

## Investigation of BMP15 and GDF9 gene polymorphisms and their effects on litter size in Anatolian sheep breed Akkaraman

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**Abstract:** This study aimed to examine the polymorphism of the BMP15 (FecX<sup>G</sup> and FecX<sup>L</sup>) and GDF9 (G1 and G4) genes and the association between genotypes and litter size in the Akkaraman breed. Blood samples were taken from 100 ewes in total. Genomic DNA was obtained from whole blood. The PCR-RFLP technique was employed to determine the polymorphisms of the genes under investigation. The G1 was the only polymorphic among the four loci analyzed. The frequencies for the GA and GG genotypes were 0.26 and 0.74, respectively, while the A and G allele frequencies were 0.13 and 0.87, respectively. The observed and expected heterozygosity values were 0.260 and 0.227, respectively. According to chi-square analysis, the tested Akkaraman population was in Hardy-Weinberg equilibrium ( $p > 0.05$ ). The association analyses showed that the genotypic structure did not significantly affect litter size in the Akkaraman ewes ( $p > 0.05$ ). However, ewes with one copy of the G1 mutation produced 0.13 more lambs than those without the mutation ( $p > 0.05$ ), suggesting that heterozygous ewes have a proclivity to increase litter size, as evidenced by previous studies.

**Key words:** Akkaraman, litter size, FecX<sup>G</sup> FecX<sup>L</sup>, G1, G4, PCR-RFLP

### 1. Introduction

The studies in the world of sheep fecundity genes have been mostly focused on the prolific sheep breeds such as Loa [1] in North-Iceland, Hu, and Smal-Tail Han in China [2,3], Garole in the Bangladesh [4]. Those and previous studies have clearly made a significant contribution to the understanding of the genetic basis of sheep reproductivity.

In recent years, studies of fecundity genes have intensified in breeds with low fertility traits, such as Ujimqin and Mongolia sheep in China [5] and Meghalaya sheep in India [6]. These studies have revealed that mutations associated with multiple births can also be found in nonprolific breeds, as opposed to what is known.

A similar tendency is also observed in some native Turkish sheep breeds with low litter size, including the Akkaraman, Awassi, Dağlıç, Karayaka, Karakaş and Norduz breeds [7–11]. Although they are all low fertility traits, researchers have provided many valuable known and novel mutations that could be applied in breeding programs [8, 10–12]. This evidence highlights the importance of studies conducted on nonprolific sheep breeds. Nevertheless, there is a great gap in the association studies for both prolific and nonprolific sheep breeds in Turkey.

The sheep genetic resources of Turkey are composed of mostly low-fecundity breeds. The total number of domestic

sheep in Turkey is about 42 million, with Akkaraman sheep accounting for approximately 45% of the whole population [13]. Akkaraman sheep are widely distributed in and around the Anatolian region of Turkey. This breed stands out due to its remarkable adaptation to a variety of conditions, disease resistance, and high production in poor feeding conditions [14,15]. In spite of its exquisite features, the twinning rate in this breed is about 1.2 per ewe, indicating low fertility [15].

Litter size is one of the most important traits that affects the income from animal product sales as it provides more lambs [16]. Bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9) are the two important genes involved in several ovarian physiological processes, such as follicular and oocyte maturation, proliferation of granulosa and theca cells, and ovulation [17]. Numerous mutations have been identified so far, including FecX<sup>R</sup>, FecX<sup>H</sup>, FecX<sup>L</sup>, FecX<sup>L</sup>, FecX<sup>G</sup>, FecX<sup>B</sup> on the BMP15 gene and, G1-G8 on the GDF9 gene, using different molecular techniques such as polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), single strand conformation polymorphism (SSCP), and DNA sequencing [18–20]. Among these techniques, in particular, DNA sequencing enabled the identification of novel [18] and breed-specific mutations such as FecX<sup>R</sup> (Rasa Aragonesa) [19].

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Fecundity gene polymorphisms, which affect litter size, have been identified in numerous studies globally [21,22]. However, most investigations of Turkish sheep fecundity gene polymorphisms were based on retrospective research, and association studies were scarce [10, 23–25]. Recent studies, however, have found GDF9 gene polymorphism in some new and local Turkish sheep breeds with low fertility features, including Akkaraman, Dağlıç, Karayaka, and Of [8–10,12], which encouraged to execute of the current study. As a result, this study investigated GDF9 and BMP15 gene polymorphisms and their relationship to litter size in the Akkaraman breed.

