

Investigation of FecB Mutation in Four Romanian Sheep Breeds

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Abstract

Hyperprolific phenotype of Booroola sheep was first discovered in the Australian Merino breed. This phenotype is due to the action of a single autosomal gene that influences the number of ovulations per estrogenic cycle. Recent discoveries have revealed that high prolificacy in Booroola Merino sheep is the result of a mutation (FecB) in the bone morphogenetic protein receptor 1B (*BMPR-1B*) gene. This mutation is located in the highly conserved kinase domain of the bone morphogenetic protein receptor 1B, and is characterized by precocious differentiation of ovarian follicles, leading to the production of large numbers of ovulatory follicles. Our objective was to develop an easy method to identify the FecB mutation in order to screen sheep populations in terms of prolificacy. We designed primers to amplify a 190 bp fragment from the *BMPR-1B* gene containing or lacking the mutation. The PCR product was cut with *AvaII* endonuclease and the restriction products were analysed by agarose gel electrophoresis. Using the PCR-RFLP technique, we established an easy and efficient method that can be used to screen the FecB mutation. Therefore, these new methods increase the panel of molecular tools available for sheep breeders to choose the most prolific genotypes for improving artificial selection.

Keywords: *FecB*, PCR-RFLP, prolificacy, screening, sheep.

1. Introduction

Booroola was the first major gene identified which increases ovulation rate in sheep [1]. The hyperprolific phenotype of Booroola ewes was first seen in the Australian Merino breed and is due to the action of a single autosomal gene that influences the number of ovulations per estrogenic cycle.

Recent discoveries have revealed that high prolificacy in Booroola sheep is the result of a mutation (FecB) in the bone morphogenetic protein receptor 1B (*BMPR-1B*) gene [2]. This mutation is located in the highly conserved kinase domain of the bone morphogenetic protein receptor 1B, and is characterized by rapid differentiation of ovarian follicles, leading to the

production of large numbers of ovulatory follicles that are smaller than wild-type follicles [3].

Knowledge of this mutation has prompted researchers to screen other prolific sheep breeds to determine presence of fertile genotypes.

The hyperprolificacy phenotype of Booroola ewes is due to the presence of the FecB⁺ allele, recently identified as a single amino acid substitution (Q249R) in the bone morphogenetic protein (BMP) type-IB receptor (*BMPR1B*), and is associated with a precocious differentiation of ovarian granulosa cells. Polymorphism is represented by a single point mutation (G turn to A) in *BMPR-1B* gene located on sheep chromosome 6 [4].

In terms of production features no major differences between the carriers and non-carriers animals were observed.

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2. Materials and methods

Sampling and DNA extraction

Blood samples were collected in EDTA-treated vacutainers from four Romanian breeds: Karakul de Botosani (SCDCOC Popauti), Palas Milk Line, Palas Meat Line and Palas Merino (ICDCOC Palas Constanta). For each breed we analysed 20 animals. The isolation of genomic DNA from fresh blood was performed with Wizard Genomic DNA Extraction Kit (Promega).

PCR-RFLP reaction

We have used a pair of primers (forward: 5'-ccagaggacaatagcaagcaaa-3'; reverse: 5'-caagatgtttcatgcctcatcaaca-3') in order to amplify a 190 bp fragment from *BMPR-1B* gene containing or not the G/A mutation.

The PCR conditions were optimized in order to determine the best annealing temperature for the two primers, between 51-61°C on a gradient thermocycler IQCycler (BioRad).

The amplification reactions were carried out in 25µL final volume and consisted of 1X PCR Buffer, MgCl₂ (1.5mM), 200µM of each dNTPs, DNA template (50ng), 0.5 units of AmpliTaq Gold DNA Polymerase, 10mM of each primer and nuclease free water. PCR amplifications were performed using a program with 45 cycles. Denaturation was performed at 95°C for 30 seconds, annealing at 59°C for 30 seconds and extension at 72°C for 1 minute. The first denaturation step was of 10 minutes at 95°C and the final extension was of 10 minutes at 72°C.

The PCR products obtained were digested with 1U of *AvaII* restriction endonuclease (Promega) for 3 hours at 37°C. The restriction fragments were directly analysed by electrophoresis in 3% agarose gels in TAE buffer, stained with ethidium bromide, and visualized under UV light. The genotypes of the analysed individuals were established using the restriction fragments observed in the gel.

Sequencing

The obtained PCR products were subjected to the sequencing reaction. In order to undergo this reaction, the amplicons were initially purified using the Wizard PCR Preps DNA Purification System Kit (Promega) according to the manufacturer's instructions. The next step was to mix them with ABI Prism®BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). The purification of the amplified

products was done using the BidDye XTerminator®Purification Kit (Applied Biosystems). The products were analysed on a ABI Prism 3130 Genetic Analyzer (Applied Biosystems) and the nucleotide sequences were aligned with the BioEdit program.

3. Results and discussion

Until now *FecB* mutation was identified in a few prolific breeds like Booroola Merino [2], Chinese Merino Meat Line [5] and Garo [6]. In particular, based on the segregation of the ovulation rate in Merino flocks, the genotypes of the ewes are classified as homozygous non-carrier ($FecB^+/FecB^+$) with an ovulation rate of one or two, heterozygous carriers ($FecB^-/FecB^+$) with an ovulation rate of three or four, and homozygous carriers ($FecB^-/FecB^-$) with more than five ovulations per cycle [7].

