Effect of Bone Morphogenetic Protein Receptor-1B (BMPR-1B) Gene Variant on Litter Size in Akkaraman Sheep Breed

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Abstract

The BMPR-1B gene, a significant fertility gene, has been examined for its relationship with fertility characteristics in various sheep populations globally. This investigation explored the impact of the BMPR-1B gene on litter size within the Akkaraman sheep population residing in the Elâzığ Region. To locate the FecB gene in Akkaraman sheep, 104 milk samples were collected, and genetic material was isolated using the salting out technique. During the 2021–2022 period, no significant correlations were observed between the genetic make-up of Akkaraman sheep and factors including age, mastitis and disease history, milk yield, birthing type, and the number of births ($p > 0.05$). However, there was a noteworthy relationship identified between the distribution of genetic make-up and live weight, indicating a potential influence of genetics on the live weight of Akkaraman sheep ($p < 0.05$). In this study, the BMPR-1B gene determined multiple births and it was determined that Akkaraman sheep were monomorphic in terms of this gene. In light of this information, it was concluded that more comprehensive studies should be carried out regarding this gene determining multiple births in the Akkaraman sheep breed.

Keywords: Akkaraman, BMPR-1B, litter size, variant

Received: 24 February 2024 **Revised:** 2 April 2024 **Accepted:** 13 May 2024

INTRODUCTION

In regions across the globe, extensive research has primarily concentrated on exploring fertility genes in sheep breeds known for their reproductive capacity, such as the Loa breed in Northern Iceland, the Hu breed in China, and the Smal Tail Han breed (Kırıkçı, 2023). Additionally, investigations have also been conducted on breeds like the Garole in Bangladesh (Davis, 2005). The findings from these studies along, with research efforts have significantly advanced our understanding of the factors underlying reproductive mechanisms in sheep (Kırıkçı, 2023). Lately, there has been a rise, in research focusing on fertility genes in breeds of sheep like Mongolian sheep in China, which are known for their low fertility traits (Wang *et al*., 2023). Similarly, investigations have been conducted on the fertility genes of productive breeds such as Meghalaya sheep in India (Khanikar *et al*., 2022). Surprisingly these studies indicate that mutations associated with births can also be found in breeds, with fertility which goes against what was commonly believed. A similar pattern can be observed in sheep breeds in Türkiye including Akkaraman, Ivesi, Dağlıç, Karayaka, Karakaş, and Norduz (Kirikci *et al*., 2021; Ağyar and Kırıkçı, 2022). These breeds tend to have several offspring. Despite their fertility traits researchers have gathered information that can be used for breeding programs using both known and newly discovered mutations. These findings highlight the significance of studying sheep breeds. Akkaraman sheep can be commonly found in the region of Türkiye, known for their remarkable adaptability to different environments, resistance to diseases, and ability to produce well even in challenging nutritional conditions. Nevertheless, each sheep generally produces 1.2 offspring on average, showing a slightly low fertility rate, despite their advantageous attributes (Kırıkçı, 2023).

Reproduction in sheep is influenced by both minor and major genes (Jamshidi *et al*., 2013). In the year 1980, researchers discovered a gene called the Booroola gene (FecB) that plays a big role in the high fertility of Merino sheep. This was the first time a single gene was found to be responsible for sheep having larger litter, and this was determined by looking at the litter size records of these sheep (Gedik, 2023). The FecB gene is thought to have first emerged in the Garole breed, also called the Bengal breed, which lives in the tough and challenging environment of the Sundarban region in India (Jansson, 2014). Extensive scientific investigations have uncovered a collection of genes referred to as the fecundity (Fec) genes, which play a crucial role in regulating the ovulation rate and litter size of sheep. Researchers have identified three distinct fecundity genes in sheep: bone morphogenetic protein receptor type 1B (BMPR-1B), also known as activin-like kinase 6 (ALK-6) or FecB, located on chromosome six; growth differentiation factor 9 (GDF-9) or FecG, found on chromosome five; and bone morphogenetic protein 15 (BMP-15) or FecX, situated on the X chromosome. These genes, through complex genetic mechanisms, exert a significant influence on the reproductive capabilities of sheep, determining the rate at which they ovulate and the size of their litter. The identification and understanding of these fecundity genes have provided valuable insights into the genetic factors that govern the reproductive performance of sheep, with potential applications in selective breeding and livestock management practices (Pramod *et al*., 2013; Bahari *et al*., 2023).