## 2. Materials and methods

### 2.1. Animals and DNA extraction

A total of 100 Akkaraman ewes, ranging in age from 2 to 6, were used as study material. Blood samples were taken into 15 mL vacuum tubes with anticoagulant ethylenediaminetetraacetic acid (EDTA) under veterinary supervision in the year of 2021 and collected from 10 flocks in which Akkaraman sheep raised as purebred in the Yozgat province of Turkey under the extensive conditions. The birth types of individuals were recorded during the sampling.

Genomic DNA from whole blood was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, Lithuania) according to the manufacturer's guidelines. The quality and purity of the obtained DNA were controlled using agarose gel electrophoresis (0.6%) at a voltage of 90 V for 30 min in  $1 \times$  TAE buffer.

### 2.2. Amplification of the GDF9 and BMP15 genes

As given in Table 1, fragments with the different lengths of the genes of GDF9 and BMP15 were amplified by polymerase chain reaction (PCR). The PCR reaction mixture consisted of 13  $\mu$ L Taq DNA polymerase 2X MasterMix (Ampliqon®, Denmark), 1  $\mu$ L of each primer, 1  $\mu$ L of DNA and ultrapure water to final volume with 25

$\mu$ L. The PCR was performed with a final volume of 25  $\mu$ L in SimpliAmp thermal cycler (Thermo Fisher Scientific, Waltham, MA, USA) following conditions; 95 °C for 5 min; 35 cycles of 94 °C for 30 s; an annealing step for 35 s (at the annealing temperatures stated in Table 1); extension at 72 °C for 30 s; followed by a final extension of 72 °C for 4 min. PCR products were visualized in EtBr (500  $\mu$ L/mL in H<sub>2</sub>O) under gel documentation system (DNR Bioimaging System, MiniLumi) following agarose gel electrophoresis with 2%.

### 2.3. PCR–RFLP analysis

To detect the G1 and G4 polymorphisms, PCR products of related gene regions were subjected to the PCR–RFLP process using enzymes with the different restriction sites, *HhaI* (GCG $\uparrow$ C to C $\downarrow$ GCG) and *Bpu14I* (TT $\uparrow$ CGAA to AAGC $\downarrow$ TT), respectively. For the polymorphisms of FecX<sup>I</sup> and FecX<sup>G</sup>, all samples were digested with the enzymes *XbaI* (T $\uparrow$ CTAGA to AGATC $\downarrow$ T) and *HinfI* (G $\uparrow$ A N T C to C T N A $\downarrow$ G), respectively. Overall RFLP reaction mixture for whole enzymes was the same in a 30  $\mu$ L final volume, consisted of 10  $\mu$ L PCR product, 2  $\mu$ L green buffer, 1  $\mu$ L enzyme (FastDigest, Thermo), and 17  $\mu$ L ultrapure water. The reaction carried out in 37.5 °C 5 or 10 min and then 65 °C for enzyme inactivation. After digestion, genotypes of individuals were determined under 3% agarose gel using 50 bp DNA ladder (Solis Bio-Dyne®).

