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Detection of Polymorphism in BMPR-IB Gene Associated with Twining in Shal Sheep using PCR-RFLP Method

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ABSTRACT

This study was carried out for detection of possible polymorphisms in BMPR-IB gene. Blood samples were collected from 239 ewes of Shal sheep. Genomic DNA was extracted using modified salting-out method. The quantity and quality of extracted DNA was examined with spectrophotometery and gel electrophoresis. The polymerase chain reaction (PCR) was carried out for amplification of a fragment with 190 bp at this locus. For genotyping of individuals at Boorola locus, the resulted amplified fragments were digested using *Ava*II restriction enzyme. *Ava*II restriction enzyme was used to detect possible mutation. All samples showed wild allele and *Fec*B^{++} genotype. Considering the phonotypic records in this breed, the obtained result indicates that the genetic factor responsible for twining or multiple lambing rate is not related to reported mutated alleles at Boorola major gene and we should search for other genes in this breed.

Key Words: Sheep; RFLP- PCR; *Fec*B; Twining gene

INTRODUCTION

The origin of the Shal sheep is the Qazvin province and it is kept mainly to produce meat. During creep feeding the lamb have an average daily gain of about 250 g. Competed to other domestic breeds of sheep of Iran this breed has higher average twining rate. It also has higher than average feed conversion ratio with higher dressing percentage. The average weight for the rams is 82 kg and for the ewes is 61 kg. The ability to adapt to different environmental circumstances is a desirable characteristic of this breed. Its reproduction season is scattered across the year and it may be bred in both fall and spring.

Improvement of reproductive traits in livestock species has become of increasing interest, especially in sheep, where small increases in litter size can equal large gains in profit. Genetic improvement of reproductive traits has traditionally been restricted to use of quantitative genetic methods but gain has been limited when using these methods. Provided that the major genes associated with reproduction are identified they can be utilized in breeding through marker-assisted selection (MAS). Reproductive traits are often suggested as prime targets for MAS for their low heritability and the fact that the trait can be measured only in one sex.

Litter size is an important economic trait in sheep breeding. Variation in litter size in sheep is controlled by both genetic and environmental factors. Most breeds of domestic sheep have one or two lambs at each lambing, although a small number of breeds, including the Booroola Merino, Cambridge, D'Man, Finnish Landrace and Romanov, consistently have litter sizes of three or more (Bindon & Piper, 1986). Attempts to increase litter size by selection within a breed result in slow progress, because the heritability of litter size is low (Morris, 1990). Therefore, the discovery of major genes (or mutations) with large effects on ovulation rate and thus litter size, has generated considerable interest among sheep breeders and scientists. The major gene (FecB) in the Booroola Merino breed was first postulated in 1980 (Piper & Bindon, 1982).

The Booroola fecundity gene (*FecB*) is a single autosomal gene, which increases ovulation rate and litter size in sheep [co-dominant for ovulation rate & partially dominant for litter size (Piper *et al.*, 1985; Montgomery *et al.*, 1992)]. The *FecB* locus is situated in the region of ovine chromosome 6, which is syntenic to human chromosome 4 (Montgomery *et al.*, 1993). Piper *et al.* (1985) and Piper and Bindon (1996) found that the effect of *FecB* mutation is additive for ovulation rate and each copy increases ovulation rate by about 1.6 and approximately one to two extra lambs in

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Booroola Merinos. Recently, Davis (2004) reported that one copy of the *Fec*B gene increases ovulation rate in Booroola Merino by about 1.5 and two copies by 3.0. These extra ovulations in turn increase litter size by 1.0 and 1.5, respectively. High prolificacy in Booroola sheep is due to a non-conservative mutation (q249r) in a highly conserved intracellular kinase signaling domain of the bone morphogenetic protein receptor-1B (BMPR-1B) expressed in the ovary and granulosa cells (Mulsant *et al.,* 2001; Wilson *et al*., 2001). The BMPR-1B, also known as ALK-6, is a member of the transforming growth factor-β (TGF-β) superfamily. These are multifunctional proteins that regulate growth and differentiation in many cell types. Members of this family play essential roles during embryogenesis in mammals, amphibians and insects as well as in bone development, wound healing, hematopoiesis, and immune and inflammatory responses (Massague, 1998; Letterio & Roberts, 1998).

MATERIALS AND METHODS

Animals and extraction of DNA. Whole blood samples (5 mL) were collected in vacutainer tube containing EDTA (1 mg mL^{-1}) from 239 Shal sheep in Qazvin province. DNA was extracted using modified salting out procedure (Miller *et al.*, 1988) and stored at -20°C till used in assay.

The purity and concentration of DNA samples was estimated using UV-visible range spectrophotometer. DNA concentration was adjusted to 50 ng μL^{-1} before PCR amplification. All the DNA samples had the 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.

