

de Resende LO, Vireque AA, Santana LF, Moreno DA, de Sa Rosa ESAC, Ferriani RA, et al. (2012). Single-cell expression analysis of BMP15 and GDF9 in mature oocytes and BMPR2 in cumulus cells of women with polycystic ovary syndrome undergoing controlled ovarian hyperstimulation. Journal of Assisted Reproduction and Genetics, 29, 1057–1065. [PubMed: 22825968]

Dey SR, Deb GK, Ha AN, Lee JI, Bang JI, Lee KL, et al. (2012). Coculturing denuded oocytes during the in vitro maturation of bovine cumulus oocyte complexes exerts a synergistic effect on embryo development. Theriogenology, 77, 1064–1077. [PubMed: 22153275]

Di Pasquale E, Beck-Peccoz P, & Persani L (2004). Hypergonadotropic ovarian failure associated with an inherited mutation of human bone morphogenetic protein-15 (BMP15) gene. American Journal of Human Genetics, 75, 106–111. [PubMed: 15136966]

Di Pasquale E, Rossetti R, Marozzi A, Bodega B, Borgato S, Cavallo L, et al. (2006). Identification of new variants of human BMP15 gene in a large cohort of women with premature ovarian failure. The Journal of Clinical Endocrinology and Metabolism, 91, 1976–1979. [PubMed: 16464940]

Dixit H, Rao LK, Padmalatha V, Kanakavalli M, Deenadayal M, Gupta N, et al. (2005). Mutational screening of the coding region of growth differentiation factor 9 gene in Indian women with ovarian failure. Menopause, 12, 749–754. [PubMed: 16278619]

Dixit H, Rao LK, Padmalatha VV, Kanakavalli M, Deenadayal M, Gupta N, et al. (2006) Missense mutations in the BMP15 gene are associated with ovarian failure. Human Genetics, 119, 408–415. [PubMed: 16508750]

Dong J, Albertini DF, Nishimori K, Kumar TR, Lu N, & Matzuk M (1996). Growth differentiation factor-9 is required during early ovarian folliculogenesis. Nature, 383, 531–535. [PubMed: 8849725]

Dragovic RA, Ritter LJ, Schulz SJ, Amato F, Thompson JG, Armstrong DT, et al. (2007). Oocyte-secreted factor activation of SMAD 2/3 signaling enables initiation of mouse cumulus cell expansion. Biology of Reproduction, 76, 848–857. [PubMed: 17192514]

Dube JL, Wang P, Elvin J, Lyons KM, Celeste AJ, & Matzuk MM (1998). The bone morphogenetic protein 15 gene is X-linked and expressed in oocytes. Molecular Endocrinology, 12, 1809–1817. [PubMed: 9849956]

Duffy DM (2003). Growth differentiation factor-9 is expressed by the primate follicle throughout the periovulatory interval. Biology of Reproduction, 69, 725–732. [PubMed: 12700191]

Dunaif A (1997). Insulin resistance and the polycystic ovary syndrome: Mechanism and implications for pathogenesis. Endocrine Reviews, 18, 774–800. [PubMed: 9408743]

Ellgaard L, & Helenius A (2001). ER quality control: Towards an understanding at the molecular level. Current Opinion in Cell Biology, 13, 431–437. [PubMed: 11454449]

Eppig JJ (2001). Oocyte control of ovarian follicular development and function in mammals. Reproduction, 122, 829–838. [PubMed: 11732978]

Eppig JJ, Pendola FL, & Wigglesworth K (1998). Mouse oocytes suppress cAMP-induced expression of LH receptor mRNA by granulosa cells in vitro. Molecular Reproduction and Development, 49, 327–332. [PubMed: 9491385]

Eppig JJ, Wigglesworth K, & Pendola F (2002). The mammalian oocyte orchestrates the rate of ovarian follicular development. Proceedings of the National Academy of Sciences of the United States of America, 99, 2890–2894. [PubMed: 11867735]

Eppig JJ, Wigglesworth K, Pendola F, & Hirao Y (1997). Murine oocytes suppress expression of luteinizing hormone receptor messenger ribonucleic acid by granulosa cells. Biology of Reproduction, 56, 976–984. [PubMed: 9096881]

Erickson GF, & Shimasaki S (2003). The spatiotemporal expression pattern of the bone morphogenetic protein family in rat ovary cell types during the estrous cycle. Reproductive Biology and Endocrinology, 1, 9. [PubMed: 12741959]

Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, et al. (2012) Consensus on women's health aspects of polycystic ovary syndrome (PCOS): The Amsterdam ESHRE/ASRM-sponsored 3rd PCOS Consensus Workshop Group. Fertility and Sterility, 97, 28–38, e25. [PubMed: 22153789]

Ferrarini E, Russo L, Fruzzetti F, Agretti P, De Marco G, Dimida A, et al. (2013). Clinical characteristics and genetic analysis in women with premature ovarian insufficiency. Maturitas, 74, 61–67. [PubMed: 23107817]

Fortuno C, & Labarta E (2014). Genetics of primary ovarian insufficiency: A review. Journal of Assisted Reproduction and Genetics, 31, 1573–1585. [PubMed: 25227694]

Franks S (1995). Polycystic ovary syndrome. New England Journal of Medicine, 333, 853–861. [PubMed: 7651477]

Galloway SM, McNatty KP, Cambridge LM, Laitinen MPE, Juengel JL, Jokiranta TS, et al. (2000). Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. Nature Genetics, 25, 279–283. [PubMed: 10888873]

Gilchrist RB, Lane M,& Thompson JG (2008). Oocyte-secretedfactors: Regulators of cumulus cell function and oocyte quality. Human Reproduction Update, 14, 159–177. [PubMed: 18175787]

Gilchrist RB, Ritter LJ, &Armstrong DT (2001). Mouse oocyte mitogenic activity is developmentally coordinated throughout folliculogenesis and meiotic maturation. Developmental Biology, 240, 289–298. [PubMed: 11784064]

