

Screening of Inverdale (FecX^I), Hanna (FecX^H), Belclare (FecX^B), Galway (FecX^G) and Boorola (Fec^B) mutations in Karayaka sheep breed

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Abstract

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The general twinning rate of the Karayaka breed, which stands out with its meat quality, ranges from 0.10 to 0.20. However, it may be encountered in the flocks with higher twinning rates. The aim of this study was to investigate five different mutations in the BMPR1B (FecB) and BMP15 (FecX^H, FecX^I, FecX^G, and FecX^B) genes. The mutations were screened directly in 100 ewes of the Karayaka breed raised in the Black Sea region of Türkiye using the restriction fragment length polymorphism (PCR-RFLP) technique. The study results showed that all the ewes examined had wild-type alleles or carried no mutations. In conclusion, the monomorphic structure of the BMPR1B and BMP15 genes concerning the loci studied suggests that these genes cannot be used as candidate genes for increasing twin births in the Karayaka breed. The findings in this study are the first for the Karayaka breed, and there is a need for comprehensive studies based on phenotypic data with more samples and other candidate genes.

Keywords: BMPR1B; BMP15; Karayaka; multiple births; polymorphism; PCR-RFLP

Introduction

The Karayaka sheep is one of Turkey's most significant sheep breeds because of its high-quality meat characteristics. It accounts for 4.5 percent of the overall sheep population in the nation (Kirikci et al., 2020) and is extensively bred in the Black Sea region for its high meat quality and adaptability. Farmers mostly prefer Karayaka for lamb meat production, which explains its popularity. The increase in litter size in Karayaka ewes will greatly enhance lamb meat production and farmers' income. Even though Karayaka is a non-prolific breed, prior studies revealed that the breed's litter size might be increased by selection (Cam et al., 2010; Cam et al., 2017).

Various factors influence the profitability of mutton production, including flushing, crossbreeding, the number of lambs at weaning age, and litter size (Cam et al., 2017).

Among these factors, litter size is one of the most crucial considerations. The Karayaka ewes' litter size was 1.01-1.60 in different studies (Akcapinar et al., 2002; Cam et al., 2017; Tamer & Sirin, 2021).

Various strategies, such as crossbreeding and selective breeding (based on phenotype or marker-assisted selection data), help sheep breeders attain the desired multiple births level in their herds. Studies of performance characteristics mainly focus on sheep's reproductive traits with low heritability (Davis, 2004; Abdoli et al., 2016). Therefore, detecting possible mutations in quantitative trait loci (QTLs) associated with fecundity will help increase lamb meat production.

The bone morphogenetic protein receptor type 1B (BMPR1B or FecB), growth differentiation factor 9 (GDF9), and bone morphogenetic protein 15 (BMP15) genes are significant major genes that affect sheep fecundity (Gootwine,

2020). These genes belong to the transforming growth factor-beta (TGF- β) superfamily, playing a pivotal role in embryo development, ovulation rate, and litter size (Fabre et al., 2006). The BMPR1B gene is situated on sheep chromosome 6 and causes enhanced ovulation in homozygous and heterozygous ewes with the mutation, resulting in increased lambing (Akhataeva et al., 2021). The BMPR1B gene influences follicular development and granulosa cells in the ovary, affecting 1.5 oocytes per estrus cycle in sheep and increasing twinning and litter size in the population (Davis et al., 1982; Piper et al., 1985; Qi et al., 2020). The BMP15 mutations, in contrast to BMPR1B, cause sterility in homozygous situations, except for FecX^{GR} and FecX^O (Calvo et al., 2020).

Given the importance of the BMPR1B and BMP15 genes, various researchers examined prolific and non-prolific sheep breeds to determine whether some relevant mutations are responsible for their high prolificacy in Turkey (Gürsel et al., 2011; Karsli et al., 2012; Kirikci et al., 2021; Kirikci, 2022). Here, we first investigated five fecundity mutations, FecB, FecX^H, FecX^I, FecX^G, and FecX^B, in BMPR1B and BMP15 genes in the Karayaka breed.

