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# Investigation of GDF9 (FecG1), BMPR1B (FecB), and BMP15 (FecX G , FecX I , FecX H , FecX B ) mutations in Kangal Akkraman rams

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## Abstract

# Background

This study aimed to characterize the alleles of fecundity genes in rams mated with female Kangal Akkaraman sheep used in an animal breeding project in Türkiye. It has been reported that heterozygous forms of the alleles of the fertility genes BMPR1B, GDF9, and BMP15 observed in different breeds are associated with multiple births. DNA isolation was performed with the phenol-chloroform-isoamyl alcohol method. The isolated DNA was genotyped using the PCR-RFLP for the BMPR1B, BMP15, and GDF9 genes.

# Results

According to the results obtained, the $FecX^{G}$, $FecX^{I}$, $FecX^{H}$ SNPs of the BMP15 gene, and the FecB SNP of the BMPR1B gene were determined to be wild type in all genotypes examined in Kangal Akkaraman sheep, while the genotypes of the FecG of the GDF9 gene and the $FecX^{B}$ of the BMP15 gene were determined to be polymorphic. The frequency of the A allele in the GDF9 gene was found to be 0.17, while the frequency of the G allele was found to be 0.83. Rams with the AA (1.54), AG (30.76), and GG (67.70) genotypes were identified. Due to X-linked inheritance in the BMP15-$FecX^{B}$ allele, the frequency of the mutant allele was found to be 0.35.

# Conclusion

These results indicate that polymorphisms in the BMP15-$FecX^{B}$ and GDF9 genes, along with high allelic diversity, provide a strong genetic foundation for breeding programs aimed at increasing multiple births. Furthermore, the high frequency of these variations in our population presents an important opportunity for identifying local genetic resources that could enhance productivity in Turkish sheep breeding.

## Background

The reproductive performance of sheep can be affected by the complex interactions of environmental conditions (i.e., climate, care, and feeding), but studies have shown that genetic factors can also play important roles in variable litter size. Studies have identified multiple mutations associated with lambing rate per pregnancy that affect reproductive performance in many different sheep breeds. However, very few mutations have been identified that are common across breeds. The production of multiple offspring per pregnancy is largely dependent on the ovulation rate. In some sheep breeds, the number of offspring per pregnancy and ovulation rate have been reported to be controlled by a single gene [1, 2, 3]. However, other studies have reported that fertility-related traits have low heritability and are controlled by multiple genes. Safari et al. [4] stated that the rate of increase in lambing rate through classical selection favoring twin births would only be 1–2%. Therefore, it has been reported that classical selection methods are limited [5, 6, 7]. Genes involved in controlling productivity in sheep include bone morphogenetic protein receptor-1B BMPR1B [8], bone morphogenetic protein 15 (BMP15) [1, 2], and Growth Differentiation Factor 9 (GDF9) [2]. The first major gene identified for productivity in sheep in 1980 was the Booroola gene (FecB-BMPR1B). When the performance of flocks crossbred with animals carrying the Booroola mutation in different countries was examined, it was found that carriers of this mutation had higher sheep productivity, leading to significant economic returns for the sheep industry. BMPR1B (FecB), located on the 6th chromosome in sheep, is known as a receptor for differentiated bone morphogenetic proteins (BMP) and is associated with twinning. Other genes identified as playing a role in controlling reproductive performance in sheep are GDF9 [2] and BMP15 [1, 2]. Mutations identified in the relevant genes tend to increase ovulation rates and