Gilchrist RB, Ritter LJ, & Armstrong DT (2004). Oocyte-somatic cell interactions during follicle development in mammals. Animal Reproduction Science, 82–83, 431–446. [PubMed: 15271471]

Gilula NB, Epstein ML, & Beers WH (1978). Cell-to-cell communication and ovulation. A study of the cumulus-oocyte complex. The Journal of Cell Biology, 78, 58–75. [PubMed: 670298]

Glister C, Groome NP,& Knight PG (2003). Oocyte-mediated suppression of follicle-stimulating hormone- and insulin-like growth factor-induced secretion of steroids and inhibin-related proteins by bovine granulosa cells in vitro: Possible role of transforming growth factor alpha. Biology of Reproduction, 68, 758–765. [PubMed: 12604623]

Gomez MN, Kang JT, Koo OJ, Kim SJ, Kwon DK, Park SJ, et al. (2012). Effect of oocyte-secreted factors on porcine in vitro maturation, cumulus expansion and developmental competence of parthenotes. Zygote, 20, 135–145. [PubMed: 21791167]

Goodenough DA, Simon AM, & Paul DL (1999). Gap junctional intercellular communication in the mouse ovarian follicle. Novartis Foundation Symposium, 219, 226–235. discussion 235–240. [PubMed: 10207907]

Gosden RG, Treloar SA, Martin NG, Cherkas LF, Spector TD, Faddy MJ, et al. (2007). Prevalence of premature ovarian failure in monozygotic and dizygotic twins. Human Reproduction, 22, 610–615. [PubMed: 17065173]

Hanrahan JP, Gregan SM, Mulsant P, Mullen M, Davis GH, Powell R, et al. (2004). Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (Ovis aries). Biology of Reproduction, 70, 900–909. [PubMed: 14627550]

Hayashi M, McGee EA, Min G, Klein C, Rose UM, van Duin M, et al. (1999). Recombinant growth differentiation factor-9 (GDF-9) enhances growth and differentiation of cultured early ovarian follicles. Endocrinology, 140, 1236–1244. [PubMed: 10067849]

Hoekstra C, Zhao ZZ, Lambalk CB, Willemsen G, Martin NG, Boomsma DI, et al. (2008). Dizygotic twinning. Human Reproduction Update, 14, 37–47. [PubMed: 18024802]

Hogan BL (1996). Bone morphogenetic proteins: Multifunctional regulators of vertebrate development. Genes & Development, 10, 1580–1594. [PubMed: 8682290]

Hussein MR (2005). Apoptosis in the ovary: Molecular mechanisms. Human Reproduction Update, 11, 162–177. [PubMed: 15705959]

Hussein TS, Sutton-McDowall ML, Gilchrist RB, & Thompson JG (2011). Temporal effects of exogenous oocyte-secreted factors on bovine oocyte developmental competence during IVM. Reproduction, Fertility, and Development, 23, 576–584.

Hussein TS, Thompson JG, & Gilchrist RB (2006). Oocyte-secreted factors enhance oocyte developmental competence. Developmental Biology, 296, 514–521. [PubMed: 16854407]

Inagaki K, & Shimasaki S (2010). Impaired production of BMP-15 and GDF-9 mature proteins derived from proproteins with mutations in the proregion. Molecular and Cellular Endocrinology, 328, 1–7. [PubMed: 20547206]

Israel DI, Nove J, Kerns KM, Moutsatsos IK, & Kaufman RJ (1992). Expression and characterization of bone morphogenetic protein-2 in Chinese hamster ovary cells. Growth Factors, 7, 139–150. [PubMed: 1419071]

Jones WK, Richmond EA, White K, Sasak H, Kusmik W, Smart J, et al. (1994). Osteogenic protein-1 (OP-1) expression and processing in Chinese hamster ovary cells: Isolation of a soluble complex

containing the mature and pro-domains of OP-1. Growth Factors, 11, 215–225. [PubMed: 7734147]

Joyce IM, Pendola FL, Wigglesworth K, & Eppig JJ (1999). Oocyte regulation ofkit ligand expression in mouse ovarian follicles. Developmental Biology, 214, 342–353. [PubMed: 10525339]

Juengel JL, Hudson NL, Whiting L, & McNatty KP (2004). Effects of immunization against bone morphogenetic protein 15 and growth differentiation factor 9 on ovulation rate, fertilization, and pregnancy in ewes. Biology of Reproduction, 70, 557–561. [PubMed: 14585806]

Kaivo-Oja N, Bondestam J, Kamarainen M, Koskimies J, Vitt U, Cranfield M, et al. (2003). Growth differentiation factor-9 induces Smad2 activation and inhibin B production in cultured human granulosa-luteal cells. Journal of Clinical Endocrinology and Metabolism, 88, 755–762. [PubMed: 12574210]

Kopito RR, & Ron D (2000). Conformational disease. Nature Cell Biology, 2, E207–E209. [PubMed: 11056553]

Kovanci E, Rohozinski J, Simpson JL, Heard MJ, Bishop CE, & Carson SA (2007). Growth differentiating factor-9 mutations may be associated with premature ovarian failure. Fertility and Sterility, 87, 143–146. [PubMed: 17156781]

Laissue P, Christin-Maitre S, Touraine P, Kuttenn F, Ritvos O, Aittomaki K, et al. (2006). Mutations and sequence variants in GDF9 and BMP15 in patients with premature ovarian failure. European Journal of Endocrinology, 154, 739–744. [PubMed: 16645022]

Laitinen M, Vuojolainen K, Jaatinen R, Ketola I, Aaltonen J, Lehtonen E, et al. (1998). A novel growth differentiation factor-9 (GDF-9) related factor is co-expressed with GDF-9 in mouse oocytes during folliculogenesis. Mechanisms of Development, 78, 135–140. [PubMed: 9858711]

Lanuza GM, Groome NP, Baranao JL, & Campo S (1999). Dimeric inhibin A and B production are differentially regulated by hormones and local factors in rat granulosa cells. Endocrinology, 140, 2549–2554. [PubMed: 10342841]

Ledig S, Ropke A, Haeusler G, Hinney B, &Wieacker P (2008). BMP15 mutations in XX gonadal dysgenesis and premature ovarian failure. American Journal of Obstetrics and Gynecology, 198(84), e81–85.

