TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2021) 45: 191-197

doi:10.3906/vet-2009-40

Investigation of G1 (c.260G>A) polymorphism in exon 1 of GDF9 gene in Turkish sheep breed Karayaka

Koray KIRIKÇI[1,*], Mehmet Akif ÇAM[2], Levent MERCAN[3]

[1]Department of Animal Science, Faculty of Agriculture, Kırşehir Ahi Evran University, Kırşehir, Turkey
[2]Department of Animal Science, Faculty of Agriculture, Ondokuz Mayıs University, Samsun, Turkey
[3]Department of Agricultural Biotechnology, Faculty of Agriculture, Ondokuz Mayıs University, Samsun, Turkey

Received: 11.09.2020 • **Accepted/Published Online:** 01.02.2021 • **Final Version:** 23.02.2021

Abstract: *GDF9* is an essential gene for normal follicular development and prolificacy in sheep. The aim of this study was to determine the genetic polymorphism and G1 mutation of *GDF9* gene exon 1 in 100 Karayaka ewes, belonging to four subpopulations in the Black Sea Region provinces of Turkey. Based on DNA sequence results, two alleles (A and G) and two genotypes (GG and GA) were determined with the restriction fragment length polymorphism analysis of PCR-amplified fragments (PCR-RFLP) method. The frequencies of mutant (A) and wild-type (G) alleles were detected as 0.10 and 0.90. The frequencies of GG and GA genotypes were calculated as 0.79 and 0.21, respectively. Twenty-one percent of the studied animals were heterozygous GA genotype. The values for observed (Ho) and expected (He) heterozygosity for *GDF9*-exon 1 were estimated as 0.210 and 0.189, respectively. DNA sequence results revealed that Karayaka breed had the mutation G1 (c.260G>A). In conclusion, this mutation might be used as a potential marker to improve litter size rate in Karayaka sheep as evidenced in other sheep breeds.

Key words: Karayaka sheep, litter size, *GDF9* gene exon 1, PCR-RFLP analysis, *Hhal* restriction enzyme

1. Introduction

Sheep farming in Turkey plays an important role in terms of sociocultural structure, animal numbers, economic output, and animal production. Although Turkey has many sheep breeds, the production has not reached the desired level. In Turkey, sheep breeding has been mainly for meat production and less for milk and wool. The fact that meat yield is closely related to lamb yield has led to studies aiming to increase the number of lambs per ewe. Improving reproductive performance is an important issue to meet human demand for sheep products [1]. For this reason, the number of lambs per birth (litter size), in other words, fertility comes to the fore [2]. The used traditional breeding strategies on fertility were insufficient for increasing lamb production due to the low heritability of fertility [3,4]. Therefore, using genetic information of the genes linked to reproductive traits could affect the selection response in animal breeding [5].

Nowadays, the studies have been focused on mutations to develop advanced breeding strategies. With the development of molecular techniques, the mutations related to multiple births have helped genetic breeding programs that aim to improve litter size [6]. The studies of inheritance patterns and DNA testing of major genes for fecundity have disclosed that major genes play a significant role in increasing the fertility performance in sheep flocks worldwide [7].

The effects of various candidate genes on multiple births in sheep have been reported. These genes are the bone morphometric protein receptor 1B (BMPR1B), bone morphogenetic protein 15 (BMP15), and growth differentiation factor 9 (*GDF9*) genes. A member of the transforming growth factor-beta (TGF β) superfamily, the *GDF9* gene is located on ovine chromosome 5 [8]. The *GDF9* gene, which is expressed by oocytes, is an essential gene for normal follicular development in sheep. It consists of 2 exons and 1 intron separated by 1126 bp and codes a propeptide with 453 amino acid residues [9]. Eight different point mutations (G1–G8) have been identified in the *GDF9* gene in Belclare and Cambridge sheep [10]. Three mutations, namely G2, G3, and G5, cause nucleotide changes that do not result in an amino acid change, but the rest of those mutations, including G1, result in a change of amino acid (Arginine (R) → Histamine (H)). A common feature of those mutations is that heterozygous ewes have been observed to have increased ovulation and birth rates in sheep [11].