The three genes mentioned are part of the transforming growth factor beta (TGF-β) superfamily, a large group of proteins known for their role in cell growth and differentiation. These particular genes, referred to as "fecundity genes," are specifically associated with the ovary and play a crucial part in regulating the organ's reproductive functions. The TGF-β superfamily is a diverse collection of signaling molecules that originate from the ovary and exert a profound influence on various biological processes, including the development and maturation of reproductive cells and tissues (Hafizuddin *et al*., 2023). By delving deeper into the characteristics and functions of these fecundity genes, researchers can gain valuable insights into the complex mechanisms underlying ovarian physiology and fertility (Çelikeloğlu *et al*., 2021).

Certain mutations in two genes, called FecG and FecX, can cause sheep that have one copy of the mutation to have higher ovulation rates. However, if a sheep has two copies of these mutations, it becomes completely infertile. On the other hand, mutations in the FecB gene have a cumulative effect, meaning they can increase the ovulation rate in sheep, but the sheep remain fertile even with two copies of the mutation (Polley *et al*., 2010). The FecB gene is the result of a small change in the DNA that leads to a different amino acid being produced in a specific part of the BMPR-1B protein. This change in the protein's structure greatly increases the rate at which the ovaries release eggs during the reproductive cycle (Liu *et al*., 2015). The BMPR-1B gene is mostly active in the ovaries of sheep, but is also found in other parts of their bodies and helps with the development of follicles. Upon deleting the BMPR-1B gene from mice, the surrounding cells failed to proliferate and the eggs were unable to fertilize, preventing the mice from bearing offspring. After the FecB mutation was discovered in Merino sheep, that same mutation was later found in different kinds of sheep all around the world (Liu *et al*., 2015).

The study examined the relationship between litter size and genetic variations in the BMPR-1B gene in Akkaraman livestock. It conducted a comprehensive analysis to explore the links between the BMPR-1B gene's genetic structure and the reproductive performance of Akkaraman animals. The study aimed to reveal the factors contributing to litter size, an important aspect of the breed's productivity and performance.

MATERIALS AND METHODS

Ethical Approval

The study conducted was approved by the ethics committee at Ondokuz Mayıs Üniversitesi,

Samsun, with approval number 10-BADK-154. It was retrospectively designed in compliance with the Declaration of Helsinki guidelines.

Study Period and Location

This study was performed at Department of Genetics, Faculty of Veterinary Medicine, Ondokuz Mayıs Üniversitesi, Samsun, Türkiye, during the 2021–2022 period.

DNA Extraction and PCR Amplification

This study utilized 104 Akkaraman sheep specimens and isolated DNA from 5 mL of milk using established protocols. The Genomic DNA isolation kit provided by Sigma-Aldrich (Taufkirchen, Germany) was employed for this purpose, with the extracted DNA then stored at -20°C. Subsequent extraction steps were conducted with the Sigma-Aldrich genomic DNA extraction kit as per the manufacturer's guidelines. A Nanodrop 2000 spectrophotometer was used to quantify and evaluate the acquired DNA's purity (Thermo Fisher Scientific, USA).

The researchers aimed to amplify the BMPR-1B gene region containing the FecB mutation site using a PCR method (Davis *et al*., 2002). For amplifying the FecB fragment of the BMPR-1B gene, a 190 bp amplicon was generated by employing specific primers: FecB-forward (5'-CCAGAGAGGACAATAGCAAAGCAAA-3') and FecB-reverse (5'-CAAGATGTTCATCATGCCTCATCAACAGGTC-3'). The PCR method was executed in Swift MiniPro thermocyclers by ESCO Healthcare in Singapore. Each reaction consisted of ~100 ng genomic DNA, 10 pmol of each primer, and 1x PCR Add Taq master mix supplied by Sigma-Aldrich (Taufkirchen, Germany) within a 16 µl. The PCR process involved an initial denaturation step at 95°C for 3 minutes followed by 35 cycles at specific temperatures for annealing and extension before concluding at 72°C for an additional 5 minutes to finalize the reaction.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows version 27.0 software. Continuous variables were presented as mean ± standard deviation ($\bar{x} \pm SD$), while categorical variables were displayed as percentage frequency (n). Statistical significance was considered for results with $p < 0.05$. The Chi-square test was employed to compare categorical variables.

