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Investigation for Mutation in BMPR-1B (FecB) Fecundity Gene in Awassi Sheep

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HIGHLIGHTS

- Ovulation rate and litter size are important economic traits in sheep breeding.
- *Booroola (FecB)* is the first major gene identified in sheep has major effects on reproduction.
- One copy of *Booroola* gene increases the ovulation rate at 1.65 and 1.0 litter size.
- To investigate *FecB* mutation in Awassi sheep, BMPR-1B gene was amplified and analyzed.

Abstract

Reproduction related traits in sheep such as ovulation rate and litter size are traits of high economic importance but as these traits are only expressed in one sex and in mature animals, thus inclusion of them in selection strategies is limited. Therefore, studying genes associated with reproductive events provides genotypic data, which is more useful in genetic improvement of sheep in a short period of time. Such reproductive traits in sheep are genetically controlled by genes having both additive and major effects. *Booroola* is the first gene identified in Booroola Merino sheep in Australia has major effects on ewe's reproduction and has gained much popularity in sheep breeding for its immense economic value. *Booroola* or *Bone Morphogenetic Protein Receptor -1B (BMPR-1B)* gene is in chromosome 6 and *FecB* allele is the result of a single mutation in this gene where one copy of this allele results in a significant increase in ovulation rate thus outcome as increased lambing per parturition. The Awassi sheep is an indigenous breed of Turkey and identifying major genes for fecundity could greatly improve their breeding program. To identify the *FecB* gene in Awassi sheep, 88 blood samples were taken, and DNA was extracted by salting out method. After PCR amplification and *Ava*Π digestion, the samples were analyzed for *FecB* mutation and all of 88 samples were found not to carry the mutant allele (*FecB*).

Keywords: Awassi; BMPR-1B gene; Booroola; Litter size; PCR-RFLP

1. Introduction

Sheep has been a prominent member of livestock since the beginning of the known history and play a significant role in agricultural production. Sheep provides meat, milk and most importantly wool for satisfying the daily needs of humans. Apart from poultry, it is considered that there are more breeds of sheep

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than any other livestock species with around 1155 different breeds worldwide (FAO 2015). Awassi is the most populous and ubiquitous fat tailed sheep breed in south-west Asia. It is the most common sheep breed in Iraq, Syria, and the sole indigenous sheep breed in Lebanon, Jordan, and Israel (FAO 1982). Awassi is used for production of meat, milk, and wool and has been brought to more than 30 countries on all seven continents (Galal et al. 2008). The superiority of Awassi from other breeds includes, it's diseases and parasites resistance, ability to walk long distances for grazing, strong flock instinct, well adaptation to management fluctuations, and tolerance to harsh environmental conditions, particularly those related to feed scarcity and hot weather conditions (Talafha and Ababneh 2011). In Turkey, Awassi sheep is known as Ivesi (FAO 1982) or Sarıbaş or Arabian sheep which constitutes 3.9 percent of the total sheep population and widely bred in Şanlıurfa, Gaziantep, Kilis and Hatay provinces (TİGEM 2020). Awassi ewes produce 196.5 ± 5.60 kg milk in a lactation period of 184.3 ± 2.11 days (Üstüner and Oğan 2013). Fertility, lambing rate, average litter size and twinning rate were found 89.8%, 108.2%, 1.30-1.40 and 20.5%, respectively in Turkish Awassi sheep populations (Üstüner and Oğan 2013; Gürsel et al. 2011). A recent report shows that the twinning rate of Awassi sheep in government farms is 20-30 percent and this rate increases to 35-40 percent in elite herds (TIGEM 2020).