multiple birth rates in sheep [9]. These genes, belonging to the TGF-β (transforming growth factor beta) superfamily, play an important role in ovulation rate and litter size [10]. These genes are produced by the ovaries and exert potent effects [11]. Furthermore, these two oocyte-specific factors induce mitosis in follicular somatic cells during follicular development, along with differentiation, via a paracrine signaling pathway [12]. The BMP15 gene significantly affects reproductive performance [13]. Also known as the GDF9B (FecX) gene, BMP15 is located on the X chromosome [1] and encodes bone morphogenetic protein 15, which plays an important role in follicular development in sheep [2]. The gene that improves reproductive performance in heterozygous Romney sheep is called the Inverdale gene ($FecX^{I}$). This mutation, which has also been identified in different sheep breeds, is referred to by breed-specific names such as $FecX^{B}$, $FecX^{G}$, $FecX^{H}$, $FecX^{I}$, $FecX^{L}$, $FecX^{R}$, $FecX^{Gr}$, and $FecX^{O}$ [14, 15]. It has been reported that sheep with two inactive copies of the BMP15 gene (homozygous animals) are sterile, while sheep with a single inactive BMP15 gene (heterozygous animals) exhibit an increased ovulation rate and incidence of twin or triplet births [1, 2, 9, 15, 16, 17, 18, 19]. The GDF9 gene, a member of the Transforming Growth Factor Beta (TGF-β) superfamily, consists of 1 intron and 2 exons located on chromosome 5 in sheep. It is a gene necessary for the regular maintenance of standard follicular development in sheep. Eight different mutations (G1-G8) have been reported on this gene [20]. Heterozygous sheep that are mutant for both the GDF9 and BMP15 genes are fertile, and the effects of these mutations on ovulation rate are additive [2]. BMP15 fertility alleles show an X-linked dominant inheritance pattern with sterility in homozygous females, while GDF9 fertility alleles show an autosomal dominant inheritance pattern with sterility in homozygous females [1, 2, 15]. It has been demonstrated that the exceptional fertility in the Cambridge and Belclare breeds is due to mutations in the X-linked BMP15 ($FecX^{G}$ and $FecX^{B}$ mutations) and GDF9 (FecGH mutation) genes. Inverdale and Hanna sheep heterozygous carriers of mutations in BMP15 exhibit an increase in ovulation rate (+ 0.9 [2]), while heterozygous sheep for FecGH show a greater increase in ovulation rate (+ 1.8; [2]). In double heterozygotes, the observed ovulation rate (e.g., $FecX^{G}/FecX^{+}$; $FecG^{H}/FecG^{+}$) reflects the additive effect of these mutations [2]. However, homozygous carriers of either the BMP15 or FecGH mutations and sheep with the $FecX^{G}/FecX^{B}$ genotype have been reported to be sterile due to arrested follicular development [29]. According to studies, sheep with a heterozygous genotype (1.88 – 1.78) have higher ovulation rates compared to sheep with a homozygous genotype (1.22 – 1.16) for the GDF9 gene [20, 21, 22]. The BMPR1B (bone morphogenetic protein receptor-1B) gene is a dominant autosomal gene located on chromosome 6 in goats and sheep, responsible for twinning and fertility [10, 15, 23]. This gene was first identified in Booroola Merino sheep [13]. Specifically, the A→G transition corresponding to nucleotide 830 of the 8th exon of mature mRNA is known as the Booroola gene or FecB. This mutation is thought to have originated in the Garole sheep, reported to be a northeast Indian breed introduced to Australia in 1792, and has since been reported to have spread to many sheep breeds worldwide [16]. There are more than 20 single nucleotide polymorphisms (SNPs) for the sheep BMPR1B gene [24]. Among these, c. 864 T > C, c. 1,113 C > A, and c.746A > G are associated with sheep reproduction [25, 26]. c.746A > G non-synonymous SNP mutation study found that damage to the BMP system during follicle development increased the average number of eggs laid in Hu sheep [27], Australian Booroola Merinos [28], and Small-tailed Han sheep [29]. This gene also has an additive effect on ovulation and lambing rate, but development has adverse effects on body mass during pregnancy and fetal growth [30]. The BMPR1B gene is considered a potential candidate gene for regulating sheep reproduction [24]. The gene has been reported to be widely expressed in sheep pituitary, hypothalamus, ovaries, and uterus [31, 32]. BMPR1B gene expression has been detected in adult pig and mouse follicles using immunohistochemistry [(Yi et al., 2001; Sun et al., 2010). Reproductive problems such as pseudopregnancy and irregular estrus cycles have been reported in mice lacking BMPR1B [33]. Performance comparisons of flocks crossed with Booroola in the sheep industry in different countries have reported that carriers of this mutation provide significant economic returns and have higher sheep productivity. Some studies have associated the FecB gene with lower birth weight in lambs and lighter mature body weight in sheep, as well as slower growth rate after weaning [30]. However, another study reported the opposite, stating that the FecB gene has a positive effect on

early postnatal body growth in the Chinese Merino productive meat breed [34]. Different alleles of genes studied in different races have been reported [1, 2, 8, 13, 15, 17, 19, 21, 24, 35–50].

In Turkiye, six different mutations (FecX$^{B}$, FecX$^{I}$, FecX$^{G}$, FecX$^{H}$, FecGH, FecB) located on the candidate genes BMP15, BMPR1B, and GDF9 in our native sheep breeds were investigated using the PCR-RFLP method [51, 52, 53, 55, 56]. The literature reports that the majority of genes belonging to the breeds studied are monomorphic or do not possess the desired mutation [51, 53, 54, 55, 56, 57]. However, heterozygous alleles were found in some of the studies examined. For example, in a study conducted by Gürsel et al. [52] in Turkey, the analysis of mutations in the BMP15, BMPR1B, and GDF9 genes, which are suggested to be associated with multiple birth in Ivesi, Sakız, Kıvırcık, and İmroz breed sheep, revealed that the genotypes in the FecB, FecX$^{I}$, FecX$^{B}$, and FecX$^{H}$ mutations in the BMP15 gene were found to be homozygous, while only the genotypes for the FecGH(AB) and FecX$^{G}$(AB) mutations in the GDF9 gene were found to be heterozygous in all breeds. Additionally, in a study conducted on the Of sheep breed, which has a twin rate of 35–40%, it was reported that genotypes belonging to the FecG$^{I}$ (GDF9) allele were found to be heterozygous as a result of the PCR-RFLP method. The polymorphism of SNPs identified in three genes associated with multiple and single birth phenotypes in Ramlıç and Dağlıç sheep was examined. In Ramlıç sheep, four SNPs and one deletion mutation in the BMPR1B gene, and in Dağlıç sheep, five SNPs were found to have significant positive regression coefficients in the desired rare allele direction as a result of statistical analyses [58]. The Kangal Akkaraman variety, a local type bred in the provinces of Sivas and Malatya, has been registered as a separate breed by the Communiqué published in the Official Gazette dated 14.08.2012 and numbered 28384, in addition to the normal genotype of the Akkaraman breed, which constitutes approximately 40% of the sheep population bred in Turkey [59]. Karslı et al. [60] (n:42) conducted PCR-RFLP analysis on the Kangal sheep breed for the FecX$^{G}$, FecX$^{H}$, and FecB alleles, but no alleles associated with multiple births were identified. The average twin birth rate in Kangal sheep has been reported as 22% [59].

