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ARAŞTIRMA MAKALESİ

Investigation of FecX^{I} Mutation by Pcr-Rflp Method in Awassi Sheep Breed

Oğuz AĞYAR[1] **Koray KIRIKÇI**[2*]

[1]Department of Veterinary, Kahta Vocational School, Adiyaman University, Adiyaman, Türkiye
[2] Department of Animal Science, Faculty of Agricultural, Kırşehir Ahi Evran University, Kırşehir, Türkiye

***Sorumlu Yazar:**
koray.kirikci@ahievran.edu.tr

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Abstract

The identification of mutations affecting multiple births in sheep is critical for the efficacy of genetic breeding studies. Before carrying out breeding studies with marker-assisted selection (MAS), it is necessary to identify major or potential novel mutations and determine their effects at the level of the breed. The propose of the study was to investigate the FecX^{I} (Inverdale) mutation in the Awassi sheep breed using the PCR-RFLP technique. The study used blood samples from 50 sheep collected from a pure breeding farm in the Adıyaman province of Turkey. To isolate genomic DNA from blood, a commercial isolation kit was utilized. The 154 base pair area of the BMP15 gene where the mutation was located was amplified by PCR, and the FecX^{I} mutation was analyzed in all PCR products using the PCR-RFLP technique. The FecX^{I} mutation was not found in the Awassi sheep tested. It is recommended that the related novel and major mutations with multiple births be investigated by different molecular methods in the Awassi sheep breed.

İvesi Koyun Irkında FecX^{I} Mutasyonunun Pcr-Rflp Yöntemi İle Belirlenmesi

Özet

Koyunlarda çoklu doğumu etkileyen mutasyonların tanımlanması genetik ıslah çalışmalarının etkinliği açısından oldukça önemlidir. Markör destekli seleksiyon (MAS) ile ıslah çalışmalarının yapılabilmesi için öncelikli olarak majör veya olası yeni mutasyonların tanımlanması ve ırk düzeyinde etkisinin ortaya konması gerekmektedir. Bu çalışmada İvesi koyun ırkında FecX^{I} (Inverdale) mutasyonunun PCR-RFLP yöntemi ile araştırılması amaçlanmıştır. Çalışmanın materyalini, Adıyaman ilinde saf yetiştiriciliği yapılan bir işletmeden alınan 50 baş İvesi koyun ırkına ait kan örnekleri oluşturmuştur. Kandan genomik DNA'nın elde edilmesinde ticari izolasyon kiti kullanılmıştır. Mutasyonun bulunduğu BMP15 geninin 154 baz çiftlik bölgesi PCR ile çoğaltılmış ve sonrasında tüm PCR ürünlerinde FecX^{I} mutasyonu PCR-RFLP yöntemi ile araştırılmıştır. PCR-RFLP sonuçları, incelenen İvesi koyunlarının FecX^{I} mutasyonunu taşımadığını göstermiştir. İvesi koyun ırkında çoklu doğum ile ilişkili majör ve günümüzde tanımlanan yeni mutasyonların farklı moleküler yöntemler ile araştırılması önerilmektedir.

Introduction

The discovery of mutations that affect twinning rates is a critical step in the advancement of genetic improvement studies. Fertility is a major contributor to good production and is determined by ovulation rates, litter size, and lamb mortality rates (Thieme et al., 1999). The litter size of sheep is an essential reproductive trait that has an economic value (Notter, 2008). There are a lot of things that can affect the size of litter, including genetic and environmental factors like feeding and shelter. However, improvement of the reproductive features is more promising than improvement of feeding and housing conditions (Rothschild et al., 1996).

The mutations responsible for multiple ovulations offer the possibility of substantial and speedy improvements in the productive capacity of sheep production (Mahdavi et al., 2014). Many mutations in three important fecundity genes, bone morphogenetic protein receptor type IB (BMPR-IB), growth differentiation factor 9 (GDF9), and bone morphogenetic protein 15 (BMP15), all of which belong to the TGF-superfamily, have been identified in the world (Nagdy et al., 2018; Davis, 2005; Mcnatty et al., 2003).

The Awassi sheep, one of the most common breeds in the Middle East, contributes significantly to milk production in sheep production systems globally, either as a pure breed or by interbreeding (Rummel et al., 2005; Galal et al., 2008). The Awassi breed accounts for about 3.5 percent of Turkey's sheep population (Gürsoy, 2005). Despite the fact that most domestic Turkish sheep breeds, with the exception of the Sakız (Chios), have one or two lambs at each lambing, it has been reported that Awassi are prolific (Gootwine et al., 2008). On the other hand, Awassi has a lowever ovulation rate with variable, imbalanced productivity compared to other nearby breeds (Abdullah et al., 2002, Üstüner and Oğan, 2013).

In recent years, substantial progress has been made in the understanding of major genes in prolific and non-prolific Turkish native sheep breeds (Kirikci et al., 2022; Kirikci et al., 2021; Gedik, 2021; Çelikeloglu et al., 2021; Kirikci and Cam, 2020). However, the major genes of the Turkish Awassi sheep breed have been investigated in a limited number of studies. The purpose of the study was to investigate the $FecX^{I}$ mutation, named Inverdale, which is located on the X chromosome and increases the ovulation rate by 1 for heterozygous genotypes compared to homozygous ones (Davis et al., 1991).