Reproduction in sheep is both influenced by minor and major genes (Jamshidi et al. 2013). In 1980, *Booroola* gene (*FecB*) was identified as the first single major gene responsible for prolificacy in Booroola Merino sheep by analyzing their litter size records (Piper and Bindon 1983) and now it is believed that *FecB* was primarily originated in India's Garole breed (also knowun as Bengal) habituated in harsh surroundings of Sundarban (Jansson 2014; Fogarty 2009; Davis et al. 2002). The following research has revealed that a group of genes known as the fecundity (*Fec*) genes can govern the ovulation rate and litter size of sheep genetically. From now three different fecundity gene have been found in Sheep naming *bone morphogenetic protein receptor type IB* (*BMPR-IB*) or *activin like kinase 6* (*ALK-6*) or FecB, growth differentiation factor 9 (*GDF9*) or *FecG* and *bone morphogenetic protein 15* (*BMP15*) or *FecX* located on chromosome number six, five and X respectively (Pramod et al. 2013). These three fecundity genes are members of the transforming growth factor beta (TGF-β) superfamily, derived from the ovary (Çelikeloğlu et al. 2021). Mutations in FecG and *FecX* belong to higher ovulation rates in heterozygous but complete sterility in homozygous, whereas *FecB* (*Booroola*) mutations have an additive influence on ovulation rate (Polley et al. 2010). One copy of *Booroola* gene increases the ovulation rate at 1.65 (Liu et al. 2014) and one litter size (Wilson et al. 2001). *Booroola* gene is a result of a point mutation that produces a glutamine to arginine amino acid substitution at base 746 of the coding area (746 A >G) in the highly conserved intracellular kinase signaling domain of the BMPR-1B, which can greatly enhance the ovulation rate (Liu et al. 2014). BMPR-1B is mainly expressed in sheep ovaries, although it is also found in other tissues and plays a role in follicle development. Due to abnormalities in cumulus growth and fertilization, BMPR-1B knockout mice were found to be infertile. After identification of *FecB* mutation in Booroola Merino sheep, this mutation was reported in different breeds worldwide (Liu et al. 2014).

This study aims to investigate *FecB* mutation in the *BMPR-1B* gene in high litter size Awassi sheep that is linked to a high ovulation rate in sheep by using the Polymerase Chain Reaction-Restriction-Fragment Length Polymorphism (PCR-RFLP) method.

2. Materials and Methods

This study used a total of 88 blood samples with high prolificacy records called "elite" group reared in Şanlıurfa province. No approval from research ethics committee was required to accomplish the goals of this study due to the experimental material were previously taken for another project (Scientific Research Projects (BAP) of Ankara University, Project No: BAP09B4347007). Blood was collected from the jugular vein of ewes into sterile tubes and maintained a cold chain until laboratory storage at -20 °C. DNA extraction was completed by following the salting-out method and gel electrophoresis (1%) and spectrophotometer (A260 / A280 nm) were used to analyze the quality and quantity of extracted genomic DNA.

The gene region of *BMPR-1B*, which contained the FecB mutation site, was intended to be amplified by PCR developed by Davis et al. (2002). *BMPR-IB* gene with *FecB* mutation carries an *Ava*II restriction site (G↓GACC), whereas the wild type has a lack of this restriction site. DNA was amplified in a 25 µl reaction volume using a forward primer 5'-CCAGAGGACAATAGCAAAGCAAA-3' and reverse primer 5'-CAAGATGTTTTCATGCCTCATCAACACGGTC-3'. PCR reaction mixture was prepared by adding 1.5 µl of gDNA, 2.5 µl of 10 X PCR buffer, 0.75 µl MgCl2, 0.25 µl forward and reverse primer, 1 U of Taq DNA polymerase, and finally added distilled water to reach a total volume of 25 µl. The amplification was carried out using 35 cycles at 94°C for 15 sec, 60°C for 30 sec, and 70°C for 30 sec followed by 72°C for 5 min and 99°C for 15 min. The *Ava*II digested the 190-base pair (bp) product, and the resultant products were separated by electrophoresis on a 3.5% agarose gel and observed with ethidium bromide. Noncarrier products stay uncut at 190 bp, whereas *Booroola* products digest to generate a 160-bp and 30bp fragment.

3. Results and Discussion

The PCR amplification was successfully produced 190 base pair long fragments of *FecB* gene and a 50 bp ladder was used to compare the amplified length. After amplification, PCR products were subjected to *Ava*II digestion and only 190 bp fragments appeared in gel electrophoresis (Figure 1). Thus, results revealed only the existence of the wild monomorphic genotype of the *Booroola* gene and indicated a lack of *FecB* mutation in the examined 88 Awassi ewe samples.

