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Investigation of BMP15 Gene Polymorphism by PCR-SSCP in Arabic Sheep Breed Population in Khuzestan Province

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ABSTRACT

The members of the transforming growth factor-beta superfamily bone morphogenetic protein 15 (BMP15), has essential roles in fertility of sheep. The present study describes polymorphism of BMP15 gene. This is first study of BMP15 gene polymorphism in Arabic sheep of Iran. We used the polymerase chain reaction single strand conformation polymorphism (PCR-SSCP) technique to screen DNA polymorphism in sheep. We amplified the 235 fragment consisting on part of exon 2. The results showed that products had good specificity and banding patterns showed by 8% polyacril amid. Two alleles were observed M and N. It gives frequencies of 0.79 and 0.21 for M and N alleles. Also genotypes MM and MN with frequencies of 58% and 42% of the total study population were diagnosed. In this population, mentioned locus did not show Hardy-Weinberg equilibrium. Also in this population BMP15 gene has medium level of heterozygosity. The result of this study confirmed that BMP-15 gene may be a strong candidate gene for further applications in marker-assisted selection (MAS) for litter size in sheep.

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INTRODUCTION

Ovulation rate is determined by a complex exchange of endocrine signals between the pituitary gland and the ovary and paracrine and probably autocrine signals in ovarian follicles involving the oocyte and its adjacent somatic cells.[1,2] Production of more than one lamb per pregnancy is obviously dependant on ovulation rate. Genetic studies have established that the litter size and ovulation rate can be genetically determined by the action of single gene(s) called Fec genes with a major effect. Three of these Fec genes identified in sheep are bone morphogenetic protein receptor type IB (BMPRIB) or Activin Like Kinase 6, known as FecB located on chromosome 6;[3,4,5] growth differentiation factor (GDF9), known as FecG located on chromosome 5 [6,7] and bone morphogenetic protein 15 (BMP15), known as FecX located on the X chromosome the full lenght.[8,6] It is a member of the transforming growth factor beta superfamily (TGFβ) which in rodents,[9,6] cattle,[10] goat [11] and sheep is expressed in the oocyte. One independent point mutation was identified causing the phenotype: a T to A transition at nucleotide position 896, substituting valine with aspartic acid at residue 299 of the unprocessed protein, called Inverdale or *FecXI* (first prolific sheep to have its genetic basis).[9] Natural occurring mutations in sheep such as FecXG, FecXB, FecXI, FecXH, FecXL [12,13,14] lead to infertility in homozygous ewes that due to defects in early folliculogenesis, whereas heterozygous ewes have increased ovulation rate and litter size. Until now, the association of BMP15 exon 2 genetic variations with ovulation rate and litter size has been reported in some sheep breeds but not in the Arabic sheep. *BMP15* has been considered as an important candidate gene for ovulation rate and high litter size productivity in domestic animals and some aquatic species such as the European sea bass[15] SNPs provide some indication of the structural diversity of a gene; extended haplotypes are typically more informative, specifically if they involve all or most of the coding region.

Considerable development in farm animal breeding has been made in the last few decades, but doing greater understanding in the development of litter size was very slow before molecular markers became an accessible technology with wide applications in breeding methods. In Iran sheep meat is a major source of animal protein and investigation for litter size and associated genes is important. Arabic sheep is a native Iranian breed and plays a great role in sheep rearing activities in the south of Iran and their main products are meat, milk

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and wool [16 **]** Therefore, it is essential to study the genetics and reproduction in sheep breeds using modern genetic methods. One of these methods is marker-assisted selection (MAS) which will be useful for increasing and accelerating the rate of genetic improvement on litter size and encourage its uptake it by commercial sheep breeders. PCR-SSCP method is a reliable method of quickly detecting a mutation. Mutation was detected from the differences in migration patterns from conformation of single strand DNA on polyacrylamide gels.[17] The aim of the present investigation was to study polymorphism in BMP15 gene of Arabic sheep.

MATERIALS AND METHODS

In this study, blood samples were randomly collected from 80 Arabic sheep in the Khuzestan province including 19, 29, 17 and 15 heads from Arabic sheep Research Center, Shoshtar; Shadegan, Dasht-e-Azadegan and Ahvaz cities, respectively (in Southwestern of Iran). From each animal, about 5 cc of blood was collected from the jugular vein with vacuum tubes coated with ethylene diamine tetra acetic acid (EDTA) and transported to the Central Laboratory of the University of Khuzestan Ramin Agriculture and Natural Resources and stored at 4 C until DNA extraction. Genomic DNA was isolated by using DNA extraction kit DIA-tom DNA Prep 100. Spectrophotometer was used investigating quality and quantity. Samples show an optical density (OD) ratio (260 nm/280 nm) ranging from 1.6 to 1.8. The PCR amplification of specific fragment DNA included SNP in 235 bp region of 2 exon. The sequence using PCR primers were designed by.[18] For-ward primer was:

5'- TACAGACCCTGGACTTTCCTCT-3' •

And reverse primer was:

5'- GCCCAACATGTTCCATGATATCC-3'.

The PCR reaction volume of 25 µL contained approximately 33.3 ng of genomic DNA, 1.25 mM Taq DNA polymerase, 2.5 µL of 1X PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTP and 10 pM of each primer. Amplification conditions included an initial denaturation at 95 C for 5 min, followed by 35 cycles at 95 C for 30 s, 56 C for 1 min and 72 C for 1 min, followed by a final extension at 72 C for 10 min. The PCR products were separated by 1% (w/v) agaros gel electrophoresis (Figure 1).