RESULTS AND DISCUSSION

According to our data, the distribution of animals with different genotypes in the sheep population was analyzed as follows: sheep with the DD genotype were 54.8%, sheep with the DI genotype were 38.5% and sheep with the II genotype were 6.7%. The birth types were as follows for sheep born in two different years: 76% of the sheep born in 2021 had a single birth, 20.2% had twins and 3.8% had a null birth. For sheep born in 2022, 89.4% had a single birth, 10.6% had twins and no empty births were reported. When the number of births of the sheep is analyzed, it is seen that 3.1% of the sheep had one birth, 32.7% had two births, 38.5% had three births, 23.1% had four births, and 1.9% had five births (Table 1).

Data were also analyzed according to age groups and genotype combinations. The distribution in age groups differed according to genotype combinations. However, this difference is not statistically significant ($p > 0.05$). This indicates that age and genotype are not interdependent (Table 2).

On the other hand, there was a relationship between mastitis and disease history. The difference in distribution between "Yes" and "No" responses for both conditions is not statistically significant ($p > 0.05$). This study reported that there is no significant relationship between mastitis and disease history (Table 2).

This study reported that there was a relationship between live weight and milk yield. Milk yield differs according to live weight categories. This difference is statistically significant ($p <0.05$) (Table 2). It can be said that live weight affects milk yield.

Based on birth types and numbers, when 2021 and 2022 birth types are examined, it is seen that "Single" birth types are more common. For

2021 births, there is no statistically significant difference between birth types ($p > 0.05$). However, for 2022 births, "Single" birth types are more common than other types and this difference is statistically significant ($p < 0.05$). Based on number of births and genotype distribution, there was no statistically significant relationship between number of births and genotype distribution ($p > 0.05$) (Table 2). Genotype distribution is similar for each number of births.

Table 1. A total of 104 female Akkaraman sheep were included in the study

Parameter		**Female Akkaraman sheep (n = 104)**
Chest circumference (cm) $\bar{x} \pm SD$		105.69 ± 6.08
Age	Two (%)	4 (3.8)
	Three (%)	33 (31.7)
	Four (%)	40 (38.5)
	Five (%)	25 (24)
	Six (%)	2 (1.9)
Mastitis history	Yes (%)	5 (4.8)
	No (%)	99 (95.2)
Disease history	Yes (%)	99 (95.2)
	No (%)	101 (95.3)
Live weight	Weak (%)	14 (13.5)
	Medium (%)	66 (63.5)
	Heavy (%)	24 (23.1)
Milk yield	Weak (%)	2 (1.9)
	Medium (%)	56 (53.8)
	Good (%)	46 (44.)
Genotype frequency	DD (%)	57 (54.8)
	DI (%)	40 (38.5)
	II (%)	7 (6.7)
Birth type 2021	Single (%)	79 (76)
	Twin (%)	21 (20.2)
	Empty (%)	4 (3.8)
Birth type 2022	Single (%)	93 (89.4)
	Twin (%)	11 (10.6)
	Empty (%)	0 (0.0)
Number of births	One (%)	4 (3.1)
	Two (%)	34 (32.7)
	Three (%)	40 (38.5)
	Four (%)	24 (23.1)
	Five (%)	2 (1.9)

The STRING database provides annotations of the functional relationships between proteins within a cellular environment. By utilizing the STRING database to study the BMPR-1B protein, a detailed examination revealed a group of predicted functional associates with a high level of certainty (score: 0.7). These partners include BMP-4, BMP-15, BMPR-1A, BMP-2, GDF-5, BMPR-2, SMAD-5, BMP-7, BMP-6, and GDF-9. The interconnected network representing the interactions among these proteins can be observed in Figure 1.