* Correspondence: koray.kirikci@ahievran.edu.tr

The mutation G1 has been reported in some Iranian sheep breeds, such as Baluchi, Sangsari, and Mehraban [11]. It was reported that the heterozygous ewes with this mutation were fertile and had a high litter size, while wild-type ewes had a smaller litter size [12]. In another study, it was found that ewes of AG genotype were the largest number of lambs (1.88) compared to AA genotypes (1.22) [3]. Paz et al. [13] reported that G1 mutation increased ovulation rate in heterozygous ewes in Chilota sheep [13]. Researchers indicated that this polymorphism could be used in the studies for improvement of fertility traits in Chilota sheep. Apart from its effects on ovulation rates, it was reported that lamb weight at birth in heterozygous ewes was higher than those in homozygous ewe as well [14]. Recently, the identification of *GDF9* gene has been of interest for some researchers [9,14]. In particular, the effects or presence of the mutations of this gene on fertility have been focused on small ruminants [9, 15–18].

In Turkish native sheep breeds, a mutation affecting fertility has not been reported on *BMP-15*, *BMPR-1B*, and *GDF9* genes [2,19–21]. Additionally, no study has included the G1 mutation in the *GDF9* gene. Numerous studies have confirmed a positive correlation between G1 mutation and litter size in sheep. Due to the important effects of *GDF9* on ovulation rates and since no studies have been performed on its polymorphism, there is clearly a need to study this gene. Therefore, we aimed to investigate the genetic polymorphism of *GDF9* exon 1 and G1 mutation in Karaya sheep.

2. Material and methods

In the present study, 100 blood samples that were previously collected from Karayaka sheep raised in the provinces of Samsun, Ordu, Giresun, and Tokat in the Black Sea region of Turkey were used. Blood samples were randomly taken from populations. Sample collecting was performed according to the Animal Care and Use Guidelines of Ondokuz Mayıs University of Local Ethical Committee (2013/64). Genomic DNA was extracted using a DNA isolation kit (IDPURE Spin Column, USA).

The samples were amplified with polymerase chain reaction (PCR) using primers (Forward;5′GAAGACT GGTATGGGGAAATG3′,Reverse;5′CCAATCTGCTC CTACACACCT 3′) [22]. PCR reactions were carried out in a final volume of 25 µL, consisting of 12 µL Tag DNA polymerase Master Mix red (2X) (1.5 mM $MgCl_2$ final concentration), 1 µL (10 pmol/µL) of each forward and reverse primer, 1.5 µL of DNA (30–50 ng), and 9.5 µL ultrapure water. The PCR reaction was performed as follows: an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 40 s, annealing at 64 °C for 40 s, extension at 72 °C for 5 min, and a final extension at 72 °C for 5 min. The PCR products of 462 bp were run in 2% agarose gel electrophoresis and visualized with EtBr (500 µL/mL in H_2O).

PCR products were digested with *Hhal* restriction enzyme in a final volume of 30 µL, consisting of 1 µL of fast digest enzyme, 10 µL of PCR product, 2 µL of green buffer, and 17 of µL ultrapure water. The reaction mix was first incubated at 37 °C for 20 min and then at 65 °C for 10 min. After digestion, the products were run in 3% agarose gel electrophoresis and visualized with EtBr (500 µL/mL in H_2O).

To confirm the existence of the mutation, a total of 8 samples, belonging to homozygous and heterozygous individuals selected according to agarose gel visuals from each population, were subjected to sequence analyses in ABI 3100 capillary electrophoresis instrument. Obtained sequence results belonging to the mutant and wild-type alleles were aligned and compared with the *GDF9* sequences of the complete DNA of *Ovis aries* (GenBank no. AF078545.2) and Norwegian White sheep (GenBank no. HE866499.1) using ExPASy translate tool [23].

The frequencies of allele and genotype and observed and expected heterozygosity values were calculated using PopGene32 software program version 1.32 [24]. The Hardy–Weinberg equilibrium for the studied populations was analyzed using chi-square (X^2) test.

3. Results

After digestion of 462 bp PCR products of the *GDF9* exon 1 gene with *Hhal* restriction enzyme, a total of four DNA fragments were obtained indicating two genotypes as follows: wild homozygous ones, GG and heterozygous ones GA as shown in Figure 1.

The frequencies of allele and genotype were calculated for the investigated populations and summarized in Table.

