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SCREENING FOR GALWAY MUTATION (*FECX^G*) IN KIVIRCIK BREED

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Abstract: High litter size or twinning is an economically important trait that enhances sheep productivity. The *FecX^G* has been influentially associated with the ovulation rate in various sheep breeds. However, there is limited information about this locus in the Kivircik sheep breed. Therefore, the aim of this study was to evaluate the presence of the Galway (*FecX^G*) mutation in Kivircik sheep (n=91) raised in Kirklareli province. Genomic DNA was isolated from whole blood using the phenol-chloroform extraction method. The genotyping was performed by the PCR-RFLP method. Results revealed that all ewes had the Galway mutation (*FecX^{GG}*) and the corresponding genotype was fixed in the studied population. The present analysis showed that the Galway mutation which is a nucleotide alteration (cytosine to thymine) at position 718 bp of the *BMP15* (also known as *FecX*) gene may be considered in enhancing twinning in the Kivircik breed. However, further analyses with larger populations are needed to confirm the present results and to provide more detailed information before focusing on this genomic region in breeding programs for purebred Kivircik sheep.

Keywords: Sheep, Kivircik, *FecX^G*, Mutation, Polymorphism, PCR-RFLP

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1. Introduction

The transforming growth factor-β (*TGF-β*) gene superfamily encodes multifunctional proteins that play fundamental roles in cell growth and differentiation (Kumar et al., 2008). These proteins have also important effects on embryogenesis in mammals. *TGF-β* consists of fecundity genes in sheep, including bone morphogenetic protein receptor IB also known as Booroola fecundity gene (*FecB*), growth differentiation factor 9 (*GDF9*) also known as *FecG*, and bone morphogenetic protein 15 (*BMP15* or *GDF9b*) also known as *FecX* (Galloway et al., 2000; Wilson et al., 2001; Hanrahan et al., 2004; Kumar et al., 2008). The mutations in two oocyte-derived growth factor genes including the X-linked *BMP15* (*FecX^G* and *FecX^B* mutations), and *GDF9* (*FecG^H* mutation) have been associated with the exceptional prolificacy in Romney, Belclare, Cambridge, and Lacaune breeds (Mullen et al., 2013). *BMP15* maps to the X chromosome and is specifically expressed in the oocytes. The BMP15 mRNA in the sheep ovary is only expressed in oocytes, and its encoded product plays an important role in oocyte development (Niu et al., 2021). Mutations in this gene [Inverdale (*FecX^I*), Hanna (*FecX^H*), Belclare (*FecX^B*), Galway (*FecX^G*), and Lacaune (*FecX^L*)] have been influentially associated with ovulation rate in various sheep breeds (Galloway et al., 2000; Hanrahan et al., 2004; Kumar et al., 2008). Among these mutations, the Galway mutation is a nucleotide alteration (C to T) at position 718 bp of the *BMP15* gene, which introduces a premature stop codon in place of glutamic acid at amino acid residue 239 of the unprocessed protein (Kumar et al., 2008).

All heterozygous ewes exhibit higher prolificacy than wild-type genotypes. The mutant type had a non-additive effect on ovulation rate, and accordingly, the homozygotes are sterile (Galloway et al., 2000; Hanrahan et al., 2004). Based on the X-linked inheritance, rams carry only one copy and pass it to all daughters.

Kivircik sheep breed is one of the most important native livestock genetic resources of Turkey. It is a thin-tailed breed and its meat is preferred widely by the consumer because of superior meat quality characteristics (Gurcan et al, 2018). There is limited information about this fecundity gene in the Kivircik sheep breed. Taken together, this research was designed to evaluate the presence of the Galway (*FecX^G*) mutation which is suggested to be associated with a high ovulation rate in Kivircik sheep.

2. Materials and Methods

2.1. Animal Material and DNA Extraction

The study was conducted on a total of 91 purebred Kivircik ewes raised in Kirklareli province, Turkey. The animals were recorded for the Pedigree Project of the Turkish Ministry of Food, Agriculture and Livestock, and Cattle Breeders Association. All animals were housed and managed according to the standard farm procedures. Blood samples (~4mL) were collected in Vacutest tubes

with a K_3EDTA (0.2 mg/mL) anticoagulant (Vacutest Kima SRL, Arzergrande, PD, Italy). Genomic DNA was isolated using the standard phenol-chloroform method as described by Green and Sambrook (2012). The concentration of total DNA samples obtained and their quality was estimated using a NanoDrop 2000c spectrophotometer (Thermo Scientific, USA).

