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Genetic markers associated to improving prolificacy of sheep. A review

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Abstract

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The application of molecular genetic markers in breeding programs is the basis for the future improvement of existing sheep breeds and preservation of genetic diversity in this animal species. This review aims to survey the literature on genetic markers suitable for inclusion in reproductive control in sheep and in particular to enhance the prolificacy of ewes. In different breeds of sheep, prolificacy can be regulated polygenically or by the action of a major segregating gene from the so-called fecundity genes (Fec). In this review, we focus on the main genes BMP15, GDF9 and BMPR1B belonging to the transforming growth factor-β (TGF-β) superfamily, as well as the gene B4GALNT2 affecting reproduction and in which polymorphisms associated with the emergence of the more prolific phenotype.

Keywords: sheep; reproduction; prolificacy; fecundity genes

Introduction

Increasing the efficiency and profitability of production is one of the main goals in animal husbandry, which in sheep breeding are directly related to animal fertility (Notter, 2000). The number of lambs born per birth is the main factor influencing sheep meat production, but most breeds of sheep usually give birth to slightly more than one lamb per birth (Kumar et al., 2017). Sheep breeds in the world number about 1155 according to the Second Report on the State of the World's Animal Genetic Resources for Food and Agriculture and possess remarkable genetic diversity in productive and reproductive traits (FAO, 2015), and among them are more productive breeds such as Thoka, Javanese, Belclare, Lacaune, Woodland, Booroola, Aragonesa, Romney (Inverdale and Hanna), Garole (Bengal), Kendrapada and others whose litter size varies from three to six lambs (Juengel et al., 2013). Reproduction is a complex process and fertility traits are under genetic control (Drouilhet et al., 2009). Selection by applying traditional methods is difficult to achieve in cases where the traits sought are of low heritability values, such as some fertility characteristics as litter size and ovulation rate having heritabilities in the range of 0.06-0.13. Rearing for high fertility is a relatively new direction in the history of sheep breeding. In sheep, there is a large range in litter size between and within breeds, providing opportunities to discover genes underlying improved reproductive success (Liu et al., 2014). Most association studies (GWAS) of the sheep genome are more frequently related with other traits such as climate adaptation, meat qualities and others (Lv et al., 2014). A wide range of genetic and non-genetic factors influence phenotypic characteristics − age, season, management, feeding, genetic effect, pre- and post-weaning period, ovulation rate, embryo survival, lamb survival, envi-

ronmental conditions, uterine capacity and milking capacity affect litter size in sheep (Janssens et al., 2004; Kumar et al., 2017). All of them usually act simultaneously and it is difficult to determine the degree of influence of each of them, so selection based on certain polymorphic genes is more effective. Most of the economically important animal traits are complex and their expression is influenced by multiple genes scattered throughout the genome and by various environmental factors (Grisart et al., 2002).

The prolificacy of domestic species is genetically influenced by multiple genes called fecundity genes and denoted as Fec genes (Gootwine, 2020). In sheep, natural genetic mutations are associated with either infertility or cycles of 20% and 40% or > 100% increased fertility (McNatty et al., 2005). Fecundity determine the profitability and efficiency of sheep farming, regardless of the productive direction (Ivanova et al., 2021). Fecundity genes have an additive effect on gene variants and litter size increasing ovulation rate (Davis, 2005). In different sheep breeds, prolificacy can be determined by many genes with small effects and sometimes by single genes with large effects, called fecundity genes (Drouilhet et al., 2009; Getmantseva et al., 2019). The study of these fertility (Fec) genes sheds light on processes regulating follicle growth and maturation (Drouilhet et al., 2013; McNatty et al., 2017), as well as pituitary functions associated with high fertility (Zheng et al., 2019).