Twenty-one percent of the studied animals were heterozygous genotype (GA), presented with four bands of 52, 156, 254, and, 410 bp. The frequency of homozygous or wild type genotype (GG) ewes was observed with the highest frequency of 79% and was denoted with three bands, 52, 156, and 254 bp.

A allele frequency mean was 0.10 through the studied populations and ranged from 0.08 in Samsun, Giresun, and Tokat to 0.16 in Ordu. As for allele G, its frequency mean was 0.90 and varied from 0.92 in Samsun, Giresun, and Tokat to 0.84 in Ordu. Consequently, the most frequent allele was G, and the lowest was A in the studied populations. Therefore, the most frequently found genotype for the Karayaka breed was homozygous or wild genotype (GG) with a frequency of 0.79. The heterozygous GA genotype was observed with a frequency of 0.21 and varied from 0.32 in Ordu to 0.16 in Samsun and Giresun. The homozygous AA genotype or mutant genotypes were not determined in the Karayaka sheep breed. Consequently,

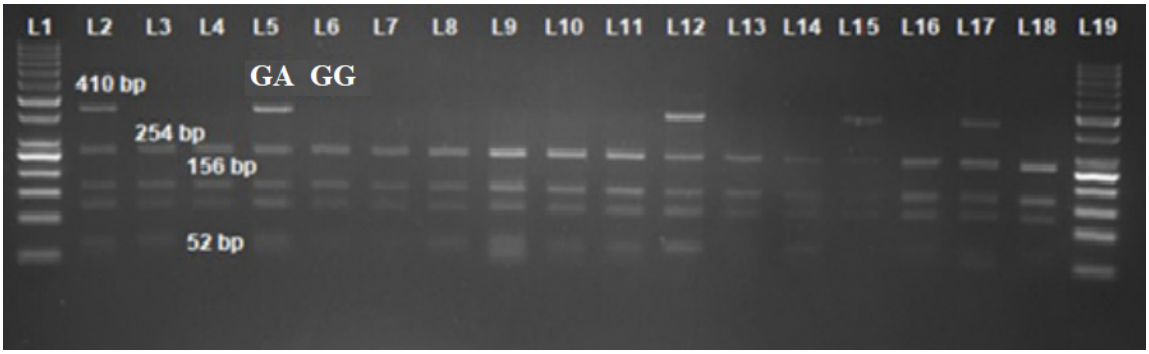

Figure 1. The determined genotypes at position c.260G>A for *GDF9*-G1 with the PCR-RFLP analysis. L1–L19 = 50 bp DNA ladder; L3, L4, L6–L11, L13, L14, L16, L18 = wild genotypes; L2, L5, L12, L15, L17 = heterozygous genotypes.

Table. Genotype and allele frequencies of *GDF9* exon 1.

Populations	N	**Allele** frequency		**Genotype** frequency		**Heterozygosity**			P-value
		G	A	GG	GA	Ho	He	X^2	
Samsun	25	0.92 (23)	0.08 (2)	0.84 (21)	0.16 (4)	0.160	0.150	0.139	0.709
Ordu	25	0.84 (21)	0.16 (4)	0.68 (17)	0.32 (7)	0.320	0.274	0.781	0.377
Giresun	25	0.92 (23)	0.08 (2)	0.84 (21)	0.16 (4)	0.160	0.150	0.139	0.709
Tokat	25	0.92 (23)	0.08 (2)	0.80 (20)	0.20 (4)	0.200	0.184	0.242	0.622
Total	100	0.90 (90)	0.10(10)	0.79 (79)	0.21 (21)	0.210	0.189	1.305	0.253

N, number of individuals; observed heterozygosity (Ho); expected heterozygosity (He); chi-square test for HWE (X^2), P-value (P).

Ordu had a higher prevalence of heterozygous genotypes and mutant alleles than other populations.

The observed (Ho) and expected (He) heterozygosity values were estimated for the studied populations and summarized in Table.

Considering all populations, Ho varied from 0.160 in Samsun and Giresun to 0.320 in Ordu with an average of 0.210, while He ranged from 0.150 in Samsun and Giresun to 0.274 in Ordu with an average of 0.189. Overall, the observed heterozygosity value was higher than expected. Consequently, the whole populations showed a low heterozygous level for the *GDF9* gene.