Research carried out on sheep populations globally has investigated the disparities in genetic factors that affect the size of litters. A specific gene, identified as FecB, has been recognized as

a key determinant of fecundity in Merino sheep. Notably, this gene is also associated with regulating ovulation rate and litter size. Mutations in significant genes related to multiple births in female sheep enhance ovulation rate and augment the number of lambs per litter (Karslı *et al*., 2012).

Table 2. Genotype distribution of Akkaraman sheep according to several parameters

Parity		Genotype n (%)			χ^2	df	p-value
		DD	DI	II			
Age	Two	1 (1.8)	2 (5.0)	1 (14.3)	13.194	8	0.105
	Three	15 (26.3)	16 (40.0)	2 (28.6)			
	Four	29 (50.9)	9 (22.5)	2 (28.6)			
	Five	10 (17.5)	13 (32.5)	2 (28.6)			
	Six	2 (3.5)	0 (0.0)	0 (0.0)			
Mastitis history	Yes	2 (3.5)	3 (7.5)	0 (0.0)	1.197	2	0.550
	No	55 (96.5)	37 (92.5)	7 (100.0)			
Disease history	Yes	2 (3.5)	3 (7.5)	0 (0.0)	1.197	2	0.550
	No	55 (96.5)	37 (92.5)	7 (100.0)			
Live weight	Weak	5 (8.8)	6 (15)	3 (42.9)	10.568	4	0.032
	Middle	34 (59.6)	29 (72.5)	3 (42.9)			
	Heavy	18 (31.6)	5 (12.5)	1 (14.3)			
Milk yield	Weak	1 (1.8)	1 (2.5)	0 (0.0)	1.061	4	0.900
	Middle	30 (52.6)	21 (52.5)	5 (71.4)			
	Good	26 (45.6)	18 (45)	2 (28.6)			
Birth type 2021	Single	45 (78.9)	28 (70)	6 (85)	5.006	4	0.287
	Twin	11 (19.3)	10 (25)	0 (0.0)			
	Empty	1 (1.8)	2 (5)	1 (14.3)			
Birth type 2022	Single	52 (91.2)	34 (85)	7 (100.0)	1.852	2	0.396
	Twin	5 (8.8)	6 (15)	0 (0.0)			
	Empty	0 (0.0)	0 (0.0)	0 (0.0)			
Number of births	One	1 (1.8)	2 (5.0)	1 (14.3)	10.907	8	0.207
	Two	16 (28.1)	16 (40.0)	2 (28.6)			
	Three	28 (49.1)	10 (25.0)	2 (28.6)			
	Four	10 (17.5)	12 (30.0)	2 (28.6)			
	Five	2 (3.5)	0 (0.0)	0 (0.0)			

The BMPR-1B gene, also known as the FecB gene, is situated on chromosome 6. This particular gene is responsible for coding the receptor for bone morphogenetic protein 1B within the ovary. Initially recognized as a mutated gene associated with multiple birth characteristics in Australia during the 1980s, it has been traced back to Garole or Bengal sheep that were introduced from India in the late 18th century. These sheep were utilized to enhance the reproductive capacity of merino flocks, leading to the establishment of the FecB lineage. Although absent in European sheep breeds, this genetic variation has been identified in short-tailed Han and Hu sheep from China, Kendrapada sheep in India, and Javanese sheep in Indonesia (Çelikeloğlu *et al*., 2018).

A multitude of international studies have investigated genetic variations linked to the reproductive success of sheep. It is crucial to highlight that the majority of investigations in this field have centered on genetic examination, with only a few association studies being accessible (Karslı *et al*., 2012; Dincel *et al*., 2015). Research has been carried out on reproductive genes; however, there is insufficient data available in indigenous sheep varieties regarding the impact of these genes on the litter size of female sheep (Çelikeloğlu *et al*., 2021).

