RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES
ISSN 2221-1896
www.roavs.com

PCR-RFLP of BMPR-IB gene in North African Barbarine sheep

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Abstract

This study reports the statue of the Booroola fecundity gene (FecB) mutation in Barbarine sheep breed. A total of 334 ewes recorded with twining births from the Tunisian fat tail Barbarine sheep were genotyped in the objective to identify the FecB mutation. The FecB genotyping was carried out by forced restriction fragment length polymorphism PCR technique. Forced PCR of the FecB gene was amplified using a specific primer designed to introduce a point mutation in the resulting PCR products with FecB non carriers sheep containing an AvaII restriction site, whereas products from non carriers lacked this site. Digestion of an amplified 140 base pair FecB gene with AvaII restriction enzyme resulted in a non-carrier 140 bp band (wild type) in all studied animals which revealed absence of the AvaII restriction site in this breed. Considering the records of twinning in this breed, it can be concluded that the genetics factor controlling twinning is not related to this mutation, which has been reported Booroola as a major gene previously.

Keywords: Barbarine; Sheep; BMPR-IB; FecB; Mutation

Introduction

Large genetic variations in the ovulation rate and litter size have been observed among different breeds and within breeds of sheep. In some instances, variation in the litter size and ovulation rate can be genetically controlled by the action of a single gene with a major effect, named fecundity gene (Davis, 2005). Three types of fecundity gene have been reported in sheep, namely Bone Morphogenetic Protein Receptor IB (BMPR-IB) also known as Booroola fecundity gene (FecB) (Wilson et al., 2001), Growth Differentiation Factor 9 (GDF9) also known as FecG (Harnahan et al., 2004) and Bone Morphogenetic Protein 15 (BMP15 or GDF9□) also known as FecX (Galloway et al., 2000; Hanrahan et al., 2004). All these fecundity genes belong to the transforming growth factor superfamily (Fabre et al., 2006). The GDF9 gene has been mapped to sheep chromosome 5 and BMP15 is located on the X chromosome. Five mutations in this gene affecting prolificacy have been described. Ovulation rates are highly increased in the heterozygotes, while in the homozygotes a primary ovarian failure results in complete sterility (Galloway et al., 2000; Hanrahan et al., 2004; Davis, 2005; Bodin et al., 2007). These mutations, named $FecX^G$ (Galway), $FecX^H$ (Hanna), $FecX^I$ (Inverdalle), $FecX^L$ (Lacaune) and $FecX^B$ (belclare), exhibit one to two additional ovulation, compared with noncarriers ewes. The Booroola (FecB) locus is located on sheep chromosome 6. This gene, identified in the Australian Booroola Merinos strain (Piper et al., 1985), was the first major gene described to have effect on ovulation rate and prolificacy in sheep. The FecB mutation increases ovulation rate about 1.5 to 3.0 in Booroola Merinos. These extra ovulations in turn increased litter size by about 1.0 and 1.5, respectively. Recently, Gootwine et al. (2008) reviewed the effect of Booroola gene on prolificacy of Awassi and Assaf sheep breeds. The FecB mutation has been identified in other prolific breeds of sheep dwarf Garole from India (Davis et al., 2002), Javanese from Indonesia (Davis et al., 2002), Hu and small tailed Han from China (Davis et al., 2006). It has been also reported that FecB mutation has been fixed in a population of Garole sheep (Davis et al., 2002), while, it is segregating in crossbreds developed from Garole inheritance (Kumar et al., 2008).

The Barbarine sheep, and specially the ewe, is well adapted to the local condition mainly because of its ability to deposit and mobiles body reserve not only from the tail (fat) but also from the rest of the body (Djemali et al., 1994; Atti et al., 2004; Bedhiaf-romdhani et al., 2006). The Barbarine sheep is a middle sized animal with a height raring between 60 and 80 cm in male and 55 and 70 cm in females. The body weight varies considerably according to nutritional conditions,

ranging between 45 and 85 kg in rams and 25 and 65 kg in ewes (Khaldi, 1989). Selection for prolificacy is of major economic interest in most husbandry situations. However, there is an optimum litter size for each environment which maximizes the return profit per ewe. Uniform optimum litter sizes result in the highest profit, and when mean prolificacy of a breed is close to this optimum, then uniformity of litter sizes will be a new additional objective.