Concerning possible deviations from Hardy–Weinberg, the exact P-values for the studied populations, were calculated as shown in Table. The chi-square analysis results indicated that whole populations were in the Hardy–Weinberg equilibrium ($P > 0.05$).

Restriction fragment length polymorphism analysis of PCR-amplified fragments (PCR-RFLP) PCR-RFLP products were confirmed by sequencing according to agarose gel visuals. Obtained sequence results belonging to the mutant and wild-type alleles were uploaded to National Center for Biotechnology Information database under accession numbers (MT295368-MT295371). DNA sequence results proved the existence of a nucleotide change (G → A) at position 260 of the 462 bp DNA fragment of the *GDF9* gene exon 1. This identified nucleotide chance or single nucleotide polymorphism (SNP) is called G1 mutation. To show the presence of nonidentified (a) and identified SNP (b) in the exon 1 of the *GDF9* gene, the chromatogram results of the DNA forward sequence are given, as seen in Figure 2. To show nucleotide change, sequencing results for both alleles were compared to the *GDF9* sequencing results for *Ovis aries* (GenBank no. AF078545.2) and Norwegian White sheep (GenBank no. He866499.1). The results proved that the Karayaka breed had A (mutant) and G (wild) alleles (Figure 3).

4. Discussion

Determination of nucleotide variations or major mutations on candidate genes affected by reproduction traits is an important subject for improving small ruminants' fertility traits. For this reason, researchers have tried to find the known or novel mutations in their native sheep breeds using different methods such as PRC-RFLP, PCR-SCCP, DNA sequencing, and GWAS analysis [11,25,26].

By using mostly the PCR-RFLP method, six different mutations (FecXI, FecXH, FecXG, FecXB, FecB, FecXG),

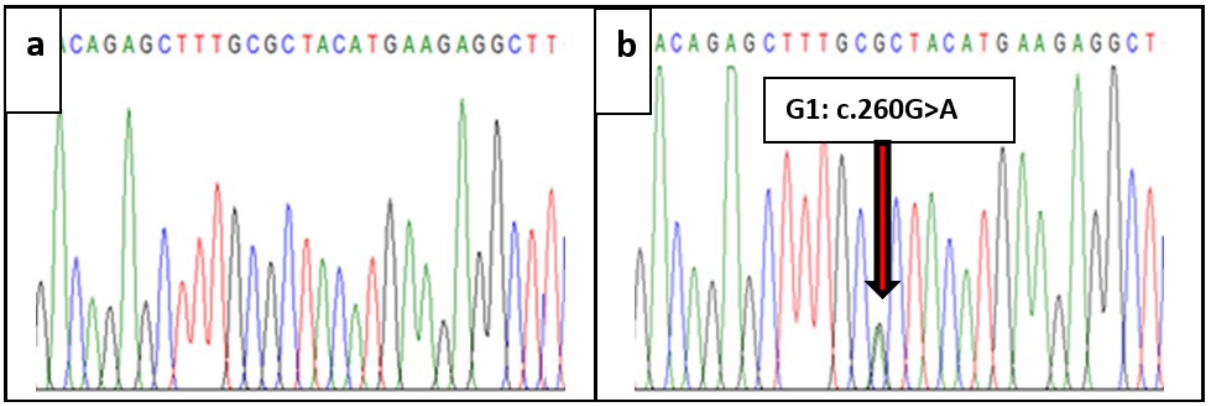

Figure 2. The nonidentified and identified SNP in the exon 1 of *GDF9* gene in Karayaka sheep breed (a: nonidentified region, b: the identified SNP).

Figure 3. Comparison of the G and A alleles of the *GDF9* gene of Karayaka sheep with those of Norwegian White and *Ovis aries* complete

which are on *BMP15*, *BMPR1B*, and *GDF9*, were investigated in some Turkish native sheep breeds [19–21,27,28]. In most of those studies, the investigated genes had monomorphic structure. Until now, a major or novel mutation, which is related to litter size or ovulation rate, has not been determined in Turkish native sheep breeds. As some reasons for these results, researchers have focused on the importance of sample size, preferred method, and whether the breed is prolific or not [29].