Legro RS, Spielman R, Urbanek M, Driscoll D, Strauss JF, 3rd, & Dunaif A (1998). Phenotype and genotype in polycystic ovary syndrome. Recent Progress in Hormone Research, 53, 217–256. [PubMed: 9769710]

Li Q, Agno JE, Edson MA, Nagaraja AK, Nagashima T, & Matzuk MM (2011). Transforming growth factor beta receptor type 1 is essential for female reproductive tract integrity and function. PLoS Genetics, 7, e1002320. [PubMed: 22028666]

Li Q, Rajanahally S, Edson MA, & Matzuk MM (2009). Stable expression and characterization of N-terminal tagged recombinant human bone morphogenetic protein 15. Molecular Human Reproduction, 15, 779–788. [PubMed: 19651638]

Li L, Wang B, Zhang W, Chen B, Luo M, Wang J, et al. (2017). A homozygous NOBOX truncating variant causes defective transcriptional activation and leads to primary ovarian insufficiency. Human Reproduction, 32, 248–255. [PubMed: 27836978]

Liao WX, Moore RK, Otsuka F, & Shimasaki S (2003). Effect of intracellular interactions on the processing and secretion of bone morphogenetic protein-15 (BMP-15) and growth and differentiation factor-9: Implication of the aberrant ovarian phenotype of BMP-15 mutant sheep. Journal of Biological Chemistry, 278, 3713–3719. [PubMed: 12446716]

Liao WX, Moore RK, & Shimasaki S (2004). Functional and molecular characterization of naturally occurring mutations in the oocyte-secreted factors BMP-15 nd GDF-9. Journal of Biological Chemistry, 279, 17391–17396. [PubMed: 14970198]

Lin JY, Pitman-Crawford JL, Bibby AH, Hudson NL, McIntosh CJ, Juengel JL, et al. (2012). Effects of species differences on oocyte regulation of granulosa cell function. Reproduction, 144, 557–567. [PubMed: 22967842]

Ling N, Ying S-Y, Ueno N, Shimasaki S, Esch F, Hotta M, et al. (1986). Pituitary FSH is released by a heterodimer of the B-subunits from the two forms of inhibin. Nature, 321, 779–782. [PubMed: 3086749]

Liu J, Wang B, Wei Z, Zhou P, Zu Y, Zhou S, et al. (2011). Mutational analysis of human bone morphogenetic protein 15 in Chinese women with polycystic ovary syndrome. Metabolism, 60, 1511–1514. [PubMed: 22014425]

Ma L, Chen Y, Mei S, Liu C, Ma X, Li Y, et al. (2015). Single nucleotide polymorphisms in premature ovarian failure-associated genes in a Chinese Hui population. Molecular Medicine Reports, 12, 2529–2538. [PubMed: 25954833]

Martin NG, Healey SC, Pangan TS, Heath AC,& Turner G (1997). Do mothers of dizygotic twins have earlier menopause? A role for fragile X? AmericanJournal of Medical Genetics, 69, 114–116. [PubMed: 9066896]

Matzuk MM, Burns KH, Viveiros MM, & Eppig JJ (2002). Intercellular communication in the mammalian ovary: Oocytes carry the conversation. Science, 296, 2178–2180. [PubMed: 12077402]

Mayer A, Fouquet B, Pugeat M, & Misrahi M (2017). BMP15 “knockout-like” effect in familial premature ovarian insufficiency with persistent ovarian reserve. Clinical Genetics, 92, 208–212. [PubMed: 28094433]

Mazerbourg S, Klein C, Roh J, Kaivo-Oja N, Mottershead DG, Korchynskyi O, et al. (2004). Growth differentiation factor-9 signaling is mediated by the type I receptor, activin receptor-like kinase 5. Molecular Endocrinology, 18, 653–665. [PubMed: 14684852]

McCracken AA, & Brodsky JL (1996). Assembly of ER-associated protein degradation in vitro: Dependence on cytosol, calnexin, and ATP. Journal of Cell Biology, 132, 291–298. [PubMed: 8636208]

McGrath SA, Esquela AF, & Lee S-J (1995). Oocyte-specific expression of growth/ differentiation factor-9. Molecular Endocrinology, 9, 131–136. [PubMed: 7760846]

McIntosh CJ, Lun S, Lawrence S, Western AH, McNatty KP, & Juengel JL (2008). The proregion of mouse BMP15 regulates the cooperative interactions of BMP15 and GDF9. Biology of Reproduction, 79, 889–896. [PubMed: 18633140]

McMahon HE, Sharma S, & Shimasaki S (2008). Phosphorylation of bone morphogenetic protein-15 and growth and differentiation factor-9 plays a critical role in determining agonistic or antagonistic functions. Endocrinology, 149, 812–817. [PubMed: 18006624]

McNatty KP, Juengel JL, Reader KL, Lun S, Myllymaa S, Lawrence SB, et al. (2005a). Bone morphogenetic protein 15 and growth differentiation factor 9 co-operate to regulate granulosa cell function. Reproduction, 129, 473–480. [PubMed: 15798022]

McNatty KP, Juengel JL, Reader KL, Lun S, Myllymaa S, Lawrence SB, et al. (2005b). Bone morphogenetic protein 15 and growth differentiation factor 9 co-operate to regulate granulosa cell function in ruminants. Reproduction, 129, 481–487. [PubMed: 15798023]

McNatty KP, Lawrence S, Groome NP, Meerasahib MF, Hudson NL, Whiting L, et al. (2006). Meat and livestock association plenary lecture 2005. Oocyte signalling molecules and their effects on reproduction in ruminants. Reproduction, Fertility, and Development, 18, 403–412.

