Methods of Improving Reproductive Parameters in Sheep and The Major Genes Associated with Prolificacy: A Review

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REVIEW

Abstract
Farm profitability is heavily influenced by reproductive capacity. Fertility, prolificacy, and fecundity are all indicators of reproductive efficiency. In sheep with high economic value, prolificacy is a key reproduction parameter (Notter, 2008). Because most sheep breeds are monotocous, similar to Mouflon wild sheep (Garel et al., 2005), improving fecundity is a serious concern (Tang et al., 2019). This review aims to study genes and the genetic means of improving sheep reproduction parameters. Numerous mutations in the transforming growth factor (TGF) superfamily have been reported to influence sheep reproductive parameters. As a result, molecular genetics and marker-assisted selection (MAS) are essential in improving reproduction efficiency. If these mutations are not present in the population, introgression of the beneficial mutations to indigenous breeds is possible. Because within-breed selection has been considered relatively inefficient, due to the low heritability of the trait, crossbreeding of native breeds with prolific breeds has been the major means of genetically improving prolificacy. Studying fecundity genes is important in order to increase production efficiency and stabilizing optimal litter sizes. Different studies based on whole-genome sequencing (WGS), which are called genome-wide association studies (GWAS), and also proteomic studies, transcriptome analysis, and mitochondrial DNA analysis have revealed further genetic variation with medium or minor effects on reproduction.

Keywords: Sheep; reproduction parameters; fecundity; genes; prolificacy.

INTRODUCTION

Developing reproductive parameters may be of great interest to sheep farmers (Kumm, 2008). Prolificacy is one of the most important reproductive characteristics in sheep, with a significant economic value (Notter, 2008). The heritability of traits related to fertility are typically low, therefore genetic improvement based on phenotypic selection linked to individual data are often limited (Jansson, 2014). Ovulation rate and litter size are also exclusively expressed in one sex and are only recorded quite late in the life of the animal. This obstructs breeding progress and limits the inclusion of these traits into selection schemes (Jansson, 2014). Fortunately, there is a wide variation in terms of litter size between and within sheep breeds (Liu et al., 2014). These sheep breeds offer the possibility of revealing genes that lead to better reproductive performance (Liu et al., 2014). Similar to the Mouflon wild sheep, ewes of low-prolific breeds produce a single lamb in most cases, and twin lambs on rare occasions (Garel et

al., 2005). Lambing of triplets and quadruplets, on the other hand, is common in prolific sheep breeds like the Finnish Landrace and Romanov breeds, which have been known to produce litters of six or even seven live-born lambs (Fahmy, 1996).

Multiple genes have been shown to have significant effects on reproduction traits, with some of the most essential ones controlling animal prolificacy (Mishra, 2014). High litter size, also known as twinning, is an economically significant aspect that maximizes sheep productivity by producing more lambs, meat, and wool (Mishra, 2014).

Follicle development is a multi-stage process that occurs in the ovaries. Oocytes, or egg cells, are the haploid female reproductive gametes that are located in folicles, being surrounded by epithelial cells (Jansson, 2014). Endocrine factors influence the development of follicles and the ovulation process (Jansson, 2014). Follicle stimulating hormone (FSH) and luteinizing hormone (LH) are key hormones in reproduction (Jansson, 2014). Both are gonadotrophins that are secreted by the anterior pituitary and induce ovulation in the ovaries (Sjaastad et al., 2003). Fecundity traits like ovulation rate and litter size can be genetically regulated by many genes with small effects, as well as by single genes with major effects, called fecundity (Fec) genes (Jansson, 2014). Reproduction is a complex process, and fecundity aspects like ovulation rate and litter size can be genetically influenced by numerous genes with little effects as well as a few major genes. (Drouilhet et al., 2009). High variation in ovulation rate and litter size along with high repeatability are indicators of the presence of a major gene regulating fertility in a population. Prolific ewes and rams are screened and their genomes mapped to detect additional genes (Jansson, 2014). Table 1 gives an overview of the mutations and their effects.

Table 1. Mutations in the fecundity genes BMPR-1B, BMP15 and GDF9, B4GALNT2 and FecX2 and their effect on prolificacy in sheep. The effect on ovulation rate and litter size is a comparison between heterozygous (A+) and homozygous (AA) to non-carriers of the mutations (++)

Gene	Founder Breed	Name, allele symbol	Chrom-osome	Effect ovulation rate	Effect litter size	Reference
BMPR-1B	Booroola Merino, (Garole)	Booroola, FecB[B]	6	B+: +1.3 BB: +3.6	B+: +0.7 BB: +0.8	Fogarty, 2009
BMP15	Romney	Inverdale, FecX[I]	X	I+: +1.0 II: infertile	I+: +0.6 II: infertile	Davis, 2005
BMP15	Romney	Hanna, FecX[H]	X	H+: +1.0 HH: infertile	H+: +0.6 HH: infertile	Davis, 2005
BMP15	Belclare	Belclare, FecX[B]	X	B+: +1.0 BB: infertile	- BB: infertile	Davis, 2005
BMP15	Belclare si Cambridge	Galway, FecX[G]	X	G+: +0.7 GG: infertile	- GG: infertile	Davis, 2005
BMP15	Lacaune	Lacaune, FecX[L]	X	L+: +2.0 LL: infertile	- LL: infertile	Bodin et al., 2007
BMP15	Rasa Aragonesa	Rasa Aragonesa FecX[R]	X	- RR: infertile	R+: +1.3 RR: infertile	Monteagudo et al., 2009
BMP15	Grivette	Grivette, FecX[Gr]	X	- -	Gr+: +1.9 GrGr: +2.5	Demars et al., 2013
BMP15	Olkuska	Olkuska, FecX[O]	X	O+: +2.0 OO: +3.3	- -	Demars et al., 2013
GDF9	Belclare si Cambridge	High Fertility, FecG[H]	5	H+: +1.4 HH: infertile	- HH: infertile	Davis, 2005
GDF9	Icelandic	Thoka, FecG[T]	5	I+: +1.2 II: infertile	I+: +0.7 II: infertile	Davis, 2005
GDF9	Santa Inês	Embrapa, FecG[E]	5	- EE: +1.0	- EE: +0.7	Demars et al., 2013
GDF9	Norwegian white sheep (Finnsheep)	-	5	- -	N+: +0.2 NN: +0.5	Våge et al., 2013
B4GALNT2	Lacaune	Lacaune, FecL[L]	11	L+: +1.5 LL: +3.0	L+: +1.0 LL: +2.0	Drouilhet et al., 2013
FecX2	Coopworth	Woodland, FecX2[W]	X	W+: +0.4 WW: ≥ +0.4	W+: +0.3 WW: ≥ +0.3	Davis, 2005

Note: Adapted from Jansson, 2014.

Sheep producers have been given the capacity to boost ovulation rate and litter sizes, and so productivity, by DNA testing rams and ewes for major genes and learning about inheritance patterns (Davis, 2005). It's worth noting that breeding for a consistent and ideal litter size is desirable in trying to increase the number of lambs born per ewe (SanCristobal-Gaudy et al., 2001).