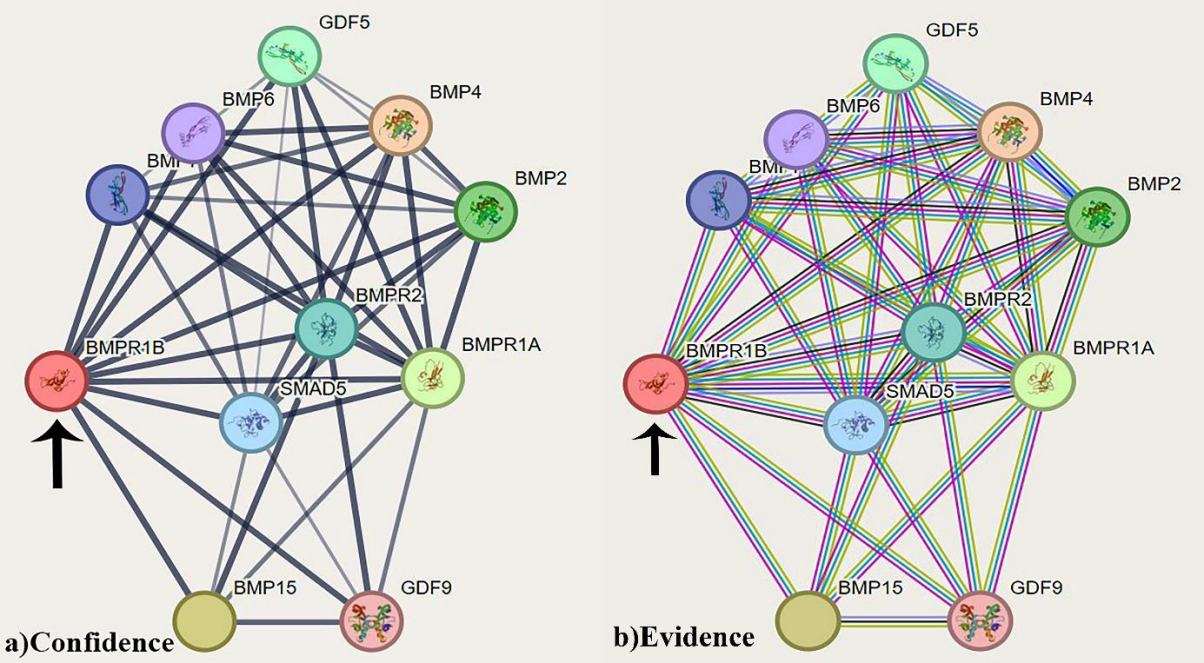

Figure 1. Interactions of BMPR-1B protein, according to STRING database predictions: a) Confidence network: stronger associations are represented by thicker lines; b) This network represents the types of evidence for the association; BMPR-1B protein is evidenced with an arrow.

Considering the retrospective nature of the studies conducted on the Akkaraman sheep population in our country, our study is the first and distinctive one. In the studies conducted so far in Türkiye, FecB allele could not be determined in Sakız and Sakız X Kıvırcık crosses. Karslı and Balcıoğlu could not detect the FecB allele in Akkaraman, Morkaraman, Dağlıç, İvesi, Karakaş, and Tuj sheep (Karslı *et al*., 2011).

In a study conducted in Türkiye, the genes BMPR-1B, BMP-15, and GDF-9, which are associated with multiple births in sheep, were analyzed. It was found that all three genes exhibited a monomorphic structure (Çelikeloğlu *et al*., 2018). A study on BMP-15 and GDF-9 gene variations and their impact on litter size in the Akkaraman Anatolian sheep breed revealed that the genotypic composition did not have a notable influence on the litter size of Akkaraman sheep ($p > 0.05$) (Kırıkçı, 2023).

The study findings can be summarized as follows: The genotype distribution of Akkaraman sheep did not show any notable correlation with factors such as age, mastitis and disease history, milk yield, birth type in 2021–2022, and number of births ($p > 0.05$). However, a significant association was observed between the genotype distribution and live weight ($p < 0.05$). It is worth mentioning that the divergence in our results from global studies can be attributed to the constraining factor of limited reproductive numbers within the selected sheep population.