This planned study aims to determine the genotypic structures of rams mating with Kangal Akkaraman sheep, as defined in the project coded TSA-2022-11745, supported by the Erciyes University Scientific Research Coordination, using the PCR-RFLP method for 6 different SNPs of the GDF9, BMP15, and BMPR1B genes.

## Discussion

Sheep farming in our country is primarily carried out for meat production. According to 2022 TÜİK data, sheep account for approximately 60% of farmed animals (cattle, sheep, goats, buffalo). According to the last 10 years of TÜİK data, sheep account for an average of 10% of red meat production and 6% of milk production. Meat yield from sheep farming is closely related to lamb production [63]. It has been reported that breeding methods for lamb yield should focus on identifying mutations in major genes that may be related, rather than selection [64]. Therefore, rather than classical selection methods, it is important to use molecular breeding methods to obtain genotypic data and correlate it with the phenotype. In the literature, there are many studies on alleles and genotypic studies determined on the BMP15, GDF9, and BMPR1B genes, which are reported to be particularly related to this trait in different sheep breeds.

According to the results obtained in our study, polymorphism was observed only in the BMP15 FecX$^{B}$ and GDF9 FecG alleles among the 6 SNPs examined. When studies conducted on these genes in the literature were reviewed, the literature reported for FecX$^{B}$ is as follows. Shi et al. [65] reported that no FecX$^{B}$ mutation was observed in the Xingjiang Cele black sheep breed in their study. Vacca et al. [66] reported in their study that the FecX$^{B}$ mutation was not found in five sheep breeds raised in Tunisia: Barbarine, Queue Fine de L'Ouest, Noire de Thibar, Sicilo-Sarde, and D'man. Gürsel et al. [52] reported that the FecX$^{B}$ allele was monomorphic in the Sakız, Kıvırcık, İvesi, and İmroz sheep breeds. Karslı et al. [54] reported that the FecX$^{B}$ allele was monomorphic in the Akkaraman, İvesi, Dağlıç, Tuj, and Kararkaş sheep breeds in their study. Mohammadi et al. [67] reported that the FecX$^{B}$ locus was monomorphic in the Lory-Bakhtiari and Arabic

sheep breeds of Iran. In a study conducted by Wan Somarny et al. [68], it was reported that there were samples that were not homozygous carriers of FecXB in Malin and Dorper sheep breeds. In a study conducted by Paz et al. [22], it was found to be monomorphic in terms of the $FecX^B$ allele. In a study conducted by Al-Barzinji et al. [69], it was reported that the Kurdi, Avasi, Arabi, and Hamdani sheep breeds were monomorphic for $FecX^B$. In a study conducted by Lassoued et al. [39], it was reported that the Tunisian Barbarine Sheep did not have the $FecX^B$ mutant allele. In a study conducted by Wang et al. [70], it was reported that $FecX^B$ was monomorphic in the Hu sheep breed. In a study conducted by Dinçel et al. [56] on Sakız sheep, it was reported that the $FecX^B$ allele was monomorphic. Mohamed et al. [71] reported that $FecX^B$ was homozygous in Watish Sudanese desert sheep.

Sasi et al. [72] reported that mutations associated with $FecX^B$ were not observed in the entire population of tropical goats (Attappady Black and Malabari goats). Niu et al. [73] reported that no polymorphism was observed on the $FecX^B$ allele. Kırıkçı et al. [74] reported that the $FecX^B$ allele was monomorphic in the Karakaya sheep breed and that they did not carry the mutated allele. In a study conducted by Sepúlveda et al. [75], it was reported that the Araucana, Austral, Corridale, Chilota Creole, and Merino sheep breeds were monomorphic for $FecX^B$. In a study conducted by Bozhilova-Sakova et al. [76], it was reported that the $FecX^B$ allele was monomorphic in Bulgarian dairy sheep. In addition to all these studies, in a study conducted by Farag et al. [77] in goats, it was reported that the $FecX^B$ allele was heterozygous in 2 of 93 samples. Additionally, in the study reported by Bekoe et al. [78], it was reported that Ghanaian sheep breeds have AA and AB genotypes for the $FecX^B$ allele. In our study, the $FecX^B$ allele was also found to be polymorphic. The frequency of the wild-type allele was found to be 0.65, while the frequency of the mutant allele was found to be as high as 0.35. The fact that the gene under investigation is located on the X chromosome and that the population consists of rams indicates that the relevant allele can be observed in many sheep and that it is highly likely to be effective in twinning.

