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Association of bone morphogenetic protein 15 and growth differentiation factor 9 with litter size in livestock: a review study

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ABSTRACT. Litter size is one of the crucial factors in livestock production and is of high economic value, which is affected by ovulation rate, hormones, and growth factors. Growth factors play a multifaceted role in reproductive physiology. This review aims to investigate the association of bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9) with litter size in livestock. The transforming growth factor β (TGF- β) superfamily includes more than 34 members; GDF9 and BMP15 are among the most significant factors for regulating fertility and litter size in most livestock species. Ovarian follicles release BMP15 and GDF9 that are involved in the maturation of primary follicles into the basal form, proliferation of granulosa and theca cells, steroidogenesis, ovulation, and formation of the corpus luteum. Besides, these factors are highly expressed in oocytes and are necessary for female fertility and multiple ovulation in several livestock species. Animals with two inactive copies of these factors are sterile, while those with one inactive copy are fertile. Thus, the present review provides valuable information on the association of BMP15 and GDF9 with litter size in livestock that can be used as biological markers of multiple ovulation or for improving fertility in livestock.

Keywords: birth type; domestic animals; ovarian follicle; reproductive performance; TGF-β.

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Introduction

As demand increases for livestock production, a need arose for livestock with larger litter size (Al-Thuwaini, 2021a; Al-Thuwaini & Kareem, 2022). Litter size is one of the most significant features of fertility in livestock (Al-Thuwaini, & Al-Hadi, 2022; Kadhem & Al-Thuwaini, 2022), which is affected by ovulation rate, hormones, and growth factors (Tesema et al., 2020; Al-Thuwaini, 2021b; Al-Thuwaini, 2022). Ovulation rate and the number of oocytes released during ovulation have an impact on litter size (Chu, Xu, Yang, & Sun, 2018). Besides, a high level of reproductive hormones strongly affects the development and maturation of ovarian follicles, which increases litter size (Mohammed, Al-Thuwaini, & Al-Shuhaib, 2021).

On the other hand, growth factors play a multifaceted role in reproductive physiology (Muñoz-García et al., 2020). They include members of the transforming growth factor β (TGF- β) superfamily that are vital intra-ovarian growth factors (Table 1) (Santibanez & Kocic, 2012). This superfamily comprises over 34 family members, the most important ones that are associated with litter size are bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9) (Abdurahman et al., 2019; Imran, Al-Thuwaini, Al-Shuhaib, & Lepretre, 2021; Ali, Kadhim, & Al-Thuwaini, 2022).

Table 1. Members of the TGF- β superfamily.

TGF-βs	BMP and GDF	Activins, inhibins	MIF
TGF-β1, TGF-β2,	BMP2, BMP3b/GDF10, BMP4, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9/GDF2, BMP10, BMP11/GDF11, BMP12/GDF7,	Activin A, Activin B, Activin AB, Activin C, Activin E	MIF/AMH
TGF-β3	BMP13/GDF6, BMP14/GDF5, BMP15/GDF9b, BMP16/Nodal, GDF1, GDF3, GDF8/myostatin, GDF9, GDF15	Inhibin A, Inhibin B, Inhibin C	

Transforming growth factor β (TGF- β), bone morphogenetic protein (BMP), growth and differentiation factor (GDF), Müllerian inhibitory factor (MIF), anti-Müllerian hormone (AMH).

Source: Adapted from Santibanez and Kocic (2012).

BMP15 and GDF9 are released by ovarian follicles, leading to the transformation of primary follicles to the basal form (Castro, Cruz, & Leal, 2016; Ajafar, Kadhim, & AL-Thuwaini, 2022), and involved in follicular and oocyte maturation, proliferation/atresia of granulosa and theca cells, steroidogenesis, ovulation, and corpus luteum formation (Chu et al., 2018). Moreover, genetic modifications in BMP15 and GDF9 play a vital role in ovarian function (Sanfins, Rodrigues, & Albertini, 2018). Mutations in *BMP15* and *GDF9* genes are associated with a higher ovulation rate and litter size in most livestock species (Wang et al., 2018; Muñoz-García et al., 2020). Although some researchers have studied the growth factors that affect the reproductive traits of sheep and cattle, their role in litter size remains unclear. Therefore, this review aims to investigate the association of BMP15 and GDF9 with the litter size in livestock.

