Molecular Study on the Exon 2 Region of the Ovis Bone Morphology Protein 15 (BMP-15) Gene in Iranian Bluchi Sheep Breed by PCR-SSCP Technique

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ABSTRACT

Litter size is one of the most important economical traits in sheep breeding industry. In addition to minor genes, litter size is under the influence of major genes. Bone morphogenetic protein 15 (BMP-15), a member of the transforming growth factor beta (TGF-ß) superfamily, which is specifically expressed in oocytes, plays a dramatic role in sheep prolificacy. Reported mutations in this gene cause increased ovulation rate and infertility in a dosage-sensitive manner. Six different point mutations have been indicated in the BMP-15 gene of sheep, each having a major effect on prolificacy. The aim of the current study was to investigate the Lacaune (FecXL) mutation in the prolificacy of the Iranian Baluchi sheep breed. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) coated tubes from jugular vein and genomic DNA was extracted from whole blood samples. Single nucleotide polymorphism of FecXL loci in BMP-15 gene were determined using polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) technique. No evidence of mutation in FecXL was observed, all of which were monomorphic for exon 2 BMP-15 gene.

KEY WORDS BMP-15, FecXL, gene, litter size, polymorphism, TGF-ß.

INTRODUCTION

There are more than fifty million head of sheep of more than twenty breeds and sub-breeds in Iran. Baluchi sheep is the most common native breed of sheep in Iran, comprising 30% of the sheep population. This breed is native to the eastern part of the country and widespread in the south parts of Khorasan, Sistan and Balouchestan, Yazd and Kerman provinces, which are mainly arid zones of the country. The breed is fat-tailed, having white fleece with black markings on the head and legs. This breed is primarily used for meat production, but also useful for wool production for the textile industry (Gholibeikifard et al. 2013). Prolificacy is measured by the ewe’s ability to produce multiple lambs e.g. twins and triplets, through high ovulation rate and high survival rate of embryos.

There are several factors affecting ovulation rate of ewes which include genetics, stress, health, pasture type and quality, and ewe age and weight (Kareta et al. 2006). Genetic studies have indicated that the litter size and ovulation rate can be genetically determined by the action of single genes with a major effect in some European sheep breeds. The TGF-ß superfamily contains over 35 members, many of which have been shown to have a vital role in fertility
regulation (Knight and Glistter, 2006). The TGF-β members are multifunctional proteins which act through specific receptors to regulate growth and differentiation in many cell types, including those within the ovary (Elvin et al. 2000). They also play key roles in fertility and also during the embryogenesis period in mammals.

So far, three prolificacy loci related oocyte derived members of the TGF-β superfamily have been discovered in sheep, namely bone morphogenetic protein receptor type 1B (BMPR1B; or activin-like kinase 6, ALK6), known as FecB (Booroola) on chromosome 6 (Souza et al. 2001) corresponding to the human chromosome 4q22-23 (Montgomery et al. 1993); growth differentiation factor 9 (GDF9), known as FecG on chromosome 5 (Hanrahan et al. 2004); and bone morphogenetic protein 15 (BMP-15; also known as GDF-9B) known as FecX on chromosome X (Galloway et al. 2000; Hanrahan et al. 2004). BMP-15, which is specifically expressed in oocytes, is essential for sheep prolificacy. Reported mutations in this gene cause increased ovulation rate and infertility in a dosage-sensitive manner. Critical roles of BMP-15 in female fertility have also been demonstrated in women (Di Pasquale et al. 2004). Lately, the roles of BMPs in embryonic development and cellular functions in postnatal and adult animals have been extensively studied (Chena et al. 2004).

In sheep, six different point mutations FecX1 (Inverdale); FecX6 (Hanna) (Galloway et al. 2000); FecX2 (Lacaune) (Bodin et al. 2007); FecX5 (Galway); FecX8 (Belclare) (Hanrahan et al. 2004) and a 17 bp deletion of the functional gene (FecX8) in Rasa Aragonesa sheep breed (Monteagudo et al. 2009) have been identified in the BMP-15 gene, each having a major effect on prolificacy. Ewes with two inactive copies of the BMP-15 gene (homozygous animals) are sterile (Galloway et al. 2000; Hanrahan et al. 2004) and have a similar ovarian phenotype, although those who have a single inactive BMP-15 gene (heterozygous animals) are fertile and have an increased ovulation rate and a higher incidence of twin or triplet births (Davis et al. 1991; Galloway et al. 2000; Hanrahan et al. 2004). The aim of the present study was to molecular study on the exon 2 region of the ovis BMP-15 gene in Baluchi sheep breed by PCR-SSCP technique.