McNatty KP, Moore LG, Hudson NL, Quirke LD, Lawrence SB, Reader K, et al. (2004). The oocyte and its role in regulating ovulation rate: A new paradigm in reproductive biology. Reproduction, 128, 379–386. [PubMed: 15454632]

McNatty KP, Smith P, Moore LG, Reader K, Lun S, Hanrahan JP, et al. (2005). Oocyte-expressed genes affecting ovulation rate. Molecular and Cellular Endocrinology, 234, 57–66. [PubMed: 15836953]

McPherron AC, &Lee S-J (1993). GDF-3 and GDF-9: Two new members of the transforming growth factor-B superfamily containing a novel pattern of cysteines. Journal of Biological Chemistry, 268, 3444–3449. [PubMed: 8429021]

Mehdizadeh A, Sheikhha MH, Kalantar SM, Aali BS, & Ghanei A (2016). Mutation analysis of exon1 of bone morphogenetic protein-15 gene in Iranian patients with polycystic ovarian syndrome. International Journal of Reproductive Biomedicine (Yazd, Iran), 14, 527–532.

Meno C, Ito Y, Saijoh Y, Matsuda Y, Tashiro K, Kuhara S, et al. (1997). Two closely-related left-right asymmetrically expressed genes, lefty-1 and lefty-2: Their distinct expression domains, chromosomal linkage and direct neutralizing activity in xenopus embryos. Genes to Cells, 2, 513–524. [PubMed: 9348041]

Mester B, Ritter LJ, Pitman JL, Bibby AH, Gilchrist RB, McNatty KP, et al. (2015). Oocyte expression, secretion and somatic cell interaction of mouse bone morphogenetic protein 15 during the peri-ovulatory period. Reproduction, Fertility, and Development, 27, 801–811.

Montgomery GW, Zhao ZZ, Marsh AJ, Mayne R, Treloar SA, James M, et al. (2004). A deletion mutation in GDF9 in sisters with spontaneous DZ twins. Twin Research, 7, 548–555. [PubMed: 15607004]

Moore RK, Erickson GF, & Shimasaki S (2004). Are BMP-15 and GDF-9 primary determinants of ovulation quota in mammals? Trends in Endocrinology and Metabolism, 15, 356–361. [PubMed: 15380806]

Moore RK, Otsuka F, & Shimasaki S (2003). Molecular basis of bone morphogenetic protein-15 signaling in granulosa cells. Journal of Biological Chemistry, 278, 304–310. [PubMed: 12419820]

Mottershead DG, Pulkki MM, Muggalla P, Pasternack A, Tolonen M, Myllymaa S, et al. (2008). Characterization of recombinant human growth differentiation factor-9 signaling in ovarian granulosa cells. Molecular and Cellular Endocrinology, 283, 58–67. [PubMed: 18162287]

Mottershead DG, Ritter LJ, & Gilchrist RB (2012). Signalling pathways mediating specific synergistic interactions between GDF9 and BMP15. Molecular Human Reproduction, 18, 121–128. [PubMed: 21911477]

Mottershead DG, Sugimura S, Al-Musawi SL, Li JJ, Richani D, White MA, et al. (2015). Cumulin, an oocyte-secreted heterodimer of the transforming growth factor-beta family, is a potent activator of granulosa cells and improves oocyte quality. The Journal of Biological Chemistry, 290, 24007–24020. [PubMed: 26254468]

Otsuka F, Moore RK, & Shimasaki S (2001). Biological function and cellular mechanism of bone morphogenetic protein-6 in the ovary. Journal of Biological Chemistry, 276, 32889–32895. [PubMed: 11447221]

Palmer JS, Zhao ZZ, Hoekstra C, Hayward NK, Webb PM, Whiteman DC, et al. (2006). Novel variants in growth differentiation factor 9 in mothers of dizygotic twins. The Journal of Clinical Endocrinology and Metabolism, 91, 4713–4716. [PubMed: 16954162]

Pangas SA, & Matzuk MM (2004). Genetic models for transforming growth factor beta superfamily signaling in ovarian follicle development. Molecular and CellularEndocrinology, 225, 83–91.

Pangas SA, & Matzuk MM (2005). The art and artifact of GDF9 activity: Cumulus expansion and the cumulus expansion-enabling factor. Biology of Reproduction, 73, 582–585. [PubMed: 15917343]

Paradis F, Moore HS, Pasternak JA, Novak S, Dyck MK, Dixon WT, et al. (2010). Pig preovulatory oocytes modulate cumulus cell protein and gene expression in vitro. Molecular and Cellular Endocrinology, 320, 87–96. [PubMed: 20138115]

Park JY, Su YQ, Ariga M, Law E, Jin SL, & Conti M (2004). EGF-like growth factors as mediators of LH action in the ovulatory follicle. Science, 303, 682–684. [PubMed: 14726596]

Peng J, Li Q, Wigglesworth K, Rangarajan A, Kattamuri C, Peterson RT, et al. (2013). Growth differentiation factor 9: Bone morphogenetic protein 15 heterodimers are potent regulators of ovarian functions. Proceedings of the National Academy of Sciences of the United States of America, 110, E776–785. [PubMed: 23382188]

Persani L, Rossetti R, Di Pasquale E, Cacciatore C, & Fabre S (2014). The fundamental role of bone morphogenetic protein 15 in ovarian function and its involvement in female fertility disorders. Human Reproduction Update, 20, 869–883. [PubMed: 24980253]

Pulkki MM, Mottershead DG, Pasternack AH, Muggalla P, Ludlow H, van Dinther M, et al. (2012). A covalently dimerized recombinant human bone morphogenetic protein-15 variant identifies bone morphogenetic protein receptor type 1B as a key cell surface receptor on ovarian granulosa cells. Endocrinology, 153, 1509–1518. [PubMed: 22294741]

Qin Y, Jiao X, Simpson JL, & Chen ZJ (2015). Genetics of primary ovarian insufficiency: New developments and opportunities. Human Reproduction Update, 21, 787–808. [PubMed: 26243799]

Reader KL, Heath DA, Lun S, McIntosh CJ, Western AH, Littlejohn RP, et al. (2011). Signalling pathways involved in the cooperative effects of ovine and murine GDF9 + BMP15-stimulated thymidine uptake by rat granulosa cells. Reproduction, 142, 123–131. [PubMed: 21474603]

Regassa A, Rings F, Hoelker M, Cinar U, Tholen E, Looft C, et al. (2011). Transcriptome dynamics and molecular cross-talk between bovine oocyte and its companion cumulus cells. BMC Genomics, 12, 57. [PubMed: 21261964]

Requena A, Cruz M, Agudo D, Pacheco A, & Garcia-Velasco JA (2016). Type of gonadotropin during controlled ovarian stimulation affects the endocrine profile in follicular fluid and apoptosis rate in cumulus cells. European Journal of Obstetrics, Gynecology, and Reproductive Biology, 197, 142–146.