Bone morphogenetic proteins and ovarian function in farm animals

Bone morphogenetic proteins (BMPs) are a group of multi-functional growth factors within the TGF-β superfamily (Tong, Guo, Glen, Morrell, & Li, 2019) that regulates animal reproductive processes (Lochab & Extavour, 2017). The BMPs system in the ovary plays a vital role in regulating granulosa cell proliferation, differentiation, and their responsiveness to follicle stimulating hormone (FSH) (Chu et al., 2018; Sanfins et al., 2018). BMPs in sheep and cattle control granulosa cells proliferation by suppressing the FSH receptors expression (Zhang, Klausen, Zhu, Chang, & Leung, 2015; Haas et al., 2019). During follicle and oocyte maturation in farm animals, BMPs signaling affects animal fertility by regulating sex hormone secretion, gene expression of gonadotropin receptors, and oocyte quality (Figure 1) (Zhang et al., 2015; Sanfins et al., 2018). BMPs signaling is also related to ovulation rate and estrus cycle (Lochab & Extavour, 2017).

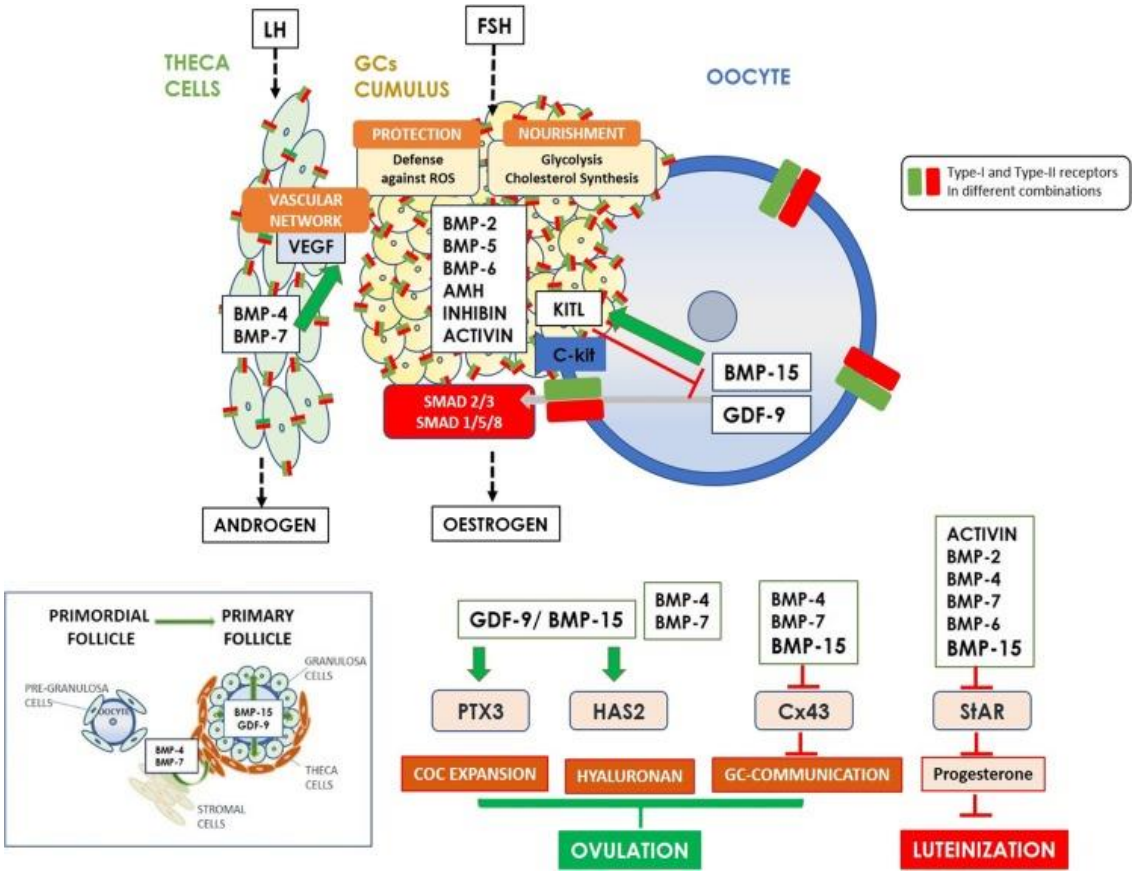

Figure 1. The main bone morphogenetic proteins (BMPs) members and the functional roles in regulating ovarian physiology.
Source: Adapted from Sanfins et al. (2018).

Among the most important members of the BMPs is BMP15, which has a vital role in the female fertility of livestock (Hernández-Montiel et al., 2020). The physiological action of the BMP15 is more effective in sheep in the first stages of follicle growth (Rossi et al., 2015), and expressed at the initial stage of follicular development in many livestock species (Sanfins et al., 2018). During the development of ovarian follicles, the expression of oocyte-secreted factors BMP15 contributes to follicular growth

through a paracrine signaling pathway (Rossi et al., 2015). Furthermore, it is involved in the development of primordial follicles into the primary follicle, and in preventing premature luteinization (Sanfins et al., 2018). BMP15 inhibits premature follicular luteinization by downregulating the steroidogenic acute regulatory protein (StAR) and suppressing the LH receptor. These effects on follicular cell functions could finally lead to the absence of a dominant follicle (Dalbies-Tran et al., 2020). After ovulation, the rapid