The main objective of the present research was to apply PCR-RFLP technique for determining BMPR-IB gene polymorphism in Tunisian Barbarine sheep breed.

Materials and Methods

Blood samples were collected in vacutainer tubes containing EDTA (1 mg mL-1) from 334 Barbarine sheep individuals. DNA was extracted using modified salting out procedure (Miller et al., 1999) and stored at -20◦C until used in assay.

The concentration of DNA samples was estimated using UV-visible range spectrophotometer and diluted to 50 ng/µL before PCR amplification. All the DNA samples had 260/280 OD ratios in the range of 1.8 to 2, indicating high purity. DNA was also examined by loading samples on 0.8% agarose gel and visualizing the band under gel documentation system. In total, 334 ewes were genotyped for the Booroola mutation. The PCR-RFLP technique used primers and restriction enzyme (AvaII) described by Wilson et al. (2001) for detection of the mutation in the Bone Morphogenetic Protein Receptor type 1B (BMPR-1B) gene.

Primers, described by Wilson et al. (2001), were used to amplify a 140 bp band of FecB gene. The amplification was carried out using 39 cycles at 94 °C for 30 sec, 60°C for 30 sec, and 72°C for 1 min, followed by 72°C for 10 min. 15 µl of PCR products was digested for 4h at 37°C with 10 units of AvaII. There was only one restriction site for AvaII (G|GACC) to recognize FecB gene.

Digested products were separated by electrophoresis on a 3% agarose gel and visualized with Ethidium bromide under gel documentation system.

To screen the mutation through the individuals of sheep, a method that deliberately introduces a point mutation into one of the primers was used so that the PCR product will contain an AvaII (Fermentas) restriction endonuclease. PCR products from non-carriers have no restriction site. After digestion with AvaII the (++) animals could have a 140 bp band, the (B+) animals would have 140, 110 and 30 bp, and the (BB) animals will have only 110 and 30 bp bands.

Results and Discussion

A total of 334 individuals from Barbarine sheep were genotyped with the PCR-RFLP approach (Figure 1 and 2). The results showed that all genotyped ewes had the wild type allele (FecB+/FecB+). The basic finding of the current study was the absence of polymorphism at the FecB gene in Tunisian Barbarine sheep.

In respect to the results of the present study, the presence of non carrier 140 pb band pattern (wild type) in all the animals studied could be explained on basis of medium size in this breed. The results found in the present research are in accordance with those obtained for Madras Red, Deccani and Bunnur breeds as well as for Egyptian sheep breed (Wilson et al., 2001; Davis et al., 2002, 2006 and Amr and El-Saadani, 2009).

PCR-RFLP is a rapid, simple and exact technique for single nucleotide polymorphism (SNP) genotyping. After a forced restriction site was introduced into one of the primers, the PCR product contained a certain restriction enzyme site. This forced PCR-RFLP approach has been used previously to genotype prolific sheep (Wilson et al., 2001). In our experiment, PCR-RFLP approach was used to detect the genotype, based on the method described by Wilson et al. (2001), in Barbarine sheep individuals. PCR-RFLP has a good

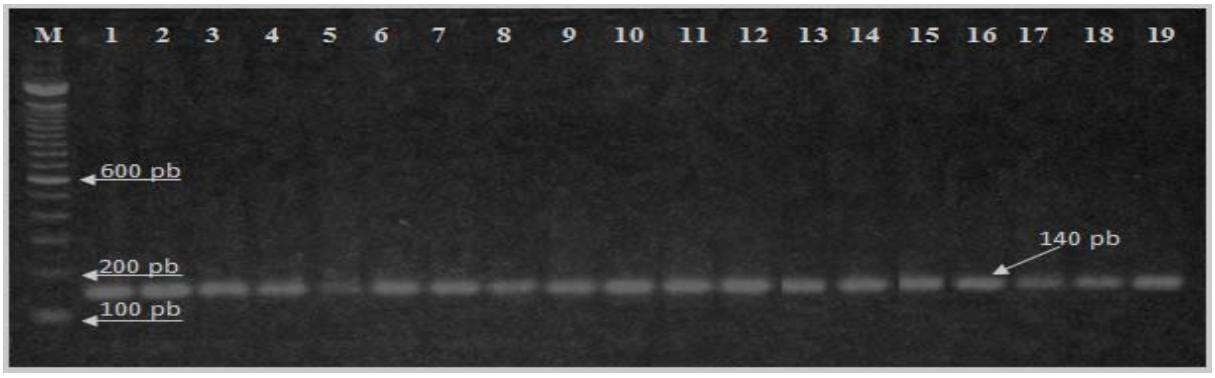

Fig. 1: Amplification of genomic DNA from different Barbarine individuals (lanes 1–19). M: 1kb Molecular weight marker (invitrogen DNA Ladder)