Optimal litter size is defined differently in various production systems (Gootwine, 2020). Under extensive management conditions, high prolificacy (an average prolificacy of 2.0 or more LB/L) is not a desirable trait because nutrient availability may not support the metabolic needs of ewes carrying multiple fetuses and because high prolificacy has a variety of negative effects on maternal lifetime productivity (Menéndez Buxadera et al., 2004). Increased prolificacy is projected to be economically beneficial in semi-intensive or intensive production systems due to increased income from lamb sales (Byrne et al., 2012).

Because of the increasing demand for animal products around the world (Alexandratos and Bruinsma, 2012), prolificacy will continue to be a key breeding goal for sheep. In these animals, different pathways lead to genetic improvements in reproductive efficiency (Notter, 2012). The purpose of this review is to study genes that affect sheep's prolificacy and the main methods of improving reproductive parameters in sheep.

THE MAJOR GENES ASSOCIATED WITH PROLIFICACY

Mutations in a closely related group of genes have been found to significantly increase the rate of ovulation in sheep (Davis, 2005). These genes are BMPR-1B, BMP15, and GDF9, which are all members of the ovary-derived transforming growth factor-β (TGFβ) superfamily. The genes code for proteins that are required for follicular development in the ovaries, such as growth factors and receptors. As a result, the genes have a significant impact on ovulation rate and litter size (Pramod et al., 2013). Growth differentiation factors (GDFs) and bone morphogenetic proteins (BMPs) stimulate granulosa cell proliferation, modulate other growth factors and hormones, and influence follicle growth and cell survival signaling (Demars et al., 2013). These proteins most likely have a biological effect after binding to a type 1 receptor in the ovaries (BMPR-1A, BMPR-1B, or TGFR1), which then combines with type 2 receptors (BMPR-2) (Mulsant et al., 2001; Feary et al., 2007). The GDF9 and BMP15 mutations cause similar ovaries phenotypes, but their inheritance patterns differ and their effects on ovulation rate and litter size in sheep are different (Nicol et al., 2009). Some mutations in the BMP15 and GDF9 genes have been suggested to cause over-dominance, or heterozygous advantage. This is because heterozygous individuals with the mutations have a higher ovulation rate and thus are more fit than homozygous individuals, who are infertile due to the majority of mutations in the BMP15 and GDF9 genes (Gemmell & Slate, 2006).

Significant breakthroughs have been made in studies on the effects of bone morphogenetic protein (BMP) family growth factors in the reproductive system (Campbell et al., 2006). The revelation of abnormal reproductive phenotypes in animals with naturally occurring mutations (Otsuka et al., 2011) or targeted deletions (Shi et al., 2009; Yan et al., 2001) of certain BMP genes has underlined its importance even more. In sheep, three important fecundity genes from the BMP family have been discovered: bone morpho-genetic protein receptor type IB (BMPR1B) or activin-like kinase 6 (ALK-6) or FecB on chromosome 6 (Souza et al., 2001), growth differentiation factor 9 (GDF9) or FecG on chromosome 5 (Hanrahan et al., 2004), and bone morphogenetic protein 15 (BMP15) or FecX on chromosome X.

Prolificacy genetic variation in sheep has been well documented, with data indicating significant differences between breeds and, in certain cases, exceptional variance within breeds/strains (Bindon et al., 1996). Segregation of a gene with a large effect on ovarian function can explain the latter phenomenon (Hanrahan et al., 2004). The remarkable prolificacy of Booroola sheep was explained by this idea (Davis et al., 1982; Piper and Bindon, 1982). Following that, putative major genes were used to explain why a variety of breeds/strains had larger litters and/or higher ovulation rates (Hanrahan et al., 2004).

The Booroola Merino was the first sheep breed to reveal that a segregating major gene influences prolificacy (Piper et al., 1985). Further key genes that improve prolificacy in sheep have been discovered using data from prolific flocks in many areas (Davis, 2005).

BMPR-1B – Booroola

The Booroola gene, also known as BMPR-1B, codes for bone morphogenetic protein 1B receptors in the ovaries and is found on ovine chromosome 6 (Davis, 2005). FecB was the name given to the mutation by the Sheep and Goat Genetic Nomenclature Committee in 1989. (Fecundity Booroola). Three groups discovered that the mutation is located in the BMPR1B gene by 2001 (Mulsant et al., 2001; Wilson et al., 2001; Souza et al., 2001). This point mutation, which causes a glutamine to arginine amino acid substitution in the highly conserved intracellular kinase signaling domain of the BMPR1B and can significantly increase the ovulation rate of ewes (Souza et al., 2001; Fabre et al., 2003), is located at base 746 of the coding region (746 A -> G) in the highly conserved intracellular kinase signaling domain of the BMPR1B (Wilson et al., 2001). The known to date mutations in BMPR1B that affect prolificacy in sheep are presented in Table 2.

A single mutation in the BMPR1B coding sequence corresponds to the FecB[B] allele (Pramod et al., 2013). Before the gonadal sex differentiation, BMPR1B is expressed precociously, as early as 25 days post-cotum (dpc) (Edson et al, 2010). At 56 dpc, at the time of germinal cell meiosis, BMPR1B expression increases significantly (Pramod et al., 2013). From the primary to late antral follicle stages, granulosa cells and oocytes, as well as the theca layer of ovine and bovine antral follicles, express the BMP receptor BMPR1B (Glister et al., 2010).

The FecB locus has the most significant physiological effects on ovulation rate, as well as the size and quantity of ovulatory follicles in the ovary (Montgomery et al., 2001). Follicles in homozygous (BB) and heterozygous (B+) carrier ewes mature and ovulate at considerably smaller diameters than in non-carrier or wild-type (++) ewes (McNatty and Henderson, 1987; Montgomery et al., 1992; Baird and Campbell, 1998). BB sheep' ovulatory follicles are smaller and have fewer granulosa cells than ++ ewes' ovulatory follicles (McNatty and Henderson, 1987; Montgomery et al., 1992).

Table 2. Mutations in BMPR1B, mapped to ovine chromosome 6, that affect ovulation rate and litter size

Allele symbol and name	Line/breed	Reference
FecB Booroola	Booroola Merino (Australia)	Piper et al.,1985.
	Garole, Bonpala, Kendrapada, Nilagiri, Shahabadi, Deccani, Nellore (India)	Davis et al., 2002; Roy et al., 2011; Dash et al., 2017; Sudhakar et al., 2013; Debnath and Singh, 2014; Praveena et al., 2017; Chaudhari et al., 2019.
	Javanese (Indonesia)	Davis et al., 2002.
	Small-Tail Han, Merinoprolific, Hu, Duolang, Zeller black, Vadi, Mongolian, Cele black, Altay, Bayanbulak (China)	Hua and Yang, 2009; Shi et al., 2010; Liu et al., 2014; Chong et al., 2019.
	Kalehkoohi (Iran)	Mahdavi et al., 2014.
	Mehraban (Iran)	Talebi et al., 2018.
	Mehraban (Iran)	Abdoli et al., 2013.
	Dorset, Mongolian, Small-Tail Han (China)	Jia et al., 2019.

Note: Adapted from Gootwine, 2020.

As a result, the total number of granulosa cells in all ovulatory follicles and total oestradiol production in B+/BB ewes' ovaries are similar to those in ++ ewes (Montgomery et al., 1992; Souza et al., 1997). Despite significant differences in follicular development, oocytes from mature follicles in BB genotypes appear fully competent and produce viable progeny, with no noticeable changes in fertility or embryo viability (Montgomery et al., 2001).