decrease of these oocyte factors in the corpus luteum led to elevate StAR expression and consequent control of follicular maturation by modulating FSH's differentiating effects (Sanfins et al., 2018). In addition, BMP15 plays a vital role in regulating ovulation rate and oocyte health along with the initiation of pregnancy (Ghoreishi, Fathi-Yosefabad, Shayegh, & Barzegari, 2019).

Several mechanisms by which BMP15 influences the ovulation rate in livestock. BMP15 participates in ovarian folliculogenesis as it regulates its action by the binding protein follistatin, which by binding to BMP15 inhibits its effect on the expression of FSH receptors in granulosa cells (Chu et al., 2018; Nagdy et al., 2018). Because more follicles can synthesize LH receptors, even when FSH levels drop, the development of more follicles continues during the preovulation period, leading to the female releasing more eggs during the anestrus period (Wang et al., 2021). In parallel, mutations in the *BMP15* gene in farm animals showed higher ovulation rates and a higher rate of twins in heterozygous animals, while homozygous animals are sterile (Nagdy et al. 2018). This mutation leads to an increase in the density of follicle-stimulating hormone receptor (FSHR) and luteinizing hormone receptor (LHR) in granulosa cells, which can reduce apoptosis of the granulosa cells and increase the ovulation rate (Abdurahman et al., 2019; Wang et al., 2021). The low BMP15 signaling is believed to contribute to the sensitization of granulosa cells to FSH, and thus to the selection and maintenance of follicles during the follicular phase, resulting in enhanced ovulation in these mutant ewes (Estienne et al., 2017). In contrast, ewes homozygous for *BMP15* mutations exhibit early inhibition of follicle growth and reduce the sensitivity of granulosa cells to FSH by inhibiting expression of the FSH receptor (Wang et al., 2021). Additionally, ewes with mutations of *BMP15* show a diminished expression of anti-Müllerian hormone receptors (AMHR2 or AMH) in the granulosa cells of their antral follicles. These mutant ewes have low levels of BMP15 and AMH signaling, which improves granulosa cell sensitization to FSH, thereby enhancing the rate of ovulation (Estienne et al., 2017).