Genetic diversity is of great importance for prolificacy, i.e. the average number of lambs born within one lambing is a key trait controlling productive efficiency (Gootwine, 2020). The high prolificacy of some of the fertile breeds is inherited qualitatively rather than quantitatively, due to the presence of major genes with large effects on ovulation and therefore litter size (Davis, 2005). Exploiting the genetic variants underlying the desired phenotypic profile is a major goal of today‘s animal breeders, and to realize it, genetic variants with a positive effect on fertility must be known. Therefore, candidate genes for traits of interest are investigated for possible association. The influence of DNA variants or polymorphisms on production traits has been identified in many such genes. Three of these have been shown to belong to the transforming growth factor β (TGF-β) superfamily (BMPRIB, BMP15 and GDF9), and another newly discovered candidate β-1,4-N-acetylgalactosaminyl transferase 2 (B4GALNT2) outside the TGF-β superfamily has been identified as having mutations that lead to changes in ovulation in sheep (Liu et al. , 2014; Wang et al., 2021). Early identification of the desired phenotype on the basis of genetic markers allows more effective management of the selection process. Genotyping for these essential genes allows the application of a marker-assisted selection approach in breeding for high productivity in these breeds and their crosses, and introgression of beneficial mutations into new breeds. Different approaches such as mitochondrial DNA studies, genome-wide association analysis, whole genome sequencing, transcriptomic and proteomic analysis in high- and low-productivity breeds allow the discovery of additional genetic variations with medium or small effects on fertility (Ibeagha-Awemu et al., 2008). Identification of different genetic variants may facilitate breeding progress in sheep using molecular markers.

This review focuses on the main genes and their mutations affecting prolificacy in sheep.

Major Genes Controlling Fecundity

Different pathways lead to improved reproductive performance in sheep. Genetic tools for increasing fertility are inbred selection, crossbreeding and using essential genes to improve fertility. To date, the morphogenetic protein receptor IB (BMPRIB), bone morphogenetic protein 15 (BMP15), growth differentiation factor 9 (GDF9), β-1, 4-N-acetylgalactosaminyl transferase 2 (B4GALNT2) and leptin receptor (LEPR) genes have been considered as major candidate genes for the prolificacy of sheep (Tong et al., 2020). The first mutation associated with an increased level of fecundity in sheep (FecB) was found in the bone morphogenetic protein receptor 1B (BMPR1B) gene (Davis, 2005) in sheep with increased levels of ovulation (Davis et al., 1982). Currently, FecB is one of the most important fertility markers in many breeds and is widely used in sheep breeding, while in other breeds, fertility is shaped by other major genes or combinations thereof, but still many breeders select and improve sheep reproductive performance according to phenotypic their characteristics (Pan et al., 2015).

BMPR1B gene

In the first major fertility gene discovered, BMPRIB (also known as activin-like kinase 6 (ALK6)), an autosomal mutation in the genome of the prolific Australian Booroola sheep, called the Booroola gene or FecB, has been identified, causing a high level of ovulation in carriers. The BMPR1B gene is localized to the ovine chromosome 6 and contains 15 exons. The mutation has an additive effect on the ovulation rate and for each copy of the gene realizes an increase of 1.5 (Davis, 2005; McNatty et al., 2005) which also reflects on litter size of carriers of one or two copies of the FecB gene with an increase of 1 and 1.5 lambs born, respectively (Wilson et al., 2001; Mulsant et al., 2001; Souza et al., 2001), in which case the influence is partly dominant (Kaczor, 2017). The mutation is thought to have entered the

Booroola Merino from the original source, the Bengal Garole sheep breed (Davis, 2005; Pramod et al., 2013). The BMPR-1B gene locus has been mapped to chromosome 6q23-31 of the *Ovis aries* sheep genome (Piper et al., 1985) and is structurally composed of 15 exons. BMPR1B is involved in the signal transduction of many factors, is found mainly in the ovine ovary but also in other tissues, and is important for follicular development (Liu et al., 2014). The FecB point mutation leading to an arginine→glutamine transition (746A→G) is located in exon 7 and is expressed in oocytes and granulosa cells (McNatty et al., 2005; Liu et al., 2014). This is the main mutation responsible for the large litter size in Merino sheep (Souza et al., 2001). In the Iranian Mehraban breed, two new SNPs in exon 7 with a possible effect on litter size were also demonstrated at the BMPR1B locus, confirming the role of the locus in controlling sheep reproduction (Talebi et al., 2018). In Mongolian sheep, litter size is mainly influenced by $FecB^{B}$ (Tong et al., 2020). Different breeds of sheep have different effects of the mutation. In the Kendrapada breed, the mean prolificacy of the non-carrier, heterozygous and homozygous FecB mutation ewes was 1.61, 1.80 and 2.06 respectively (Dash et al., 2017). A total of 41 polymorphisms in the BMPRIB gene have been identified, among which eight affect litter size and most notably the p.Q249R SNP mutation which is widespread in Asian countries and contributes to the exceptional productivity of sheep (Akhatayeva et al., 2021).

