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GENETIC SCREENING OF *FecX*^{G} POLYMORPHISM IN SAANEN GOAT (*Capra Hircus*) BREED IN TÜRKİYE

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Abstract: Determination of fecundity gene mutations and utilizing them in farm animals programs offers the opportunity to improve productivity. The BMP15/FecX gene is one of the candidate genes with significant effects on multiple births in sheep. Studies in small ruminant have shown that BMP15 gene mutations increase the rate of multiple births, although the effect of BMP15 gene mutations varies at the breed level. Although there are many studies on sheep fecundity in Türkiye, there are no studies on goat. Therefore, the objective of the current study was to investigate FecX^{G} mutation in the exon 2 region of BMP15 gene in Saanen goats (*Capra hircus*). A total of 24 samples were used to investigate the FecX^{G} mutation in Saanen goats raised in the Muş Plain of Türkiye. A fragment of 141 bp of BMP15 gene was amplified by PCR and then products subjected to the digestion of restriction enzyme *HinfI*. This preliminary study's findings showed that there is no FecX^{G} mutation in Saanen goats.

Keywords: Goat, Saanen, BMP15, FecX^{G}, PCR-RFLP, *HinfI*

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1. Introduction

The bone morphogenetic proteins (BMPs), which belong to the transforming growth factor β (TGF β) superfamily, have significant influence on cellular processes such as growth, proliferation, and apoptosis within different kinds of cells, with particular relevance to mammalian fertility (Massague, 1998; Galloway et al., 2000). The BMP15 gene was first detected in the Romney sheep and revealed its role in sheep fertility (Davis et al., 1991). The BMP15 gene, located in goat X chromosome (Farhadi et al., 2013), is responsible for regulating the proliferation and differentiation of granulosa cells. It achieves this by stimulating mitosis in granulosa cells, suppressing the expression of FSH receptors, and promoting the expression of ligands. These functions are crucial for maintaining female fertility in mammals (Moore and Shimasaki, 2005).

In Türkiye, as in the world, fecundity gene research has been focused more on native sheep breeds (Karslı et al., 2012; Çelikeloğlu et al., 2022; Gedik 2021; Kırıkçı, 2023a; Kırıkçı, 2023b), while there are significant shortcomings in the research on goat fecundity genes. Recently, there has been an increasing interest in fecundity genes in goats (Maitra et al., 2023; Song et al., 2023; Abuzahra et al., 2023). A study by Maitra et al. (2023) to understand the genetic mechanism of prolificacy in goats found that the genes BMPR1B, BMP15 and GDF9 are highly expressed in goat ovaries. The BMP15 gene and its effects on goat prolificacy were detected in Chinese goat breeds; Funiu white and Taihang black (Wang et al., 2011; Wang et al., 2015).

This study aimed to investigate the Galway (FecX^{G}) mutation on BMP15 gene in Saanen goats, which are well adapted to the ecological conditions of Türkiye (Özkaya et al., 2017).

2. Materials and Methods

A total of 24 blood samples were collected for the study from the enterprise located in the plain of Muş. Genomic DNA was isolated using a kit, IDPURE™ Spin Column (Empire Genomics, Buffalo, NY). Following the manufacturer's instructions, the DNA isolation process was carried out. The PCR reaction for amplifying a 141 bp region of the BMP15 gene was performed in a 25 µl final volume with 1µl of genomic DNA, 12µl of Taq DNA Polymerase 2X Master Mix Red (1.5 mM $MgCl_2$ final concentration, AMPLIQON), 1µl of each primer at a concentration of 10 pmol/µl and water. The PCR analysis was carried out at the following temperatures and times: initial denaturation at 95 °C for 3 minutes, followed by 35 cycles of 95 °C for 30 seconds (denaturation), 55 °C for 30 seconds (annealing), and 72 °C for 30 seconds (elongation), with a final extension step at 72 °C for 5 minutes. The goat BMP15 gene exon 2 region was amplified with the primer pairs; 5′ CACTGTCTTCTTGTTACTGTA TTTCAATGAGACG 3′ and 5′ GATGCAATACTGCCTGCTTG 3′ using SimpliAmp thermal cycler (Applied Biosystem). Samples were analyzed for

the presence of the FecX^G mutation by using PCR-RFLP technique described by Galloway et al. (2000). Digestion was performed in a final volume of of 30 µL, which included of 1 µL of fast digest enzyme (*HinfI*), 10 µL of PCR product, 2 µL of green buffer, and 17 of µL deionized water. They were incubated at 37 °C for 10 min and inactivated at 65 °C for 20 min. After the PCR-RFLP analysis, all of the samples were run on a 2% high-resolution agarose gel electrophoresis, stained with a green safe dye, and then visualized under a UV transilluminator for evaluation (Kiraz and Ağyar, 2016). A DNA ladder of 100 bp was used as molecular size marker.