In this study we have analysed 100 animals from four Romanian sheep breeds. In order to evaluate the presence or absence of *FecB* carriers, the amplified products were subjected to enzymatic digestion using the *AvaII* restriction enzyme. The possible polymorphism will generate a restriction site of this enzyme and thus we will be able to differentiate between carriers and non-carriers.

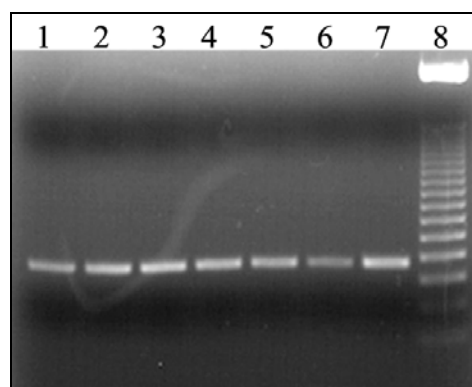


Figure 1. Results after *AvaII* digestion of a 190 bp fragment of *BMPR-1B* gene. 1-2: Karakul de Botosani; 3-4: Palas Merino; 5: Palas Milk Line; 6: Palas Meat Line; 7: uncut fragment; 8: 50 bp molecular weight marker (Promega).

After PCR amplification, enzymatic digestion with *AvaII* and agarose gel electrophoresis, the wild type individuals (non-carriers) yields one fragment of 190 bp, the homozygous individuals for *FecB* mutation yields two fragments of 60 and

130 bp, while the heterozygotes have all three fragments. The restriction products obtained were separated on 3% agarose gel and the results showed in Figure 1.

After the digestion with *Ava*II restriction enzyme, we have obtained in all four sheep breeds only the wild allele which represents the non-carrier animals.

In order to confirm the results obtained using the PCR-RFLP technique we have sequenced the amplicons. After aligning the obtained sequences with the reference sequence from GenBank database we have obtained a homology of 99% (Figure 2). The single polymorphism observed is situated at the position 816 from the gene and leads to the replacement of Arg with Gln at the protein level.

The existence of *FecB* polymorphism causes positive but also negative effects. The positive effect is the increasing of lamb numbers per birth, with about 0.9 [1] while negative effects are observed in the body weight, body size and development during pregnancy. Body weight during pregnancy is lower at homozygous or heterozygous carriers compared with wild type and also they grow poorly and the body length is smaller [3].

Our results showed that, by using this technique, it is easy to identify the animals which have the favourable allele for reproduction. The method presented is reliable, fast and can be successfully applied in the wide-scale screening of different sheep populations.

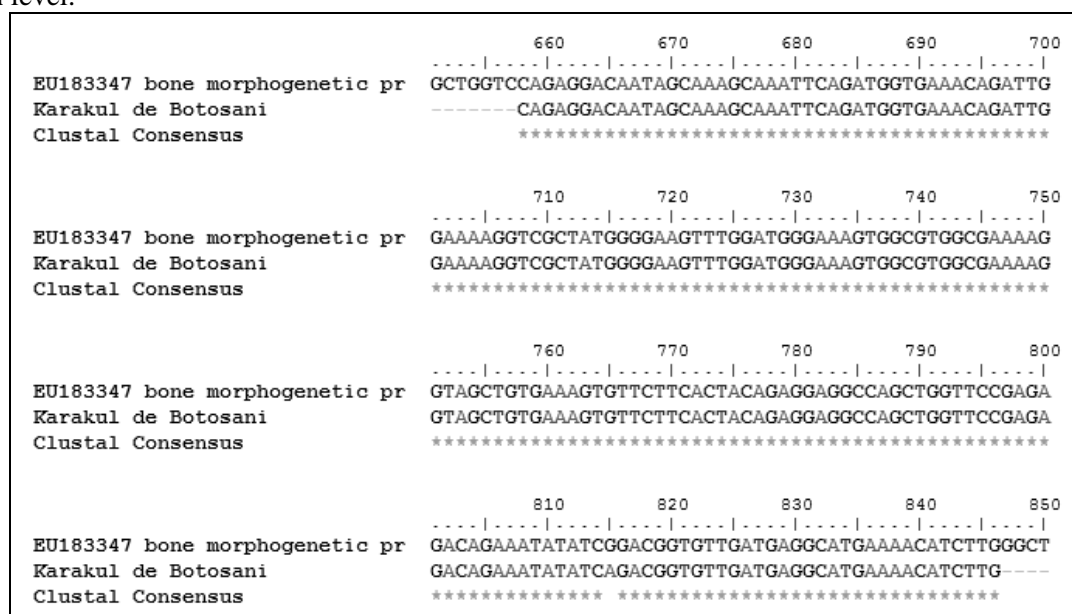


Figure 2. BioEdit alignment between a reference sequence (GenBank Database Accession Number EU183347) and the sequence we obtained for Karakul de Botosani.

4. Conclusions

FecB mutation can have a positive effect on ovine prolificacy and production. For this reason we wanted to use a modern, efficient and economic method in order to identify the animals carrying the G/A substitution in the ovine *BMPR-1B* gene. The results obtained highlighted the absence of *FecB* carriers in the analysed Romanian sheep breeds.

This method increases the panel of molecular tools available for sheep breeders to choose the most prolific genotypes for improving the artificial selection.

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