Materials and Methods

DNA extraction and amplification of BMPR1B and BMP15 genes

Blood samples were previously collected according to the Guidelines for the Care and Use of Animals of the Local Ethics Committee of Ondokuz Mayıs University (2013/64). Genomic DNAs were isolated from the whole blood of 100 randomly selected Karayaka ewes belonging to four different regions (Samsun, Ordu, Giresun, and Tokat) of Turkey using a commercial DNA extraction kit (GeneJET™ Whole Blood Genomic DNA Purification Mini Kit, Lithuania). The isolated DNA samples were stored at -20°C until PCR amplification.

The PCR amplifications were carried out in a 25- μ L final reaction volume, containing 1 μ l of genomic DNA, 12 μ L

Tag DNA polymerase Master Mix red (2X) (Ampliqon, Denmark) (1.5 mM final concentration), 1 μ L (10 pmol/ μ L) of each forward and reverse primer and 9.5 μ L ultrapure water.

The primers detailed in Table 1 were used to amplify the studied loci. PCR conditions were as follows: one initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55-63°C for 30 s and extension at 72°C for 30 s. The final elongation step was performed at 72°C for 5 min.

Digestion of PCR products with the restriction enzymes

PCR products from BMPR1B and BMP15 genes were digested with restriction enzymes listed in Table 2, under restriction conditions; a final volume of 30 μ L, consisting of 1 μ L of fast digest enzyme, 10 μ L of PCR product, 2 μ L of green buffer, and 17 of μ L deionized water. They were incubated at 37°C for 10 min and inactivated at 65°C for 20 min. The PCR-RFLP products were visualized under a UV transilluminator with the dye of nucleic acid staining (Red-Safe™, iNtRON Biotechnology Co.). In the study, a 100-bp DNA ladder was utilized as a molecular size marker.

Table 2. PCR products sizes for mutant/wild alleles and the restriction enzymes

Loci	PCR product (bp)	Mutant (bp)	Wild (bp)	RE
FecB	190	160 and 30	190	<i>AvaII</i>
FecX ^B	153	153	122, 31	<i>DdeI</i>
FecX ^H	240	218 and 22	240	<i>SpeI</i>
FecX ^G	141	141	112, 29	<i>HinfI</i>
FecX ^I	154	124 and 30	154	<i>XbaI</i>

Results

The PCR-RFLP approach explored five mutations (FecB, FecX^I, FecX^H, FecX^G, and FecX^B) in BMP15 and BMPR-1B genes in Karayaka sheep. The analyzed loci were successfully amplified by PCR, as seen in Figure 1.

Table 1. Genes, mutations, primer sequences, and annealing temperatures of the primers

Genes	Mutations	Primers	T _a (°C)	References
BMPR-1B	FecB	5'-CCAGAGAATAGCAAAGCAAA-3' 5'-CAAGATGTTTTTCATGCCTCATCAACACGGTC-3'	62	Davis et. (2006)
BMP15	FecX ^H	5'-TATTTCAATGACACTCAGAG-3' 5'-GAGCAATGATCCAGTGATCCCA-3'	55	Hua et al. (2008)
	FecX ^I	5'-GAAGATAACAGTGTTCCCTCCACCCTTTTCT-3' 5'-CATGATTGGGGAGAATTGAGACC-3'	55	Davis et. (2006)
	FecX ^G	5'-CACTGTCTTCTTGTTACTGTATTTCAATGAGAC-3' 5'-GATGCAATACTGCCTGCTTG-3'	63	Hanrahan et al. (2004)
	FecX ^B	5'-GCCTTCTGTGTCCCTTATAAGTATGTTCCCTTA-3' 5'-TTCTTGGGAAACCTGAGCTAGC-3'	62	Hanrahan et al. (2004)

