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An Overview of Major Genes Affecting Prolificacy in Sheep and Related Mechanisms

Review Article

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ABSTRACT

The objective of this review is to explore the current developments on major genes working on prolific sheep breeds and the mechanism behind it, while identifying the future working points. Productivity is the ultimate goal of farm animal production and prolificacy is a key feature in determining productivity in farm animals. Ovulation rate in mammals is an intricate process involving genetics and endocrine pathways. Exceptional reproductive capabilities along with higher ovulation rates were observed in many breeds of sheep from different parts of the world since the discovery of Booroola Merino sheep. These naturally occurring mutations acting on prolificacy are found in chromosomes 5, 6, 11 and X but speculations are around about the presence of more mutations on these genes or different genes on multi ovulating sheep breeds. The exact control mechanism of multiple ovulations and multiple births in prolific sheep breeds is poorly understood. Over the years it has been repeatedly shown that gonadotropins and intra ovarian factors play vital and variety of roles. More specifically, follicular stimulating hormone regulation during folliculogenesis could be a promise for the future studies. Among those intra ovarian factors, bone morphogenic protein system is one of the indispensable components, which exerts enormous enthusiasm among the scientific community towards manipulating ovarian folliculogenesis. Rather surprisingly, biological and physiological roles of bone morphogenic protein subfamily are not thoroughly elucidated and contradictory findings among the mammals make further twists, which will be the gaps to be filled in the near future. Presence of a regulatory control loop between oocyte, granulosa and theca cells through transforming growth factor B (TGFB) superfamily is proposed here.

KEY WORDS BMPs, follicular selection, mutated genes, ovulation rate, prolificacy.

INTRODUCTION

Prolificacy is defined as the number of progenies born per parturition. Fertility is often used as synonym of prolificacy; however, prolificacy is slightly different from fertility, nevertheless to be prolific, an animal must be highly fertile. High fecundity should reflect the high prolificacy as well due to the linear relationship (i.e., fecundity=fertility×prolificacy). Generally, prolificacy is assessed as ovulation rate (number of mature oocytes released during one reproductive cycle). Whereas, ovulation rate is the primary source of variation in prolificacy, both within and between breeds (Webb *et al.* 2007) but unfortunately, it is poorly understood in female mammals (Shimasaki *et al.* 2004; Fabre *et al.* 2006; Vireque *et al.* 2008). It is a complex trait influenced by genetic and multiple transection of endocrine signals between ovary and the pituitary gland (McNatty *et al.* 2001). The complex signals do involve

paracrine and autocrine within the ovarian follicles involving the oocyte and its adjacent somatic cells (Campbell and McNeilly, 1996; Bodensteiner *et al.* 1999). The functional unit of female gonad is ovarian follicle which includes oocyte, surrounding granulosa cells and external theca cells (Knight and Glister, 2003; Orisaka *et al.* 2009).

Major genes that increase prolificacy exceptionally on sheep flocks have reported throughout the world (McNatty *et al.* 2001; Davis, 2005; Nassiry *et al.* 2006). Current understanding of major genes affecting prolificacy in sheep falls into three categories:

Mutation has been identified in genes and the DNA testing is also available for them. This category includes ALK6 (activin receptor like kinase) or BMPR-1B (bone morphogenetic protein receptor type 1B), GDF9 (growth differentiation factor 9) and BMP15 **(**born morphometric protein 15) (Table 1).

Mode of inheritance of the genes has been described but the mutation has not been identified. Woodlands gene, Thoka gene and Lacaune are falls into this group. It is necessary to point out that until now Lacaune had two types of mutations one at chromosome X and another one at chromosome 11 (Davis, 2005; Drouilhet *et al.* 2009).

Putative genes where there is evidence of apparent genetic segregation but there are insufficient records to ascertain the mode of inheritance. This segment includes Olkuska, Belle-Ile and New Zealand Longwool breeds (Davis, 2004).

Mutated genes

ALK6 or BMPR-IB mutation

ALK6 was first found in Booroola ewes (FecB) at nucleotide position 830 (point mutation) leading to an arginine replacing glutamine amino acid (Q249R) in a highly conserved region of the intracellular kinase domain (Mulsant *et al.* 2001). This mutation was mapped in sheep chromosome 6 (Montgomery *et al.* 2001). Ovulation rates are usually > 5.0 and in some cases it goes up to 15 (McNatty *et al.* 2005a).