For instance, the presence of FecB allele was not reported by Polat [30] who studied Chios sheep (406 samples), a prolific breed, while Davis et al. [31] found FecB allele in only twelve samples per breed in Hu and Han sheep. Similarly, Amini et al. [26] reported a novel T755C mutation associated with a litter size of Iranian Lori-Bakhtiari sheep (96 samples) by using the PCR-SCCP and DNA sequencing methods, while it was not reported in the same breed by Abdoli et al. [15] who conducted a study on ten ewes.

Consequently, it can be said that many factors will affect the succession in this kind of studies. Karslı and Balcıoglu [19] pointed out that it is necessary to study with purebred sheep breeds and the maximum sample number. On the other hand, Dincel et al. [21] and Gursel et al. [20] emphasized that different mutations should be studied with an intensive screening.

In the present study, we tried to study Karayaka sheep bred as pure with an average sample number higher than those in some previous studies of native sheep breeds in Turkey. Moreover, it was considered studying with more populations because the genetic variations changed from one population to another, as evidenced by the previous studies on Karayas sheep [32,33]. Maybe, this strategy provided determination of mutant allele in this study because the mutation was observed in only Tokat population.

There have been almost no studies of Turkish sheep breeds on exon 1 of the *GDF9* gene. A study carried out

by Çelikeloğlu et al. [34] investigated the *GDF9* exon 1 gene with eight samples, and the studied gene was monomorphic. This may be due to the used sample size and other factors as described above. In another study, Gursel et al. [20] found heterozygous genotypes for $FecX^G$ and $FecG^H$ mutations on the genes of *BMP15* and *GDF9* in Awassi, Chios, Imrose, and Kivircik sheep breeds while it was monomorphic for the mutations $FecX^I$, $FecX^H$, $FecX^B$, and FecB on *BMP15* gene in the mentioned breeds.

Up to now, G1 mutation, also called $FecG^1$, has not been determined on Turkish native sheep breeds. It shows that there has been a need to study this gene. Therefore, in the present study, a comparison of allele and genotype frequencies was not established for exon 1 of *GDF9* gene with Turkish sheep breeds.

In the study, the highest frequency genotype for the Karayaka breed was wild homozygous genotype GG (0.79) due to the low frequency of the A allele. This result was similar to the findings for Rusian sheep breeds Salsk (0.88) and Volgograd (0.84) [3]. The frequency (0.21) of heterozygous genotypes (GA) in Karayaka sheep were similar to those of Colombian Creole hair sheep (0.24) and Volgograd sheep (0.16) [3,35]. Moreover, the frequencies of A (0.10) and G (0.90) alleles of *GDF9* exon 1 gene are consistent with those of the same studies (G: 0.89–0.94, A: 0.08–0.11). The allele and genotypic frequencies from this study are also in line with reports on Moghani sheep in which the allele frequencies were 0.78 for $FecG^+$ and 0.22 for $FecG^-$, the genotype frequencies were 0.66 for $FecG^+$ / $FecG^+$ and 0.24 for $FecG^+/FecG^-$ [36].

Mutant AA genotypes of the *GDF9* gene were not determined in the present study. This finding was similar to those reported by some researchers [12,28,29]. The absence of a mutant genotype can be explained by the low frequency (0.10) of A allele in the Karayaka breed, and mutant genotypes could suffer from embryonic death and reproductive defects [28]. This obtained result might also be due to differences in the biological effects of mutations, changing by species [37]. Similar results were observed in Chilean [12] and Iran fat-tailed sheep breeds [29].

There are conflicting results in the literature on associations of homozygous and heterozygous genotypes with litter size or ovulation rate. For example, a study conducted in Bangladesh sheep indicated the homozygous ewes (AA) had a higher lambing rate (2.00 ± 0.41) than heterozygous ones (GA) (1.59 ± 0.41). On the contrary, Rusian Salsk and Volgograd breeds showed higher lambing rates (1.80 ± 0.12 to 1.88 ± 0.17) in heterozygous (AG) sheep compared to homozygous (GG) sheep (1.13 ± 0.09 to 1.22 ± 0.11) [3]. Jawasreh et al. [38] reported that the homozygous ewes (MM) of the *GDF9* gene produced 0.792 more lambs than that of heterozygous genotypes (NM). These results showed that prolificacy seemed to be affected differently by genetic variants of *GDF9* gene exon 1.