### 2.4. Statistical analysis

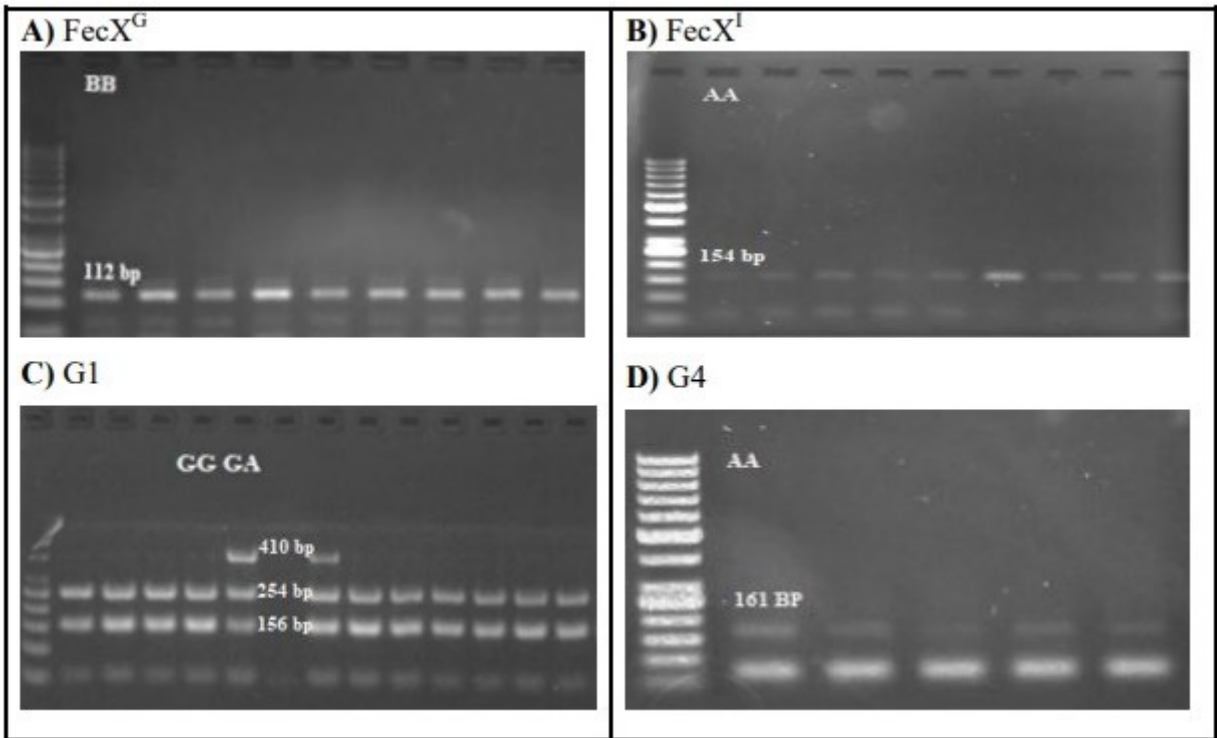
The frequencies of allele and genotype for the detected polymorphism were calculated using PopGene32 software, as well as the values of observed and expected heterozygosity [26]. The chi-square ( $X^2$ ) test was used to determine whether the population were in Hardy-Weinberg equilibrium (HWE).

The general linear model (GLM) was used to explore the genotype effect on litter size using SPSS 22 (IBM SPSS Inc., Chicago, IL, USA). The p value was considered statistically significant when its value was less than 0.05.

**Table 1.** Primer sequence, annealing temperature (T<sub>m</sub>) for the loci in the study.

Loci	Primers	T <sub>m</sub> (°C)	Fragment lengths (Bp)	References
FecX <sup>I</sup>	5'- GAAGTAACCAGTGTTCCTCCACCCTTTTCT - 3' 5'- CATGATTGGGAGAATTGAGACC - 3'	55	154	[21]
FecX <sup>G</sup>	5'- CACTGTCTTCTTGTACTGTATTCAATGAGAC - 3' 5'- GATGCAATACTGCCTGCTTG - 3'	63	141	[22]
G1	5'- GAAGACTGGTATGGGGAAATG - 3' 5'- CCAATCTGCTCCTACACACCT - 3'	58	462	[45]
G4	5'- GGAATATTCACATGTCTGTAAATTTTACATGTTGG - 3' 5'- GAGGGAATGCCACCTGTGAAAAGCC - 3'	63	161	[22]

T<sub>m</sub>: Temperature, Bp: Base pair



**Figure.** The PCR-RFLP results of the amplified loci (A–D). Identified genotypes: BB, homozygous (one band of 112 bp) for  $FecX^G$  locus; AA, homozygous (one band of 154 bp) for  $FecX^I$  locus; GG, homozygous (two bands of 254 and 156 bp) and GA, heterozygous (three bands of 410, 254, and 156 bp) for G1 locus; AA, homozygous (one band of 161 bp) for G4 locus.

### 3. Results

This study explored the gene polymorphisms of GDF9 and BMP15 and their effects on litter size in Akkaraman sheep. DNA fragments of 462 (G1) and 161 (G4) bp in the exon 1 and exon 2 parts of the GDF9 gene and 154 ( $FecX^I$ ) and 141 bp ( $FecX^G$ ) in the BMP15 gene were successfully amplified by PCR. After that, all PCR products were subjected to the RFLP process. The results showed the only G1 mutation to be present in Akkaraman sheep, and no other mutations were observed in the study, as shown in Figure. Genotype frequencies for the studied loci are given in Table 2. In the study, the  $X^2$  values were not calculated due to the structure of monomorphic for the analyzed loci, except for G1.