In the study, research on the GDF9 G1 allele, another gene found to be polymorphic, is as follows. In a study conducted by Wan Somarny et al. [68], it was reported that there were samples that were not homozygous carriers for FecG in Malin and Dorper breed sheep. In a study conducted by Al-Barzinji et al. [69], it was reported that the Kurdi, Avasi, Arabi, and Hamdani sheep breeds were monomorphic for FecG. In a study conducted by Wang et al. [70], it was reported that GDF9 alleles were monomorphic in the Hu sheep breed. In a study conducted by Sasi et al. [72] on tropical goats (Attappady Black and Malabari goats), it was reported that mutations associated with GDF9-G1 alleles were not observed in the entire population. However, in a study conducted by Pineda et al. [79], it was reported that A and G alleles were present in the population in the FecGI analysis of the Colombian Crole sheep breed. In a study conducted by Paz et al. [22], heterozygous individuals were reported in Chilota and Araucana sheep breeds in terms of the GDF9 allele. In a study conducted by Mohamed et al. [71], it was reported that heterozygous individuals of FecG1 were present in the population of Watish Sudanese desert sheep. In another study conducted by Kırıkçı, [80], it was determined that the GDF9-G1 (FecG1) allele was polymorphic in the Akkaraman sheep breed, with the A and G alleles being 0.13 and 0.87, respectively. In a study conducted by Sepúlveda et al. 75], it was reported that the GDF9 G1 polymorphism was polymorphic in the Araucana, Austral, Corridale, Chilota Creole, and Merino sheep breeds. In our study, the frequency of the A allele of the GDF9 G1 allele was determined to be 0.17, while the frequency of the G allele was 0.83. Aksel et al. [81] found the SNP to be polymorphic in their study. The frequency of the A allele was reported to be 0.11 and the frequency of the G allele was reported to be 0.89 in the relevant study. In our study, the homozygous AA genotype was found in one ram sample, but no AA genotype was found in the study conducted by Aksel et al. [81]. Considering that the rams in question will mate with many ewes and be effective in the gene pool, the importance of the study becomes even more apparent.

## Conclusion

These results indicate that the significant polymorphisms detected in the BMP15 $FecX^B$ and GDF9 FecG genes in our study, when evaluated together with the high allele diversity observed in the ram population, provide an important genetic foundation for molecular breeding programs aimed at increasing multiple birth rates in flocks; Furthermore, despite these genetic variations being reported as monomorphic in many sheep breeds, their relatively high frequencies in our population indicate a noteworthy opportunity for identifying local genetic resources with the potential to increase productivity in Turkish sheep farming and for developing sustainable breeding strategies.

## Methods

# Identification of animals

In the planned study, Kangal Akkaraman rams that mated sheep identified as samples in the project with code TSA-2022-11745, supported by the Scientific Research Coordination Unit of Erciyes University and completed in December 2023, were identified. This study aimed to genotype the rams that mated with the sheep identified in the BAP project with code BAP TSA-2022-11745 (according to 2021 data) for SNPs related to the relevant genes. According to the results of the power analysis, the minimum number of animals to be included in the study was determined to be 65, with an effect size of w = 0.35, α = 0.05, β (test power) = 0.80, and sd = 1. In order to ensure access to the animals to be included, the selection of rams mated with ewes that had twin and single births, especially in the 2021 data, was targeted.

## Blood Sample Collection and DNA Isolation

Blood samples used in molecular studies were obtained from blood samples collected from rams for routine disease control. Blood collected in $K_3$EDTA vacuum tubes was transported to the laboratory via a cold chain and stored at -20°C until the DNA isolation stage. DNA was isolated using the phenol-chloroform-isoamyl alcohol method. The quality control of the DNA was determined using a Biospech-nano (Shimadzu Ltd, Japan) spectrophotometer.

## PCR-RFLP

For the PCR process, a total reaction volume of 20 µl will be prepared by adding 1 X buffer solution, MgCl2 (2.0 mmol/L), 0.5 U Taq DNA polymerase, dNTP (0.25 mmol/l), and 3 µl DNA (50 ng/µl). The PCR reaction was completed by performing a total of 35 cycles at 95˚C for 5 minutes pre-denaturation, 95 ˚C for 30 seconds annealing temperature (according to the annealing temperatures of the primers), 72˚C for 30 seconds, followed by holding at 72 ˚C for 7 minutes.

The band sizes of the obtained PCR products, restriction enzymes, and fragment sizes are reported in Table 1. The restriction endonuclease digestion protocols for the obtained PCR products were performed according to the manufacturer's protocol. The obtained digestion and PCR products were run on a 2% agarose gel electrophoresis and visualized under UV light.

Table 1
Genes, regions, primers, and band sizes for PCR-RFLP regions.

| Gene | Allele | Primerler (Forward- Reverse) | Restriction Enzyme | Bant Sizes | References |
|---|---|---|---|---|---|
| GDF9 | FecG (G1) | F: GAAGACTGGTATGGGGAAATG<br>R: CCAATCTGCTCCTACACACCT | Hha*I* | AA: 410, 52 bp<br>GG: 254, 156,52 bp<br>AG: 410, 254, 156, 52 bp | [2] |
| BMP15 | $FecX^B$ | F: GCCTTCCTGTGTCCCTTATAAGTATGTTCCCCTTA<br>R: TTCTTGGGAAACCTGAGCTAGC | BstDE*I* | Wild: 122, 31 bp<br>Mutant:153 bp | |
| | $FecX^G$ | F: CACTGTCTTCTTGTTACTGTATTTCAATGAGAC<br>R: GATGCAATACTGCCTGCTTG | Hinf*I* | Wild type: 112, 29 bp<br>Mutant: 141 bp | |
| | $FecX^I$ | F: GAAGTAACCAGTGTTCCCTCCACCCTTTTCT<br>R: CATGATTGGGAGAATTGAGACC | Xba*I* | Mutant: 124, 30 bp<br>Non-carrier:154 bp | |
| | $FecX^H$ | F: TATTTCAATGACACTCAGAG<br>R: GAGCAATGATCCAGTGATCCCA | Ah1*I* | Mutant: 218, 22 bp<br>Non-carrier: 240 bp | [61] |
| BMPR1B | FecB | F: CCA GAG GAC AAT AGC AAA GCA AA<br>R: CAAGATGTTTTCATGCCTCATCAACACGGT C | Ava*II* | Mutant: 160 bp<br>Non-carrier: 190 bp | [62] |

Table 1 **will be insert here…**

# Statistical Analysis

Both genotypic and allelic frequencies were calculated for the obtained data. The Hardy-Weinberg equilibrium of the population was determined using chi-square analysis.

