Genetic Polymorphism of Fertility Genes in Lori Sheep Breed

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ABSTRACT

The aim of this study was to verify polymorphism of the BMP-15 and GDF9 genes in Lori sheep breed. These loci are located in the region of ovine chromosome X and 5, respectively. In this study, blood samples were randomly collected from 123 ewe Lori sheep breed in Lorestan province and subsequently their DNA content were extracted using modified salting-out method. Single nucleotide polymorphisms of BMP-15 and GDF9 genes were detected in Lori ewes by PCR-RFLP using HinfI and HhaI restriction enzymes, respectively. In both loci were detected two genotypes wild type (+/+) and carrier-mutant (+/-). The results of the present study revealed, heterozygous genotype frequencies for both loci lower than homozygous genotypes. Unlike GDF9 locus, the population was found to follow Hardy-Weinberg equilibrium for BMP-15 locus. In view of our results, a while polymorphism of these loci in Lori sheep breed.

Keywords: BMP-15, GDF9, Lori sheep, PCR-RFLP

INTRODUCTION

The reproductive potential of the flock is determined by genetics and modified by flock management - namely ewe nutrition and selection. Trying to improve the reproductive efficiency of a sheep herd by genetic selection is slow and difficult because heritabilities for most reproductive traits are low. Various mutations influencing ovulation rate and litter size in sheep provide additional opportunities to rapidly adjust genetic potentials but require careful breeding management [1]. Two key oocyte molecules incorporated 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 [2,3]. BMP15 and GDF9 are growth factors secreted by oocyte and regulate growth and differentiation of prophase ovarian follicle. Investigation on the major genes impressing fertility in sheep will increment the yean proficiency and it will help comprehend basis the mechanism of reproduction in mammals.

Several researches have recently been shown, a number of natural genetic mutations in BMP15 and GDF9 genes [4-13]. It was certificated that these mutations had great association to the ovulation rate of different sheep breeds.

The BMP15 gene has been mapped to sheep chromosome X and contains 2 exons with over-dominant inheritance pattern gene [2,14]. All mutations in this gene show the same phenotype: homozygous carrier ewes are sterile and heterozygous carriers show increased ovulation rate [3]. It

Research Article, Acta Biologica Indica 2015, 4(1):101-106

pISSN 2319-1244, eISSN 2279-0160

has been shown that effect of BMP15 is caused by a point mutation (C to T) leading to a Glutamic acid → Stop codon.

The GDF9 is an autosomal over-dominant inheritance pattern gene that has been located to the sheep chromosome 5. It has been shown to be essential for growth and differentiation of early ovarian follicles [15]. The mutation in this gene is caused by a point mutation (G to A) leading to an Arginine → Histidine transition, that reason increased ovulation rate and twin or triplet births in heterozygotes, but animals homozygous for GDF9 mutation are sterile due to arrested follicular development from the primary stage of growth in some prolific breeds of sheep [3].

The Lori sheep is meat breed with low lambing rate that may be explained by the harsh mountain environment for rearing of this breed in Iran. The origin of this sheep is the Lorestan province. The objective of the current study was to detect the single nucleotide polymorphisms of the BMP-15 and GDF9 genes in Lori sheep breed by PCR-RFLP.

MATERIALS AND METHODS

Animals and data collection

Lori sheep examined in this study were fat-tailed sheep, with large size and meat type. This sheep have strong constitution, good traveling ability with suitable conformation as a mountain sheep. Blood samples were collected randomly from 123 ewe Lori sheep breed from jugular vein, using vacuum blood collection tubes containing EDTA and stored at 4°C.

Genomic DNA extraction

Genomic DNA was extracted by salting out procedure with minor modifications [16]. DNA quantity and purity of each sample were assessed by spectrophotometer and agarose gel electrophoresis, which were proper for a PCR protocol application.