No alterations in hypothalamic function have been seen in Booroola sheep, indicating that the FecB gene's major effects are likely to be downstream of the hypothalamus, most likely at the pituitary gland and ovary (Montgomery et al., 1992). The only gene-specific effect on pituitary hormones was observed for FSH, but gene-specific differences were not found for LH (McNatty et al., 1994).

The higher ovulation rate in BB sheep [homozygous for a mutation in the bone morphogenetic protein receptor type 1B (BMPR1B)] is due, at least in part, to lower oocyte-derived BMP15 mRNA levels combined with the granulosa cells sheep's earlier onset of LH-responsiveness (Crawford et al., 2011).

This gene (FecB) is a dominant autosomal gene that has an additive effect on ovulation rate (Piper et al., 1985). One copy of the Booroola gene increases ovulation rate (number of eggs released per ewe ovulating) by about 1.5, whereas two copies increase by about 3.0 (Davis, 2005). These extra ovulations usually increase litter size (number of lambs born per ewe lambing) by 1.0 to 1.5 (Davis, 2005). Because embryonic losses induce partial failure of multiple pregnancy, the influence on litter size is semi-dominant (Piper et al., 1985). The effect of the Booroola gene has been linked to a mutation in the bone morpho-genetic protein 1B receptor (BMPR-1B), which is found on chromosome 6 and expressed in oocytes and granulosa cells (Wilson et al., 2001). The prolificacy of carriers and non-carriers of mutations in BMPR1B is illustrated in Table 3.

During the late 1980s, various laboratories began looking for markers linked to the FecB gene (Montgomery et al., 1992; Lanneluc et al., 1994). An anonymous microsatellite marker associated to secretory phosphoprotein 1 (SPP1) was used to first detect linkage to the FecB gene (Montgomery et al., 1993). The FecB locus was localized to sheep chromosome 6 after further genes from the same area were examined (Montgomery et al., 1994). Although

the chromosome position for the gene could not be found using these markers, a DNA fingerprinting technique could find markers connected to the FecB locus in Booroola flocks in France (Lanneluc et al., 1994).

For the mutation in the Booroola gene, a high-accuracy DNA test has been developed, needing just few drops of blood on a specially designed absorbent paper (Davis, 2005). The results of these DNA tests can be used into marker-assisted selection breeding programs (Davis, 2005).

Table 3. Prolificacy of carriers and non-carriers of mutations in BMPR1B

| Gene | Allele symbol and name | Line/breed | Average litter size | | | Reference |
			Wild type	Heterozy-gous	Homozy-gous	
BMPR1B (Cr. 6)	FecB[B]	Booroola Merino	1.51	2.39	2.56	Davis et al., 1982.
		Mérinos d'Arles	1.21	2.10		Teyssier et al., 2009.
		Small-Tail Han	1.50	2.05	2.47	Hua and Yang, 2009.
		Hu	1.75	2.16	2.46	Wang et al., 2018b.
		Chinese Merino linia prolifică	1.60	2.11	3.00	Hua and Yang, 2009.
		Tan	1.27	1.35	1.43	Chong et al., 2019.
		Wadi	1.96	2.49	2.81	Chong et al., 2019.
		Zeller black	1.98	2.66	3.00	Chong et al., 2019.
		Garole	1.03	1.58	1.65	Nimbkar et al., 2009.
		Cele black	1.62	2.17	2.23	Shi et al., 2010.
		Javanese	1.24	1.95	2.59	Inounu et al., 1993.
		Kalehkoohi	1.38	1.72	1.90	Mahdavi et al., 2014.
		Kendrapada	1.61	1.80	2.06	Dash et al., 2017.
		Bonpala	1.50	1.61	1.69	Roy et al., 2011.
		Deccani	1.00	1.20		Praveena et al., 2017.
		Nellore	1.00	1.40		Praveena et al., 2017.
		Nilagiri	1.23	1.44		Chaudhari et al., 2019.
		Mehraban	1.05	1.32		Abdoli et al., 2013.
Mean			1.41	1.86	2.30	
SD			0.30	0.43	0.52	
Min.			1.00	1.20	1.43	
Max.			1.98	2.66	3.00	

Note: Adapted from Gootwine, 2020.

BMP15

The bone morphogenetic protein 15 gene (also known as FecX or GDF9B, located on the X-chromosome) codes for an ovary-derived growth factor that is required for follicular development in sheep (Hanrahan et al., 2004). The protein is produced in the ovaries from the primary stage of follicular development onwards (Nicol et al., 2009). BMP15 is only found in the ovary's oocyte, and its expression increases in tandem with follicle development and

growth (Otsuka et al., 2011). Follistatin, a binding protein that binds to BMP15 and inhibits its activity regulates BMP15's actions (Otsuka et al., 2011). Atretic follicles often have low or undetectable levels of follistatin, whereas dominant follicles have very high levels (Pramod et al., 2013). Follistatin regulation of BMP15 actions is likely important for maintaining granulosa cell responsiveness to FSH because BMP15 inhibits FSH receptor expression (Pramod et al., 2013).

Inverdale, Hanna, Belclare, Galway, Lacaune, Rasa Aragonesa, Grivette, and Olkuska (Table 4) are the mutations in the BMP15 gene. A signal peptide, a large proregion, and a mature peptide make up BMP15, which is a pre-proprotein (Jansson, 2014). Two of the mutations are in the proregion (Rasa Aragonesa and Galway), while the other six are in the mature peptide (Jansson, 2014). The type and effect of the BMP15 mutations differ slightly. The Rasa Aragonesa mutation is a deletion of one amino acid, whereas the Galway and Hanna mutations are premature stop codons (Jansson, 2014). The other mutations are most likely amino acid substitutions that change the shape of the proteins and thus their function (Demars et al., 2013).

Table 4. Mutations in BMP15 and Woodland genes, mapped to ovine chromosome X, that affect ovulation rate and litter size

Allele symbol and name	Line/breed	Homozygous	Reference
FecX^{Bar}	Lori-Bakhtiari (Iran)		Abdoli et al., 2018.
	Mehraban, Lori (Iran)		Nadri et al., 2016.
	Barbarine (Tunisia)	Sterile	Lassoued et al., 2017
	Barbarine (Tunisia)		Vacca et al., 2010.
FecX^{R}	Rasa-Aragonesa (Spain)	Sterile	Martinez-Royo et al., 2008.
			Monteagudo et al., 2009.
FecX^{G} B2 (Galway)	Belclare (Ireland)	Sterile	Hanrahan et al., 2004.
	Cambridge (England)		Hanrahan et al., 2004.
	Small-Tail Han (China)		Wang et al., 2015.
	Hu (China)		Wang et al., 2015.
	Moghani Ghezel (Iran)		Barzegari et al., 2010.
	Afshari, Baluchi, Makui, Mehraban (Iran)		Javanmard et al., 2011.
	Shal, Ghezel, Afshari, Lori-Bakhtiari (Iran)		Amini et al., 2018.
FecX^{H} (Hanna)	Romney (New Zealand)	Sterile	Davis et al., 1991.
			Galloway et al., 2000.
FecX^{I} (Inverdale)	Romney (New Zealand)	Sterile	Davis et al., 1991.
			Galloway et al., 2000.
FecX^{Gr} (Grivette)	Grivette (France)		Demars et al., 2013.
	Mouton Vendéen (France)		Chantepie et al., 2018.
	Romanov, Dorper, Ovella Galega (Spain)		Vera et al., 2018.
FecX^{L}	Lacaune (France)	Sterile	Bodin et al., 2007.
FecX^{O}	Olkuska (Poland)		Demars et al., 2013.
FecX^{RA}	Rasa-Aragonesa (Spain)		Calvo et al., 2020.
FecX^{B} B4 (Belclare)	Belclare (Ireland)	Sterile	Hanrahan et al., 2004. FecX2W Coopworth (New Zealand)
FecX2^{W} (Woodlands)	Coopworth (New Zealand)		Davis et al., 2001b. Feary et al., 2007.