## Results

Images of PCR reactions established for 6 SNPs of the GDF9, BMP15, and BMPR1B genes in the 65 samples examined are reported (Fig. 1-A, Fig. 2-A, Fig. 3-A, Fig. 4-A, Fig. 5-A, Fig. 6-A). According to the gel electrophoresis results obtained from the cutting reactions established with the restriction enzymes reported in the literature for each SNP, the genotypes associated with the FecG (Fig. 1-B) SNP of the GDF9 gene and the $FecX^B$ (Fig. 2-B) SNP of the BMP15 gene

were found to be polymorphic. However, the $FecX^G$ (Fig. 3-B), $FecX^I$ (Fig. 4-B), and $FecX^H$ (Fig. 5-B) SNPs of the BMP15 gene and the FecB (Fig. 6-B) SNP of the BMPR1B gene were determined to be wild type and monomorphic in all genotypes examined in Kangal Akkaraman sheep.

Figure 1 **will be insert here…**

Figure 2 **will be insert here…**

Figure 3 **will be insert here…**

Figure 4 **will be insert here…**

Figure 5 **will be insert here…**

Figure 6 **will be insert here…**

Since the $FecX^B$ allele of the BMP15 gene is located only on the X chromosome and exhibits a sex-linked inheritance pattern, each ram carries only the W or M alleles. The frequency table is reported in Table 2.

Table 2
Genotypic and allelic frequencies of the FecG allele of the GDF9 gene in the population results of the Hardy-Weinberg equilibrium chi-square analysis and BMP15- $FecX^B$ genotypes.

| Gene | Genotypes | Expected Value | Observed Value (% Frequency) | Frequency of A Allele | Frequency of G Allele | Chi-square, *P* value |
|---|---|---|---|---|---|---|
| **GDF9** | AA | 1.86 | 1 (1.54) | | | 0.5777 P = 0.4472 |
| | AG | 18.28 | 20 (30.76) | 0.1692 | 0.8308 | |
| | GG | 44.86 | 44 (67.70) | | | |
| | **Observed W (Allele frequency)** | | | **Observed M (Allele frequency)** | | |
| **BMP15- Fec $X^B$** | 42 (0.6462) | | | | 23 (0.3538) | |

Table 2 **will be insert here…**

# Abbreviations

GDF9 Growth Differentiation Factor 9

BMPR1B Bone Morphogenetic Protein Receptor Type 1B

BMP15 Bone Morphogenetic Protein 15

DNA Deoxyribonucleic Acid

SNP Single Nucleotide Polymorphism

PCR-RFLP Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

TGF-β Transforming Growth Factor Beta

TÜİK Turkish Statistical Institute (Turkish Statistical Institute, TSI)

## Declarations

**Ethics approval and consent to participate**

The animal studies were reviewed and approved on May. 3rd, 2023 by the Animal Ethics Committee of Erciyes University Animal Experiments Local Ethics Committee (ERU-HADYEK. (Approval no: 23/102 – 03.05.2023).

**Consent to publication**

Not applicable.

**Competing Interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Author Contribution

E.G.A: Writing–original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Funding acquisition, Project administration. E.Ç.G: Writing–review and editing, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Funding acquisition. M.A: Writing–review and editing, Data curation, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. Ö.O.D: Writing–review and editing, Project administration, Visualization, Validation, Supervision, Methodology, Conceptualization.

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# Data Availability

This research was supported by the Erciyes University Scientific Research Projects Coordination Unit, project number: TSA2023-13002.