Allele frequencies were estimated as 0.87 (G) and 0.13 (A), whereas genotype frequencies were calculated 0.26 for GA and 0.74 for GG. Estimated observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity values were determined as 0.260 and 0.227, respectively. The  $X^2$  values for G1 locus displayed that investigated Akkaraman population were in HWE ( $p > 0.05$ ,  $p = 0.1437$ ), indicating that the flocks being studied were not influenced by breeding and sampling etc. Akkaraman sheep had a monomorphic structure in terms of the mutations of  $FecX^I$ ,  $FecX^G$ , G4 and thus the genotypes were wild-type, indicating noncarriers.

The litter size averages of ewes with the GG and GA genotype were  $1.41 \pm 0.06$  and  $1.54 \pm 0.10$ , respectively, indicating that heterozygous ewes produced 0.13 more lambs on average than homozygous ewes. As seen from here, the litter size of heterozygous ewes was higher than that of wild or homozygous. The effect of genotype on litter size was not significant ( $p > 0.05$ ) in Akkaraman sheep.

### 4. Discussion

Akkaraman sheep, Turkey's most populous breed, have plenty of importance for breeders due to their primarily meat production and high adaptability to poor nutrition conditions, but farmers suffer from their low litter size under extensive conditions. Despite the existence of data from studies investigating fecundity genes in Turkish native sheep breeds [7–9, 12, 23, 25], there has been almost no data demonstrating the effects of fecundity genes on sheep litter size [10]. The study aimed to determine the frequency of allele and genotype in four fecundity loci (G1; G4;  $FecX^I$ ; and  $FecX^G$ ) and their effects on litter size in the analyzed Akkaraman individuals. G1 was the only polymorphic locus among those examined. Therefore, the association analysis was performed for this locus, as well as the estimation of allele and genotype frequencies.

**Table 2.** Genotype frequencies of the studied loci.

Loci	Genotype	N	Frequency	X <sup>2</sup>	P-value
G1	GG	74	0.74	2.1377	0.1437
	GA	26	0.26		
	AA	0	0.00		
G4	CC	1.00	1.00	NC	NC
	CT	0.00	0.00		
	TT	0.00	0.00		
FecX <sup>I</sup>	AA	1.00	1.00	NC	NC
	AB	0	0.00		
	BB	0	0.00		
FecX <sup>G</sup>	AA	0	0.00	NC	NC
	AB	0	0.00		
	BB	1.00	1.00		

N: Number of individuals, NC: Not calculated, X<sup>2</sup>: Chi-square, P: Probability

The BMP15 and GDF9 genes cause sterility in homozygous mutant animals but increase the rate of ovulation in heterozygous mutant animals [21,22]. A study on the GDF9 knockout mice revealed sterility due to a lack of follicular formation [27]. A similar effect was also observed in homozygous inverdale (FecX<sup>I</sup>) animals [28] and in homozygous Belclare and Cambridge ewes for the FecG<sup>H</sup> (G8) mutation [22]. In this study, Akkaraman ewes had a polymorphic structure for the GDF9 gene, which was consistent with the previous studies [10, 12, 29]. As for other loci, all animals analyzed were wild-type or nonhomozygous mutants. This was the expected result since the animals examined were all fertile.

The genotype GA was found in 0.26 of the analyzed ewes, demonstrating a moderate genetic variation within the GDF gene. This could be due to inbreeding or the use of the same ram, which resulted in a loss of genetic variation [30] in the herds studied. Similar results were also observed in the study by Berra et al. [31] who reported low genetic variation as a result of inbreeding in Chilota and Romney sheep.

The frequency of GA genotype ewes was more than that of some breeds; Akkaraman (0.16) [8] and Karayaka (0.21) [12] in Turkey, Salsk (0.12) and Volgograd (0.16) in Russia [32], and was comparable to that of Sudanese Desert sheep (0.30) [33]. Since this gene-related study was virtually nonexistent in Turkey, the obtained findings were not comparable to other native breeds in the country. Regarding the frequency of the mutant A allele, Akkaraman sheep exhibited a higher frequency (0.13) than numerous sheep breeds: Watish (0.04) [34], Barki (0.00), and Rahmani (0.00) [35], Awassi (0.02) [36] and

MEGA (Merino × Garut) sheep developed in Indonesia (0.02) [37].