The role of BMPs/GDF9 system in ovarian follicle growth and differentiation

A combination of autocrine/paracrine factors is involved in ovarian follicle growth and differentiation (Kawashima & Kawamura, 2018). In livestock ovaries, the expression pattern of BMPs ligands and receptors indicates that the BMPs system and GDF9 form a vital part of this intra-follicular network (Rossi et al., 2015). The BMPs system and GDF9 regulate granulosa cell proliferation, differentiation, and apoptosis during follicle formation through autocrine/ paracrine action in the ovary (Shimasaki, Moore, Otsuka, & Erickson, 2004) (Figure 2). The oocytes express BMP6, BMP15, and GDF9 (Belli & Shimasaki, 2018), granulosa cells express BMPR-II and ALK3 and ALK6 receptors, and theca cells express the ALK3, and ALK6 receptors (Liu, Qin, Qu, Wang, & Yan, 2020). Whereas theca cells, granulosa cells, and oocytes express BMP3b, BMP4, and BMP7 (Juengel, Smith, Quirke, French, & Edwards, 2018).

Oocyte derived growth factors, GDF9 and BMP15 are expressed selectively by oocytes from the primordial stage in ruminants and interact during follicular development, thereby affecting litter size (Heath, Pitman, & McNatty, 2017). These oocyte growth factors are essential in the early stages of follicle development and then in late follicular development (Sanfins et al., 2018). Furthermore, it plays a fundamental role in the differentiation of the different granulosa cell types (Alam & Miyano, 2020) and regulates the basic functions of granulosa cells (Sanfins et al., 2018). Therefore, these factors control the differentiation and function of granulosa cells and the patterns of gene expression in somatic follicular cells (Alam & Miyano, 2020). BMP15 and GDF9 have been implicated in stimulating the expansion of cumulus cells by stimulating the expression of hyaluronan synthase 2, which is crucial in the matrix formation needed for cumulus expansion (Sanfins et al., 2018). In addition, to regulate ovulation rate both GDF9 and BMP15 may strongly contribute to oocyte health and pregnancy establishment (Ghoreishi et al., 2019).

While BMP15 is responsible for granulosa cells proliferation, differentiation, and follicle development, GDF9 plays a critical role in regulating ovarian function and enhancing oocyte developmental competence (Wang et al., 2021). This suggests that BMP15 and GDF9 work synergistically to promote ovulation, oocyte health, and pregnancy establishment (Alam & Miyano, 2020).

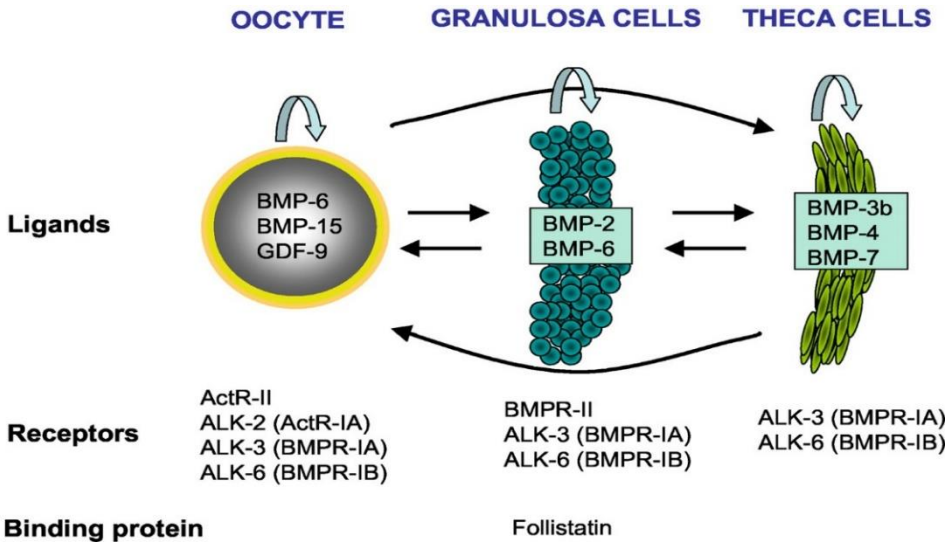

Figure 2. The role of growth factors in the ovarian follicle.
Source: Adapted from Shimasaki et al. (2004).