Note: Adapted from Gootwine, 2020

The Inverdale gene (FecX), an X-linked gene that increased ovulation rate by approximately 1.0 but induced sterileity in homozygous carrier females, was first discovered in Romney sheep (Davis et al., 1991, 1992). The ovaries of infertile ewes were tiny and underdeveloped, and they never ovulate (Davis, 2005). Ovarian hypoplasia occurs in homozygous BMP15 carrier ewes because ovarian follicles fail to progress beyond the primary stage of follicle development, resulting in sterileity (Davis et al., 1992). The ovulation rate increases as the concentration of biologically active BMP15 decreases until it is too low to support follicular growth (McNatty et al., 2004). Because the gene is located on the X-chromosome, males only have one copy of it, which they pass on to all daughters but not sons (Davis, 2005). Galloway et al. (2000) observed that Inverdale sheep have a mutation in the bone morphogenetic protein 15 gene, which is an oocyte-derived growth factor (BMP15; also known as GDF9B). BMP15, a paracrine factor, boosts follicle development, granulosa cell proliferation, and cell survival signaling via its protein product (Demars et al., 2013).

BMP15 is essential for female fertility, according to the results of mutation studies in Inverdale sheep, and natural mutations in an ovary-derived factor can cause both increased ovulation rate in heterozygotes and infertility in homozygotes (Galloway et al., 2000). Oocytes and granulosa cells express the BMP receptor, which binds to BMP15 from the primary to late antral stages of follicle development. Follicles mature and ovulate at a lower size and in larger numbers for the carriers of the mutation (Jansson, 2014). This is due to a higher concentration and response to FSH at an earlier stage in carriers of the mutation compared to non-carriers (Jansson, 2014). Granulosa cells are likewise in decline, despite the fact that they express LH receptors earlier (Montgomery et al., 2001).

The effects of a major gene affecting prolificacy, located on the X-chromosome, have been observed in Inverdale sheep (FecX^I) (Davis et al., 1991). FecX^I-positive ewes (I+) have higher ovulation rates, whereas FecX^I-negative ewes (II) have small non-functional streak ovaries and are infertile (Davis et al. 1992). Crossbreeding between FecX^I-positive Romney rams and prolific Romney ewes from the unrelated Hanna family line resulted in females with streak ovaries, leading to the conclusion that sheep in the Hanna flock either carried FecX^I or another allele of the same gene with similar effects (Davis et al. 1994).

One copy of the allele FecX^I (Inverdale) or FecX^H (Hanna) leads to an increase in ovulation level of +0.8–1.0 CL, affecting litter size by +0.6 lamb (Kaczor, 2017). Homozygous FecX^{II} and FecX^{HH} Hanna ewes are Sterilee, with a single layer of granulosa cells lining follicles in their small, underdeveloped ovaries (Davis et al., 2001c). Follicles produced in FecX^{I+} sheep's ovaries were found to be smaller in diameter than those produced in homozygotes without the mutation. FecX^{I+} ewes, on the other hand, have a higher number of mature preovulatory dominant follicles with smaller diameters, and their levels of estradiol and inhibin are comparable to nonmutated ewes (Shackell et al., 1993).

Compared with wild-type ewes, in Inverdale ewes, FecX^I heterozygotes have more differentiated follicles in the ovary, fewer granulosa cells in these follicles, increased sensitivity of granulosa cells to LH in the early stage of follicle, and a smaller corpus luteum (Liu et al., 2014). FecX^H, FecX^G, and FecX^B have similar characteristics to FecX^I. This suggested that all four mutations might share the same mechanism of action (Hanrahan et al., 2004). Granulosa cells in heterozygous ewes with BMP15 mutations were thought to develop an earlier responsiveness to LH for most follicles, increasing ovulation rate in sheep (McNatty et al., 2009).

Major fecundity genes were investigated in a highly prolific population of Belclare sheep in Ireland that showed an inconsistent inheritance pattern of fertility traits (Davis, 2005). Infertile ewes were also prevalent in the population, implying that they carried more than one major gene (Davis, 2005). Belclare sheep have three fecundity gene mutations, including two different mutations of the BMP15 gene, the Belclare (FecXB) and the Galway (FecXG) mutations, according to studies (the third mutation being the high fertility mutation in GDF9) (Davis, 2005). Ewes who are heterozygous for one of the BMP15 gene mutations have a higher fertility rate (Davis, 2005). Ewes that are heterozygous for both the Galway and the Belclare mutations, on the other hand, are infertile. Ewes with homozygous ewes are also infertile (Hanrahan et al., 2004). The homozygous phenotype includes reproductive tract abnormalities such as inactive or undeveloped ovaries (Davis, 2005).

The BMP15 mutation found in Lacaune sheep is a missense non-conservative substitution resulting in an amino acid change at the FecXL allele (Bodin et al., 2007). The mutation causes a large increase in ovulation rate in heterozygous ewes but infertility in homozygous ewes. The Lacaune mutation causes an impairment in the maturation of BMP in the ovaries in homozygous individuals (Bodin et al., 2007). The primary stage of folliculogenesis is prematurely blocked as a result, and the follicle does not develop beyond the primordial phase (Bodin et al., 2007).

The Rasa Aragonesa mutation was the sixth of its kind discovered in the BMP15 gene (Jansson, 2014). The allele is FecXR, and the mutation results in a deletion of 17 base pairs, or 6 amino acids, in the bone morphogenetic protein, resulting in a premature stop codon (Jansson, 2014). The protein loses its functionality as a result of this. Heterozygous ewes are more prolific and have larger litters, while homozygous ewes are expected to have primary ovarian failure (Monteagudo et al., 2009).

Through genome-wide association studies, two novel non-conservative mutations of BMP15 called FecX^{Gr} and FecX^O, which lead to hyper prolificacy, have been identified in the French Grivette and the Polish Olkuska breeds

(Demars et al., 2013). It's worth noting that homozygous ewes with these mutations had a higher ovulation rate while remaining sterile (Demars et al., 2013). Their findings suggest that the BMP15 protein plays a secondary role in ovarian folliculogenesis. The prolificacy of carriers and non-carriers of mutations in BMP15 is shown in Table 5.

Table 5. Prolificacy of carriers and non-carriers of mutations in BMP15.