Paz et al. [13] showed that mutation of *GDF9*-G1 or $FecG^1$ led to the increased litter size in heterozygous ewes (1.56 ± 0.08) than that of the wild-type genotype (1.25 ± .09) in Chilota sheep breed. Similarly, Javanmard et al. [39] reported that heterozygous ewes for G1 had a litter size of 1.78, while the wild genotypes had an average litter size of 1.16 in Iran fat-tailed sheep breed. A similar result was also reported by Gorlov et al. [3]. However, in another study, it was reported that G1 mutation did not affect the reproduction of Mehraban sheep in Iran [12]. Similar effects were reported for Afshari, Ghezel, Lori-Bakhtyari, or Shal sheep breeds in Iran as well [29].

There was only one study on the polymorphism of *GDF9* exon 1 based on DNA sequencing carried out by Çelikeloğlu et al. [34]. The author did not report a mutation in Pırlak sheep. In the present study, DNA sequencing allowed the detection of nucleotide variation on *GDF9* gene. A SNP (c.260G>A known as G1 mutation) was found for the first time at the *GDF9* gene, in a Turkish sheep breed Karayaka.

In this breed, the existence of the G1 mutation might be due to more and large sampling from geographically distinct regions. Similar findings were observed in Belclare, Cambridge, and Mehraban ewes [10,30] but not in Rhmani × Barki, Barki, Ossimi Suffolk × Awassi, or Rhmani breeds (GenBank no; KT357481.1, KT357482.1, KT357484.1, KT357485.1, KT357486.1).

The G1 mutation causes an increase in ovulation rate or multiple births in heterozygous ewes. However, in homozygous ewes, it causes sterility due to the arrested follicular development [10]. On the other hand, some studies demonstrated that genotypes of *GDF9* exon 1 (AA, AG, and GG) have no significant effect on fertility traits [10,12,40]. Briefly, it can be said that ovulation rates in ewes heterozygous for the *GDF9* gene are mostly higher than those of homozygous animals [41,42]

A previous study demonstrated that Karayaka sheep flocks' twinning birth rate could be increased by approximately 3–6-fold using selection in favor of twin births [43]. Considering this and the findings obtained from the present study, it can be said that fertility traits might be improved in the Karayaka breed as evidenced in other sheep breeds. However, it is necessary to perform further studies in order to provide exact evidence the effects of G1 mutation on fertility traits in Karayaka and other Turkish sheep breeds.

In conclusion, we determined the exon 1 of *GDF9* gene was polymorphic in Karayaka sheep breed and found out G1 mutation (c.260G>A) on this gene. There were evidenced relationships between this mutation and litter size in other sheep breeds. For this reason, the similar

relationships should be investigated in native sheep breeds in Turkey.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgements

This study was financially supported by the Scientific Research Project Fund of Kırşehir Ahi Evran University under the project number ZRT.A3.17.009.