Furthermore, there is evidence of the combination of *BMP15* and *GDF9* mutations, as evidenced with ewes who have mutations in both *BMP15* and *GDF9* ovulate more frequently than heterozygotes with mutations in one of them alone (Ghoreishi et al., 2019). Thus, it is crucial to consider BMP15 and GDF9 interactions in follicular development that further affect livestock litter size.

The oocyte-specific growth factors and litter size

The oocytes secrete many essential growth factors into granulosa cells to regulate the growth and development of follicles through paracrine regulation (Figure 3) (Piotrowska et al., 2013; Castro et al., 2016). BMP15 is specialized cell-expressed in the ovaries of livestock and plays major roles in regulating follicle formation, ovulation, and luteal functions (Rossi et al., 2015). Besides, GDF9 promotes the proliferation of theca cells and reduces steroid formation, thus it may prevent theca cells from early differentiation during follicle formation (Souza, McNeilly, Benavides, Melo, & Moraes, 2014).

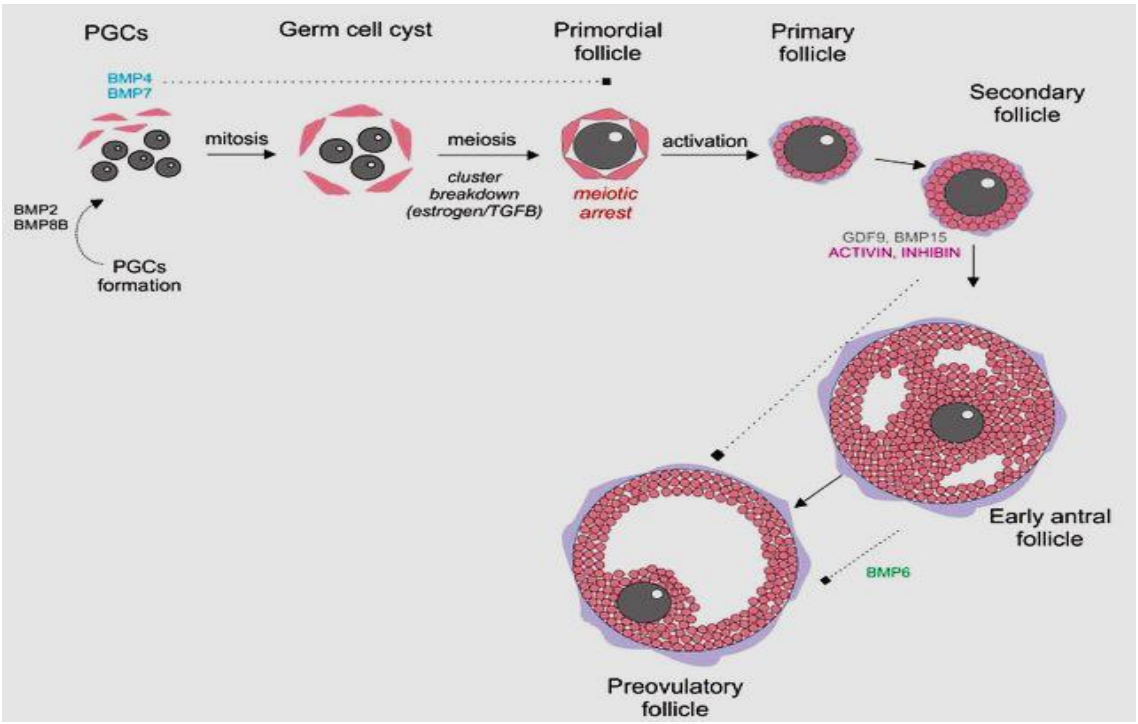

Figure 3. Oocyte growth factors and follicular growth.
Source: Adapted from Piotrowska et al. (2013).