Gene	Allele symbol and name	Line/breed	Wild type	Hete-rozy-gous	Homozy-gous	Reference
			Average litter size			
BMP15 (Cr. X)	FecX[Bar]	Mehraban, Lori	1.08	1.17		Nadri et al., 2016.
		Tunisian-Barbarine	1.12	1.43	Sterile	Lassoued et al., 2017.
	FecX[R]	Rasa-Aragonesa	1.36	2.66	Sterile	Monteagudo et al., 2009.
	FecX[G]	Small-Tail Han	2.06	2.61	Sterile	Chu et al., 2007.
		Hu	1.66	2.18		Wang et al., 2015.
		Fat-tail breeds: Afshari, Baluchi, Makui, Mehraban	1.21	1.62		Javanmard et al., 2011.
	FecX[H] (Hanna)	Romney	1.63	1.98	Sterile	Davis et al., 2001a.
	FecX[I] (Inverdale)	Romney	1.63	2.12	Sterile	Davis et al., 2001a.
	FecX[Gr] Grivette	Grivette	1.83	1.93	2.50	Demars et al., 2013.
		Mouton Vendéen	1.68	1.98	1.99	Chantepie et al., 2018.
	FecX[O]	Olkuska	1.84	2.46	3.05	Demars et al., 2013.
Mean			1.55	2.01	2.51	
SD			0.32	0.47	0/53	
Min.			1.08	1.17	1.99	
Max.			2.06	2.66	3.05	

Note: Adapted from Gootwine, 2020

GDF9

The GDF9 gene, also known as FecG, codes for oocyte-derived growth differentiation factor 9 and is required for normal folliculogenesis. It is found on chromosome 5 and codes for oocyte-derived growth differentiation factor 9 (Hanrahan et al., 2004). From the first stage of follicular development until ovulation, the growth factor is present in oocytes (Hanrahan et al., 2004). GDF9 is highly similar to BMP15 in terms of amino acid sequence and ovarian expression pattern (Dube et al., 1998). GDF9 suppresses both basal and FSH-stimulated progesterone production by ovine granulosa cells from small antral follicles in sheep, while leaving proliferation unaffected (Shi et al., 2009; Fabre et al., 2006).

Hanrahan et al. (2004) discovered eight mutations in the GDF9 (Table 6) gene in Cambridge and Belclare sheep (G1–G8). To date, five non-conservative amino acid changes in GDF9 have been linked to sheep prolificacy: High Fertility (FecG[H]) (Hanrahan et al., 2004), Thoka (FecG[T]) (Nicol et al., 2009), Embrapa (FecG[E]) (Silva et al., 2011), G7 (FecG[F]) (Vage et al., 2013; Mullen et al., 2014), and Vacaria (FecG[V]) (Souza et al., 2014). The polymorphisms in GDF9 are unusual in that FecG[E] and FecG[F] have additive effects on prolificacy, whereas FecG[H], FecG[T], and FecG[V] result in increased ovulation rate and litter size in heterozygotes and sterileity in homozygotes (Souza et al., 2014).

A single point mutation in GDF9 was discovered in some Cambridge ewes (Hanrahan et al., 2004). The ovarian phenotype in animals homozygous for this mutation is different follicles continue to develop to the antral stages, though most, if not all, are abnormal in terms of oocyte morphology and the arrangement and appearance of the granulosa and cumulus cell-types (Hanrahan et al., 2004). The biological roles and importance of GDF9 actions in

follicle growth and development at all stages of folliculogenesis have been clarified in vitro studies using recombinant GDF9 protein (Pramod et al., 2013). GDF9 is required for early stages of follicle development in several mammalian species, according to compelling evidence (Pramod et al., 2013).

Table 6. Mutations in GDF9, mapped to ovine chromosome 5, that affect ovulation rate and litter size in sheep

Allele symbol and name	Line/breed	Homozygous	Reference
FecG G1	Awassi (Iraq)		Al-Mutar et al., 2018.
	Belclare (Ireland)		Hanrahan et al., 2004.
	Cambridge (England)		Hanrahan et al., 2004.
	Romanov (Jordan)		Jawasreh et al., 2017.
	Chilota (Chile)		Paz et al., 2015.
	Araucana creole (Chile)		Paz et al., 2015.
	Baluchi (Iran)		Moradband et al., 2011.
	Lori-Bakhtiari (Iran)		Abdoli et al., 2018.
	Moghani, Ghezel (Iran)		Barzegari et al., 2010.
	Afshari, Baluchi, Makui, Mehraban (Iran) Dalagh (Iran)		Javanmard et al., 2011; Talebi et al., 2018. Khanahmadi et al., 2014.
	Bonpala (India)		Roy et al., 2011.
	Garole (India)		Polley et al., 2010.
	Salsk, Volgograd (Russia)		Gorlov et al., 2018.
FecG G4	Belclare (Ireland)		Hanrahan et al., 2004.
	Cambridge (England)		Hanrahan et al., 2004.
	Kermani (Iran)		Khodabakhshzadeh et al., 2016.
	Bonpala (India)		Roy et al., 2011.
	Kendrapada (India)		Dash et al., 2017.
	Afshari (Iran)		Eghbalsaied et al., 2012.
	Lori-Bakhtiari (Iran)		Abdoli et al., 2018.
	Mehrabam (Iran)		Talebi et al., 2018.
	Salsk, Volgograd (Russia)		Gorlov et al., 2018.
	Small-Tail Han (China)	Sterile	Chu et al., 2011.
	Thoka (Iceland)		Nicol et al., 2009.
	Bagnolese (Italy)		Albarella et al., 2015.
FecG^V (Vacaria)	Ile-de-France (Brazil)	Sterile	Souza et al., 2014.
FecG G6	Belclare (Ireland)		Hanrahan et al., 2004.
	Cambridge (England)		Hanrahan et al., 2004.
	Araucana (Chile)		Bravo et al., 2016.
FecG$^{E (SI)}$ (Embrapa)	Santa lnês (Brazil)		Silva et al., 2011. Melo et al., 2008.
FecG^F G7	Belclare (Ireland)		Mullen and Hanrahan, 2014.
	Cambridge (England), Norwegian white (Norway), Finnsheep (Finland)		Våge et al., 2013.
FecG^H G8	Belclare (Ireland)	Sterile	Hanrahan et al., 2004.
	Cambridge (England)		Mullen and Hanrahan, 2014.

FecG[T] (Thoka)		Icelandic (Iceland)		Sterile		Jónmundsson and Adalsteinsson, 1985; Nicol et al., 2009.
		Hu (China)				Wang et al., 2018a, 2018b.
		Garut (Indonesia)				Rahmawati et al., 2019.

Note: Adapted from Gootwine, 2020

Heterozygous ewes with mutations in both BMP15 and GDF9 had higher fertility than heterozygous ewes with mutations in only one of these genes (Liu et al., 2014). When mutant GDF9 and mutant BMP15 were co-expressed, the secretion levels of both proteins were significantly lower than when wild-type GDF9 and mutant BMP15 were co-expressed, suggesting a possible mechanism for the compound heterozygous mutant sheep's extreme fertility (Liao et al., 2004). The prolificacy of carriers and non-carriers of mutations in GDF9 is illustrated in Table 7.