Twinning rates (%) of the Akkaraman breed were reported to be at range from 29.5 to 43.1 [38]. Another study found that the litter size was an average of 1.27 [39]. Akkaraman sheep's litter size in this study was  $1.44 \pm 0.05$  on average. The study's association analysis was limited to the G1 polymorphism since other loci were monomorphic. The genotypes did not impact litter size means statistically ( $p > 0.05$ ), even if heterozygous ewes produced 0.13 more lambs than homozygous ewes ( $1.54 \pm 0.10$  – GA vs.  $1.41 \pm 0.06$  – GG). Similarly, most Iranian sheep breeds with varying sample sizes, including Afshari (145), Ghezel (126), Lori-Bakhtyari (171), and Shal (54), exhibited nonsignificant results [40]. In opposition to these findings, Al-Khuzai and Ahmed (2019) [36] reported significant differences between genotypes in 50 Awassi sheep (GA;  $1.33 \pm 0.08$  vs. GG;  $1.23 \pm 0.07$ ), which is a 0.10 difference in litter size. In Baluchi sheep, this value was 0.15 (GG;  $1.24 \pm 0.03$  vs. GA;  $1.39 \pm 0.05$ ) [41]. These values were comparable to the result (0.13) of the present study. The discrepancies in levels of significance between these studies may be due to genotypic distributions and sample size. In a related study, Gorlov et al. [32] revealed the polymorphism of the GD9 gene, and demonstrated that the G1 and G4 polymorphisms have significant effects on litter size in the Salsk and Volgograd breeds. Consequently, while the results of GDF9 gene mutations were incompatible among the studies, generally higher lambs could be obtained from heterozygous ewes compared to homozygous ones, as in this study. The study of El-Fiky et al. [42] who investigated the GDF9/*Msp*I polymorphism in a number of 47 animals

belonging to the breeds of Saidi and Ossime, pointed out the litter size differences between the two breeds instead of genotypes. As in the study of El-Fiky et al. [42], litter size depends on breed, age, and some other factors, which highlights the complex mechanism of ovulation rate in sheep.

Regarding the mutations of FecX<sup>I</sup> and FecX<sup>G</sup> in the BMP15 gene, none of them was detected in the Akkaraman samples being studied. These results were consistent with the findings reported for other sheep breeds; Awassi and Sakız (Chios) in Turkey [7,25,43], Santa Ines and Morada Nova in Brazil [44], and in 21 prolific breeds [21]. Gursel et al. [24] examined a total of six mutations (FecX<sup>I</sup>, FecX<sup>H</sup>, FecX<sup>G</sup>, FecX<sup>B</sup>, FecB, and FecG<sup>H</sup>) on BMP15, BMPR1B, and GDF9 genes and all were wild-type except FecX<sup>G</sup>. These results were similar or different for the FecX<sup>I</sup> and FecX<sup>G</sup> mutations compared to the findings obtained from the present study.

In conclusion, the current study was the first to examine whether the investigated genes have an impact on litter size in the Akkaraman breed. The Akkaraman ewes with one copy of the GDF9 gene mutation produced 0.13 more lambs than those without the mutation. Apparently, G4, FecX<sup>I</sup>, and FecX<sup>G</sup> mutations can be not responsible in Akkaraman sheep for multiple births as evidenced in many Turkish sheep breeds, as well as prolific breeds [23–25, 43]. This study's molecular approach was the PCR-RFLP. Apart from this method, DNA sequence analysis is a highly informative method for determining other possible

or novel mutations. As in the studies by Koyun et al. [11] and Çelikeloğlu et al. [10], DNA sequencing revealed many major and novel SNPs in the fecundity genes in nonprolific Turkish sheep breeds: Dağlıç, Norduz, and Karakaş. As a result of these findings, Akkaraman and other native sheep breeds should be studied using phenotypic records of large sample sizes and comprehensive, informative molecular methods.

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#### Ethical statement

This study's ethical permission (31/03/2021, 68429034/04) was taken from the Ethical Committee of Ahi Evran University (AEUHAYDEK), Turkey.

#### Conflict of interest

The author has no conflict of interest.