In Bulgarian sheep breeds, the mutation in BMPRIB is absent (Bozhilova-Sakova & Dimitrova, 2021a), only in the Northeast Bulgarian Merino breed it was observed in heterozygous state at separate individuals (Bozhilova-Sakova et al., 2020).

BMP15 gene

The bone morphogenetic protein − 15 (BMP15) gene is located on the X chromosome and is composed of two exons and has a 1182 bp CDS. BMP15 regulates the traits of fertility and as a result of the many mutations (mostly SNPs) occurring in it, the rate of ovulation increases. The first mutation described in Romney sheep was called Inverdale (Davis et al., 1992) and was known as $FecX^{I}$ (Galloway et al., 2000). The other seven well-known fecundity-related mutations are Hanna ($FecX^{H}$), also found in Romney sheep (Galloway et al., 2000), Galway ($FecX^{G}$) – in the Belclare and Cambridge breeds (Hanrahan et al., 2004), Belclare ($FecX^{B}$) in the Belclare breed (Hanrahan et al., 2004), Lacaune ($FecX^{L}$) in Lacaune sheep (Bodin et al., 2007), Rasa ($FecX^{R}$ – epresenting a 17 bp deletion in exon 2) in the Rasa Aragonesa breed (Martinez-Royo et al., 2008), $FecX^{GR}$ and $FecX^{O}$. The BMP15 gene shows association with infertility and hyperfertility mechanisms in a dose-dependent manner. For the first six mutations, heterozygous carriers were found to show the same phenotype and exhibit one to two additional ovulations and an increase in litter size compared to noncarriers, while homozygous carriers were found to be sterile. With the remaining two mutations, $FecX^{GR}$ – found in the French Grivette breed, and $FecX^{O}$ − observed for the first time in the Polish Olkuska breed, homozygous sheep were also highly fertile (Wilson et al., 2001; Vacca et al., 2010; Demars et al., 2013).

A new mutation located in the regulatory region of the BMP15 gene (as opposed to others located in the coding region of the gene) on the X chromosome and named $FecX^{N}$ has been identified in the French Noire du Velay sheep breed. It increases litter size by + 0.2 lambs per lambing in the heterozygous state, possibly by inhibiting BMP15 expression in the oocyte, and homozygous for the mutation also have increased fertility (Chantepie et al., 2020).

A mutation in Xinjiang Cele black Chinese sheep is associated with a T > C base change at position 755 of exon 2, resulting in a leucine to proline substitution at this position of the BMP15 protein (p.L252P). Two genotypes were identified in the flock: heterozygous (E +) and wild-type genotype (+ +), with heterozygous (E +) ewes having significantly larger litter sizes than wild-type genotypes (Niu et al., 2021). A new study shows 6 new variant polymorphisms within the BMP15 gene, including four SNPs (c.352 + 342C > A, c.352 + 1232T > C, c.352 + 1165A > G and c.353-2036T > A) , which were significantly associated with litter size in the Luzhong sheep breed. These results suggest that BMP15 is a critical gene for litter size in Luzhong sheep and these SNPs are novel candidates for improving prolificacy (Di et al., 2021).

In our country, in the study of sheep from the Northeast Bulgarian Merino breed, two alleles − + and G, and two genotypes − + + and G + were found at the mutation point of $FecX^{G}$ (Bozhilova-Sakova & Dimitrova, 2021b).