Table 7. Prolificacy of carriers and non-carriers of mutations in GDF9

			Average litter size			
Gene	**Allele symbol and name**	**Line/breed**	**Wild type**	**Heterozy-gous**	**Homozy-gous**	**Reference**
GDF9 (Chr. 5)	FecG G1	Romanov	–	1.94	2.73	Jawasreh et al., 2017.
		Chilota	1.25	1.56	–	Paz et al., 2015.
		Araucana	1.35	1.55	–	Paz et al., 2015.
		Fat-tail breeds: Afshari, Baluchi, Makui, Mehraban	1.16	1.78	–	Javanmard et al., 2011.
		Baluchi	1.23	1.38	1.03	Moradband et al., 2011.
		Salsk	1.13	1.80	–	Gorlov et al., 2018.
		Volgograd	1.22	1.88	–	Gorlov et al., 2018.
	FecG G4	Kendrapada	1.63	2.00	1.91	Dash et al., 2017.
		Salsk	1.13	1.80	–	Gorlov et al., 2018.
		Volgograd	1.22	1.88	–	Gorlov et al., 2018.
	FecG[V] (Vacaria)	Ile-de-France	1.29	1.61	Sterile	Souza et al., 2014.
	FecG G5,G6	Araucana creole	1.19	1.68	1.85	Bravo et al., 2016.
	FecG[E (SI)] (Embrapa)	Santa lnês	1.13	1.44	1.78	Silva et al., 2011.
	FecG[F] G7	Belclare	2.16	2.29	2.85	Mullen and Hanrahan, 2014.
		Small-Tail Han	2.11	2.88	Sterile	Chu et al., 2011.
	FecG[T] (Thoka)	Icelandic	1.74	2.28	Sterile	Jónmundsson and Adalsteinsson, 1985.
		Garut	–	1.37	2.09	Rahmawati et al., 2019.
		Hu	2.20	2.30	2.26	Wang et al., 2018b.
Mean			1.24	1.72	1.86	
SD			0.14	0.20	0.60	
Min.			1.13	1.38	1.03	
Max.			1.63	2.00	2.73	

Note: Adapted from Gootwine, 2020

Genes that are not related to the TGFβ superfamily

Mutations associated with major effects on prolificacy has also been observed in genes not related to the TGFβ superfamily: The B4GALNT2 mutation was first described in the French Lacaune breed and segregates with the

FecX^L mutation in that breed, and mutations in FSHR, COIL, GUCY1A1, KISS1, HIRA, LEPR, INHBB, NELFE, SLC5A1, SmaD1, PRLR, and PRL have been identified in Chinese sheep breeds (Gootwine, 2020).

Mutations in the leptin receptor (LEPR) gene had a minor effect on prolificacy, as the mutations decreased rather than increased prolificacy when compared to the wild-type allele (Gootwine, 2020). The biological significance of ovine leptin (LEP) gene mutations, as well as interactions between various LEP and LEPR mutations and their putative effects on prolificacy in sheep, are of interest (Reicher et al., 2011). The prolificacy of carriers and non-carriers of mutations close or within the B4GALNT2 gene is illustrated in Table 8.

Table 8. Prolificacy of carriers and non-carriers of mutations close or within the B4GALNT2 gene

			Average litter size			
Gene	Allele symbol and name	Line/breed	Wild type	Heterozy-gous	Homozy-gous	Reference
Mutații associate cu gena B4GALNT2	FecL^L	Lacaune	1.67	2.14		Martin et al., 2014.
		Noire du Velay	1.57	1.99	2.12	Chantepie et al., 2018.
		D'man	1.93	2.23	2.47	Ben Jemaa et al., 2019.
	FSHR	Small-Tail Han	1.70	1.88	2.33	Wang et al., 2015.
		Hu	1.49	1.75	2.29	Wang et al., 2015.
	LEPR	Davisdale	2.48	2.25	1.96	Juengel et al., 2016.
	KiSS-1	Small-Tail Han	1.98	2.49	2.86	Chu et al., 2012.
	SLC5A1	Small-Tail Han	1.34	1.96	2.29	La et al., 2019.
Mean			1.77	2.09	2.33	
SD			0.36	0.24	0.28	
Min.			1.34	1.75	1.96	
Max.			2.48	2.49	2.86	

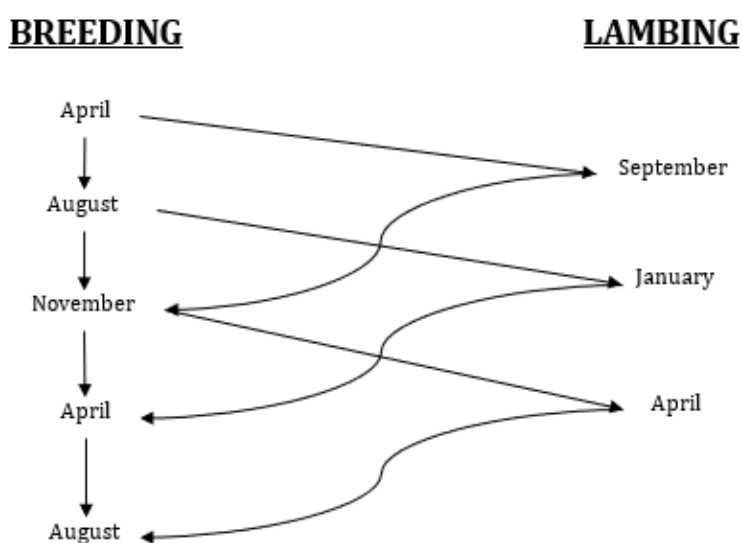Note: Adapted from Gootwine, 2020

BREEDING PATTERN

Figure 1. A breeding schedule to allow 3 lambing in 2 years (Notter and Copenhaver, 1980). Straight lines indicate movement of open or pregnant ewes after breeding, curved lines indicate pattern of return to breeding after lambing.

METHODS OF IMPROVING REPRODUCTIVE PARAMETERS

Within-breed selection

Prolificacy has long been thought to be a quantitative polygenic trait with low heritability in sheep (Safari et al., 2005), indicating a slow annual increase of 1–2% using within-breed selection (Gootwine, 2020). The high heritability values reported for the Lacaune breed (SanCristobal-Gaudy et al., 2001) and Swedish Sheep (Gates and Urioste, 1995) were most likely the result of major gene segregation for high prolificacy in those populations (Gootwine, 2020).

Prolificacy has been used as a breeding goal in various breeding schemes despite its low heritability (Bradford, 1985). Individual performance is recorded, and breeding values are estimated based on the individual and its relative performances. According to Elsen et al., 1994, the statistical models used to calculate prolificacy breeding values must account for the fact that prolificacy is a maternal trait, is a discrete trait, and that the statistical models must include fixed effects such as the way the sheep conceived (natural or induced estrus) and parity number, among other things. In a number of breeding programs, genomic selection has been used to assign for larger litter size (Bolormaa et al., 2017).

Monitoring and further optimisation of litter size can be achieved by relatively simple recording of lambs at birth and weaning in pedigree flocks once breed composition has been optimized and strategies to use or avoid major genes have been implemented (Gootwine, 2020). Because of the high genetic correlation between ovulation rate and litter size, direct assessment of ovulation rate is usually not feasible, necessary, or recommended (Waldron and Thomas, 1992). It is strongly advised to keep track of the litter size at weaning, especially if the incidence of triplet or larger births is high (Gootwine, 2020).

Improved fertility can be achieved in winter and spring lambing by selecting appropriate breeds and crosses, as well as paying close attention to breeding management (Notter, 2012). Spring lambing fertility estimates are generally low in heritability, mean fertility is generally high, and there is little room for genetic improvement (Notter, 2012).