Materials and Methods

This study was carried out in accordance with the approval (Protocol No: 2022/011) of Adıyaman University, Animal Experiments Local Ethics Committee (ADYÜ-HADYEK). A total of 50 blood samples were collected for the study from a purebred farm in Türkiye Adıyaman district.

Genomic DNA was isolated using a kit, IDPUR E^{TM} Spin Column (Empire Genomics, Buffalo, NY). Following the manufacturer's instructions, the DNA isolation process was carried out.The PCR reaction for amplifying a 154-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 MgCl2 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.

Samples were analyzed for the presence of the $FecX^I$ mutation in the BMP15 gene 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 (*Xba*l), 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. A DNA ladder of 100 bp was used as moleculer size marker.

Results and Discussion

In order to determine if the $FecX^I$ mutation is present in the studied samples, all PCR products were digested using the *Xba*I enzyme. For the wild-type genotypes, we expected a 154-bp fragment, two fragments (124 and 30 bp) for homozygous genotypes, and all tree fragments for heterozygous genotypes. The results following restriction revealed that all individuals had wild genotypes (non-carriers). The obtained results are shown in Figure 1.

It was determined that the investigated Awassi ewes have a monomorphic structure. In other words, all individuals in the study did not carry the $FecX^I$ mutation. This finding was similar to the results of Gedik (2021), who investigated the mutation in the same breed. The previous studies conducted by various researchers also reported the same result with this study for the Awassi breed (Karslı et al., 2010; Gürsel et al., 2011; Karslı et al., 2012). To summarize, not only has this mutation not been recorded in the Awassi breed but also in the prolific Sakız breed and other sheep breeds in Turkey. Morever, the BMP15 $FecX^I$ mutation was also not reported in 21 high prolific sheep breeds and strains from 13 different nations (Davis et al., 2006).

Today, efforts to identify gene regions associated with multiple births in sheep are increasing across the world. In a study by Abdoli et al. (2018), no mutations were discovered in three candidate genes in the Iranian Lori breed. However, when the same researchers conducted the study using comprehensive approaches such as GWAS, they discovered that several gene regions are associated with multiple births (Abdoli et al., 2018). To our knowledge, it is difficult to claim that Awassi sheep have been tested extensively for BMP15, GDF9, and BMPR1B genes in Turkey. In the Awassi breed, an intogression study was carried out by Gootwine et al. (2008) in 1986 with the goal of introducing the FecB (Booroola) gene B allele to Awassi ewes in order to increase profitability

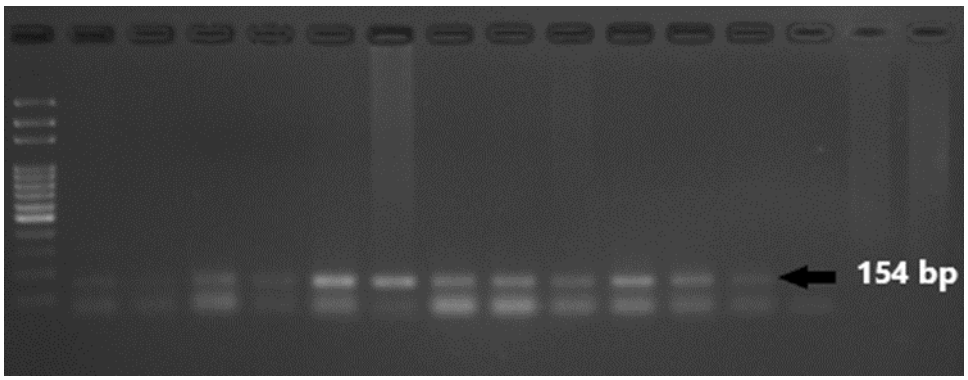

Figure 1. The result of digestion with the *Xba*l enzyme of PCR products in the Awassi breed

As evidenced by the above information, studies aiming at identifying mutations causing multiple births in the Awassi breed have been continued from the past to the present. In a recent study, Mohammed et al. (2022) reported a novel mutation that demonstrated a significant association ($P < 0.01$) between the p. K116Q SNP in the OLR1 (oxidized low-density lipoprotein receptor) gene and fertility characteristics, in that ewes with this SNP had a smaller litter size and a lower twinning rate than those with the CC genotype. A latest study by Al-Nafie et al. (2022) reported a novel mutation on the exon 2 region of Hemoglobin subunit beta (HBB) gene. Al-Nafie et al. (2022) found c.51983514 A>G polymorphism; indicated ewes with the AA genotype (64.61 %) exhibited superior performance in terms of twining ratio compared to those with the AG genotype (29.55 %).

Conclusions

This study and previous research findings indicate that the FeX^I mutation is most likely not responsible for multiple births in the Awassi sheep breed; nevertheless, the fecundity mutation in the Awassi breed in Turkey has not been extensively studied. In recent years, several mutations associated with prolificacy have been identified in the Awassi breed. As a result, more extensive studies with a large phenotypic sample size are required for both the most recently discovered novel mutations and other major gene mutations in the Awassi breed.

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