Paracrine oocyte growth factors BMP15 and GDF9 are essential components of intra-follicular communication (Andrade, Collado, Meirelles, Silveira, & Perecin, 2019). Oocytes express GDF9 in developing ovarian follicles, which is essential for early ovarian follicle growth and differentiation (Ghoreishi et al., 2019). Then, BMP15 plays a vital role in female fertility in livestock through its role in regulating granulosa cell proliferation and differentiation by suppressing the expression of FSH receptors and enhancing ligand expression (Qin et al., 2019). This paracrine interaction between the oocyte and the surrounding follicular cells promotes the growth and development of both the oocyte and follicle (Andrade et al., 2019). In addition, BMP15 and GDF9 act as paracrine/autocrine agent to stimulate follicle growth and has a great role in regulating ovulation rate and litter size (Ghoreishi et al., 2019). They are highly expressed in oocytes and are necessary for female fertility and multiple ovulation in several species of livestock (Hernández-Montiel et al., 2020).

Moreover, these factors allow more follicles to synthesize LH receptors even when FSH levels drop this enables a developing female to release more eggs during its anestrus phase (Figure 4). Variant *BMP15* and *GDF9* expressions affect the paracrine actions of these oocyte-secreted factors and altered follicle development that blocks the biological actions of BMP15 or GDF9, suggesting potential as contraceptives or sterilizing agents (Niu et al., 2021).

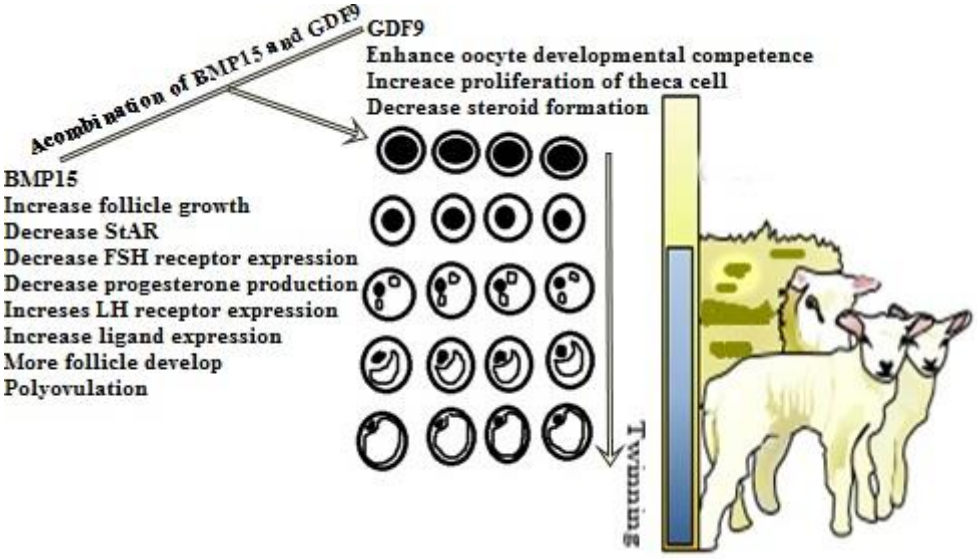

Figure 4. The association of BMP15 and GDF9 with litter size.

Conclusion

The present review provides valuable information on the association of BMP15 and GDF9 with litter size in livestock that can be used as biological markers of multiple ovulation or for improving fertility in livestock. Mutations in the *BMP15* and *GDF9* genes provide valuable genetic resources for livestock production. These mutations affect growth factor profiles, affecting litter size. The effects of BMP15 and GDF9 interactions on follicular development on livestock litter size require further consideration. Breeders need to pay attention to the importance of BMP15 and GDF9 as fertility markers since abnormalities in their expression may result in animal infertility.

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