Al-Shorepy and Notter (1997), on the other hand, used procedures to improve fertility in annual autumn lambing that were effective but not practical in commercial production. A more realistic production system would include some form of multi-season lambing, such as Notter and Copenhaver's system (1980) of three lambings every two years (Fig. 1). Accelerated lambing programs can boost profits, but they only work if ewe fertility is acceptable during the least favorable mating seasons (Notter, 2012). It has been difficult to identify effective selection criteria for genetic improvement of reproductive performance in these systems (Notter, 2012).

Lewis et al. (1998) found heritabilities of 0.34 and 0.21 for the first two lambing intervals in a system of up to five lambings in three years, but heritabilities for lambing intervals fell to zero at later ages. Vanimisetti (2006) discovered that heritabilities for lambing intervals in a commercial Polypay flock did not differ from 0 at any age, implying that management practices in commercial flocks with accelerated lambing, such as avoiding mating of first and second parity ewes in undesirable seasons and delaying mating for ewes that wean very young lambs, can complicate selection protocols. In this flock, Vanimisetti (2006) found heritability estimates of 0.14 for ewe-lamb fertility and 0.31 for age at kth parity, suggesting that these variables could be used as selection criteria in accelerated lambing programs.

Lambs born in the autumn should be genetically superior; however, regardless of their genetic merit, if mated for the first time when 7 months old in April, they have very low fertility (Lewis et al., 1996) and are unlikely to conceive until the following autumn, when they are approximately 12-months old. ewe-lamb dams born in January or April, on the other hand, are unlikely to be genetically superior for out-of-season lambing, but their offspring can be mated when 7 to 9 months old in a favorable season, increasing their chances of lambing when 12- to 14 months old (Notter and Cockett, 2005). The solution to this conundrum (Fig. 1) is to identify ewes that lamb in the autumn but wait until their daughters' lamb in April to select them, maximizing both their genetic merit and the seasonally mediated probability of lambing (Notter, 2012). Mating superior dams to rams with a high estimated breeding value can improve the genetic merit of their daughters even more (Notter, 2012).

Crossbreeding

The genetic regulation of ovulation rates in many prolific breeds is polygenic, including a large number of genes with minor individual effects (Fahmy, 1996). However, sheep have several important mutations in genes related to ovarian function, which have an effect on ovulation rates in homozygotes with two or more ova compared to wild type ewes (Notter, 2012).

Prolific breeds can be crossed with native breeds to produce new ones with modest increases in prolificacy, and prolific breeds can be found adapted to a wide range of environmental conditions, including damp temperate (Finnish Landrace, Romanov), warm arid (D'Man), and humid subtropical (Barbados Blackbelly) (Notter, 2012).

If a significant increase in prolificacy is desired, crossbreeding with prolific breeds or introgression of major

genes for ovulation rate is recommended (Notter, 2012). Ovulation rate has a heritability of 0.15, litter size has a heritability of 0.13, and the number of lambs weaned has a heritability of 0.05. (Safari et al., 2005). These are low values, and even though these traits have a lot of phenotypic variances, the potential increase in litter size from selection rarely exceeds 0.02 lambs per year, and 0.01 lambs per year with multiple-trait selection is probably more realistic (Notter, 2012). Within-breed selection for litter size is thus only recommended as a primary genetic improvement strategy if environmental adaptation is well-established and still important, or if a litter size increase of 0.10 lambs or less is sufficient to optimize production (Notter, 2012).

The European Finnsheep, Romanov, Chios, and East Friesian breeds, the Small-Tail Han and Hu breeds in China, and the tropical Barbados Black Belly sheep are all known to be highly prolific breeds with average prolificacy of 2.0–3.0 LB/L. (Fahmy, 1996). In fact, pure prolific sheep breeds are not widely used outside of their native countries (Gootwine, 2020). Because of the difficulty in managing their extremely high prolificacy in most production systems, their relatively low meat, milk, and wool production in comparison to local breeds, and, in some cases, their lack of adaptability to new climate conditions, such as the Finnsheep (Aboul-Naga, 1988) and East Friesian (Gootwine and Goot, 1996) breeds imported from Europe.

The prolific breeds' high prolificacy has been exploited through a variety of mating systems, including the production and use of F1 crosses, the production of three-way crosses, and the development of composite breeds with varying levels of prolific breed contribution (Gootwine, 2020). Heterosis and maternal effects, which may influence crossbred sheep's reproductive performance, were not found to be significant in terms of prolificacy (Boylan, 1985).

To develop composite breeds, local breeds have been crossed with prolific breeds, followed by multiple generations of *inter-se* matings (Gootwine, 2020). Higher prolificacy is combined with the advantages of local breeds in other beneficial traits in composite breeds. In the United States (US), it was observed that for every 1% increase in Finnsheep breeding in ewes, 0.01 extra lambs were produced each ewe lambing (Thomas, 2010).

Composite breeds with a 25-49% Finnsheep contribution have been developed in the United States (Polypay), Canada (Rideau Arcott and Outaouais Arcott; Thomas, 2010), and England, among other places (Cambridge; Ap Dewi et al., 1996). By crossing the Romanov and Berrichon du Cher breeds in France, the composite INRA 401 breed, later named Romane, was created (Razungles et al., 1985). Crossbreeding with the East-Friesian breed produced the Assaf dairy breed in Israel (Gootwine and Goot, 1996), which was later exported to a number of other countries (Rummel et al., 2006).

Several crossbreeding studies with the Awassi and other breeds have been done in ME nations and internationally to boost lamb and milk production (Galal et al., 2008). By far the most successful attempt was the Improved Awassi East Friesian cross in Israel (Gootwine and Goot, 1996), which led in the development of the Assaf breed. The Assaf has become a major dairy breed not only in Israel, but also in Spain, because to its relatively high prolificacy and milk production, moderate seasonality, and good lamb growth ability (Pollott and Gootwine, 2004). The Assaf was first introduced to Spain in 1977, and its rapid proliferation was fueled by the establishment of F1 populations with local breeds like the Castellana, Churra, and Manchega, as well as continuous upgrades to the Assaf utilizing Assaf rams.

While selection has improved milk production in the Awassi, prolificacy remains low in the improved lines, at around 1.3 LB/EL (Gootwine and Pollott 2000). With the importation of five homozygous BB Booroola Merino rams from New Zealand in 1986, breeding for high prolificacy through introgression of the B allele of the FecB locus (Piper et al. 1985) into the Improved Awassi and Assaf breeds began in Israel. After the "Booroola mutation" was introduced, the high-prolific Afec Awassi and Afec Assaf strains resulted, with average prolificacies of 1.9 and 2.5 LB/EL, respectively (Gootwine et al. 2008). The Afec strains are managed under intensive conditions because to their high prolificacy, where animals are fed to meet all of their metabolic needs and ewes are assisted at lambing in the case of large litters. In the Afec strains, heterozygous B+ ewes are preferred at the FecB gene, as homozygous BB ewes have disadvantages in terms of prolificacy and growth (Gootwine et al. 2006, 2008). Because the "Booroola mutation" is segregating in the Afec strains, genotyping for the FecB locus is used to select ewe lambs as replacements (Gootwine, 2011).

The Afec Assaf's naturally high prolificacy revealed latent heterogeneity in uterine capacity (Gootwine, 2011). The maximum number of fetuses that the uterine environment can support for birth is known as uterine capacity (Bazer, 1969). While litters of four or more are uncommon in the Afec Awassi (approximately 2%), they account for roughly 15% of litters in the Afec Assaf (Gootwine et al., 2008). Only half Afec Assaf ewes have been shown to successfully carry big litters of four or more lambs to term (Gootwine, 2011). Further research is needed to identify the genetic and managerial variables driving the phenomena of intra-uterine fetal growth limitation in order to fully utilize the economic benefits of the Afec Assaf's high prolificacy (Gootwine et al. ,2007).