2.2. PCR-RFLP Analyzes and Genotyping

The Galway (*FecX*^{G}) mutation was screened in Kivircik sheep by the PCR-RFLP. The primers used in the amplification of the target gene region was presented in Table 1. The amplification of genomic DNA was carried out with a total volume of 25 µL in PCR reaction, including 1 µL (0.025 µM) of each primer (forward and reverse), 12.50 µL PCR master mix (OneTaq Quick-Load 2x MM with Standard Buffer, New England BioLabs Inc., Ipswich), 3 µL of the purified DNA sample, and 7.5 µL of nuclease-free water (Thermo Scientific). The PCR amplification program was 94 °C for 5 min, 35 cycles of 94°C for 30 s, 62.3°C for 30 s, 72°C for 45 s, and a final extension of 72 °C for 5 min. 10 µL of amplified PCR products were digested with *Hin*fI restriction enzyme *(*Thermo Fisher Scientific Inc., USA) at 37°C for about 4 h to determine the allelic polymorphism.

Table 1. Primers sequences (from 5' to 3') for Galway mutation (*FecX*^{G})

Primer sequences	Reference
F: CACTGTCTTCTTGTTACTGTATTTCAATGAGAC R: GATGCAATACTGCCTGCTTG	Hanrahan et al., 2004

F= forward, R= reverse.

Briefly, PCR amplified and digested DNA fragments were separated on 2% and 3% agarose gels, respectively, and stained with ethidium bromide. The gels were scored for the presence or absence of the mutations by a gel imaging system (DNR-Minilumi, DNR Bio-Imaging Systems, Israel). In this respect, the PCR product from noncarriers (wild type genotype) has a *Hin*fI site, while carrier individuals (mutant genotype) lack this restriction site. After digestion, wild type individuals (*FecX*$^{++}$) should have 111 bp and 30 bp fragments, heterozygotes should have (*FecX*$^{G+}$) 141 bp, 111 bp, and 30 bp fragments, and homozygous individuals (*FecX*^{GG}) are recognizable with an uncut 141 bp fragment (Kumar et al., 2008).

2.3. Statistical Analysis

The *FecX*^{GG} genotype was fixed in the studied animals. Hence population genetics parameters and Hardy-Weinberg equilibrium could not be estimated.

3. Results and Discussion

In small ruminant breeding, there has been increasing interest in the evaluation of prolificacy (Gedik, 2021). The trends in selection procedures have gradually changed from traditional phenotype-based applications to genotypic considerations by the identification and utilization of major genes for prolificacy (Davis, 2004). The Booroola Merino was the first breed in which ovulation rate and litter size were shown to be affected by a segregating major gene in sheep (Piper et al., 1985). It was demonstrated that *FecB*, which is a dominant autosomal gene, has an additive effect on ovulation rate. On the other hand, an X-linked gene was associated with an increase in the ovulation rate and it was first described in Romney sheep and named the Inverdale gene (*FecX*). It is important to note that homozygous carrier females exhibit sterility. Therefore, this gene has been suggested to be a pivotal genetic marker for prolificacy in sheep (Davis, 2004).

This study focuses on the analysis of the Galway mutation (*FecX*^{G}) in the Kivircik breed which is one of the most important sources of Turkey's national livestock. In this sense, the 141 bp fragment in the *BMP15* gene [genomic location: X: 56594565-56601245 (-)] was amplified (Figure 1).

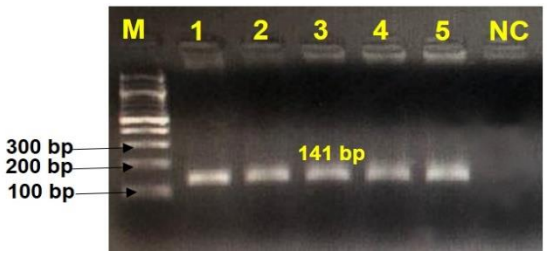

Figure 1. The electrophoresis pattern of PCR amplification for the Galway mutation (FecXG). M: Marker (100 bp).