The BMP15 genotypes of 77 fertile Chios sheep were investigated by PCR-RFLP method. Monomorphism was established in all studied individuals who showed a wild type genotype and did not carry the $FecX^{B}$ mutation. In conclusion, it is believed that the high fertility of Chios sheep may be based on a different region of the BMP15 gene or a different major gene (Dinçel et al., 2018).

Studies have shown that BMP15 and GDF9 proteins act synergistically in the process of oocyte maturation, ovarian cumulus expansion and ovulation, generally heterozygous sheep with mutations in both BMP15 and GDF9 genes show higher fertility than those with the mutation in only one of these genes (Liu et al., 2014).

GDF9 gene

The growth differentiation factor 9 (GDF9) gene, a member of the transforming growth factor β (TGF-β) superfamily, has important functions in ewe reproduction (Al-Mutar & Younis, 2020; Wang et al., 2021). The GDF9 gene, also called FecG, has been mapped to chromosome 5 of the *Ovis aries* genome, spans approximately 2.5 kb, and contains two exons and one intron, of which exon 1, exon 2, and the single intron span 397, 965, and 1126 bp, respectively (Sadighi et al. 2002). GDF9 is mainly expressed in oocytes and plays an important role in follicular development and ovulation in sheep (Tang et al., 2018) and has a major influence on surrounding somatic cells, especially granulosa, cumulus and theca cells (Otsuka et al., 2011). In mammals, the presence of Growth Differentiation Factor 9 is necessary for normal maturation of the oocyte and further development of the embryo, while in its absence the embryos stop developing until they reach the blastocyst stage (Sudiman et al., 2014). A total of 15 mutation sites have been identified in this gene in sheep (Margawati et al., 2023). Eight SNPs were initially identified, known as G1, G2, G3, G4, G5, G6, G7 and G8 (Hanrahan et al., 2004). Identified polymorphisms in the GDF9 gene in sheep cause variation in ovulation rate and litter size (Paz et al., 2014). In the study by Nicol et al. (2009) is presented a detailed characterization of a novel growth differentiation factor 9 mutation found in the Icelandic Thoka sheep (FecG^H). This mutation changes one base (A1279C) and results in a non-conservative amino acid change (S109R) at the C-terminus of the mature GDF9 protein, which is normally expressed in oocytes at all developmental stages. Genotyping of all animals with reproductive records confirmed that Thoka mutation is associated with increased prolificacy in heterozygous sheep and infertility in homozygotes.

Similarly, in the Finnish Landrace sheep breed, a relatively high frequency missense mutation FecG^F (c.1111G > A) associated with high fertility in this breed, responsible for a Val→Met substitution at position 371 (V371M) was identified (Våge et al., 2013; Mullen et al., 2014).

In Brazil, a point mutation called FecG^V(c.943C > T) was found in flocks of fertile Ile-de-France ewes (frequently giving birth to triplets). It leads in an amino acid change (Arg-315Cys) in the cleavage site of the propeptide. This mutation results in increased ovulation rate and litter size (Souza et al., 2014) in heterozygous individuals and sterility in sheep homozygous for the mutation.

A specific mutation in the GDF9 gene, called FecG^E (Embrapa), display a phenotypic behavior contrary to other mutations (Våge et al., 2013] − ewes homozygous for the mutant allele E show increased ovulation rate (82%) and prolificacy (50%) (Silva et al., 2011). In ewes Fec$G^{E/E}$ of Santa Inês breed show a higher number of ovulated follicles as ewes with both genotypes − Fec$G^{E/E}$ and Fec$G^{+/E}$, have smaller diameter of ovulatory follicles (Chaves et al., 2019).

