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Full Length Research Paper

Study of *BMP-15* gene polymorphism in Iranian goats

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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. To test the polymorphisms of genes in goat, which have been demonstrated as major genes of fecundity in sheep, the genetic polymorphism of *FecX^B* and *FecX^G* loci in *BMP-15* gene were studied in 109 Iranian native goats. Blood samples were collected in EDTA coated tubes from Jugular vein and genomic DNA was extracted from whole blood samples. Single nucleotide polymorphism of *FecX^B* and *FecX^G* loci in *BMP-15* gene were determined using PCR-RFLP technique. There was no evidence of mutation in *FecX^B* and *FecX^G* in these goats, all of which were monomorph for exon 2 *BMP-15* gene.

Key words: Goat, ***FecX^B***, ***FecX^G***, ***BMP-15***, polymorphism.

INTRODUCTION

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. So far, two keys oocyte molecules identified is growth differentiation factor-9 (GDF-9) and bone morphogenic protein-15 (BMP-15; also known as GDF-9B), two members of the transforming growth factor-ß (TGF-ß) super family (Galloway et al., 2000; Hanrahan et al., 2004). These oocyte growth factors are critical for progression of the very earliest stages of folliculogenesis (Dong et al., 1996; Galloway et al., 2000), and then in late follicular development. These oocyte-secreted factors play an important role in the differentiation of different granulosa cell lineages (Eppig, 2000; Li et al., 2000) and in the regulation of key granulosa cell functions (Elvin et al., 1999; Joyce et al., 2000; Otsuka et al., 2001). Thus, oocyte controls the differentiation and function of granulosa cells and their influence on gene expression patterns in follicular somatic cells is one mechanism to achieve this (Sugiura and Eppig, 2005).

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. 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; Hanrahan et al., 2004).

Five naturally occurring mutations in exon 2 of the sheep ***BMP-15*** gene have been described. One of these mutations in ***BMP-15*** gene was found in Belclare sheep breed in Ireland and was called ***FecX^B*** that shows very high ovulation rates (Davis, 2005).

GDF-9-deficient female mice (Dong et al., 1996) and ***BMP-15***-deficient ewes (Galloway et al., 2000) are infertile due to a complete block in folliculogenesis at the primary follicle stage. These animals exhibit remarkably similar ovarian phenotypes, characterized by “streak ovaries” containing a multitude of primordial and primary follicles and also tumor-like, oocyte-free granulosa cell nests (Dong et al., 1996; Juengel et al., 2002). Moreover, recent findings in sheep, humans and rodents show that ***BMP-15*** and ***GDF-9*** genes can be considered to be new targets for fertility regulation in mammals (McNatty et al., 2005).

The ***BMP-15*** gene is located on the X chromosome and in rodents its mRNA and protein are expressed in oocytes from the primary stage through ovulation (Dube et al.,

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Table 1. Primer sequences and PCR amplification parameters.

Gene	Forward and reverse Primers (5' to 3')	Amplicon size (bp)	Annealing temperature (°C)	Enzyme
FecX^B	GCCTTCCTGTGTCCCTTATAAGTATGTTCCCCTTA	153	57.5	***Dde*I**
	TTCTTGGGAAACCTGAGCTAGC			
FecX^G	CACTGTCTTCTTGTTACTGTATTTCAATGAGAC	141	63	***Hinf*I**
	GATGCAATACTGCCTGCTTG			

1998; Otsuka et al., 2001). Silva et al. (2004) reported that the mRNAs and proteins of ***GDF-9*** and ***BMP-15*** are expressed in goat ovarian follicles at all stages of their development. Goats are among the first mammalian species that have been domesticated. They are economically important because of their milk, meat and fiber production, being a major source of protein in the tropics. The population of goat in Iran is about 25.8 million, distributed mainly in arid and semiarid regions of the country (FAO STAT, 2007). Information on genes affecting the fertility of Iranian goats is scarce. Molecular analysis of ***BMP-15*** gene in Markhoz goats was recently investigated by Arefnezhad (2007). The goal of the present work was to examine the polymorphism of the ***FecX^B*** and ***FecX^G*** loci in ***BMP-15*** gene in Iranian native goats.