In ewes carrying FecB induces not only precocious maturation of ovarian follicles when compared to their wild type or non-carrier counterparts (McNatty *et al.* 2005a) but also ovulate at significantly smaller diameters in BB and B+ carriers (McNatty *et al.* 1986a). Granulosa cell populations show that the ALK6 mutation influences granulosa cell development both before as well as after antrum formation (McNatty *et al.* 2003). Within the ovary ALK6 mutation affects both oocyte and granulosa cell maturation from the earliest stages of follicular development. Secondary effects observed most likely due to ALK6 mRNA presence in wide range of tissues such as ovaries, brain, pituitary, kidney, skeletal muscle, uterus, prostrate and testes.

Comparisons of ALK6 mutation in sheep with the ALK6 knockout mice to explore the species differences in ovulation rate are premature (Bodensteiner *et al.* 1999; McNatty *et al.* 2005a).

Possibility for a functional interaction between BMP15 and ALK6 could not be ruled out in the current contexts of understanding (Davis *et al.* 1999). Further findings on this interaction could add more enthusiasm into the highly enthralling field of prolific sheep breeds.

GDF9 mutation

Transforming growth factor-B (TGFB) superfamily comprises of more than 35 different factors (Figure 1) such as GDF9, activin, inhibin, anti-mullerian hormone (AMH) and BMPs that influence oocyte growth and function (Knight and Glister, 2001; Chang *et al.* 2002; Wu and Matzuk, 2002; Knight and Glister, 2003; Pangas and Matzuk, 2004; Lin *et al.* 2006).

Members of TGFB superfamily signals through two types (Type 1 and Type 2) of membrane bound receptors. Type 1 receptors comprises of seven members (ALK 1-7) while Type 2 has five members (ActR2, ActR2B, BMPR2, TGFBR2 and AMHR2).

GDF9 mutation corresponds to a non-conservative AA replacement at position 77 of the mature protein region found in chromosome number 5 which is an autosomal gene (Davis, 2004). Ewes homozygous for GDF9 mutation are anovulatory therefore sterile, whereas heterozygous animals have mean ovulation rate > 2.0 (McNatty *et al.* 2005b). Many Iranian sheep breeds showed clear mutation on GDF9 and BMP15 (Nassiry *et al.* 2006; Deldar-Tajangookeh *et al.* 2009; Ghaderi *et al.* 2010; Javanmard *et al.* 2011).

BMP15 mutation

Another member of TGFB superfamily, BMP15 (also known as GDF9B) mutation located in X chromosome (Davis *et al.* 1991; Davis *et al.* 2001) has five separate point mutations in which two of them have premature stop codon; one at amino acid position 29 of proregion of exon 2 and the other one at amino acid position 23 of mature protein. Another two mutations are non conservative amino acid substitutions within the mature proteins at amino acid positions 31 and 99. The other mutation of BMP15 is a co-dominant mutation in autosomal gene affecting ovulation rate (Davis, 2004; McNatty *et al.* 2005a). Apart from these mutations, more mutations of these genes or in different genes are likely to be present in other prolific breeds (Galloway *et al.* 2000; Davis *et al.* 2002; Hanrahan *et al.* 2004; Martinez-Royo *et al.* 2008). Research on other mutations would certainly shift the gears towards new paradigm in mutated fecundity genes.

Table 1 Major genes affecting ovulation rate in sheep

Name	Gene	Allele	Base Change	AA Change	Mutation	Founder breed
Inverdale	BMP15 (X)	$FecX^{I}$	T-A	Val-Asp	V299D / V31D	Romney
Hanna	BMP15 (X)	$FecX^{H}$	C-T	Glu-Stop	Q291stop / Q23stop	Romney
Belclare	BMP15 (X)	$FecX^{B}$	G-T	Ser-Ile	S3671 / S991	Belclare
Galway	BMP15 (X)	$FecX^{G}$	C-T	Gln-Stop	T239stop / no	Belclare and Cambridge
Lacaune	BMP15 (X)	$FecX^{L}$	G-A	Cys-Tyr	C321Y / C53Y	Laucane
Booroola	ALK6 (6)	$FecB^{B}$	A-G	Glu-Arg	Q249R	Merino, Garole, Javanese, Hu and Han
High Fertility	GDF9 (5)	$FecG^{H}$	C-T	Ser-Phe	S395F / S77F	Belcalre and Cambridge
Lacaune	-	$FecL^{L}$	?	?	?	Laucane

AA: amino acid; Arg: arginine; Asp: aspartic acid; Cys: cysteine; Glu: glutamic acid; Gln: glutamine; Ile: isoleucine; Phe: phenylalanine; Ser: serine; Stop: stop codon; Tyr: tyrosine and Val: valine.