In the present analysis, all animals remained undigested with the *Hin*fI restriction enzyme (Figure 2). This suggests that Kivircik sheep were found to be *FecX*^{GG} genotype. Hence, the corresponding genotype was fixed in studied ewes, and accordingly, population genetics parameters and Hardy-Weinberg equilibrium could not be estimated.

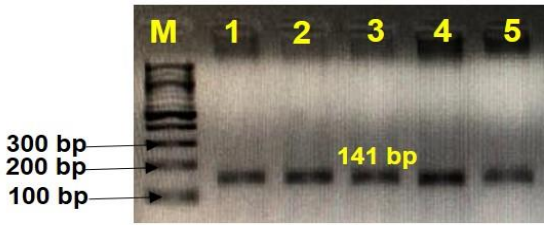

Figure 2. The electrophoresis pattern of restriction enzyme digestion of PCR product with *Hin*fI for the Galway mutation (*FecX*^{G}). M: Marker (100 bp). The PCR product remained undigested, and hence, all ewes were genotyped as (*FecX*^{GG}).

This result demonstrated that admissible prolific characteristics of the Kivircik breed can be partially explained by the presence of the *FecX*^{G} mutation. However, it is worth noting that, undigested fragments in the PCR-RFLP analyses may cause unreliable or controversial results. To overcome this limitation, RFLP analysis was performed in duplicate in this study.

High litter size or twinning is a crucial economically important indicator that determines the productivity of the herd, concerning the number of lambs, meat, and wool (Ardicli et al., 2021). The presence of *FecX*^{G} mutation has been shown to be an important genetic factor to achieve high prolificacy in sheep (Davis, 2004; Kumar et al., 2008). Concerning different breeds of sheep, the results of *FecX*^{G} mutation analyses revealed mostly controversial suggestions. But this situation is a common circumstance in genotypic evaluation. Different breeds or different individuals of the same breed may exhibit distinctive genotypic distributions. Hereupon, previous analyses revealed remarkable differences in genotypic frequencies in various breeds of sheep. Gursel et al. (2011) found that all of the investigated Kivircik sheep were heterozygous for the *FecX*^{G} locus. These researchers suggested that Kivircik, Imrose, Awassi, and Chios breeds had an advantage for fertility due to heterozygosity for *FecX*^{G} mutation. Moreover, the *FecX*^{G} mutation was identified in Belclare and Cambridge sheep (Davis, 2004). On the other hand, Dincel et al. (2015) suggested that the high prolificacy of the Sakiz breed does not result from *Fec*^{B}, *FecX*^{G}, and *FecX*^{I} mutations. Another important point is that the presence of inbreeding should be considered when evaluating the variability in litter size among different sheep breeds (Doekes et al. 2021; Tao et al., 2021).

There are certain limitations to the genetic studies conducted on native sheep breeds in Turkey. On one hand, unconscious crossbreeding and importation have resulted in a decrease or loss of diversity in Turkish native sheep breeds without genetic characterization. This situation has also resulted in difficulties in finding purebred individuals. On the other hand, population sizes or the number of genotyped individuals in these studies are quite low, similar to those in the current study. These limitations prevent providing reliable suggestions or achieving the concrete data to use in gene-assisted selection. Moreover, pedigree data is mostly far from trustworthiness to be applicable in sheep breeding management. Kivircik breed is one of the most important native livestock genetic resources of Turkey and its meat is preferred widely by the consumer because of superior meat quality characteristics. Hence, further molecular genetic analyses should be performed in larger populations.

4. Conclusion

The present analysis showed that all ewes were the Galway mutation (*FecX*^{GG}) carriers. This suggests that selected Kivircik individuals have an advantage for fecundity due to the desired genotype. Consequently, ovine *FecX*^{G} may be considered to achieve high litter size in the Kivircik breed. Nevertheless, the other fecundity genes should be analyzed.

Author Contributions

All authors had equal contributions and all authors reviewed and approved the manuscript.

Ethical Approval

The experimental procedures were approved by the Local Animal Care and Ethics Committee of Namik Kemal University, Tekirdag, Turkey, ensuring compliance with EC Directive 86/609/EEC for animal experiments (NKUBAP.00.24.YL.11.01).

Conflict of Interest

The authors declared that there is no conflict of interest.

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