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

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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 (TGFβ) 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 transaction 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 (TGFβ) 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 TGFβ 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 TGFβ 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 prorogation 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.
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 oocytes 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 dependent 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).

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**Table 1 Major genes affecting ovulation rate in sheep**

<table>
<thead>
<tr>
<th>Name</th>
<th>Gene</th>
<th>Allele</th>
<th>Base Change</th>
<th>AA Change</th>
<th>Mutation</th>
<th>Founder breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inverdale</td>
<td>BMP15</td>
<td>FecX</td>
<td>T-A</td>
<td>Val-Asp</td>
<td>V299D / V31D</td>
<td>Romney</td>
</tr>
<tr>
<td>Hanna</td>
<td>BMP15</td>
<td>FecX</td>
<td>C-T</td>
<td>Glu-Stop</td>
<td>Q291stop / Q23stop</td>
<td>Romney</td>
</tr>
<tr>
<td>Belclare</td>
<td>BMP15</td>
<td>FecX</td>
<td>G-T</td>
<td>Ser-Ile</td>
<td>S367I / S99I</td>
<td>Belclare</td>
</tr>
<tr>
<td>Galway</td>
<td>BMP15</td>
<td>FecX</td>
<td>C-T</td>
<td>Glu-Stop</td>
<td>T2396stop / T27stop</td>
<td>Belclare and Cambridge</td>
</tr>
<tr>
<td>Lacaune</td>
<td>BMP15</td>
<td>FecX</td>
<td>G-A</td>
<td>Cys-Tyr</td>
<td>C321Y / C53Y</td>
<td>Laucane</td>
</tr>
<tr>
<td>Booroola</td>
<td>ALK6</td>
<td>FecB</td>
<td>A-G</td>
<td>Glu-Arg</td>
<td>Q249R</td>
<td>Romney, Han, Javanese</td>
</tr>
<tr>
<td>High Fertility</td>
<td>GDF9</td>
<td>FecG</td>
<td>C-T</td>
<td>Ser-Phe</td>
<td>S3955 / S77F</td>
<td>Belclare and Cambridge</td>
</tr>
</tbody>
</table>

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.
Overview of Major Genes Affecting Prolificacy in Sheep

Figure 2 Localization of selected members (TGFβ, BMP6, GDF9 and BMP15) of the TGFβ 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 steroidogenesis 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).

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 TGFβ / BMP ligands, BMPR2, ALK6, ALK3, ALK5 and betaglycan mRNA in oocytes (Wilson et al. 2001; Souza et al. 2002). ALK3 and BMPR II 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, TGFβ1, TGFβ2, ALK3, ALK5, ActR2B and TGFβR2 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 TGFβ1 or activin signaling pathway (Fabre et al. 2003). TGFβ superfamily do interplays between oocyte, granulosa and theca cell types to control folliculogenesis in the ovary (Webb et al. 2004; Knight and Gli ster, 2006) (Figure 4).

![Figure 4](Members of the TGFβ 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)](image)

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 TGFβ 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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