Ritter LJ, Sugimura S, & Gilchrist RB (2015). Oocyte induction of EGF responsiveness in somatic cells is associated with the acquisition of porcine oocyte developmental competence. Endocrinology, 156, 2299–2312. [PubMed: 25849729]

Roh J-S, Bondestam J, Mazerbourg S, Kaivo-Oja N, Groome N, Ritvos O, et al. (2003). Growth differentiation factor-9 stimulates inhibin production and activates Smad2 in cultured rat granulosa cells. Endocrinology, 144, 172–178. [PubMed: 12488343]

Romaguera R, Morato R, Jimenez-Macedo AR, Catala M, Roura M, Paramio MT, et al. (2010). Oocyte secreted factors improve embryo developmental competence of COCs from small follicles in prepubertal goats. Theriogenology, 74, 1050–1059. [PubMed: 20542547]

Rossetti R, Di Pasquale E, Marozzi A, Bione S, Toniolo D, Grammatico P, et al. (2009). BMP15 mutations associated with primary ovarian insufficiency cause a defective production of bioactive protein. Human Mutation, 30, 804–810. [PubMed: 19263482]

Rossetti R, Ferrari I, Bonomi M, & Persani L (2017). Genetics ofprimary ovarian insufficiency. Clinical Genetics, 91, 183–198. [PubMed: 27861765]

Saito S, Yano K, Sharma S, McMahon HE, & Shimasaki S (2008). Characterization of the post-translational modification of recombinant human BMP-15 mature protein. Protein Science, 17, 362–370. [PubMed: 18227435]

Shimasaki S, Moore RK, Otsuka F, &Erickson GF (2004). The bone morphogenetic protein system in mammalian reproduction. Endocrine Reviews, 25, 72–101. [PubMed: 14769828]

Simpson CM, Robertson DM, Al-Musawi SL, Heath DA, McNatty KP, Ritter LJ, et al. (2014). Aberrant GDF9 expression and activation are associated with common human ovarian disorders. The Journal of Clinical Endocrinology and Metabolism, 99, E615–624. [PubMed: 24438375]

Simpson CM, Stanton PG, Walton KL, Chan KL, Ritter LJ, Gilchrist RB, et al. (2012). Activation of latent human GDF9 by a single residue change (Gly 391 Arg) in the mature domain. Endocrinology, 153, 1301–1310. [PubMed: 22234469]

Sproul K, Jones MR, Mathur R, Azziz R, & Goodarzi MO (2010). Association study of four key folliculogenesis genes in polycystic ovary syndrome. British Journal of Obstetrics and Gynaecology, 117, 756–760. [PubMed: 20236105]

Su J, Hu G, Wang Y, Liang D, Gao M, Sun H, et al. (2014). Recombinant human growth differentiation factor-9 improves oocyte reprogramming competence and subsequent development of bovine cloned embryos. Cellular Reprogramming, 16, 281–289. [PubMed: 24840335]

Su YQ, Sugiura K, Li Q, Wigglesworth K, Matzuk MM, & Eppig JJ (2010). Mouse oocytes enable LH-induced maturation of the cumulus-oocyte complex via promoting EGF receptor-dependent signaling. Molecular Endocrinology, 24, 1230–1239. [PubMed: 20382892]

Su J, Wang Y, Zhang L, Wang B, Liu J, Luo Y, et al. (2014). Oocyte-secreted factors in oocyte maturation media enhance subsequent development of bovine cloned embryos. Molecular Reproduction and Development, 81, 341–349. [PubMed: 24420374]

Su YQ, Wu X, O'Brien MJ, Pendola FL, Denegre JN, Matzuk MM, et al. (2004). Synergistic roles of BMP15 and GDF9 in the development and function of the oocyte-cumulus cell complex in mice: Genetic evidence for an oocyte-granulosa cell regulatory loop. Developmental Biology, 276, 64–73. [PubMed: 15531364]

Sudiman J, Ritter LJ, Feil DK, Wang X, Chan K, Mottershead DG, et al. (2014). Effects of differing oocyte-secreted factors during mouse in vitro maturation on subsequent embryo and fetal development. Journal of Assisted Reproduction and Genetics, 31, 295–306. [PubMed: 24408183]

Sugimura S, Ritter LJ, Rose RD, Thompson JG, Smitz J, Mottershead DG, et al. (2015). Promotion of EGF receptor signaling improves the quality of low developmental competence oocytes. Developmental Biology, 403, 139–149. [PubMed: 25981108]

Sugimura S, Ritter LJ, Sutton-McDowall ML, Mottershead DG, Thompson JG, & Gilchrist RB (2014). Amphiregulin co-operates with bone morphogenetic protein 15 to increase bovine oocyte developmental competence: Effects on gap junction-mediated metabolite supply. Molecular Human Reproduction, 20, 499–513. [PubMed: 24557840]

Sugiura K, Su YQ, Diaz FJ, Pangas SA, Sharma S, Wigglesworth K, et al. (2007). Oocyte-derived BMP15 and FGFs cooperate to promote glycolysis in cumulus cells. Development, 134, 2593–2603. [PubMed: 17553902]

Tagliabracci VS, Engel JL, Wen J, Wiley SE, Worby CA, Kinch LN, et al. (2012). Secreted kinase phosphorylates extracellular proteins that regulate biomineralization. Science, 336, 1150–1153. [PubMed: 22582013]