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## Figures

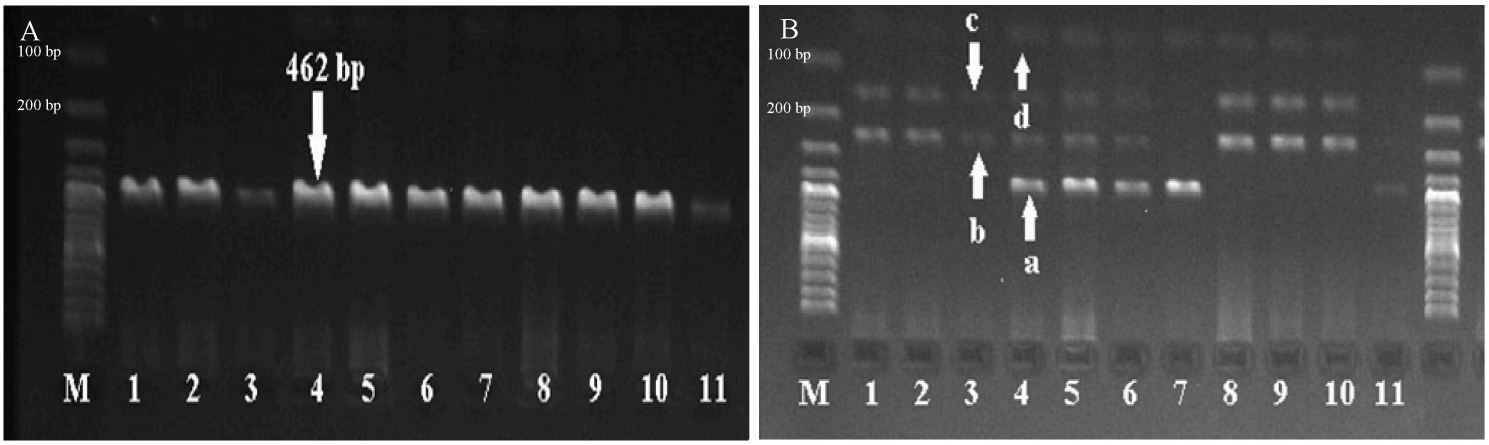



**Figure 1**

(A) Gel-electrophoresis image of PCR products for GDF9 gene FecG SNP (462 bp). (B) Gel-electrophoresis image of restricted PCR products for GDF9 gene FecG SNP; M: Marker with 100 base pairs; a: 410 bp, b: 254 bp, c: 156 bp, d: 52 bp; GG: 1, 2, 3, 8, 9, 10 (254, 156, 52 bp) AG: 4, 5, 6, 11 (410, 254, 156, 52 bp); AA: 7 (410, 52 bp).

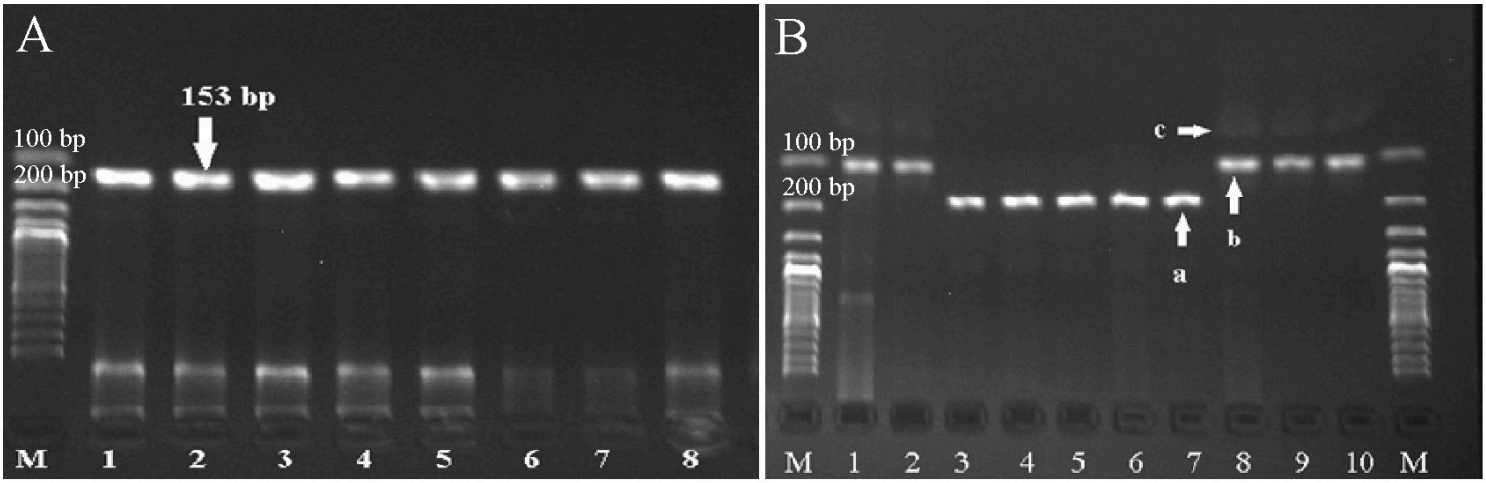



**Figure 2**

(A) Gel-electrophoresis image of PCR products for BMP15 gene $FecX^B$ SNP (153 bp). (B) Gel-electrophoresis image of restricted PCR products for BMP15 gene $FecX^B$ SNP; M: Marker with 100 base pairs; a: 153 bp, b: 122 bp, c: 31 bp; Wild type RFLP products: 1, 2, 8, 9, 10 (122, 31bp). Mutant products: 3, 4, 5, 6, 7 (153 bp).