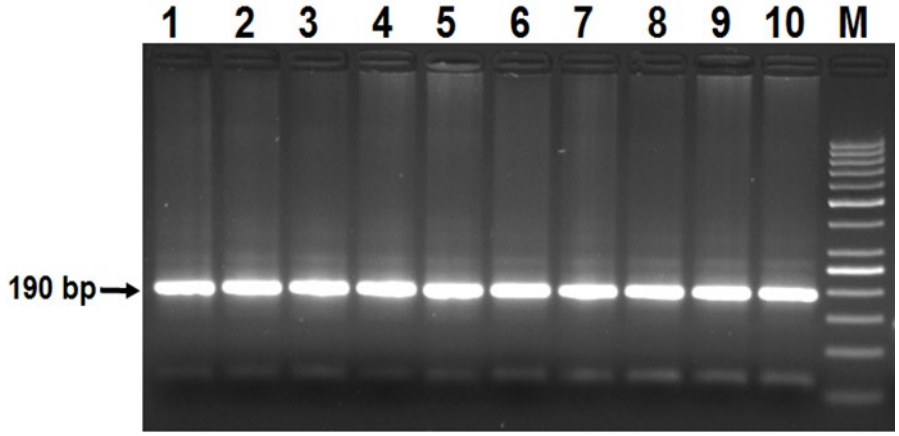

Figure 1. PCR-RFLP results of *FecB* gene by *Ava*II restriction enyzme on 3.5% agorose gel. M; Fermentas GeneRuler™ 50 bp DNA Ladder.

The results showed that there was no *FecB* mutation in the Awassi ewe samples studied, therefore all sheep were monomorphic in terms of *FecB* locus. These same samples were also investigated for Inverdale ($FecX^I$) mutation on *BMP-15* gene and reported no mutation (Gedik 2021). This finding is supported by past study of Gürsel et al. (2011) and Karslı and Balcıoğlu. (2010) where along with Awassi *FecB* was absent in indigenous Chios, Kivircik, Imrose Akkaraman, Morkaraman, Dağlıç, Tuj and Karakaş sheep breeds of Turkey. By using the PCR-RFLP method, Dinçel et al. (2015), Karslı et al. (2011), and Polat (2006) were unable to detect *FecB* gene in 71 Sakiz, 42 Kangal and 29 Güney Karaman and 406 Sakiz and Sakiz-Kivircik cross samples respectively. A recent study by Çelikeloğlu et al. (2018), used DNA base sequencing but *FecB* was not identified in 16 blood samples of Pirlak sheep. Apart from Turkey, *FecB* was absent in many prolific breeds. For example, in a study comprising samples from the world's most prolific 21 breeds and strains, the *FecB* mutation was detected exclusively in two Chinese sheep breeds Hu and Han (Davis et al. 2006). Presence of *Booroola* mutation was found in some Indian sheep breeds such as Garole, Bonpala, Kendrapada, Nilagiri, Shahabadi, Deccani, Nellore Sheep (Gootwine 2020; Liu et al. 2014). *FecB* also exists in Indonesian Javanese breed and Iranian

Kalehkoohi breed. In addition to these, some Chinese breeds such as Small-Tail Han, Merino prolific, Hu, Duolang, Zeller black, Vadi, Mongolian, Cele black, Altay, Bayanbulak have also been reported to be carriers of the productive *FecB* gene (Gootwine 2020). Thus, most *Booroola* carriers are from Asian origin whereas there is a lack of *FecB* mutation in European sheep breeds (Jansson 2014).

4. Discussion

Three different major genes were identified regulating the litter size in ewes, thus the absence of one major gene does not mean absence of other genes in a specific breed. As a result of study Awassi sheep have a lack of Booroola gene, so the reason for prolificacy in Awassi sheep with respects to major genes needs to be elucidated as some genes such as BMP15, and GDF9 not only increase litter size but also cause sterility when homozygous. When the genetic basis of Awassi sheep will be noticed it will be helpful in terms of breeding decisions. If the Awassi breed does not convey any major gene, breeding with major gene carrier breeds will also be a solution to improve production in a short period of time.

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