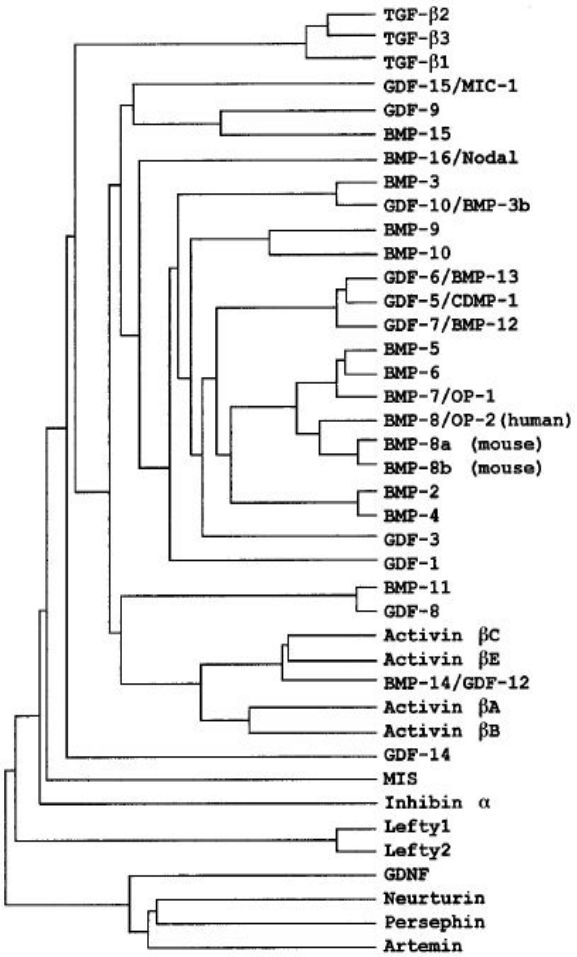

Figure 1 Dendogram of TGFB superfamily members (Chang *et al.* 2002)

Inverdale sheep was the first prolific sheep breed to have the genetic basis identified, which results from mutations in the BMP15 gene and GDF9 (Galloway *et al.* 2000) and both of them were exclusively secreted by ovaries during follicular development (Dong *et al.* 1996; Juengel *et al.* 2006; Juengel *et al.* 2004; Souza *et al.* 2004). These mutations have increased prolificacy in heterozygous ewes and infertility in homozygous animals (Galloway *et al.* 2000; Hanrahan *et al.* 2004), nevertheless, discrepancies have been observed between species (Souza *et al.* 2002; Shimasaki, 2006; Edson *et al.* 2009; Otsuka *et al.* 2011). Not only the effect of GDF9 and BMP15 mutation is additive for ovulation rate in sheep (Hanrahan *et al.* 2004), but also they form BMP15 / GDF9 heterodimers (Liao *et al.* 2003; McIntosh *et al.* 2008). Normal folliculogenesis in sheep is highly depends on bioavailability of BMP15 and GDF9 (Galloway *et al.* 2000; Juengel *et al.* 2002; Shimasaki *et al.* 2004). GDF9 mutation in sheep may enhance the sensitivity of the ovarian follicles to FSH and thereby increase the ovulation rate (Vitt *et al.* 2000; Hanrahan *et al.* 2004). It was believed that the mutations in the BMP15 gene may actually affect the level of GDF9 secretion and the abnormal concentrations of GDF9 are the cause of amino acid substitution in sheep. The mRNA of GDF9 is found in oocytes from primordial to large antral follicles (Bodensteiner *et al.* 1999), in contrast BMP15 gene expression begins in oocytes from primary follicles. Afterwards, within the ovary BMP15 is found exclusively in most of the growing follicles (Galloway *et al.* 2000; Otsuka *et al.* 2000; Juengel *et al.* 2002) (Figure 2).