Most studies have been conducted on the presence of mutations in G1 of the GDF9 gene. When studying the Greek breeds Chios and Karagouniki, the more prolific Chios (with 1.77 mean prolificacy) in the G1 point mutation of the GDF9 gene showed a higher frequency of the mutant allele A − 0.24, while in the less fertile Karagouniki (with 1.27 mean prolificacy) − allele A is rare (frequency is 0.03) (Liandris et al., 2012). In the G1 mutation point of the GDF9 gene in the Karayaka breed from the Black Sea Region provinces of Turkey, 21% sheep with the heterozygous genotype AG were also found (Kirikçi et al., 2021). In studies at the G1 of the GDF9 gene in four Bulgarian merino breeds − Ascanian, Caucasian, Karnobat and Northeast Bulgarian (Dimitrova et al., 2020; Bozhilova-Sakova & Dimitrova, 2021b), and Synthetic Population Bulgarian Dairy (Dimitrova et al., 2021) the presence of two alleles was found in all breeds and two genotypes (GG and AG) with the exception of Karnobat merino, in which all three possible genotypes are observed, and animals with genotype AA are fertile. In a study also in the G1 of 126 animals from 5 local breeds of sheep in Bangladesh, in 3 of the breeds, fertile animals with genotype AA were found, in which the fertility was the highest (2.0) compared to heterozygotes AG (1.83) and homozygotes GG (1.59) (Hossain et al., 2020). Two genotypes each in G1 (G260A) and G4 (G721A) of the GDF9 gene have been identified in the Russian Salskaya and Romanov breeds, with higher diversity found in the more prolific Romanov breed (Kolosov et al., 2015). In investigation of the Volgograd breed in Russia, it was found that weight of lamb at birth in ewes with the AG genotype in G1 of GDF9 gene was 0.156 kg larger than in ewes of the GG genotype (Getmantseva et al., 2019).

Twin births are common in some Iranian sheep breeds, and research has shown Afshari sheep to have three mutations in GDF9 − G2, G3 and G4. Of these, G2 (C471T) and G3 (G477A) do not cause a substitution in the translated amino acid and consequently have no phenotypic effect (Eghbalsaied et al., 2012). However, G4 is considered to be the second most important mutation in the ovine GDF9 protein and its occurrence causes a glutamic acid to lysine substitution at amino acid residue 241 (Hanrahan et al., 2004). The latter is directly related to the increased prolificacy of Afshari, while in other Iranian breeds such as Moghani and Ghezel, the G1 mutation in GDF9 (Barzegari et al., 2010) is associated with prolificacy, in which an arginine to histidine substitution is observed.

B4GALNT2 gene

Another fecundity gene B4GALNT2, also known as

FecL, encodes a glycosylating enzyme (beta-1,4-N-acetyl-galactosaminyl transferase 2) and is unrelated to the transforming growth factor β (TGF-β) family. B4GALNT2 was detected in French Lacaune meat sheep as a single nucleotide substitution (SNP) 803A > G in association with its function to regulate ovulation rate (Drouilhet et al., 2009). It is located on chromosome 11 of the sheep genome and is composed of 15 exons separated by introns (Drouilhet et al., 2013; Guo et al., 2018). The mutation located in intron 7 of the B4GALNT2 gene is associated with ovarian expression, increasing ovulation rate and fertility (Drouilhet et al., 2013). In the Lacaune meat breed population, the effect of $FecL^{L}$ on ovulation rate inheritance is additive, as one copy increases ovulation rate by approximately 1.5 ova and litter size by 0.5 lambs compared with the wild-type allele (Martin et al., 2014). Significantly fewer studies have been conducted on this gene compared to the other major genes. Three specific mutation sites associated with the FecL mutation were identified in Small Tail Han sheep that were not found in previous studies of 11 sheep breeds (Small Tail Han, Hu sheep, Cele Black sheep, Tan sheep, White Suffolk sheep, Black Suffolk sheep, East Friesian sheep, Dorset sheep, Mutton Merino sheep, Dorper sheep, and Corriedale sheep). However, two of the g.36946470C > T and g.36933082C > T mutations in the B4GALNT2 exon were found to have a significant effect on litter size in the first parity of Small Tail Han sheep and play an important role in their reproduction (Guo et et al., 2018).

Conclusion

Genes of the TGF-β superfamily are key in the control of ovulation rate and folliculogenesis, and as a result of ovine fertility, as well as some other genes such as B4GALNT2, open new opportunities for ewe fertility research. The implementation of effective reproductive management programs involving such high-performance genetic markers with elucidated breed-specific influence can maximize the profitability of sheep farming.

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