References

1. Tang JS, Hu WP, Di R, Wang XY, Zhang XS et al. Expression analysis of BMPR1B, BMP15, and GDF9 in prolific and non-prolific sheep breeds during the follicular phase. Czech Journal of Animal Science 2019; 64: 439-447.
2. Karsli T, Sahin E, Karsli BA, Eren MG, Balcioglu MS. An investigation of presence of FecB, FecXG, FecXH allele in Kangal and Güney Karaman sheep using PCR-RFLP method. Journal of Lalahan Livestock Research Institute 2011; 51 (2): 71-80.
3. 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 Fisiche e Naturali 2018; 29 (1): 61-66.
4. Notter D. Genetic improvement of reproductive efficiency of sheep and goats. Animal Reproduction Science 2012; 130 (3-4): 147-151.
5. Abdoli R, Zamani P, Mirhoseini SZ, Ghavi Hossein-Zadeh N, Nadri S. A review on prolificacy genes in sheep. Reproduction in Domestic Animals 2016; 51 (5): 631-637.
6. Holanda GML, Oliveira 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 Veterinaria e Zootecnia 2017; 69 (4): 1047-1053.
7. Davis GH. Major genes affecting ovulation rate in sheep. Genetic Selection Evolution 2005; 37 (1): 1-14.
8. Sadighi M, Bodensteiner KJ, Beattie AE, Galloway SM. Genetic mapping of ovine growth differentiation factor 9 (GDF9) to sheep chromosome 5. Animal Genetics 2002; 33 (3): 244-245.
9. Mohamed SEI, Ahmed RM, Jawasreh KI, Salih M, Abdelhalim DM, et al. Genetic polymorphisms of fecundity genes in Watish Sudanese desert sheep. Veterinary World 2019; 13 (4): 614-621.
10. 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.
11. Vage DI, Husdal M, Kent MP, Klemetsdal G, Boman IA. A missense mutation in growth differentiation factor 9 (GDF9) is strongly associated with litter size in sheep. BMC Genetics 2013; (14): 1.
12. Abdoli R, Zamani P, Deljou A, Rezvan H. Association of BMPR-1B and GDF9 genes polymorphisms and secondary protein structure changes with reproduction traits in Mehraban ewes. Gene 2013; 524 (2): 296-303.
13. Paz E, Quiñones J, Bravo S, Montaldo H, Sepúlveda N. Genotyping of BMPR1B, BMP15 and GDF9 genes in Chilean sheep breeds and association with prolificacy. Animal Genetics 2015; 46 (1): 98-99.
14. Getmantseva L, Bakoev N, Shirokova N, Kolosova M, Bakoev S et al. Effect of the GDF9 gene on the weight of lambs at birth. Bulgarian Journal of Agricultural Science 2019; 25 (1): 153-157.
15. Abdoli R, Mirhoseini SZ, Hossein-Zadeh NG, Zamani P. Screening for causative mutations of major prolificacy genes in Iranian fat-tailed sheep. International Journal of Fertility & Sterility 2018; 12 (1): 51-55.
16. Latifi M, Rashidi A, Abdollahi-Arpanahi R, Razmkabir M. Comparison of different selection methods for improving litter size in sheep using computer simulation. Spanish Journal of Agricultural Research 2020; 18 (1): 0403.
17. Hua GH, Chen SL, Ai JT, Yang LG. None of polymorphism of ovine fecundity major genes FecB and FecX was tested in goat. Animal Reproduction Science 2008; 108 (3-4): 279-286.
18. Polley S, De S, Batabyal S, Kaushik R, Yadav P et al. Polymorphism of fecundity genes (BMPR1B, BMP15 and GDF9) in the Indian prolific Black Bengal goat. Small Ruminant Research 2009; 85 (2-3): 122-129.
19. Karsli T, Balcioglu MS. An investigation of presence of FecB allele on BMPR-IB(Boroola) gene raised in Turkey in six local sheep breeds using PCR-RFLP method. Kafkas Universitesi Veteriner Fakültesi Dergisi 2010; 16 (6): 1033-1036.
20. Gursel FE, Akis I, Durak H, Mengi A, Oztabak K. Determination of BMP-15, BMPR-1B and GDF-9 gene mutations of the indigenous sheep breeds in Turkey. Kafkas Universitesi Veteriner Fakültesi Dergisi 2011; 17 (5): 725-729.
21. Dincel D, Ardicli S, Soyudal B, Er M, Alpay F et al. Analysis of FecB, BMP15 and CAST gene mutations in Sakiz sheep. Kafkas Universitesi Veteriner Fakültesi Dergisi 2015; 21 (4): 483-488.
22. Kasiriyan MM, Hafazian SH, Hassani N. Genetic polymorphism BMP15 and GDF9 genes in Sangsari sheep of Iran 2011; 3 (1): 31-34.
23. Grosdidier, A. ExPASy: SIB bioinformatics resource portal. Nucleic Acids Research 2012; 40 (W1): W597-W603.
24. 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.

25. Xu SS, Gao L, Xie XL, Ren YL, Shen ZQ, Wang F, Kantanen J. Genome-wide association analyses highlight the potential for different genetic mechanisms for litter size among sheep breeds. Frontiers in Genetics 2018; 9, 118.

26. Amini HR, Ajaki A, Farahi M, Heidari M., Pirali A, Forouzanfar M, Eghbalsaied S. The novel T755C mutation in BMP15 is associated with the litter size of Iranian Afshari, Ghezel, and Shal breeds. Archiv fuer Tierzucht 2018: 61 (1): 153.

27. Karsli T, Sahin E, Karsli B A, Alkan S, Balcioglu MS. An investigation of mutations ($FecX^{G}$, $FecX^{I}$, $FecX^{H}$, $FecX^{B}$) on BMP-15 gene in some local sheep breeds raised in Turkey. Akdeniz Üniversitesi Ziraat Fakültesi Dergisi 2012; 25 (1): 29-33.