PCR-RFLP for *Fec*B gene. Primers were synthesized by Metabiyon Company based on the sequences described by Wilson *et al.* (2001) and the amplification procedure was carried out based on the method described by Davis *et al.* (2002). The primers were designed as follows:

Forward primer:
5'-CCAGAGGACAATAGCAAAGCAAA-3'
Reverse primer:
5'-CAAGATGTTTTCATGCCTCATCAACAGGTC-3'

The reverse primer was deliberately introduced a point mutation resulting in PCR products with FecB carrier sheep containing an *Ava*II restriction site (G|GACC), whereas products from non-carriers lacked this site.

Total volume of 25 µL of each PCR reaction contained 1.5 µL of genomic DNA, 2 mm of MgCl2, 0.25 µL each of forward and reverse primers, 0.5 µL of dNTPs, 1 unit of Taq DNA polymerase (CinnaGen, Iran), 1X PCR buffer and distilled water.

The amplification was carried out using 35 cycles at 95°C for 30s, 60°C for 30s and 72°C for 30s followed by 72°C for 5 min. The PCR products were digested with *Ava*II over night at 37°C. The resulting products were separated by electrophoresis on a 2.5% agarose gel and visualized with ethidium bromide under gel documentation system.

Genotype analysis. The PCR product of the *Fec*B gene produced a 190 bp band. After digestion with *Ava*II (Fermentas), the *FecB* gene homozygous carriers should produce a 160 bp band (BB), the non-carrier should produce a 190 bp band (++), whereas heterozygotes should produce both 160 and 190 bp bands (B+).

RESULTS AND DISCUSSION

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 (Souza *et al.,* 2001; Davis *et al.,* 2002) and swine (Kim *et al.*, 2003) to decide whether they had the same mutation as FecB in Booroola Merino sheep. In our experiment, PCR-RFLP approach was used to detect the genotype based on the method described by Souza *et al.* (2001) and Davis *et al.* (2002). RFLP has a good 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. We compared several of these factors 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.

A total of 239 individuals from Shal sheep were genotyped with the PCR-RFLP approach (Fig. 1). The results show that the frequency of polymorphism distributions of *FecB* gene was imbalanced in breed (Table I). All genotyped sheep had the wild type allele (++).

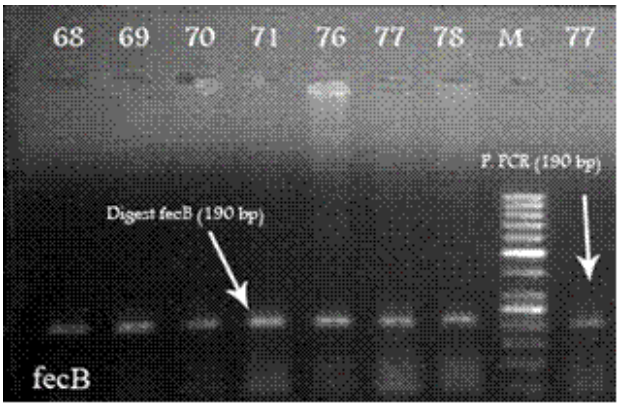
The most striking physiological effects of the FecB locus are on ovulation rate and the size and number of ovulatory follicles in the ovary. Follicles mature and ovulate at significantly smaller diameters in homozygous (BB) and heterozygous (B+) carrier ewes compared with non-carrier or wild-type (++) ewes (McNatty & Henderson, 1987; Montgomery *et al.*, 1992; Baird & Campbell, 1998). The smaller ovulatory follicles of BB ewes contain fewer granulosa cells than ovulatory follicles in ++ ewes.

The ovaries of BB/B+ ewes contain less inhibin than ovaries of ++ ewes.

Table I. The frequency distributions of *FecB* gene in Shal sheep breed

Gene frequency		Genotype frequency		
B	+	BB	B+	++
0	1	0	0	1

Fig. 1. PCR-RFLP analysis of Shal sheep samples, M= Molecular weight marker



These results are in agreement with reports in Romanov, Finn, East Friesian, Teeswater, Blueface Leicester, D'Man, Chios, Mountain Sheep, German Whiteheaded Mutton, Lleyn, Loa, Galician, Barbados Blackbelly (Davis 2006), Mulpura sheep (Kumar 2006), Suffolk sheep, Dorset sheep, Charolais sheep, Chinese Merino and Romney Hills. However, results contrast with the other reports in Hu and Han sheep (Davis, 2006), Garole and Garole * Malpura (Kumar, 2006), Hu sheep and Chinese Merino prolific meat strain (Guan *et al.*, 2007).

CONCLUSION

In the present study the results of PCR showed the same band pattern in all samples, implying no mutation in *Fec*B locus in our 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 may 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 investigate this.

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