PCR-RFLP Analysis

The Primer sequences used for the BMP15 HinfI site: F: 5′-CAC TGT CTT CTT GTT ACT GTA TTT CAA TGA C-3′ and R: 5′-GAT GCA ATA CTG CCT GCT TG-3′. A primer pair was also designed to detect SNP of the GDF9 with HhaI: F: 5'-GAA GAC TGG TAT GGG GAA ATG-3' and R: 5'-CCA ATC TGC TCC TAC ACA CCT-3'. Polymerase chain reactions were performed in a 25 µL reaction mixture containing approximately 2.5 µL of 10X PCR buffer, 2 mM of $MgCl_2$, 200 µM of each dNTP, 10 pmol from each primer, 50-100 ng of ovine genomic DNA, and 1 U of Taq DNA polymerase. The amplification conditions for primers of the BMP15 gene were as follows: denaturation at 95°C for 5 min; followed by 30 cycles of denaturation at 95°C for 45 s, annealing at 61°C for 30 s, and extension at 72°C for 60 s; with a final extension at 72 °C for 10 min. At the same time, the amplification conditions for primers of the GDF9 gene were carried out using 35 cycles of denaturation at 94° C for 5 min; followed by denaturation at 94 °C for 45 s, annealing at 60°C for 40 s, and extension at 72°C for 1 min; and final extension at 72°C for 10 min.

The PCR products were digested with 10U HinfI restriction at $37^{\circ C}$ for 12 h. After digestion, the homozygous non-carriers (wild type) should produce fragments of 106 and 35 bp (+/+), and homozygous carriers remain uncut at 141 bp (-/-). Heterozygotes should produce fragments of 141, 106 and 35 bp (+/-). Also, the 462 bp PCR products were digested at 37°C for 9 h with 10U Hin6I restriction enzyme. After digestion with restriction enzymes, all products were separated by using electrophoresis in 2% agarose gel and visualized with ethidium bromide. Digestion of the 462 bp fragment in GDF9 gene with HhaI restriction enzyme can reveal three genotypes. Homozygous

carriers should produce fragments of 410 and 52 bp (-/-), the homozygous non-carriers (wild type) should produce fragments of 254, 156 and 52 bp (+/+) and heterozygotes should produce fragments of 410, 254, 156 and 52 bp (+/-).

Statistical Analysis

Genetic diversity parameters, genotype and allele frequencies of BMP15 and GDF9 genes were calculated using POPGEN software (version 1.32). Hardy-Weinberg equilibrium for the population was also analyzed using Chi-square (X^2) test [17].

RESULTS AND DISCUSSION

A DNA fragment with the size of 141 bp was amplified from exon 2 of BMP15 and 462 bp from exon 1 of GDF9 genes successfully. We analyzed the status of the BMP15 and GDF9 mutations in Lori breed and none of the individuals carried homozygous genotype for both BMP15 and GDF9 variants in this breed. Two genotypes, +/+, +/- for both gene were detected in Lori sheep (Figure 1, Figure 2). Also, RFLP pattern of BMP15 and GDF9 genes are given in figure 1 and figure 2, respectively.

As proteins regulate oocyte secretion, the change of important sites in BMP15 and GDF9 can significantly affect ovulation in ewe. C/T mutation in BMPl5 carried ewe led to the change of Glutamic acid at amino acid 23 into termination codon [2]. We detected SNP sites in BMP15 and GDF9 genes which could affect litter size. Allelic and genotypic frequencies of the BMP15 gene and the GDF9 gene are presented in table 1. In both loci, wild types were dominant genotypes.

Similar results have been reported in some previous researches with other breeds. Javanmard et al. [6] and Bahrami et al. [11] reported that none of the individuals carried homozygous genotype for the BMP15 and GDF9 variant in the Afshari, Baluchi, Makui and Mehraban Iranian sheep breeds and Hisari Tajikistan sheep breed. The results of the present experiment also are consistent with Barzegari et al. [8] suggested that -/- genotypes for BMP15 and GDF9 locus not found in Moghani and Ghezel sheep breeds.

In contrast to these reports, Moradband et al. [4] observed the all three possible genotypes for the GDF9 in Baluchi sheep population. Also, our findings are in conflict with Chu et al. [9] that genotypic frequency of heterozygotes was more than wild type homozygote at the BMP15 locus in Small Tailed Han ewes. Beside, Shafieiyan et al. [12] reported that all of the 50 individuals were wild homozygote for BMP15, therefore, none of the samples carried the mutation in this gene.