28. Dinçel D, Ardıçlı S, Şamlı H, Balcı F. Genotype frequency of FecXB (Belclare) mutation of BMP15 gene in Chios (Sakiz) sheep. Uludağ University Journal of the Faculty of Veterinary Medicine 2018; 37 (2): 87-91.

29. Eghbalsaied S, Khorasgani FR, Amini HR, FarahibM, Davari M, Pirali A, Pourali S, Vatankhah M, Rostami M, Atashi H. Variant GDF9 mRNA is likely not the main cause of larger litter size in Iranian Lori-Bakhtyari, Shal, Ghezel, and Afshari sheep breeds. Archiv fuer Tierzucht 20177; 60 (2): 119.

30. Polat YO. Sakız koyun ırkında BMPR-IB geninde çoklu doğuma neden olabilecek FecB allele varlığının PCR-RFLP yöntemi ile araştırılması. PhD, Uludağ University, Bursa, 2006 (in Turkish).

31. Davis G H, Galloway SM, Ross IK, Gregan SM, Ward J, Nimbkar BV, Tiesnamurti B et al. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (FecB) mutation. Biology of Reproduction 2002; 66 (6): 1869-1874.

32. Kirikci K, Noce A, Cam MA, Mercan L, Amills M. The analysis of mitochondrial data indicates the existence of population substructure in Karayaka sheep. Small Ruminant Research 2018; 162: 25-29.

33. Kirikci K, Cam MA, Mercan L. Genetic diversity and relationship among indigenous Turkish Karayaka sheep subpopulations. Archives Animal Breeding 2020; 63 (2): 269-275.

34. Çelikeloğlu K, Erdoğan M, Hacan Ö, Koçak S, Bozkurt Z et al. Pırlak koyunlarında BMPR1B, BMP15 ve GDF9 genlerinde olası polimorfizmlerin araştırılması. Kocatepe Veteriner Dergisi 2018; 11 (4): 356-362 (in Turkish).

35. Hernández LE, Montes JL, Otero ER, Prieto JB, Espitia JR et al. Polimorfismos FecXR, FecGI y FecGH en el ovino de pelo colombiano. Archivos de Zootecnia 2019; 68 (262): 192-197 (in Spanish).

36. Ala Noshahr F, Rafat A. Genetic polymorphism of GDF9 gene in Iranian Moghani sheep breed. Iranian Journal of Applied Animal Science 2014; 4 (4); 887-890.

37. Basheer A, Shareef M, Islam M, Zahoor I. Study of $FecX^{G}$ Polymorphism in Beetal Goat and its phylogenetic relationship. Kafkas Ünivesitesi Veteriner Fakültesi Dergisi 2019; 25 (2): 239-244.

36. Jawasreh, KI, Ismaıl ZB. Polymorphism of prolactin, growth differentiation factor 9, and calpastatin genes and their effects on weight traits in Awassi lambs. Journal of Advanced Veterinary and Animal Research, 2019; 6 (1): 86.

37. Javanmard A, Azadzadeh N, Esmailizadeh AK. Mutations in bone morphogenetic protein 15 and growth differentiation factor 9 genes are associated with increased litter size in fat-tailed sheep breeds. Veterinary Research Communications 2011; 35 (3): 157-167.

38. Barzegari A, Atashpaz S, Ghabili K, Nemati Z, Rustaei M et al. Polymorphisms in GDF9 and BMP15 associated with fertility and ovulation rate in Moghani and Ghezel sheep in Iran. Reproduction Domestic Animals 2010; 45 (4): 666-669.

39. Juengel JL, Hudson NL, Heath DA, Smith P, Reader KL et al. Growth differentiation factor 9 and bone morphogenetic protein 15 are essential for ovarian follicular development in sheep. Biology of Reproduction 2002; 67 (6): 1777-1789.

40. Yan C, Wang P, DeMayo J, DeMayo FJ, Elvin JA et al. Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. Molecular Endocrinology 2001; 15 (6): 854-866.

41. Çam MA, Olfaz M, Kırıkçı K, Soydan E, Kuran M. Correctional potential in terms of Karayaka race fertility. International Animal Science Congress of Turkish and Relatives Communities 2012; 29.