## Materials and Methods

### Sample collection and DNA isolation

Two hundred Iranian Baluchi sheep from animal breeding station of Abbasabad in northeast of Khorasan province in Iran were used in this study. The animals were used in accordance with the guidelines for the use and care of experimental animals and approved by the animal ethical committee of Tehran university, Iran, Islamic Republic of Iran. Blood samples (5 mL) were collected in EDTA vacu-
Each PCR reaction was diluted in denaturing solution, denatured at 95 °C for 5 min, cooled on ice and resolved by polyacrylamide gel electrophoresis. The electrophoresis was carried out in a vertical unit (160×140×1.0 mm spacers), in 1X TBE buffer. The gels were stained with silver nitrate. The conditions of SSCP analysis in the current study are described in Table 2.

**DNA sequencing**

Twenty randomly selected samples of PCR products from homozygote and heterozygote animals were used for sequencing. Primers, dNTP, buffer ingredients and nonspecific products were isolated, and then sequencing was done using a 3730 sequencer (Applied Biosystems 3730×1 DNA Analyzer, Bioneer Company, South Korea). The sequenced fragments were aligned next to each other and single nucleotide polymorphism (SNP) was identified by sequence traces in contrast to original sequences in a livestock genomics database: (http://www.livestockgenomics.csiro.au/blast).

The generated sequence data was further analyzed by using laser gene software (Burland, 2000). Multiple sequence alignment was performed using MegAlign program of laser gene software.

**RESULTS AND DISCUSSION**

The fragment of the BMP-15 gene (310 bp) was successfully amplified from the DNA of each sample (200 samples) used in the present study. Figure 1 shows agarose gel electrophoresis of the PCR amplified 310 bp fragment from exon 2 of ovine BMP-15 gene of Baluchi breed. After optimization of the parameters which affect the detection of SSCP’s, the PCR products (n=200) animals were analyzed under the conditions described in Table 2. In the PCR-SSCP analysis of BMP-15 gene (exon 2) revealed one allele was identified and designated as A on the basis of electrophoretic mobility in the gel. We obtained one conformational pattern (genotype), AA (Figure 2). The amplification was not possible in ten samples (5%).

**Allele and genotype frequencies**

The allele and genotype frequencies of this gene in Iranian Baluchi sheep breed are shown in Table 3. For BMP-15 gene, the genotype frequencies were 100 and 0.0% for AA and AB, respectively; the allele frequencies for the A and B were 1.0 and 0.0, respectively.

**Sequencing results**

The results obtained after SSCP analysis were confirmed by sequencing twenty randomly selected DNA samples for each primer pair, in both forward and reverse directions for BMP-15. In the present study, DNA sequencing analysis showed that the sequences of PCR products corresponded to the sequences (Figure 3) in the GenBank. In SSCP analysis and sequencing nucleotide variation for the BMP-15 gene was not detected. In this case, more studies are needed in order to better understanding the importance of this gene, which has never been described in other breeds.

Mutations at five different points in exon 2 of BMP-15 gene are associated with prolificacy in some breeds of sheep (Montgomery et al. 2001). Mutations in fecundity genes like BMP-15 have important economic values in sheep breeding and probably ruminant reproduction (Galloway et al. 2000; Hanrahan et al. 2004; McNatty et al. 2005).
Naturally occurring heterozygous mutations in BMP-15 in sheep increase the ovulation rate and prolificacy, whereas homozygous mutations yield infertile animals (Galloway et al. 2000; Hanrahan et al. 2004).

The results of our study detected no polymorphism in FecX1 gene (having a major effect on litter size) in Baluchi sheep and all of them were monomorphic for this locus. These results are in agreement with reports of Romanov, Finn, East Friesian, Teeswater, Blueface Leicester, D’Man, Chios, Mountain Sheep, German Whiteheaded Mutton, Lleyn, Loa, Galician, Barbados Blackbelly sheep (Davis et al. 2006), and Iranian goats (Deldar-Tajangookeh et al. 2009). In the present study, tests were carried out only for the Lacaune FecX L mutation and we did not find any genetic variations within the BMP-15 gene by PCR-SSCP among 200 individual Baluchi sheep. Zare et al. (2007) also detected no mutations in two points of BMP-15 gene (FecXG and FecXCl) from 240 blood samples of Shal ewes by using of PCR-RFLP and PCR-SSCP techniques. Nejati-Javaremi et al. (2007) using PCR-SCCP techniques investigated the polymorphism in FecXL gene associated with twinning in Iranian Lori-Bakhtiari sheep but found no differences in the band pattern of denatured PCR products.

CONCLUSION

The results of the present study show that there is no genetic polymorphism of FecX1 loci in BMP-15 gene in Iranian Baluchi sheep. Further investigation should be directed at other loci of BMP-15 gene or other genes, using larger sample sizes.

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