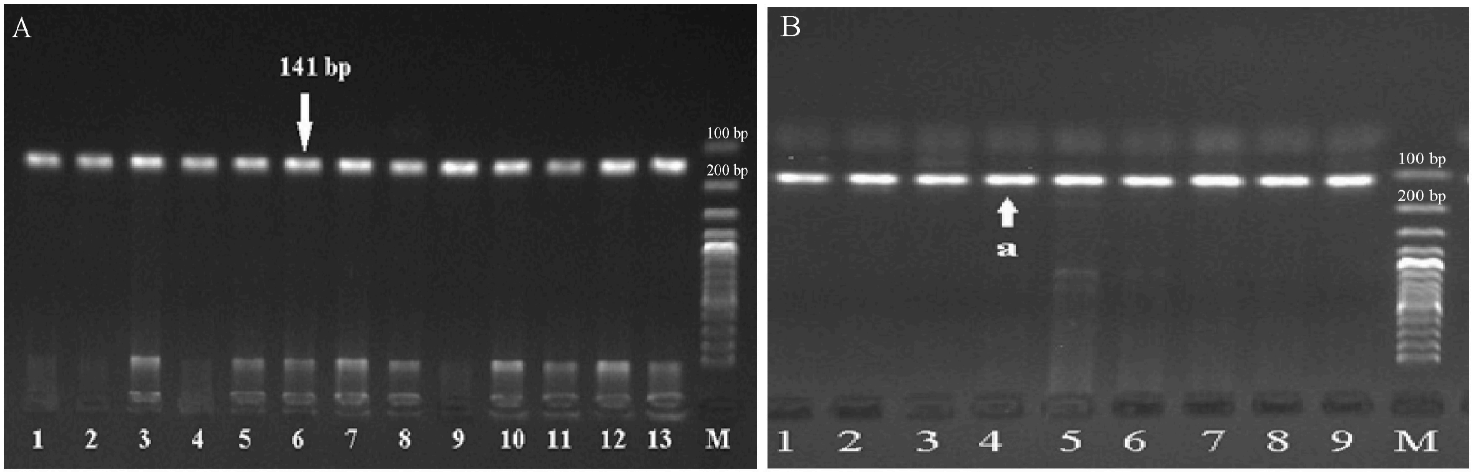



**Figure 3**

(A) Gel-electrophoresis image of PCR for BMP15 gene $FecX^G$ SNP (141 bp). (B) Gel-electrophoresis image of restricted PCR products for BMP15 gene $FecX^G$ SNP; M: Marker with 100 base pairs; a: 141 bp, b: 112 bp; Wild type RFLP products: 1-9 (112 bp).

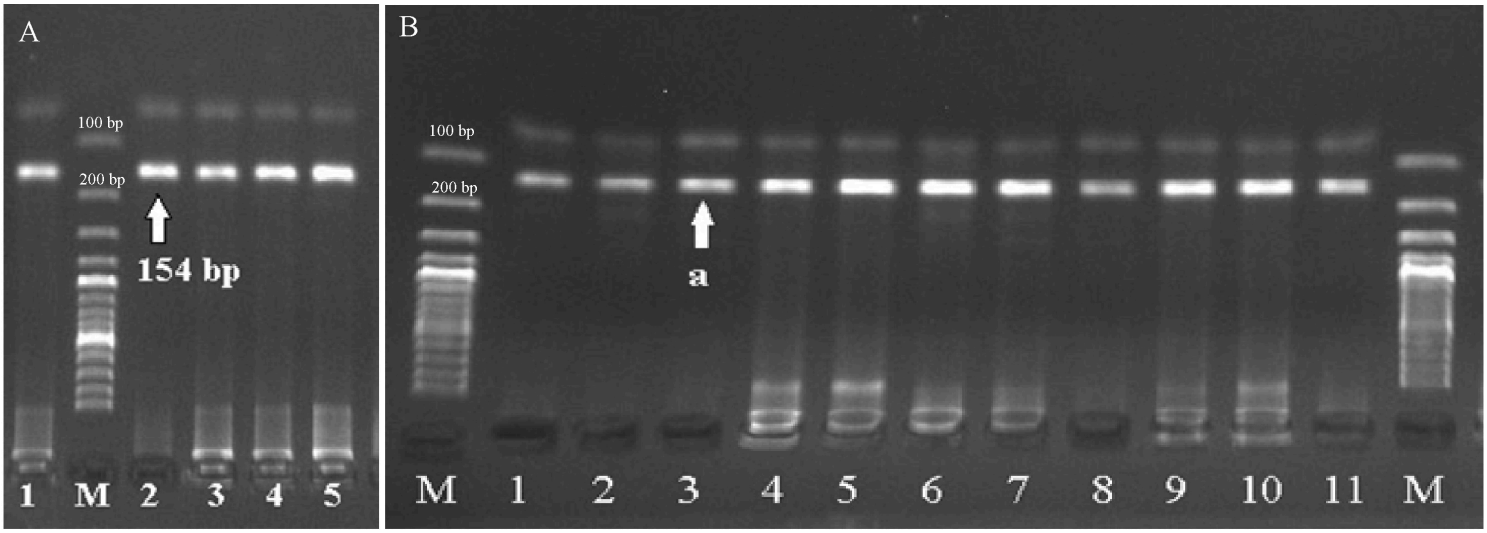



**Figure 4**

(A) Gel-electrophoresis image of PCR products for BMP15 gene $FecX^I$ SNP (154 bp). (B) Gel-electrophoresis image of restricted PCR products for BMP15 gene $FecX^I$ SNP; M: Marker with 100 base pairs; a: 154 bp; Non-carrier RFLP products: 1-10 (154 bp).