## References

- Holm LE, Bendixen C, Eythorsdottir E, Hallsson JH. A frameshift deletion in the GDF9 gene in Icelandic Loa prolific sheep. *Animal Genetics* 2022; 53 (2): 220-223. <https://doi.org/10.1111/age.13168>
- Wang X, Guo X, He X, Di R, Zhang X et al. Integrated proteotranscriptomics of the hypothalamus reveals altered regulation associated with the FecB mutation in the BMPR1B gene that affects prolificacy in small Tail Han Sheep. *Biology* 2023; 12 (72): 1-19. <https://doi.org/10.3390/biology12010072>
- Yang Z, Yang X, Liu G, Deng M, Sun B et al. Polymorphisms in BMPR-IB gene and their association with litter size trait in Chinese Hu sheep. *Animal Biotechnology* 2022; 33 (2): 250-259. <https://doi.org/10.1080/10495398.2020.1789158>
- Mony SI, Maruf TM, Desha NH, Pabitra MH, Ahmed S et al. Validation study of c. 746A>G SNP of BMPR1B gene associated with litter size in sheep genetic resources of Bangladesh. *Journal of Advanced Biotechnology and Experimental Therapeutics* 2022; 5 (3): 712-720. <https://doi.org/10.5455/jabet.2022.d149>
- Wang Y, Chi Z, Jia S, Zhao S, Cao G et al. Effects of novel variants in BMP15 gene on litter size in Mongolia and Ujimqin sheep breeds. *Theriogenology* 2022; 198 (1): 1-11. <https://doi.org/10.1016/j.theriogenology.2022.12.005>
- Khanikar D, Phookan A, Kalita D, Zaman GU, Das A et al. Polymorphism and phylogenetic analysis of FecB and FecG genes in native sheep of Meghalaya, India. *Tropical Animal Health and Production* 54 (2022): 367 <https://doi.org/10.1007/s11250-022-03366-6>
- Ağyar O, Kırıkçı K. Investigation of FecXI mutation by PCR-RFLP method in Awassi sheep breed. *Bahri Dağdaş Hayvancılık Araştırma Dergisi* 2022; 11 (2): 88-93.
- Kırıkçı K. Polymorphism of the Calpastatin (CAST) and Growth Differentiation Factor 9 (GDF9) genes in Akkaraman sheep breed. *Hayvansal Üretim* 2022; 63 (1): 21-26. <https://doi.org/10.29185/hayuretim.1008768>
- Kırıkçı K, Cam M. Türkiye yöresel yeni koyun tipi Of koyunlarında GDF9 (FecG1) gen polimorfizmin PCR-RFLP yöntemi ile araştırılması. *Manas Journal of Agriculture Veterinary and Life Sciences* 2020; 10 (2): 98-102.
- Çelikeloğlu K, Tekerli M, Erdoğan M, Koçak S, Hacı Ö et al. An investigation of the effects of BMPR1B, BMP15, and GDF9 genes on litter size in Ramlıç and Dağlıç sheep. *Archives Animal Breeding* 2021; 64 (1): 223-230. <https://doi.org/10.5194/aab-64-223-2021>