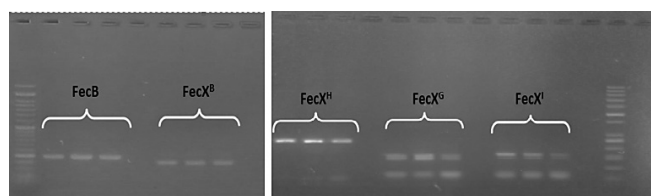


Fig. 1. The PCR products of studied loci

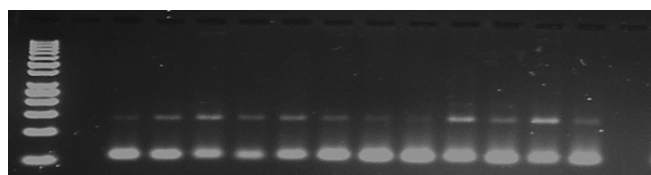
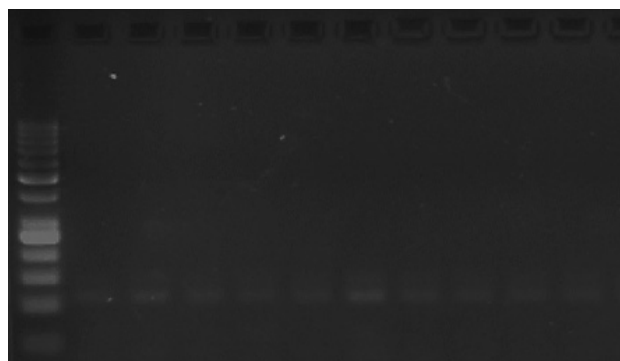


Fig. 2. PCR-RFLP results of the *FecB* mutation on **BMPR1B**

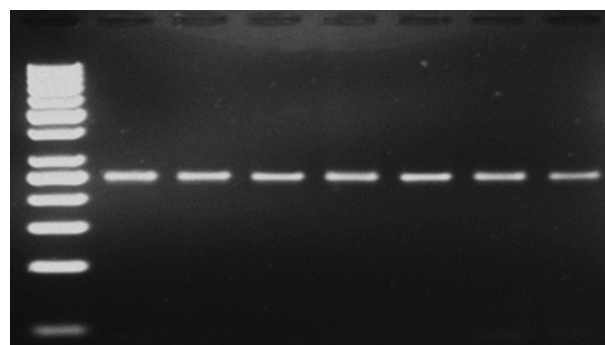
The current study showed that all animals were monomorphic concerning the investigated mutations on the *BMPR1B* and *GDF9* genes, as shown in Figures 2 and 3, respectively. All individuals in the study did not produce products with the predicted sizes given in Table 2 after RFLP. For this reason, allele and genotype frequencies were stable, and any diversity was not able to be detected for these loci in the Karayaka breed in the present study.



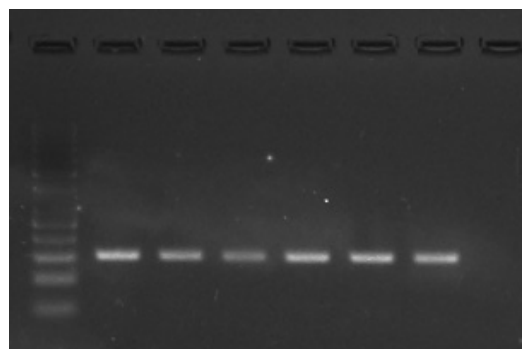
a) *FecX^G*



c) *FecX^B*



b) *FecX^H*



d) *FecX^I*

Fig. 3. PCR-RFLP results of the *BMP15* mutations in the study

Discussion

The present study focused on detecting five major mutations associated with litter size in sheep due to the lack of research on this subject in the Turkish sheep breed, Karayaka.