Takebayashi K, Takakura K, Wang HQ, Kimura F, Kasahara K, & Noda Y (2000). Mutation analysis of the growth differentiation factor-9 and −9b genes in patients with premature ovarian failure and polycystic ovary syndrome. Fertility and Sterility, 74, 976–979. [PubMed: 11056243]

Teixeira Filho FL, Baracat EC, Lee TH, Suh CS, Matsui M, Chang RJ, et al. (2002). Aberrant expression of growth differentiation factor-9 in oocytes of women with polycystic ovary syndrome. The Journal of Clinical Endocrinology and Metabolism, 87, 1337–1344. [PubMed: 11889206]

Thies RS, Chen TJ, Davies MV, Tomkinson KN, Pearson AA, Shakey QA, et al. (2000). GDF-8 propeptide binds to GDF-8 and antagonizes biological activity by inhibiting GDF-8 receptor binding. Growth Factors, 18, 251–259.

Tiotiu D, Alvaro Mercadal B, Imbert R, Verbist J, Demeestere I, De Leener A, et al. (2010). Variants of the BMP15 gene in a cohort of patients with premature ovarian failure. Human Reproduction, 25, 1581–1587. [PubMed: 20364024]

Tong S, & Short RV (1998). Dizygotic twinning as a measure of human fertility. Human Reproduction, 13, 95–98. [PubMed: 9512236]

Tucker EJ, Grover SR, Bachelot A, Touraine P,& Sinclair AH (2016). Premature ovarian insufficiency: New perspectives on genetic cause and phenotypic spectrum. Endocrine Reviews, 37, 609–635. [PubMed: 27690531]

Vanderhyden BC, Cohen JN, & Morley P (1993). Mouse oocytes regulate granulosa cell steroidogenesis. Endocrinology, 133, 423–426. [PubMed: 8319589]

Vanderhyden BC, Telfer EE, & Eppig JJ (1992). Mouse oocytes promote proliferation of granulosa cells from preantral and antral follicles in vitro. Biology of Reproduction, 46, 1196–1204. [PubMed: 1391318]

Vitt UA, Mazerbourg S, Klein C, & Hsueh AJ (2002). Bone morphogenetic protein receptor type II is a receptor for growth differentiation factor-9. Biology of Reproduction, 67, 473–480. [PubMed: 12135884]

Wang TT, Ke ZH, Song Y, Chen LT, Chen XJ, Feng C, et al. (2013). Identification of a mutation in GDF9 as a novel cause of diminished ovarian reserve in young women. Human Reproduction, 28, 2473–2481. [PubMed: 23851219]

Wang EA, Rosen V, D'Alessandro JS, Bauduy M, Cordes P, Harada T, et al. (1990). Recombinant human bone morphogenetic protein induces bone formation. Proceedings of the National Academy of Sciences of the United States of America, 87, 2220–2224. [PubMed: 2315314]

Wang B, Wen Q, Ni F, Zhou S, Wang J, Cao Y, et al. (2010). Analyses of growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) mutation in Chinese women with premature ovarian failure. Clinical Endocrinology, 72, 135–136. [PubMed: 19438907]

Wang B, Zhou S, Wang J, Liu J, Ni F, Yan J, et al. (2010). Identification of novel missense mutations ofGDF9 in Chinese women with polycystic ovary syndrome. Reproductive Biomedicine Online, 21, 344–348. [PubMed: 20705511]

Wei LN, Huang R, Li LL, Fang C, Li Y, & Liang XY (2014). Reduced and delayed expression of GDF9 and BMP15 in ovarian tissues from women with polycystic ovary syndrome. Journal of Assisted Reproduction and Genetics, 31, 1483–1490. [PubMed: 25172094]

Wei LN, Liang XY, Fang C, & Zhang MF (2011). Abnormal expression of growth differentiation factor 9 and bone morphogenetic protein 15 in stimulated oocytes during maturation from women with polycystic ovary syndrome. Fertility and Sterility, 96, 464–468. [PubMed: 21669410]

Weibezahn J, Bukau B, & Mogk A (2004). Unscrambling an egg: Protein disaggregation by AAA+ proteins. Microbial Cell Factories, 3, 1. [PubMed: 14728719]

Wu YT, Tang L, Cai J, Lu XE, Xu J, Zhu XM, et al. (2007). High bone morphogenetic protein-15 level in follicular fluid is associated with high quality oocyte and subsequent embryonic development. Human Reproduction, 22, 1526–1531. [PubMed: 17347167]

Yan C, Wang P, DeMayo J, DeMayo FJ, Elvin JA, Carino C, et al. (2001). Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. Molecular Endocrinology, 15, 854–866. [PubMed: 11376106]

Yeo CX, Gilchrist RB, Thompson JG, & Lane M (2008). Exogenous growth differentiation factor 9 in oocyte maturation media enhances subsequent embryo development and fetal viability in mice. Human Reproduction, 23, 67–73. [PubMed: 17933754]

Yoshino O, McMahon HE, Sharma S, & Shimasaki S (2006). A unique preovulatory expression pattern plays a key role in the physiological functions of bmp-15 in the mouse. Proceedings of the National Academy of Sciences of the United States of America, 103, 10678–10683. [PubMed: 16818886]

Zhang P, Shi YH, Wang LC, & Chen ZJ (2007). Sequence variants in exons of the BMP-15 gene in Chinese patients with premature ovarian failure. Acta Obstetricia et Gynecologica Scandinavica, 86, 585–589. [PubMed: 17464588]

Zhao ZZ, Painter JN, Palmer JS, Webb PM, Hayward NK, Whiteman DC, et al. (2008). Variation in bone morphogenetic protein 15 is not associated with spontaneous human dizygotic twinning. Human Reproduction, 23, 2372–2379. [PubMed: 18614612]

Zhao SY, Qiao J, Chen YJ, Liu P, Li J, & Yan J (2010). Expression of growth differentiation factor-9 and bone morphogenetic protein-15 in oocytes and cumulus granulosa cells of patients with polycystic ovary syndrome. Fertility and Sterility, 94, 261–267. [PubMed: 19376510]