11. Koyun H, Kiraz S, Karaca S, Koncağül S, Yılmaz A et al. Single nucleotide polymorphisms of GDF9 gene/exon 2 region and their associations with milk yield and milk content traits in Karakaş and Norduz sheep breeds. *Turkish Journal of Veterinary & Animal Sciences* 2021; 45 (5): 881-889. <https://doi.org/10.3906/vet-2010-26>
12. Kirikçi K, Cam MA, Mercan L. Investigation of G1 (c.260G > A) polymorphism in exon 1 of GDF9 gene in Turkish sheep breed Karayaka. *Turkish Journal of Veterinary & Animal Sciences* 2021; 45 (1): 191-197. <https://doi.org/10.3906/vet-2009-40>
13. Turksat. Turkish Statistical Institute. Livestock Statistics. 2022 [cited 2022 January 4].
14. Akçapınar H. Koyun yetiştiriciliği. İsmat Maatbacılık, Ankara [inTurkish], 2000.
15. Yalcin BC. Sheep and goats in Turkey. 1986: FAO Rome, Italy.
16. Roy J, Polley S, De S, Mukherjee A, Batabyal S et al. Polymorphism of fecundity genes (FecB, FecX, and FecG) in the Indian Bonpala sheep. *Animal Biotechnology* 2011; 22 (3): 151-162. <https://doi.org/10.1080/10495398.2011.589239>
17. Ajafar MH, Kadhim AH, Al-Thuwaini TM, Al-Shuhaib MBS, Hussein TH. Association of bone morphogenetic protein 15 and growth differentiation factor 9 with litter size in livestock: a review study. *Acta Scientiarum Animal Sciences* 2023; 45: 1-8. <https://doi.org/10.4025/actascianimsci.v45i1.57927>
18. Zamani P, Nadri S, Saffaripour R, Ahmadi A, Dashti F et al. A new mutation in exon 2 of the bone morphogenetic protein 15 gene is associated with increase in prolificacy of Mehraban and Lori sheep. *Tropical Animal Health and Production* 2015; 47: 855-860.
19. Calvo JH, Chantepie L, Serrano M, Sarto MP, Iguacel LP et al. A new allele in the BMP15 gene (FecX<sup>RA</sup>) that affects prolificacy co-segregates with FecX<sup>R</sup> and FecX<sup>GR</sup> in Rasa aragonesa sheep. *Theriogenology* 2020; 144 (1): 107-111. <https://doi.org/10.1016/j.theriogenology.2020.01.010>
20. Bodin L, Pasquale ED, Fabre S, Bontoux M, Monget P et al. A novel mutation in the bone morphogenetic protein 15 gene causing defective protein secretion is associated with both increased ovulation rate and sterility in Lacaune sheep. *Endocrinology* 2007; 148 (1): 393-400.
21. Davis GH, Balakrishman L, Ross IK, Wilson T, Galloway SM et al. Investigation of the Booroola (FecB) and Inverdale (FecX(I)) mutations in 21 prolific breeds and strains of sheep sampled in 13 countries. *Animal Reproduction Science* 2006; 92 (1-2): 87-96. <https://doi.org/10.1016/j.anireprosci.2005.06.001>
22. Hanrahan JP, Gregan SM, Mulsant P, Mullen M, Davis GH et al. Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (Ovis aries). *Biology of Reproduction* 2004; 70 (4): 900-909. <https://doi.org/10.1095/biolreprod.103.023093>
23. Karşlı T, Şahin E, Karşlı BA, Alkan S, Balcıoğlu MS. An investigation of mutations (FecXG, FecXI, FecXH, FecXB) on BMP-15 gene in some local sheep breeds raised in Turkey. *Akdeniz Üniversitesi Ziraat Fakültesi Dergisi* 2012; 25 (1): 29-33.
24. Gursel FE, Akış I, Durak H, Mengi A, Öztabak K. Determination of BMP-15, BMPR-1B and GDF-9 gene mutations of the indigenous sheep breeds in Turkey. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* 2011; 17 (5): 725-729.
25. Dincel D, Ardıçlı S, Soyudal B, Mehlika E, Alpay F et al. Analysis of FecB, BMP15 and CAST gene mutations in Sakız sheep. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* 2015; 21 (4): 483-488. <https://doi.org/10.9775/kvfd.2014.12680>
26. Yeh FC, Yang R, Boyle TJ, Ye Z, Xiyan JM. PopGene32, Microsoft Windows-based freeware for population genetic analysis, version 1.32. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alberta, Canada, 2000.
27. Dong J, Albertini DF, Nishimori K, Kumar TR, Lu N et al. Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature* 1996; 383: 531-535.
28. Davis GH, McEwan JC, Fennessy PF, Dodds KG, McNatty KP et al. Infertility due to bilateral ovarian hypoplasia in sheep homozygous (FecX1 FecX1) for the Inverdale prolificacy gene located on the X chromosome. *Biology of Reproduction* 1992; 46 (4): 636-640. <https://doi.org/10.1095/biolreprod46.4.636>
29. Aboelhassan DM, Darwish AM, Ali NI, Ghaly IS, Farag IM. A study on mutation points of GDF9 gene and their association with prolificacy in Egyptian small ruminants. *Journal of Genetic Engineering and Biotechnology* 2021; 19 (85): 1-11. <https://doi.org/10.1186/s43141-021-00181-8>
30. Molotsi A, Dube B, Oosting S, Marandure T, Mapiye C et al. Genetic traits of relevance to sustainability of smallholder sheep farming systems in South Africa. *Sustainability* 2017; 9 (8): 1225. <https://doi.org/10.3390/su9081225>
31. Barra R, Uribe H, Latorre E, Primitivo FS, Arranz J. Genetic structure and diversity of four Chilean sheep breeds. *Chilean Journal of Agricultural Research* 2010; 70 (4): 646-651.
32. Gorlov IF, Kolosov YA, Shirokova NV, Getmantseva LV, Slozhenkina MI et al. GDF9 gene polymorphism and its association with litter size in two Russian sheep breeds. *Rendiconti Lincei-Scienze Fisiche E Naturali* 2018; 29 (1): 61-66. <https://doi.org/10.1007/s12210-017-0659-2>
33. Abdelgadir AZ, Musa LMA, Jawasreh KI, Saleem AO, El-Hag F et al. G1 point mutation in growth differentiation factor 9 gene affects litter size in Sudanese desert sheep. *Veterinary World* 2021; 14 (1): 104-112. <https://doi.org/10.14202/vetworld.2021.104-112>
34. Mohamed SEI, Ahmed RM, Jawasreh KIZ, Salih MAM, Abdelhalim DM et al. Genetic polymorphisms of fecundity genes in Watish Sudanese desert sheep. *Veterinary World* 2020; 13 (4): 614. <https://doi.org/10.14202/vetworld.2020.614-621>
35. Elaraby I, Magdy S, Awad A, Saleh A. Polymorphisms of growth differentiation factor 9 (GDF 9) and bone morphogenetic protein 15 (BMP15) genes in Barki and Rahmani sheep breeds. *Zagazig Veterinary Journal* 2019; 47 (2): 160-167.
36. Al-Khuzai FLJ, Ahmed JR. Polymorphism of GDF9 (exon 1) gene and its association with milk production and prolificacy of awassi sheep. *Plant Archives* 2019; 19 (2): 4037-4040.