MATERIALS AND METHODS

Jugular blood samples (7 ml) were randomly collected from 109 Iranian native goats (mixed breeds) using EDTA coated tubes, immediately placed on ice and transported to the laboratory in a thermos flask, within 2 h. The samples were recovered from a local slaughterhouse in Tehran province. Caprine genomic DNA was isolated from whole blood samples by using a commercially available kit (Cinnagen Co., Tehran). The final DNA pellets were resuspended in 50 µl of sterile distilled water and stored in -20°C for use.

Recovery and purity of each DNA sample was estimated by UV spectrophotometery. Separation and purification of DNA fragments were done by electrophoresis through an agarose gel. Electrophoresis was carried out at 7 to 8 volt/cm of the gel and the migration was monitored using an UV transilluminator.

The ***FecX^B*** allele in exon 2 ***BMP-15*** gene was amplified using the polymerase chain reaction (PCR) (Hanrahan et al., 2004). Amplification carried out for 30 cycles in a 25 µl reaction mixture, containing 100 ng of caprine genomic DNA, 1U of Taq DNA polymerase (Cinnagen Co., Iran), and 2.5 mM magnesium chloride. The PCR products were analyzed in 6% polyacrylamide gel electrophoresis (PAGE). The PCR products were digested with 10 U of ***Dde*I** enzyme (C/TNAG) (Fermentase Co.) overnight at 37°C, 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 and 31 bp fragments and the mutation type animals with ***FecX^B*** variant will have a 153 bp fragment (Table 1).

A primer pair was also used to detect SNP of the ***FecX^G*** allele in ***BMP-15*** gene with ***Hinf*I** (Hanrahan et al., 2004). Polymerase chain reactions were carried out in a 25 µl reaction mixture containing approximately 2.5 µl of 10X PCR buffer, 1 mM of $MgCl_2$, 200 µM of each dNTP, 4 µM of each primer, 100 ng of caprine genomic DNA, and 1U of Taq DNA polymerase (Cinnagen Co., Iran). The PCR products were analyzed in 6% polyacrylamide gel electrophoresis. The PCR products of 8 µl were digested separately with 10 U of ***Hinf*I** (Metabion Co., Germany) 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. The wild type products could be cleaved by ***Hinf*I** (G/ANTC) with a 112 and 29 bp fragments, the mutation type with ***FecX^G*** remained uncleaved. The primers were synthesized by Metabion Company (Germany).

RESULTS AND DISCUSSION

In the present study, PCR-RFLP with ***Dde*I** and ***Hinf*I** digestion was used to investigate the polymorphism of ***FecX^B*** and ***FecX^G*** in exon 2 ***BMP-15*** gene. The PCR products were separated by 6% PAGE (Figure 1) and following digestion with restriction enzymes were separated by 10% PAGE (Figure 2). 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.

Naturally occurring heterozygous mutations in ***BMP-15*** and ***GDF-9*** in sheep increase the ovulation rate and prolificacy, whereas homozygous mutations yield infertile animals (Galloway et al., 2000; Hanrahan et al., 2004). So far, no mutation in ***BMP-15*** gene affecting prolificacy has been reported in goats. The findings of the present study are in line with those of Hua et al. (2007) in Chinese goats who reported that none of the polymorphism of the ovine fecundity major genes ***FecB*** and ***FecX*** was tested in 550 goats from six breeds (or flocks). Also, none of the polymorphisms were found in the coding region of ***BMP-15*** mature peptide of Markhoz goats of Iran by using of PCR-SSCP with three primer pairs, PCR Sequencing and PCR- RFLP with ***Dde*I** digestion (Arefnezhad, 2007). Guan et al. (2006) found no ***FecB*** mutations in the highly fertile Hu sheep in China. The absent of mutation in ***BMP-15*** of six breeds of Chinese goats was reported by He et al. (2006).

There are no attestation of mutations in ***GDF-9*** and ***FecB*** genes in Shal sheep, a native breed of Iranian sheep (Ghaffari et al., 2007a, b). Zare et al. (2007) also detected no mutations in two points of ***BMP-15*** gene (***FecX^G*** and ***FecX^L***) from 240 blood samples of Shal ewes by using of PCR- RFLP and PCR-SSCP techniques. A study by Nejati-Javaremi et al. (2007) using PCR-SCCP technique investigated the polymorphism in ***FecX^L*** gene associated with twinning in Iranian Lori-Bakhtiari sheep but found no differences in the band pattern of denatured PCR products. Farajzadeh et al. (2007) found no muta-