Single-Stranded conformation polymorphism

The SSCP analysis of the PCR products was carried out following the protocol of Sambrook and Russell[19] with some modifications. The 8% SSCP gel mixture was prepared with acrylamide: bisacrylamide (38:2) g, TBE 5**x(**6cc**)**, Ammonium per sulfate(300µl) and TEMED(50 µl). Autoclaved twice distilled water to make up the volume. 5 µl PCR products were mixed with 5 µl denaturing solution (98% formamide, 0.025% xylenecyanoleand, 0.025%, bromophenol blue and 10 mM EDTA), incubated at 98 c for 10 min and then chilled on ice Denatured DNA was loaded on 8% PAGE gel in 0.5X TBE buffer and constant voltage 160v for 3-4 h. The gel after its run was silver-stained as per the protocol of with some modifications.[20] We used 2 g/l silver nitrate. The SSCP banding patterns of different samples were visualized with the naked eye in normal light and the gel was photographed in a gel documentation system. Gen Alex 6/3 software were applied to estimate the gene and genotype frequencies, the heterozygosity and effective number of alleles. Expected theoretical heterozygosity from Hardy-Weinberg assumption was calculated.

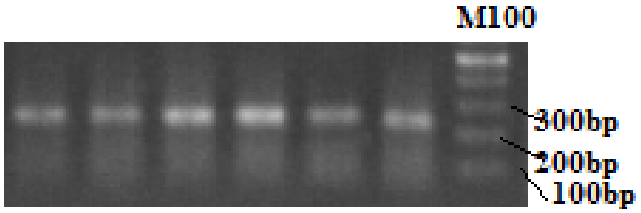

Fig. 1: PCR products of BMP15 gene exon 2 amplificated by the primer.

Results:

PCR-SSCP (polymerase chain reaction-single strand conformation polymorphism) was used to scan and genotype the single nucleotide polymorphism (SNP) in the sheep *BMP15* gene. All samples were evaluated with PCR- Single-Stranded conformation polymorphism (SSCP) technique (Figure 2). In this study, two alleles were observed, M and N. It gave frequencies of 0.79 and 0.21 for M and N alleles, respectively. The frequencies of genotypes were 0.58, 0.42 for MM, MN respectively. Chi square χ^2 test was used to evaluate Hardy-Weinberg equilibrium (HWE). Deviations between observed genotypic frequencies and those expected under Hardy-Weinberg equilibrium were not significant. It suggested that the Arabic sheep population sampled is in disequilibrium for the BMP15 locus (χ^2=5.401). Average genetic diversity in Arabic sheep breed population in Khuzestan province was observed. Effective allele and true allele are estimated 1.48 and 2.000, respectively.

Discussion:

Our results show that BMP15 gene had approximately similar value for frequency mutant N and M allele in the population of Arabic sheep. Genotype and gene frequencies of four regions BMP15 gene were average in this population. Our findings are the first report of BMP15 gene polymorphism in Arabic sheep and the results are presented in Table 1.

Each four population follows Hardy-Weinberg disequilibrium. This confirmed that factors leading to disequilibrium, especially selection, may affect the genetic structure of the population. Average heterozygosity for four regions was estimated. The results indicated that average heterozygosity of Shadegan was the highest and Ahwaz was the least. Effective allele for four regions was estimated beetween1.48 and 1.57. The obtained results indicated that Effective allele of Ahvaz was the least. The BMP15 allele frequencies estimated in the present study were approximately similar to the wang *et al*. 2011, Soleimani *et al*. 2011, Hanrahan *et al*. 2004.[18,21,6] In previous study of BMP15 gene in another animals: identified two SNP in exon 2 of the BMP15 gene in anglo-nubian goat identified a 4-bp deletion in the coding region of the bovine BMP15 gene. Identified two SNP in intron 1 of the BMP15 gene in corriedale sheep kashmir valley sheep.[22,23,24]

Table 1: Genotype and gene frequencies of the PCR-SSCP in different regions sheep

Region	Genotype ferquencies		Gene ferquencies	
	MM	MN	M	N
Shoshtar	0.52	0.47	0.763	0.273
Shadegan	0.51	0.48	0.759	0. 241
susangerd	0.58	0.41	0.794	0.206
Ahvaz	0.8	0.2	0.9	0.1

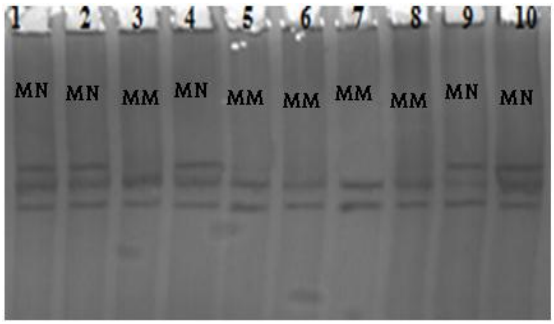

Fig. 2: SSCP analysis of PCR products of primer

Conclusion:

The findings presented in this study indicate that the 235 bp BMP15 gene fragment is polymorphic in this population. These results showed that BMP-15 gene is a genetic marker and closely linkage to the litter size trait and consequently can be used as a marker-assisted selection (MAS) for high litter size productivity in sheep.

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