3. Results

In the current study, the FecX^G mutation was screened in the Saanen goats. A fragment of 141 bp of BMP15 gene was amplified successfully and then all PCR products were subjected to the digestion of *HinfI* enzyme. After digestioning, samples were run on 2% agarose gel electrophoresis (Figure 1).

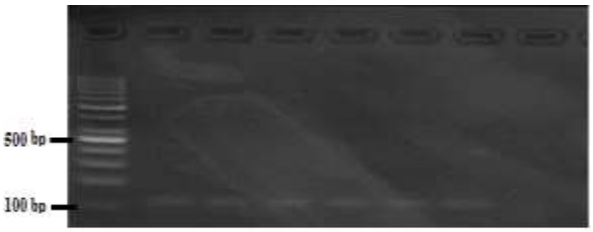

Figure 1. PCR-RFLP result for FecX^G locus in Saanen goat.

According to literature, heterozygous mutant individuals were expected to have two fragments of 111 and 54 bp, while individuals that not carrying the mutation were expected to have a single fragment of 141 bp (Basheer et al., 2019) According to the results of the RFLP analysis, not all goats examined carried the FecX^G mutation, which displayed a band of 141 bp in length as shown in Figure 1.

4. Discussion

As a candidate gene, BMP15 were well documented in the world for sheep fecundity. In general, the interest in fecundity gene research has been more on sheep in Türkiye, as is the case all over the world (Hanrahan et al., 2004; Wang et al., 2015; Çelikeloğlu et al., 2021; Kırıkçı, 2023a; Kırıkçı, 2023b). The main reason for this may be the consumption of more sheep meat within small ruminants. Recently, the study of the fecundity gene in goats has drawn more interest (Basheer et al., 2019; Dangar et al., 2022; Gujarmale et al., 2023). Nevertheless, more research is need that reveals the genetic structures of goats in terms of fertility genes.

Maskur et al. (2023) reported that the mutations of FecX^G (c.718C>T) and FecB (c.746A>G) were in Indonesian Kacang and Boerka goats. Another study examining the S32G mutation in the BMP15 gene in 18 breeds of goats, reported a monomorphic structure in Saanen goats (Feng et al., 2014). This monomorphic result was consistent with the findings of the present study. Similar result was also observed in different goat breeds; Markhoz (Shokrollahi, 2015) and Guizhou (Lin et al., 2007). Hua et al. (2007) reported the same results even when the FecX mutation was examined in a large sample (506) in six goat breeds. Contrary to these findings, Abdel-Rahman et al. (2013) using a total of 25 animals with the highest and lowest litter sizes, reported that goats with the BB genotype for the BMP15 gene produced a larger litter size than goats with other genotype (Abdel-Rahman et al., 2013) According to these findings, prolificacy inheritance differs between sheep and goats, and most likely between breeds (Shokrollahi, 2015).

There are differences between studies in terms of twin birth rates in Saanen goats reared in Türkiye. In a study by Bolacalı and Küçük (2012), the mean number of Saanen goats per birth was given as 1.59 young, while in the studies by Taşkın et al. (2003), Ulutaş et al. (2010) and, Ceyhan and Karadağ (2009) were given as 71.43%, 58.83% and 44.2%, respectively. From the results of these studies it can be concluded that there are large differences in the number of kids per birth. In this study, mean number of kids per birth was 1.64. Apart from its effects on fertility, BMP15 have been reported to be a candidate gene in terms of growth and development in goats (Ahlawat et al., 2016). Therefore, fecundutiy gene mutations should be investigated for economic traits such as growth and fertility in goats. The present study reported that Saanen goats did not carry the mutation of FecX^G. In the study, the sample size was small. Therefore, conducting similar studies with more samples and phenotypic data will give more reliable results.

5. Conclusion

The current analysis showed that not all Saanen Goat breed are carriers of the Galway mutation (FecX^G). It can be assumed that selected goat individuals have no advantage for the examined mutation. Therefore, this and other fertility genes should be investigated in comprehensive studies.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	O.A.	K.K.
C	60	40
D	100	
S	100	
DCP	50	50
DAI	50	50
L	50	50
W	80	20
CR	80	20
SR	80	20
PM	80	20
FA	80	20

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

This study was carried out in accordance with the approval (approval date: June 20, 2023, protocol code: 2023/011-E19057416-125.02.02-10311949) of Republic of Türkiye, Ministry of Agriculture and Forestry, Directorate of Muş.

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