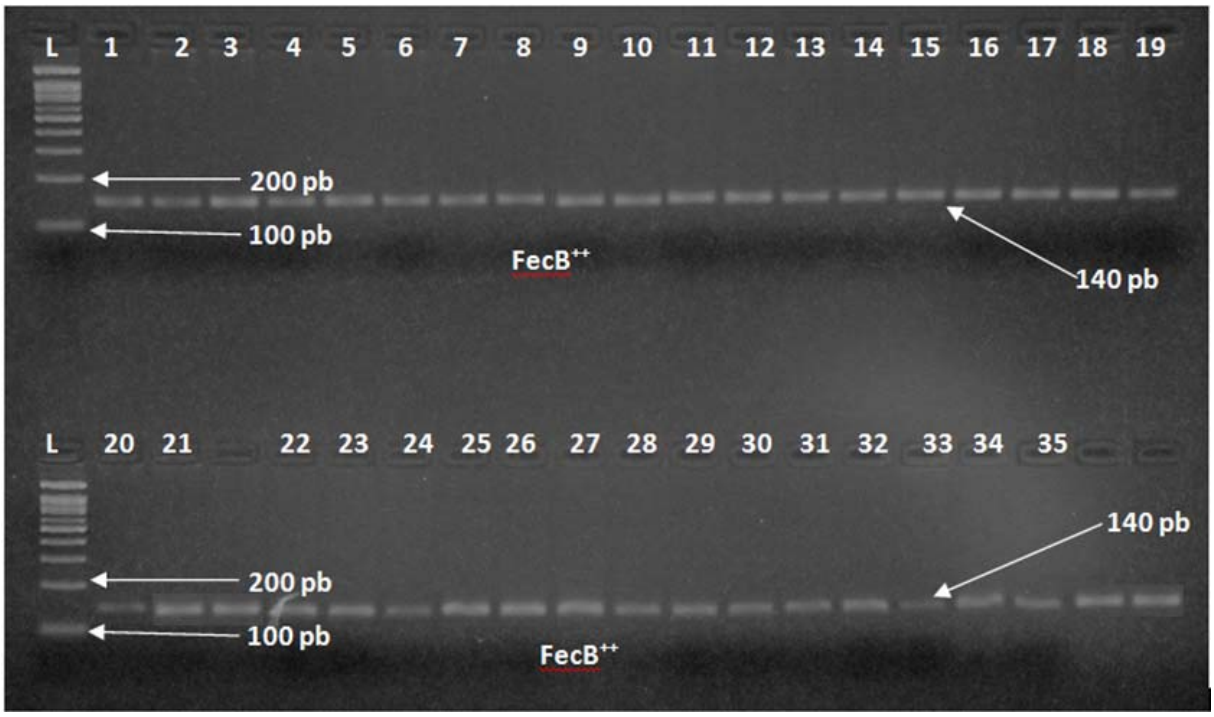

Fig. 2: Detection of FecB mutation by digested RFLP with *AvaII*. (lanes 1–35 uncut 140 bp band). M: 1kb Molecular weight marker (invitrogen DNA Ladder)

repeatability and stability, but its results were affected by several factors, such as enzymes from different companies, time of digestion and volume of electrophoresis and concentration of gel. Several of these factors were compared by adding various concentrations of ingredients to select the optimal reaction conditions to maintain repeatability and veracity. Detections with illegible results were repeated until the genotyping was clear.

Conclusion

In the present study the results of PCR-RFLP showed the same band pattern in all samples, implying no mutation in FecB locus in North African Barbarine ewes. Regarding the records of twinning in this breed it is concluded that the genetics factor controlling twinning is not related to the mutation, which is reported in Booroola major gene. It might be concluded that litter size in this breed is either not affected by major genes or it is possible that some other major genes control twinning in this breed. Further research is recommended to be conducted.

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