Zhao H, Qin Y, Kovanci E, Simpson JL, Chen Z-J, & Rajkovic A (2007). Analyses of GDF9 mutation in 100 Chinese women with premature ovarian failure. Fertility and Sterility, 88, 1474–1476. [PubMed: 17482612]

Table 1

Prevalence of Carriers With BMP15 and GDF9 Nonsynonymous Variants in Cohorts of Mothers of DZ Twins and Respective Controls

Variants			No. of Cases/Total			
DNA	**Protein**	**Domains**	**DZ Twinning**	**Normal**	**Countries**	**References**
BMP15 in DZ twinning						
c.520C > T	P174S	Proregion	2/1693	0/1512	Australia	Zhao et al. (2008)
c.581T > C	F194S	Proregion	5/1693	11/1512	Australia	Zhao et al. (2008)
c.931G > A	A311T	Mature region	3/1693	0/1512	Australia	Zhao et al. (2008)
c.1175G > C	R392T	Mature region	1/1693	0/1512	Australia	Zhao et al. (2008)
Total			11/1693	11/1512	$P = 0.8328$ by Fisher's exact test	
GDF9 in DZ twinning						
c.207_210delATCT	L69LfsTer88	Proregion	1/449	NA	Australia	Montgomery et al. (2004)
c.307C > T	P103S	Proregion	30/1693	13/1512	Australia	Palmer et al.(2006) *
c.362C > T	T121L	Proregion	4/1693	1/1512	Australia	Palmer et al.(2006)
c.392_393insT	Frame shift	Proregion	NA	NA	Australia	Palmer et al.(2006)
c.1121C>T	P374L	Mature region	4/1693	0/1512	Australia	Palmer et al.(2006) *
c.1268_1269delAA	Frame shift	Mature region	4/1693	0/1512	Australia	Palmer et al.(2006) *
c.1360C > T	R454C	Mature region	22/1693	12/1512	Australia	Palmer et al.(2006)
Total			64/1693	26/1512	$P = 0.0006$ by Fisher's exact test	

Asterisks denote a significance ($P < 0.05$) that was calculated for the likelihood ratio test (MENDEL binomial link measured genotype model) (Palmer et al., 2006). *NA* denotes unavailable data, and thus the respective frequency numbers were excluded from the calculation. The total number of women analyzed in the same study was only counted once.

Table 2

Prevalence of Carriers With BMP15 and GDF9 Nonsynonymous Variants in Cohorts With POI and Respective Controls

Variants			No. of Cases/Total			
DNA	**Protein**	**Domains**	**POI**	**Normal**	**Countries**	**References**
BMP15 in POI						
c.l3A > C	S5R	Signal peptide	1/300	0/216	Italy	Rossetti et al. (2009)
c.l3A > C	S5R	Signal peptide	4/100	NA	France	Bouilly et al. (2016)
c.151_152delGA	E51IfsTer27	Proregion	NA	NA	France	Mayer et al. (2017)
c.181C > T	R61W	Proregion	2/133	0/197	India	Dixit et al. (2006)
c.182G > A	R61E	Proregion	1/133	0/197	India	Dixit et al. (2006)
c.189_198delAGGGCATTCAinsTG	E64AfsTer12	Proregion	NA	NA	France	Mayer et al. (2017)
c.202C > T	R68W	Proregion	2/300	0/216	Italy	Rossetti et al. (2009)
c.202C > T	R68W	Proregion	1/160	0/181	Italy	Di Pasquale et al. (2006)
c.226C > T	R76C	Proregion	3/133	0/197	India	Dixit et al. (2006)
c.227G > A	R76H	Proregion	1/133	0/197	India	Dixit et al. (2006)
c.242A > G	H81R	Proregion	1/50	0/214	Belgium	Tiotiu et al. (2010)
c.308A > G	N103S	Proregion	13/133	10/197	India	Dixit et al. (2006)
c.308A > G	N103S	Proregion	11/160	16/181	Italy	Di Pasquale et al. (2006)
c.308A > G	N103S	Proregion	7/50	25/214	Belgium	Tiotiu et al. (2010)
c.308A > G	N103S	Proregion	NA	NA	France	Bouilly et al. (2016)
c.413G > A	R138H	Proregion	1/300	0/216	Italy	Rossetti et al. (2009)

Variants			No. of Cases/Total			
DNA	**Protein**	**Domains**	**POI**	**Normal**	**Countries**	**References**
c.443T > C	L148P	Proregion	1/203	0/54	France	Laissue et al. (2006)
c.443T > C	L148P	Proregion	2/300	0/216	Italy	Rossetti et al. (2009)
c.443T > C	L148P	Proregion	1/50	1/214	Belgium	Tiotiu et al. (2010)
c.443T > C	L148P	Proregion	2/100	NA	France	Bouilly et al. (2016)
c.538G > A	A180T	Proregion	1/133	0/197	India	Dixit et al. (2006)
c.538G>A	A180T	Proregion	2/203	0/54	France	Laissue et al. (2006)
c.538G>A	A180T	Proregion	5/160	0/181	Italy	Di Pasquale et al. (2006)
c.538G>A	A180T	Proregion	2/20	1/93	Germany	Ledig et al. (2008)
c.538G>A	A180T	Proregion	6/300	0/216	Italy	Rossetti et al. (2009)
c.538G>A	A180T	Proregion	2/50	2/214	Belgium	Tiotiu et al. (2010)
c.538G>A	A180T	Proregion	1/50	0/150	Italy	Ferrarini et al. (2013)
c.538G>A	A180T	Proregion	NA	NA	France	Bouilly et al. (2016)
c.538G > T + 539C > T	A180F/S+V	Proregion	1/133	0/197	India	Dixit et al. (2006)
c.581T > C	F194S	Proregion	1/50	1/214	Belgium	Tiotiu et al. (2010)
c.588T>A	N196K	Proregion	1/133	0/197	India	Dixit et al. (2006)
c.595G>A	G199R	Proregion	1/50	0/214	Belgium	Tiotiu et al. (2010)
c.598C > T	H200Y	Proregion	5/100	1/100	China	Wang et al. (2010)
c.617G > A	R206H	Proregion	1/133	0/197	India	Dixit et al. (2006)
c.631C > T	E211X	Proregion	1/133	0/197	India	Dixit et al. (2006)
c.661T > C	W221R	Proregion	1/133	0/197	India	Dixit et al. (2006)