Despite the fact that Afec Assaf ewes are fed intensively to suit their metabolic needs, pregnancy toxemia affects some of the animals (PT) (Gootwine, 2011). Drenching the affected ewe in propylene glycol is the most typical treatment for PT (Gootwine, 2011). It is rare, however, to be able to save the female and return her to regular lambing (Gootwine, 2011). Zamir et al. (2009) discovered that combining the propylene glycol treatment with

flunixin meglumine, a potent analgesic and antipyretic non-steroidal anti-inflammatory drug, dramatically improves both ewe and lamb survival rates, with the goal of developing appropriate management for handling high-prolific Afec Assaf ewes.

Using major genes to improve prolificacy

Due to the presence of major genes with large effects on ovulation rate and thus on litter size, it became clear in the 1980s that the high prolificacy of some prolific breeds is inherited as a qualitative, rather than quantitative trait (Davis, 2005). The study of those genes, known as fecundity (Fec) genes, revealed intraovarian processes regulating follicular growth and maturation (Drouilhet et al., 2013; McNatty et al., 2017), as well as pituitary functions linked to high prolificacy (Zheng et al., 2019).

The benefit of incorporating major genes for high prolificacy into a breeding program is the possibility of introducing desired mutations into other breeds through cross-breeding, which could dramatically improve prolificacy and lamb production in a short period of time (Gootwine, 2020). The result of such breeding strategies is the preservation of the native breed's adaptability and production traits, as well as increased prolificacy (Gootwine, 2020). This is in contrast to composite breeds, in which the manifestation of desired traits is largely dependent on the relative contributions of the parental breeds (Gootwine, 2020).

The use of major genes to increase prolificacy has its own set of advantages and disadvantages (Notter, 2012). Several ovulation rate mutations increase litter size by 0.5–1.0 lambs in heterozygotes and can be introgressed into any breed through repeated backcrossing and DNA testing, resulting in improved ovulation rates in a genetic background that is otherwise unmodified (Notter, 2012). Because homozygous ewes are infertile, using mutations in BMP15 (FecX) or GDF9 is particularly difficult, and usually entails mating carrier males and females with DNA testing of offspring to identify the desired heterozygous replacement females (Notter, 2012). Individual animal identification (at least in terms of genotype), regular DNA testing, and mating control are thus required, which may preclude the effective use of these genes in large or poorly controlled breeding programs (Notter, 2012).

Booroola Merino homozygous rams have been crossed with many breeds around the world to take advantage of their high prolificacy (Fogarty, 2009). The average prolificacy of the F1 generation of heterozygous females with the Booroola mutation is about 0.5 LB/L higher than that of local breeds of ewes, according to research.

Individuals with two copies of the FecB allele are fertile, but increases in ovulation rates can exceed two, resulting in litter sizes of more than one (Notter, 2012). High rates of triplet births or higher numbers of births in homozygous ewes, compared to non-carrier ewes, may result in an excess of neonatal deaths, necessitating specialized breeding programs to generate heterozygous FecB ewes and excluding homozygous ewes from the breeding flock (Notter, 2012). The prolificacy of the recipient breeds determines the suitability of heterozygous ewes (Notter, 2012). Adult ewes of common commercial breeds have a prolificacy of 1.75 to 1.95 in the United States (Notter et al., 2009). A single copy of FecB inserted into this genetic background increases litter size by about one lamb, but with unacceptable increases in the frequency of large (>3) litters and little or no increase in the number of lambs weaned (Notter et al., 2009).

However, once the Booroola mutation was introduced into the Awassi and Assaf breeds in Israel, the prolific "Afec" strains were developed, native breed lamb production improved to reasonable levels of 1.90 and 2.40 in heterozygotes and 1.92 and 2.55 in homozygotes, respectively (Gootwine, 2009). This was also done to improve lamb production in India's Deccani breed (Nimbkar et al., 2009) and France's Mérinos d'Arles breed (Nimbkar et al., 2009), both of which had low prolificacy normally (Teyssier et al., 2009). The use of FecB in low-input smallholder flocks in India allowed for a rapid increase in prolificacy in Deccani ewes with low prolificacy (mean litter size of 1.03) (Nimbkar et al., 2009). In that experiment, heterozygous ewes had an average litter size of 1.5 live lambs at birth, while homozygous ewes had an average litter size of 1.65. When combined with outreach and training activities to achieve incremental improvements in management and nutrition appropriate to this level of prolificacy, heterozygous ewes produced 7.5 percent more lamb and had a 37–50 percent higher gross margin per breeding ewe than wild type ewes. Furthermore, in Deccani ewes, a second copy of FecB had a much smaller impact than a single copy, suggesting that homozygous ewes may be less important in lowly prolific ewe programs (Notter, 2012). However, when the Booroola mutation was introduced into Australian (Walkden-Brown et al., 2009) and American (Notter et al., 2009) sheep breeds, lamb mortality increased significantly in the prolific ewes managed under extensive conditions.

Introgression the FecG^T Thoka mutation improved prolificacy of the Cheviot breed in England (Walling et al., 2003; Nicol et al., 2009), despite the fact that carrying the mutation resulted in sterileity in homozygous ewes. In this regard, the BMP15 FecX^L mutation, which caused homozygous sterility in the Lacaune breed in France, was eliminated (Martin et al., 2014).

Due to the high lamb loss in ewes homozygous for BMPR1B, or sterility in ewes homozygous for mutations in BMP15 or GDF9, breeding homozygous ewes for the major gene mutations is not recommended in commercial flocks following gene introgression (Gootwine, 2020). Furthermore, homozygozygosity for the BMPR1B gene has a negative impact on lamb birth weight, post-weaning growth rate, and mature body weight (Gootwine et al., 2006).

As a result, the system for maintaining flocks of prolific gene-introgressed ewes must include the selection of replacement ewes that are determined to be heterozygous based on molecular genotyping (Gootwine, 2020). For controlling the heterozygous advantage of a major gene in sheep, novel mating techniques have been developed (Raoul et al., 2018).

CONCLUSIONS

Understanding the genetic processes and genetic background of different phenotypic traits in sheep has certainly benefited from the availability of accessible sequencing technology and tools for massive data processing. In animal breeding, identifying and characterisation of candidate genes and genetic variations linked to economically relevant phenotypic traits is critical.

Most breeding programs meant to increase prolificacy rely on crossbreeding and within-cross or within-breed selection (Gootwine, 2020). Because of the possibility of rapid progress in lamb production, the revelation of major genes affecting prolificacy, as well as the development of molecular methods to identify carriers for the desired mutations, has been intriguing for sheep farmers, because increased prolificacy will result in higher ewe productivity, and lamb production will become more efficient as a result of producing more lambs per ewe.

The reproductive traits of Romanian sheep breeds can be studied using a variety of new approaches and methods. The majority of previous research on Romanian native sheep breeds used single gene association analysis. No genome-wide association study (GWAS) was conducted. As a result, GWAS in sheep breeds in order to detect SNPs linked to prolificacy is required.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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