Study on polymorphism of BMP-15 gene in Iranian native goats

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Introdction Different mutations in the bone morphogenetic protein-15 (*BMP-15*) and the Growth Differentiation Factor-9 (*GDF-9*) genes have increased ovulation rate and infertility in a dosage-sensitive manner in sheep (McNatty *et al*, 2005). Five naturally occurring mutations in exon 2 of the sheep *BMP-15* gene have been described. These mutations produce increased ovulation rate and twin and triplet births in heterozygotes, and complete primary ovarian failure in homozygotes resulting in total infertility in some prolific breeds of sheep (Galloway *et al.*, 2000). However, information on genes affecting the fertility of Iranian goats is scarce. The goal of the present study was to examine the polymorphism of the $FecX^B$ and $FecX^G$ loci in *BMP-15* gene in Iranian native goats.

Material and methods Jugular blood samples (7 ml) were randomly collected from 109 Iranian native goats by using of EDTA coated tubes. Caprine genomic DNA was isolated from whole blood samples by using a commercially available kit. The final DNA pellets were resuspended in 50 µl of sterile distilled water and stored in -20 ºC for use. The $FecX^B$ allele in exon 2 *BMP-15* gene was amplified using the polymerase chain reaction (PCR) with primers forward: 5′-GCCTTCCTGTGTCCCTTATAAGTATGTTCCCCTTA-3′ and reverse: 5′-TTCTTGGGAAACCTGAGCTAGC-3′ to amplify a 153 bp PCR product (Hanrahan *et al.*, 2004). Amplification was for 30 cycles in a 25 µl reaction mixture, with 100 ng of caprine genomic DNA, 1U of Taq DNA polymerase and 2.5 mM magnesium choloride at an annealing temperature of 57.5°C. The PCR products were digested with 10 U of *Dde*I enzyme (C/TNAG) overnight at 37 ºC, and the resulting products were separated by 10% PAGE gel and visualized by silver staining. The resulting products of wild type animals will have a 122 bp and 31 bp fragments and the mutation type animals with $FecX^B$ variant will have a 153 bp fragment. A primer pair was also designed to detect SNP of the $FecX^G$ allele in *BMP-15* gene with *Hinf*I. The primer sequences were as follows; Forward: 5′-ACTGTCTTCTTGTTACTGTATTTCAATGAGAC-3′; and Reverse: 5′-GATGCAATACTGCCTGCTTG-3′(Hanrahan *et al.*, 2004). Polymerase chain reactions were carried out The amplification conditions for primers of the $FecX^G$ allele were as follows: denaturation at 94°C for 5 min; followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 63°C for 30 sec, and extension at 72°C for 20 sec; with a final extension at 72°C for 5 min. The PCR products of 8 µl were digested separately with 10 U of *Hinf*I overnight at 37°C in a 20 µl reaction mixture, and the resulting products were separated by 10 % PAGE gel and visualized by silver staining. Primers amplified a 141 bp band. The wild type products could be cleaved by *Hinf*I (G/ANTC) with a 112 bp and 29 bp fragments, the mutation type with $FecX^G$ remained uncleaved.

Results.In the present study the PCR products were separated by 6% PAGE and following digestion with restriction enzymes were separated by 10% PAGE (Figure 1). The basic finding of the current study was the absence of polymorphism at the $FecX^B$ and $FecX^G$ loci of *BMP-15* gene in Iranian native goats. All goats were monomorph for exon 2 *BMP-15* gene.

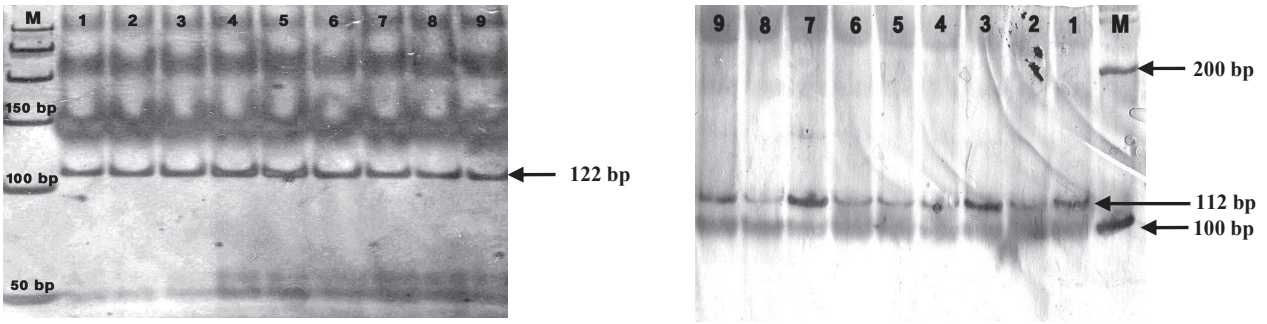

Figure 1 Polyacrylamide gel electrophoresis (10%) images for PCR product of the $FecX^B$ and $FecX^G$ digested with *Dde*I and *Hinf*I, respectively.

Conclussion These results showed that there was no genetic polymorphism of $FecX^B$ and $FecX^G$ loci in *BMP15* gene in Iranian native goats. Further investigation should be directed at other loci of *BMP-15* gene or other genes, using larger sample sizes.

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