In fact, because that heterozygous genotype frequency for GDF9 locus was 1/3 of the population, mutant allelic frequency achieved 0.185 (Table 1). Moradband et al. [4] pointed out that the mutation in GDF9 gene associated with litter size in Baluchi sheep. Also, they explained that Preliminary polymorphism analysis performed on mutation in GDF9 locus suggested a major gene inheritance of prolificacy in Baluchi population. Similarly, it seems that there is a homological situation in Lori sheep breed.

The result of chi-square analysis indicated that population of Lori sheep for BMP15 locus was in Hardy-Weinberg equilibrium. Although, due to the low samples and so on, genotype (-/-) were not recognized. In turn GDF9 locus was not in agreement with Hardy-Weinberg equilibrium ($p<0.05$). According to our findings, Shi et al. [10] reported that Hardy-Weinberg equilibrium for the BMP15 locus in Cele black sheep. In contrast to this report, Kasiriyan et al. [7] and Bahrami et al. [11] observed for populations of Hisari and Sangsari sheep were in the Hardy-Weinberg equilibrium at the GDF9 locus.

The observed and expected heterozygosity, effective number of alleles, Nei index and Shannon's Information index for BMP15 and GDF9 genes are shown in table 2. Average heterozygosity, as

well as Nei index, showed this breed had not an appropriate genetic variation. As shown in table 2, the lower heterozygous level of both genes especially BMP15 locus in studied population. According to the present data, it seems that because of closed herd and the limited number of males used for reproduction. So, reduce the level of heterozygosity is observed. The observed number of alleles at the two investigated loci was similar to that pervious reported by Hanrahan et al. [3]. But the notable thing is that in the present study, the effective number of alleles at the BMP15 locus was near to 1 (1.10), this means that only one allele of the two alleles (wild allele) in the population plays an important role. This is due to the major differences in the frequencies of alleles in a population. This is true for loci GDF9 with less intensity (1.43). Also, Shannon's Information index for BMP15 and GDF9 Loci were low (Table 2).

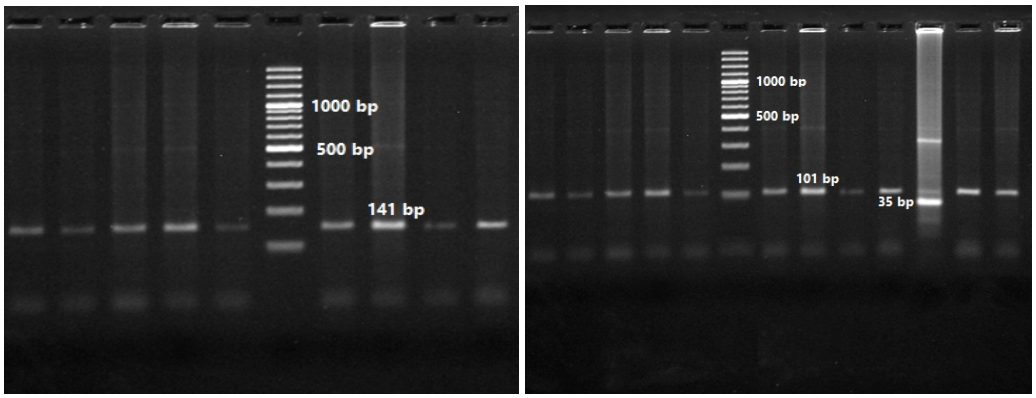

Figure 1. RFLP patterns of PCR amplification of BMP15. Left: PCR product of BMP15 gene; right: Different genotypes of BMP15 gene, (+/+):106 and 35 bp, and (+/-):141, 106 and 35 bp.