Models of follicular selection

Follicular selection indicates that multiple ovulations and multiple births are controlled by the concentrations of gonadotropins and by intra-ovarian factors (Hunter *et al.* 2004; Souza *et al.* 2004; McNatty *et al.* 2005b; Fabre *et al.* 2006; Vireque *et al.* 2008; Campbell, 2009). The gonadotrophins include FSH and LH while the intra ovarian factors include vast variety of BMP subfamily. This subfamily has a paramount role in manipulating proliferation and differentiation responses of both granulosa and theca cells (Monget *et al.* 2002; Knight and Glister, 2003; Shimasaki *et al.* 2004; Drouilhet *et al.* 2010; Trombly *et al.* 2010). Scaramuzzi *et al.* (1993) proposed a novel model on multi-ovulatory ewes with possible mechanisms based on the responsiveness of gonadotropins.

The BMP system influence granulosa and theca cells through the gonadotropic stimulation with multiple intra-follicular pathways (Souza *et al.* 2004).

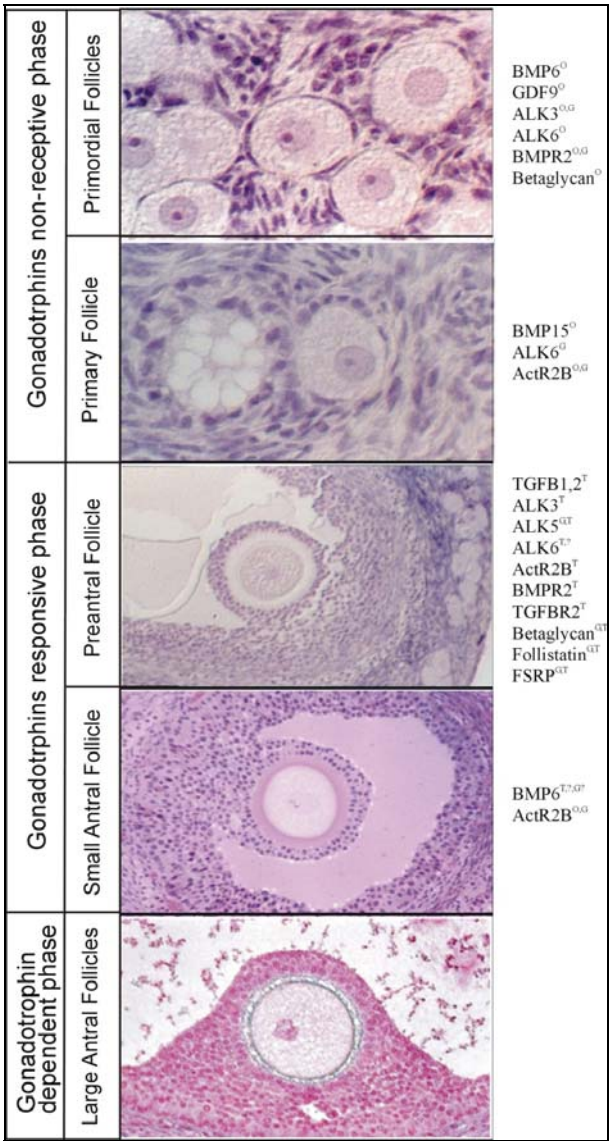

Figure 2 Localization of selected members (TGFB, BMP6, GDF9 and BMP15) of the TGFB superfamily in sheep. Their receptors, signaling proteins and binding proteins during follicular development in sheep has denoted. 'O', 'G' and 'T' indicate expression in the oocyte, granulosa cells and thecal cells, respectively. Gene expression patterns including a '?' indicate that reports of expression of this gene / protein are inconsistent in the literature

The mechanism of BMPs that affects on ovarian steroidogenisis is complex and not fully understood (Findlay and Drummond, 1999; Shimasaki *et al.* 1999; Monget *et al.* 2002; Souza *et al.* 2004; McNatty *et al.* 2005a; Fabre *et al.* 2006; Findlay *et al.* 2009). A schematic concept regarding the BMP activity which has been proposed by Fabre *et al.* (2006) can be found in Figure 3. According to this hypothesis, a loss in BMP system function guides to a raise in ovulation rate (Fabre *et al.* 2006). This loss function in BMP system implies a decrease in the proliferating capacity of granulosa cells (Monget *et al.* 2002).