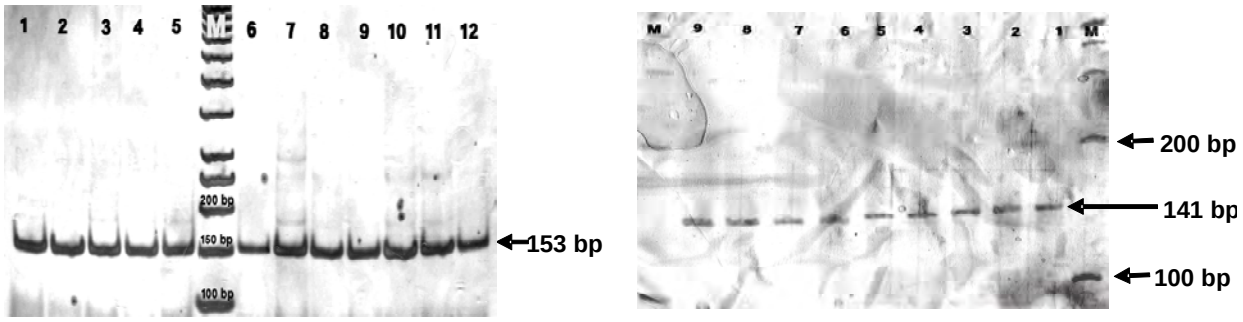

Figure 1. Polyacrylamide gel electrophoresis (6%) images for PCR product of the ***FecX^B*** from 12 samples (left picture; M: GenRuler™ 50 bp DNA Ladder Mix, MBI Fermentase®, Cat. No. SM1133) and ***FecX^G*** from 9 samples (right picture; M: GenRuler™ 100 bp DNA Ladder Mix, MBI Fermentase®, Cat. No. SM0338).

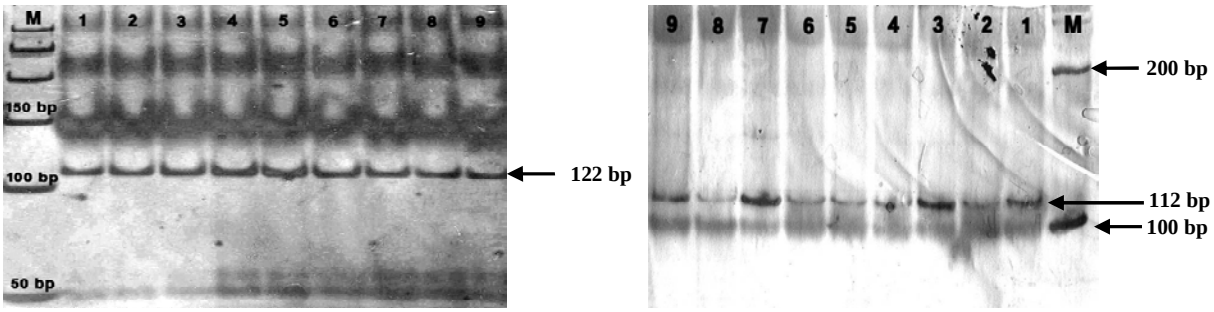

Figure 2. Polyacrylamide gel electrophoresis (10%) images for PCR product of the ***FecX^B*** from 9 samples digested with ***Dde***I (Left picture; M: GenRuler™ 50bp DNA Ladder Mix, MBI Fermentase®, Cat. No. SM1133) and ***FecX^G*** from 9 samples digested with ***Hinf***I (right picture; M: GenRuler™ 100 bp DNA Ladder Mix, MBI Fermentase®, Cat. No. SM0338).

tions in ***GDF-9*** gene in Arkha Merino sheep using RFLP-PCR. The genotyped samples of ***FecB*** and ***FecX^I*** genes in Lori-Bakhtiari sheep did not confirm the incidence of mutant allele, since all samples showed monomorph genotypes (Amiri et al., 2007).

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 ***GDF-9*** and ***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).

Action of a single major gene respond high ovulation rate in the Booroola Merino, Inverdale, Belclare and Cambridge sheep but there is no evidence of major gene for litter size in other prolific sheep such as Finish Landrace and Romanov; these findings indicate at least two genetic control mechanisms for high prolificacy operate in sheep (Gordon, 2004). The biological effect of the mutations in mammals varies among species (Yan et al., 2001). Furthermore, Hashimoto et al. (2005) recently suggested that species-specific differences in ***BMP-15*** processing may be associated with the differences in ovulation rate amongst species.

The results of the present study show that there is 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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