The BMPR-1B gene is widely expressed in various tissues such as the ovary, brain, pituitary, kidney, skeletal muscle, uterus, prostate, and testis However, most studies on BMPR-1B have focused on the ovary or a single tissue and have not considered the HPG axis. This may result in many meaningful genes being missed (Ma *et al*., 2023; Belgania *et al*., 2023). Research conducted to analyze the impact of the FecB mutation in the BMPR-1B gene on ovulation rate and litter size revealed that each instance of the FecB mutation was found to elevate both ovulation rate and litter size, as documented by Fabre and colleagues (Fabre *et al*., 2006). Previous studies have shown that this mutation was found in the Australian Merino (Davis, 2004), Indian Garole and Nilagiri sheep (Sudhakar *et al*., 2013), Indonesian Javanese (Davis *et al*., 2006), Chinese Small-tailed Han and Hu sheep (Jia *et al*., 2005), Iranian Baluchi and Kalehkoohi sheep (Mahdavi *et al*., 2014). These findings suggest that the FecB

mutation is predominant in different Asian sheep breeds and supports this productivity increase.

A research analysis focused on the manifestation, alteration, and correlation of BMPR-1B with litter size in small-tailed Han sheep revealed that the AG-GG, AA- GG, and GG-GG genotypes exhibited notably higher levels within the BMPR-1B gene when contrasted with alternative genotypes ($p < 0.05$) (Wen *et al*., 2021). An examination of Mongolian sheep with the ability to give birth to one or two offspring, and Little Tail Han sheep capable of bearing multiple offspring, revealed a correlation between blood protein concentration linked to BMPR-1B mRNA expression during various life stages and reproductive phases of both Mongolian and Little Tail Han sheep. This association was found to correspond with periods of estrus and anaestrus (Hanifah *et al*., 2020; Zhang *et al*., 2020). An investigation was conducted on potential variations in the BMPR-1B gene using 100 samples of Markhoz goats from Iran. The findings indicated that none of the individuals examined possessed the known FecB mutant allele. However, the study uncovered two novel mutations (775A > G and 777G > A) in exon 8 of the BMPR-1B gene, warranting additional scrutiny (Pourali Dogaheh *et al*., 2020; Amrullah *et al*., 2023). An examination of the polymorphism of the BMPR-1B gene in Assam hill goats was conducted using the PCR-RFLP technique. The results of this study explicitly show that the FecB mutation is absent from Assam hill goats (Dutta *et al*., 2014).

In the study, the BMPR-1B gene, which determines multiple births, was analyzed and it was determined that Akkaraman sheep were monomorphic in terms of this gene. In light of this information, it was concluded that more comprehensive studies should be carried out regarding this gene determining multiple births in the Akkaraman sheep breed and the breed should be examined in terms of other genes affecting multiple births.

The main theme of this study can be expressed as follows. Reproductive traits such as ovulation rate and litter size in sheep are traits of high economic importance, but as these traits are expressed only in one sex and in mature animals, their inclusion in selection strategies is limited. Therefore, the study of genes associated with reproductive events provides genotypic data that are more useful for the genetic improvement of sheep in a short time. Such reproductive traits in sheep are genetically controlled by genes with both additive and major effects. The first gene identified in Merino sheep in Australia has significant effects on sheep reproduction and has gained much popularity in sheep breeding due to its enormous economic value.

CONCLUSION

Bone morphogenetic protein receptor-1B (BMPR-1B) gene is on chromosome 6 and the FecB allele is the result of a single mutation in this gene, one copy of this allele causes a significant increase in ovulation rate, thus increasing lambing per litter.

ACKNOWLEDGEMENTS

This study received no funding from public agencies.

AUTHORS' CONTRIBUTIONS

RA: Conceptualization, Project administration, Resources, Validation, Writing – original draft. ED: Conceptualization, Formal Analysis, Resources, Software, Visualization, Writing – review and editing. SK: Data curation, Formal Analysis, Resources. SY: Data curation, Formal Analysis, Resources. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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