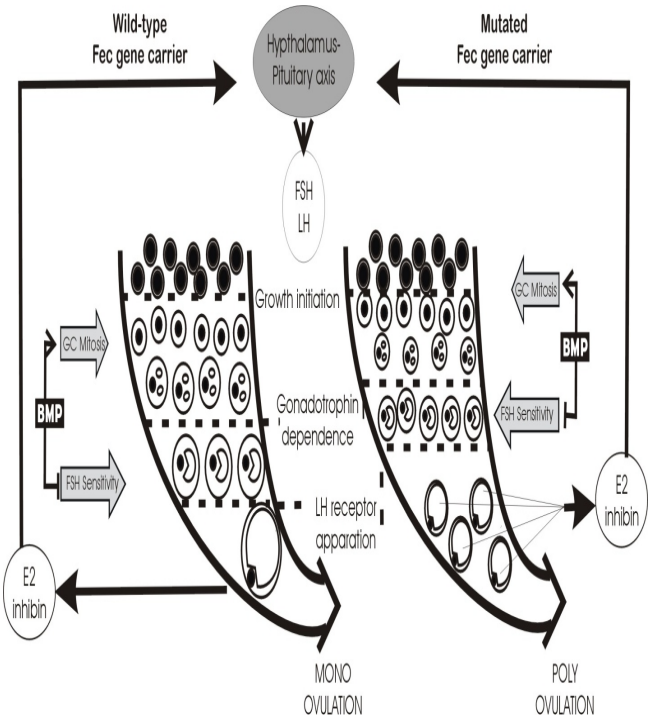

Figure 3 Schematic representations of the affects of Fec gene on folliculogenesis and ovulation. The reduced activity of the BMP signaling system in the ovary of mutated Fec gene carrier (right) compared to non-carrier (left) ewes leads to decrease both the positive action of BMP on granulosa cell (GC) mitosis and its inhibiting action on FSH sensitivity. The consequence is the presence of smaller antral follicles with a reduced number of granulosa cells exhibiting a higher FSH sensitivity, leading to an advance in follicular maturation as attested by precocious LH receptor expression. The smaller matured follicles present in mutated Fec gene carrier each produce reduced amounts of oestradiol (E2) and inhibin, but altogether they produce the same amounts than one larger wild-type follicle. Consequently, the same endocrine dialog can establish between the ovaries and the central nervous system of both genotypes that leads to the selection and ovulation of numerous smaller follicles in mutated Fec gene carrier (Fabre *et al.* 2006)

Thereby, in the mutated fecundity gene carrier ewes have formed follicles with lower number of granulosa cells in their ovaries (Montgomery *et al.* 2001). BMP may reduce the sensitivity of granulosa cells to FSH by inhibiting expression of the FSH receptor (Otsuka *et al.* 2001). Thus, lower than normal concentrations of BMP would result in higher FSH induce granulosa cell responsiveness / sensitivity (McNatty *et al.* 1986b; Otsuka *et al.* 2001; Young *et al.* 2008). All stages of follicular growth are constituted of receptors for the TGFB / BMP ligands, BMPR2, ALK6, ALK3, ALK5 and Betaglycan mRNA in oocytes (Wilson *et al.* 2001; Souza *et al.* 2002). ALK3 and BMPRII are present from primordial follicle to late antral follicle in granulosa cells while ALK6 and ActRIIB are present since primary follicle. In some of the other members of TGFβ family such as ALK5, betaglycan, follistatin and follistatin related protein (FSRP) are expressed from preantral follicle in

granulosa cells. In theca cells, BMPR2, TGFB1, TGFB2, ALK3, ALK5, ActR2B and TGFBR2 are present from the growth of the large preantral follicle but in case of ALK6 there are some conflicting reports. However, a low level of ALK6 protein has been detected in theca by immune histochemistry suggesting that this receptor might be present (Souza *et al.* 2002). ALK6 and BMPR-II mRNA have been identified in ovine oocytes of primordial follicles and expression levels for both remain high throughout the primordial follicular to large preantral growth. From onwards, the levels of ALK6 in oocytes decline in large antral follicles but not BMPR2 mRNA (Wilson *et al.* 2001). In ALK6 mutation carriers BMP signaling pathway is altered in granulosa cells but not the TGFB1 or activin signaling pathway (Fabre *et al.* 2003). TGFB superfamily do interplays between oocyte, granulosa and theca cell types to control folliculogenesis in the ovary (Webb *et al.* 2004; Knight and Glister, 2006) (Figure 4).