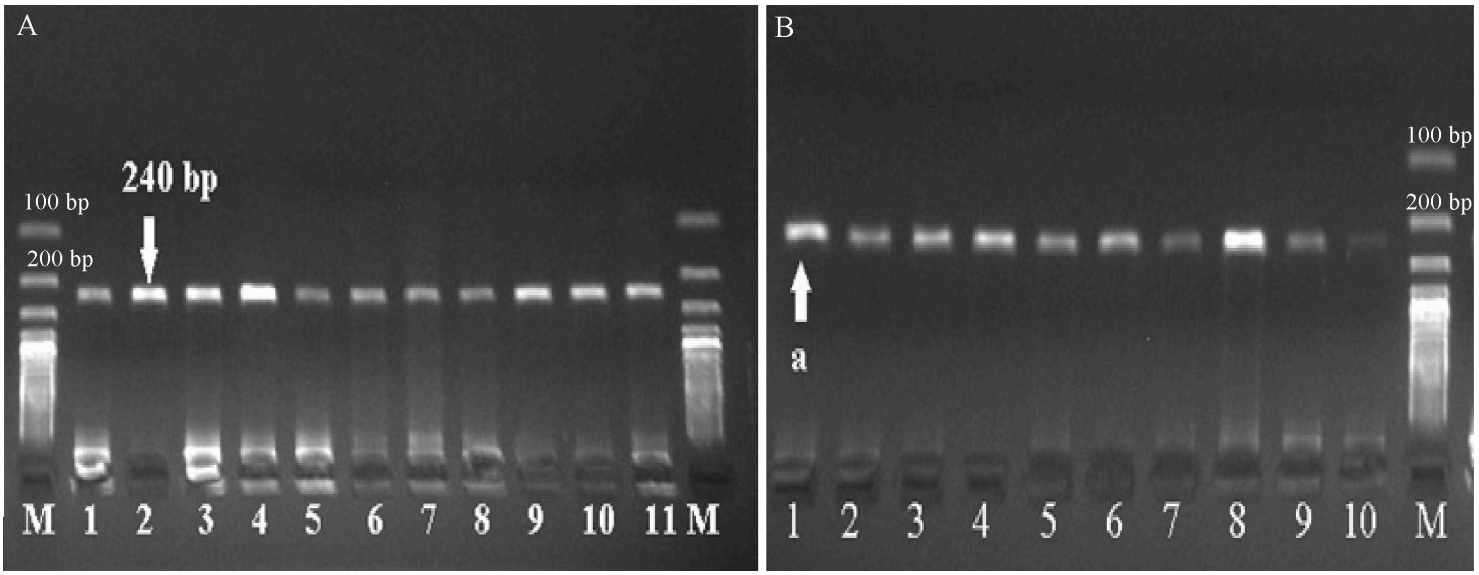



**Figure 5**

(A) Gel-electrophoresis image of PCR products for BMP15 gene $FecX^{H}$ SNP (240 bp). (B) Gel-electrophoresis image of restricted PCR products for BMP15 gene $FecX^{H}$ SNP; M: Marker with 100 base pairs; a: 240 bp; Non-carrier RFLP products: 1-10 (240 bp).

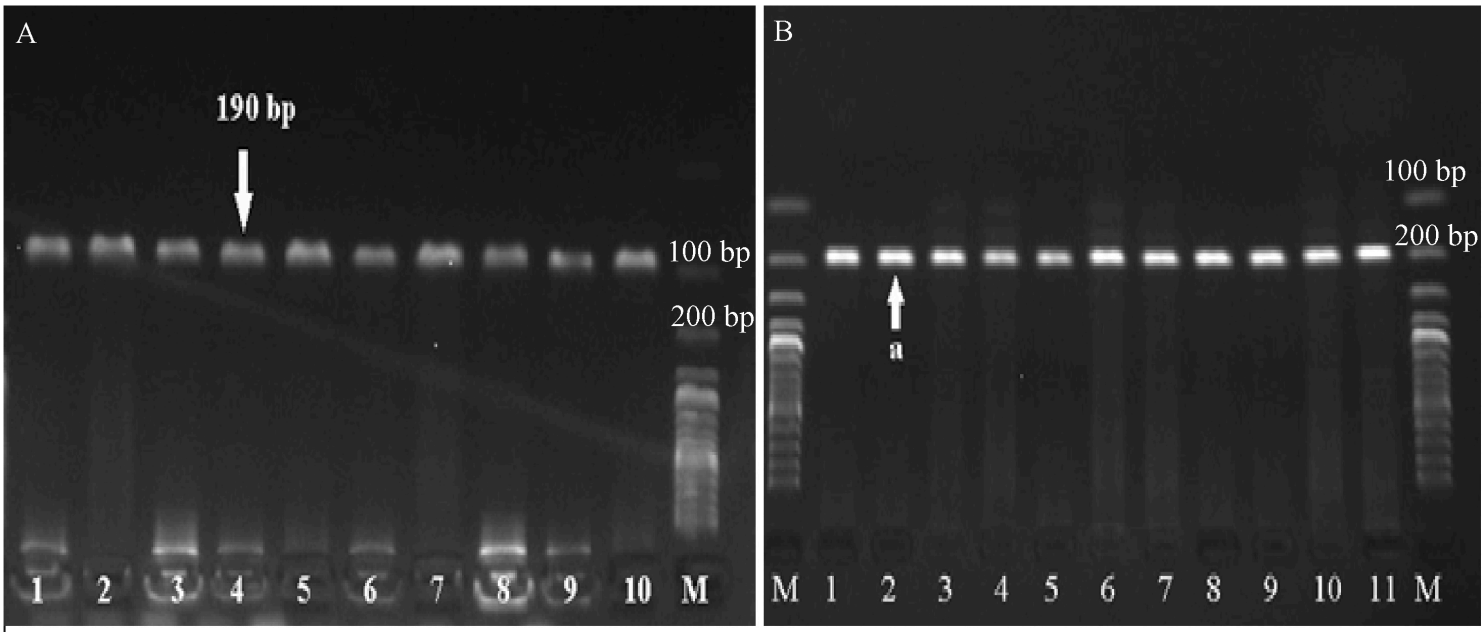



**Figure 6**

(A) Gel-electrophoresis image of PCR products for BMPR1B gene FecB SNP (190 bp). (B) Gel-electrophoresis image of PCR and restricted PCR products for BMPR1B gene FecB SNP; M: Marker with 100 base pairs; a: 190 bp; Non-carrier RFLP products: (1-11) (190 bp).

## Supplementary Files

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- Fig.4PCR.jpg
- Fig.5RFLPandPCR.jpg
- Fig.6RFLP.jpg
- Fig.6PCR.jpg