The *FecB* mutation in the *BMP15* gene was initially found in Booroola Merino sheep and examined in many sheep breeds worldwide. The mutation has been documented to have an effect on litter size in a variety of sheep breeds, including Assaf, Garole, Javanese, Small Tail Han, and Chinese Hu sheep (Davis et al., 2002; Davis, 2005; Chu et al., 2007; Yang et al., 2020) and its presence has also been reported in Bulgarian Merino sheep (Bozhilova-Sakova, 2020). However, several investigations did not report the mutation or demonstrate that it impacted litter size in some sheep breeds (Hernández et al., 2020; Saleh et al., 2020). These findings agreed with the absence of *FecB* mutation in the Karayaka sheep breed. This finding was also consistent with the results reported by Abdoli et al. (2018) for Iranian Fat-Tailed sheep and with the results reported by Karsli et al. (2012) for five Turkish sheep breeds, namely, Akkaraman, Daglic, Ivesi, Tuj, and Karakas. As in the present study, Mohammadi (2016) reported similar findings for Iranian Arabic sheep.

The BMP15 gene is involved in granulosa cell proliferation and differentiation during ovarian follicular development (Tang et al., 2019). It increases granulosa cells' mitosis and decreases FSH receptors' expression. Mutations of FecX^H, FecX^I, FecX^G, and FecX^B, located on the Ovis aries BMP15 gene, affect sheep reproduction. Heterozygous sheep with these mutations have been shown to have higher granulosa cell sensitivity to LH in the initial stages of the follicular development, fewer granulosa cells, a smaller follicle, and a smaller corpus luteum. This gene was studied in the Karayaka breed due to its importance in sheep litter size and considerable variances compared to other candidate genes.

In the present study, PCR-RFLP results for all studied loci of the BMP15 gene revealed one pattern, meaning non-digested fragments or monomorphic structure. None of the mutations in the BMP15 gene was detected for the Karayaka sheep breed. Thus, allelic and genotypic frequencies were stable in the study. Similar findings for the FecX^I mutation were also reported in the Egyptian sheep breeds, Barki, Ossimi, and Rahmani (Nagdy et al., 2018). Unlike the non-prolific Karayaka sheep, the FecX^I mutation has not also been reported in some prolific sheep breeds. The result of this study means that different genes or mutations could be responsible for prolificacy except for the investigated genes. Paz et al. (2012) investigated the same mutations and found none in the breeds; Chilota, Araucana, and Austral, consistent with our findings. The inverdale gene has been linked to infertility in homozygous carrier sheep (McLeod et al., 1997). In this investigation, the Karayaka sheep breed did not reveal the homozygous inverdale gene, and all ewes were fertile. This result was consistent with the study on İvesi sheep by Agyar & Kirikci (2022).

The present study did not detect the FecX^G mutation, which is in agreement with the findings of Kumar et al. (2008) studied prolific (Garole and Kendrapada) and non-prolific breeds (Malpura and Deccani). All of the BMP15 genes' analyzed sites in this study were monomorphic, and the results were consistent with Niu's (2021) study, which studied the same mutations in prolific Cele Black sheep.

None of the five mutations analyzed were found in the Karayaka breed. Several factors, including the breed effect, sample size, and sampling method, might explain this finding. Previous studies have shown that some significant mutations are not responsible for multiple births in some prolific breeds, indicating that additional genes may be involved in multiple births (Shi et al., 2010). As a result of these studies, more profound research into other gene locations and more extensive investigations into the Karayaka breed will be beneficial. Nevertheless, the findings obtained are essential in partially revealing the genetic structure of the Karayaka breed.

Conclusions

The current research focused on the genetic structure of the BMP1B and BMP15 genes in the Karayaka sheep breed. Considering studied samples, FecB and FecX loci, indicating that these sites are conserved and can be used for studies on sheep evolution. This finding is tentative, and further research is needed on other essential genes involved in reproduction. Comprehensive phenotypic data about the reproductive performance of the animals is also required to determine the relationship between genes and fecundity.

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Competing interests

The authors declare that they have no competing interests.

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