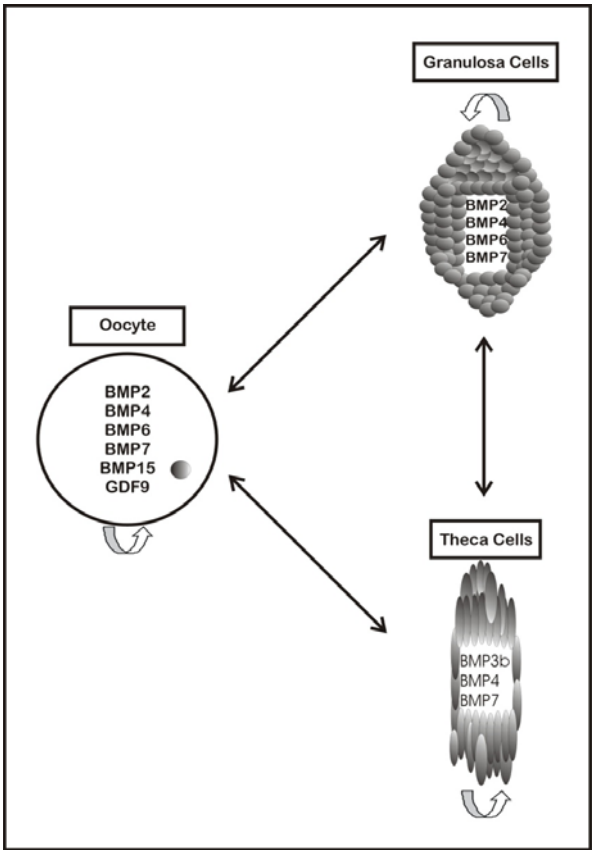

Figure 4 Members of the TGFB superfamily and the bidirectional communication between theca and granulosa cells, and granulosa cells and oocyte. Both autocrine (grey arrows) and paracrine (black arrows) signaling events are likely, depending on the expression of appropriate combinations of type1 and type2 receptors on the cell surface (Shimasaki *et al.* 2004)

There is no obvious effects on granulosa cell proliferation / survival by BMPs 2, 4, 6 and 7 even in culture conditions where insulin like growth factor (IGF) and FSH concentrations are low (Campbell *et al.* 1996; Souza *et al.* 2002; Juengel *et al.* 2006). This observation was also confirmed in Hu sheep breed of China. Furthermore, BMP4 could be a candidate gene for high fecundity in Hu sheep since it plays a vital role in manipulating ovulation rate (Xu *et al.* 2010).

A significant interaction between IGF1 and these BMPs was observed. Besides, in theca cells at very low doses of all BMPs stimulated proliferation, even in the presence of IGF1 (Campbell *et al.* 2006).

Souza *et al.* (2002) reported that granulosa cell culture of immature follicles with BMP2 under the influence of FSH; intensify inhibin A and oestradiol production, without affecting cell proliferation, whereas BMP4 reduced progesterone production owing to a reduction in side-chain cleavage expression (Mulsant *et al.* 2001; Fabre *et al.* 2003). BMP6 mRNA has been expressed in all stages of follicles and it is most likely to ligand with ALK6 (Bodensteiner *et al.* 1999; Elvin *et al.* 2000; Juengel *et al.* 2006).

Granulosa cells selectively express BMP6 mRNA while it has inhibitory effects on LH-stimulated androgen production by cultured theca cells at high doses in sheep (Campbell *et al.* 2006).

On the other hand, detection of BMP6 on granulosa and theca cells is confusing since the detection varied between species and within same species between experiments (Juengel and McNatty, 2005).

Some of the literatures relate that the exact role of BMP6 and BMP7 on granulosa in sheep is not exactly known but it's believed that similar to the activities of BMP2 and BMP4 inhibits progesterone production in ovine granulosa cells (Juengel *et al.* 2006).

CONCLUSION

In summary, the fecundity gene mutation in sheep increases ovulation rate and litter size. The mutations at GDF9, BMP15 and ALK6 have opened up many new paradigms for further research in this area. Apart from these mutations number of other genes in prolific sheep breeds yet to be recognized. Therefore, it remains to be one of the major goals of the reproductive biologists all over the world in order to regulate fertility in mammals. Intra-ovarian factors communicate between oocyte, granulosa and theca cells to control folliculogenesis. Among these factors TGFB superfamily members (BMPs and GDFs) and their receptors have a big opportunity and the future challenge is to pin point the exact pathways of interaction. It is really a daunting task to check every developmental stage of folliculogenesis since it involves numerous players and as well as stages. However, the clear understanding of each and every stage would enhance ovulation rate and ultimately pave way for increased productivity.

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