Variants			No. of Cases/Total			
DNA	**Protein**	**Domains**	**POI**	**Normal**	**Countries**	**References**
c.704A > G	Y235C	Proregion	NA	NA	Italy	Di Pasquale et al. (2004)
c.727A > G	I243G	Proregion	1/133	0/197	India	Dixit et al. (2006)
c.788insTCT	Ins263L	Proregion	4/133	4/197	India	Dixit et al. (2006)
c.788insTCT	Ins263L	Proregion	10/203	9/54	France	Laissue et al. (2006)
c.788insTCT	Ins263L	Proregion	2/160	5/95	Italy	Di Pasquale et al. (2006)
c.788insTCT	Ins263L	Proregion	17/300	5/216	Italy	Rossetti et al. (2009)
c.788insTCT	Ins263L	Proregion	3/50	5/214	Belgium	Tiotiu et al. (2010)
c.788insTCT	Ins263L	Proregion	5/100	15/100	China	Wang et al. (2010)
c.985C > T	R329C	Mature region	1/100	0/100	China	Wang et al. (2010)
None	—	—	0/92	0/76	China	Zhang, Shi, Wang, and Chen (2007)
None	—	—	0/38	0/51	New Zealand	Chand, Ponnampalam, Harris, WinshiP, and Shelling (2006)
None Total	—	—	0/15 122/1161	0/3 100/1335	Japan P = 0.0171	Takebayashi et al. (2000) by Fisher's exact test
GDF9 in POI						
c.169G > T	D57Y	Proregion	8/100	11/100	China	Wang et al. (2010)
c.169G > T	D57Y	Proregion	6/139	2/152	China	Wang et al. (2013)
c.199A > C	K67E	Proregion	4/127	0/220	India	Dixit et al. (2005)
c.307C > T	P103S	Proregion	1/60	0/60	USA	Kovanci et al. (2007)
c.307C > T	P103S	Proregion	1/100	NA	France	Bouilly et al. (2016)
c.436C > T	R146C	Proregion	1/100	1/96	China	Zhao et al. (2007)

Variants			No. of Cases/Total			
DNA	**Protein**	**Domains**	**POI**	**Normal**	**Countries**	**References**
c.436C > T	R146C	Proregion	3/139	0/152	China	Wang et al. (2013)
c.442A > G	T148A	Proregion	NA	NA	France	Bouilly et al. (2016)
c.557C > A	S186Y	Proregion	1/203	0/54	France	Laissue et al. (2006)
c.646G > A	V216M	Proregion	2/127	0/220	India	Dixit et al. (2005)
c.712A > G	T238A	Proregion	1/100	0/96	China	Zhao et al. (2007)
c.1283G > C	S428T	Mature region	2/100	2/96	China	Zhao et al. (2007)
c.1360C > T	R454C	Mature region	1/100	NA	France	Bouilly et al. (2016)
None	—	—	0/50	0/150	Italy	Ferrarini et al. (2013)
None	—	—	0/15	0/3	Japan	Takebayashi et al. (2000)
Total			29/794	16/835	$P = 0.0484$ by Fisher's exact test	

NA denotes unavailable data, and thus the respective frequency numbers were excluded from the calculation. The total number of women analyzed in the same study was only counted once.

Table 3

Prevalence of Carriers With BMP15 and GDF9 Nonsynonymous Variants in Cohorts With PCOS and Respective Controls

Variants			No. of Cases/ Total			
DNA	**Protein**	**Domains**	**PCOS**	**Normal**	**Countries**	**References**
BMP15 in PCOS						
c.34C > G	L12V	Signal peptide	1/216	0/200	China	Liu et al. (2011)
c.109G > C	A37P	Proregion	1/216	0/200	China	Liu et al. (2011)
c.169C > G	P57A	Proregion	1/216	0/200	China	Liu et al. (2011)
c.288G > C	R96S	Proregion	1/216	0/200	China	Liu et al. (2011)
c.308A>G	N103S	Proregion	1/216	0/200	China	Liu et al. (2011)
c.308A>G	N103S	Proregion	2/70	NA	Iran	Mehdizadeh et al. (2016)
c.598C > T	H200Y	Proregion	1/216	2/200	China	Liu et al. (2011)
c.788insTCT	Ins263L	Proregion	21/216	30/200	China	Liu et al. (2011)
None	—	—	0/38	0/3	Japan	Takebayashi et al. (2000)
Total			27/254	32/203	$P = 0.1665$ by Fisher's exact test	
GDF9 in PCOS						
c.15C > G	N5L	Signal peptide	1/216	0/200	China	Wang et al. (2010)
c.118T > G	L40V	Proregion	4/216	0/200	China	Wang, Zhou et al. (2010)
c.133A > G	M45V	Proregion	1/216	0/200	China	Wang, Zhou et al. (2010)
c.169G > T	D57Y	Proregion	12/216	24/200	China	Wang, Zhou et al. (2010)
c.436C > T	R146C	Proregion	2/216	0/200	China	Wang, Zhou et al. (2010)
c.1025A > T	Y342F	Mature region	1/216	0/200	China	Wang, Zhou et al. (2010)
c.1275C > A	S425R	Mature region	1/216	0/200	China	Wang, Zhou et al. (2010)
c.1283G > C	S428T	Mature region	2/216	0/200	China	Wang, Zhou et al. (2010)
None	—	—	0/38	0/3	Japan	Takebayashi et al. (2000)
Total			24/254	24/203	$P = 0.5422$ by Fisher's exact test	

NA denotes unavailable data, and thus the respective frequency numbers were excluded from the calculation. The total number of women analyzed in the same study was only counted once.