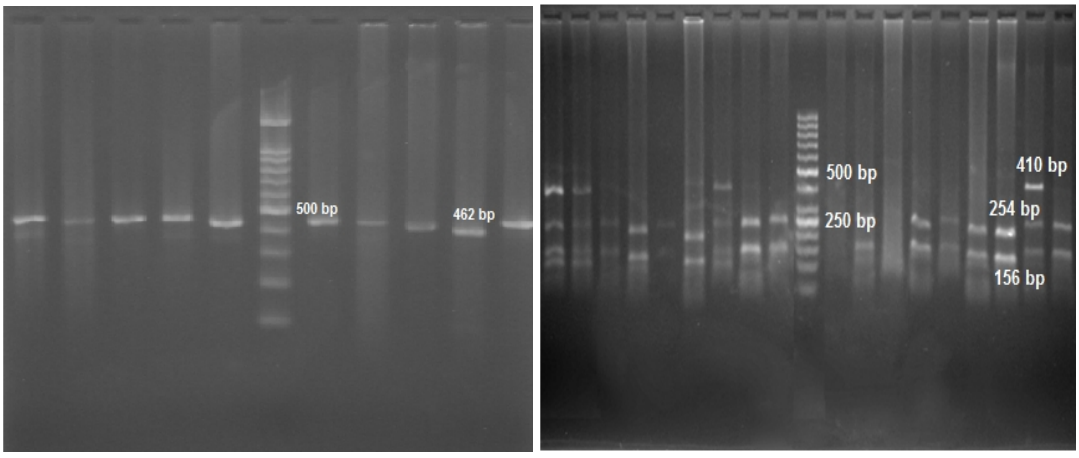

Figure 2. RFLP patterns of PCR amplification of GDF9. Left: PCR product of GDF9 gene (462 bp band); right: different genotypes of GDF9 gene; (+/+): 254, 156, and 52 bp, and (+/-):410, 254, 156, and 52 bp.

Notter et al. [1] showed that there may be some possible exceptions, but we should not ignore that, generally, there is ample evidence that increasing lambing rate leads to more profit and so is desirable. There is substantial evidence to suggest that the effects of the BMP15 and GDF9 genes on ovulation rate are additive. Nevertheless, some progeny test data demonstrate a smaller response

to a copy of GDF9 when the BMP15 mutation is also present [3,18]. In all case, the individuals with mutations in GDF9 and BMP15 had higher ovulation rates than those with either mutation alone [18].

Table 1. Allelic and genotypic frequencies of the BMP15 and GDF9 genes in Lori sheep breed.

Gene	Number of animals	Allelic frequency		Genotypic frequency			P-Value
BMP15	123	+	-	+/+	+/-	-/-	0.587^{ns}
		0.951	0.049	0.902	0.098	0.00	
GDF9	123	+	-	+/+	+/-	-/-	0.041^{*}
		0.815	0.185	0.631	0.369	0.00	

-: mutation; +: wild-type; ns: non-significant; *: P<0.05

Table 2. Genetic diversity Parameters for BMP15 and GDF9 genes in Lori sheep breed.

Gene	Effective number of alleles	Observed heterozygosity	Expected heterozygosity	Nei index	Shannon's Information index
BMP15	1.10	0.098	0.093	0.093	0.195
GDF9	1.43	0.369	0.303	0.301	0.478

Besides, it was concluded that there may be different mutations significantly affecting fertility rates in Lori sheep breed. There have been several recent research findings in relation to inheritance patterns and DNA testing of major genes for prolificacy that have the potential to significantly increase the reproductive performance of sheep flocks throughout the world. These findings will also enhance knowledge of the control of reproduction regulation mechanisms, for example studies on the Arylalkylamine N-acetyltransferase (AA-NAT) gene [19], melatonin receptor 1A gene (MTNR1A) [20], inhibin α gene (INHA) [21], and the prolactin gene (PRL) [22]. Therefore, the prolificacy of the ewes could not be totally accurately predicted by the BMP15 and GDF9 genotypes alone. More extensive screening is required to fully reveal the mechanisms underlying the prolificacy of Iranian sheep.

This study documented awhile polymorphism at the BMP15 and GDF9 loci and the low frequencies of the currently known prolificacy genotypes in fat-tailed native sheep breed. However, future investigations including complete gene sequencing and molecular marker analysis are essential.

Acknowledgments: This study was supported by Islamic Azad University, Boroujerd Branch, Iran. The authors would like to acknowledge staffs of university. This work resulted from research project no. 845.

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