37. Putra WPBB, Margawati ET, Pardede S, Soetrisno E, Raadsma HW. Detection of G1 point mutation (c. 2039G> A) in GDF9 gene of MEGA (Merino × Garut) sheep. *Veterinaria* 2022; 71 (3): 357-364. <https://doi.org/10.51607/22331360.2022.71.3.357>
38. Çolakoğlu N, Özbeyaz C. Comparison of some production traits in Malya and Akkaraman sheep. *Turkish Journal of Veterinary & Animal Sciences* 1999; 23 (4): 351-360.
39. Güngör ÖF, Ünal N. Some production characteristics of Bafra, Akkaraman, Bafra × Akkaraman F1 and B1 sheep genotypes. *Ankara Üniversitesi Veteriner Fakültesi Dergisi* 2020; 67 (4): 335-342. <https://10.33988/auvfd.478778>
40. Eghbalsaied S, Khorasgani FR, Amini HR, Farahi M, Davari M et al. Variant GDF9 mRNA is likely not the main cause of larger litter size in Iranian Lori-Bakhtyari, Shal, Ghezel, and Afshari sheep breeds. *Archives Animal Breeding* 2017; 60 (2): 119-129. <https://doi.org/10.5194/aab-60-119-2017>
41. Moradband F, Rahimi G, Gholizadeh M. Association of polymorphisms in fecundity genes of GDF9, BMP15 and BMP15-1B with litter size in Iranian Baluchi sheep. *Asian-Australasian Journal of Animal Sciences* 2011; 24 (9): 1179-1183.
42. El Fiky ZA, Hassan GM, Nassar MI. Genetic polymorphism of growth differentiation factor 9 (GDF9) gene related to fecundity in two Egyptian sheep breeds. *Journal of Assisted Reproduction and Genetics* 2017; 34 (12): 1683-1690. <http://10.1007/s10815-017-1007-2>
43. Gedik Y. Screening for Inverdale (FecXI) Mutation in BMP15 Gene in Prolific Turkish Awassi Sheep. *Black Sea Journal of Agriculture* 2021; 4 (4): 130-132. <https://doi.org/10.47115/bsagriculture.988347>
44. Holanda GML, Oliveria JC, Silva DMF, Rocha SSN, Pandolfi V et al. Survey of mutations in prolificacy genes in Santa Ines and Morada Nova sheep. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 2017; 69 (4): 1047-1053. <https://doi.org/10.1590/1678-4162-9339>
45. Kasiriyani MM, Hafezian SH, Hassani N. Genetic polymorphism BMP15 and GDF9 genes in Sangsari sheep of Iran. *International Journal of Genetics and Molecular Biology* 2011; 3 (1): 31-34.