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Survey of FecX^{L} Locus of BMP15 Gene and Growth Hormone (GH) Gene and Their Effects on Lambing Rate in Zel Sheep

Research Article

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ABSTRACT

The GH gene and BMP15 gene have been used as candidate genes for marker-assisted selection in different livestock species. Random blood samples were obtained from 180 Zel sheep breed to study genetic polymorphism of these genes. DNA was extracted from blood samples and a 365 bp fragment from the exon V of the ovine growth hormone gene and a 312 bp region from exon II of BMP15 gene were amplified by polymerase chain reaction. SSCP analysis showed three conformational patterns (A, B and C) for GH gene but for FecX^{L} locus of BMP15 no banding patterns were observed in the animals tested. Results indicated that there were no significant associations (P>0.05) between polymorphism of the GH loci and lambing rate.

KEY WORDS FecX^{L}, GH, lambing rate, PCR-SSCP, Zel sheep.

INTRODUCTION

It is well documented that growth hormone (GH) influences animal processes such as growth (Breier, 1999), lactation (Baldi, 1999), reproduction (Scaramuzzi *et al*. 1999) and metabolism (Bauman, 1999), since its finding in the 1920 s. In most mammals, GH is product of a single gene and is normally secreted in a pulsatile manner by the pituitary gland (Veldhuis *et al*. 2001). GH affects cell growth and proliferation either directly or indirectly through stimulation of the insulin-like growth factor (IGF) system. GH activity is first detected in the fetal pituitary and in circulation of fetal lambs around days 50-60 of pregnancy (Gluckman *et al*. 1979). In the ovine growth hormone (oGH) gene, restriction fragment length polymorphisms (RFLP) using restriction endonucleases TaqI and PvuII (Gootwine *et al*. 1996; Ofir and Yossefi, 1996) and EcoRI (Barracosa, 1996; Gootwine *et al*. 1998) and PCR-SSCP polymorphisms (Bastos *et al*. 2001; Marques *et al*. 2001; Santos *et al*. 2004) have been reported.

The BMP15 (Bone Morphogenetic Protein 15) gene, is located on chromosome X and contains 2 exons (Galloway *et al*. 2000). Six mutations, are labeled included FecX^{R} (Rasa) (Monteagudo *et al*. 2009), FecX^{H} (Hanna) and FecX^{I} (Inverdale) (Galloway *et al*. 2000), FecX^{L} (Lacaune) (Bodin *et al*. 2007), FecX^{G} (Galway) and FecX^{B} (Belclare) (Hanrahan *et al*. 2004) have been detected within the BMP15 gene. Bodin *et al*. (2007) for the first time found FecX^{L} mutation, they described the phenotypic and molecular characterization of a new C53Y mutation identified in the BMP15 gene in the Lacaune sheep, named FecX^{L}. FecX^{L}, as other FecX mutations, is associated with increased ovulation rate or sterility depending on its presence at the heterozygous or homozygous state, respectively (Bodin *et al*. 2007). The biological role of BMP15 is not completely understood but the immunization studies

showed that BMP15 is essential for follicular development in sheep (Bodin *et al*. 2007). The Zel sheep is a small meat-type animal and is the only thin tailed sheep breed in Iran which instead of having a fat-tail has a tail of 10-12 cm in length. It`s main distribution is on the northern of Iran in the provinces of Mazendaran and Golestan. Fecundity and prolificacy of this breed is approximately 10%. The coat of Zel sheep can vary from white to black or brown and hands and feet are drawn without wool (Saadat noori and Siah mansoor, 1990).

The aim of the present study was to investigate the genetic association of GH gene and FecX^{L} variant of BMP15 gene with lambing rate in Zel sheep by PCR-SSCP method. This intends to be a first step for a deeper study on Zel Sheep to establish a breeding program based on marker-assisted selection.

MATERIALS AND METHODS

Blood samples and DNA extraction

The blood samples were collected randomly from 180 Zel sheep (Shirang Research Station, Golestan, Iran) from jugular venipuncture, using vacuum tubes treated with 0.25% ethylene diamine tetr acetic acid (EDTA). DNA was extracted from 100 µL of blood, using a commercial kit (Diatom DNA Prep100, ISO Gene, Moscow) following the manufacturer's protocol. The quality and quantity of extracted DNA were measured spectrophotometically and on 1% agarose gel electrophoresis.

DNA amplification by PCR

Polymerase Chain Reaction (PCR) was performed, using the Personal Cycler™ thermocycler (Biometra, Germany) and the PCR Master Kit (Cinna Gen Inc., Iran). The kit master mix consisted of 0.04 U/µL of Taq DNA polymerase, 10X PCR buffer, 3 m*M* $MgCl_2$ and 0.04 m*M* dNTPs (each). Each reaction mixture consisted of 12.5 µL of the master mix, 1 µL of the DNA solution (50 to 100 ng/µL), 1 µL of each primer (5 pmol/µL) and some deionized water making up a final volume of 25 µL.

For amplifying a 365 bp fragment from the exon V of the ovine growth hormone gene performed following primers described by Barracosa (1996) were used:

GH-F (5'-GAAACCTCCTTCCTCGCC C-3')
GH-R (5'-CCAGGGTCTAGGAAGGCACA-3')

For amplifying a 312 bp region from exon II of BMP15 gene, specific primers as described by Bodin *et al*. (2007) were used:

FecX^{L}- F (5'-CATGATGGGCCTGAAAGTAAC-3')
FecX^{L}- R (5'-GGCAATCATACCCTCATACTCC-3')

The amplification reaction for both genes were performed at following conditions: an initial denaturation step at 95 ˚C for 5 min followed by 35 cycles of denaturation at 95 ˚C for 30sec, annealing at 63 ˚C for 40sec and extension at 72 ˚C for 45sec, and a final extension of 72 ˚C for 5 min.

PCR-SSCP

For SSCP analysis, 8µL of each amplification product was added to 10 µL of denaturizing solution (95% formamide, 10 m*M* NaOH, 0.05% xylene cyanol and 0.05% bromophenol blue). The samples were heat-denatured at 95 ˚C for 5-min, immediately chilled on ice and loaded onto 8% and 12% polyacrylamide gel (39:1) for GH and BMP15, respectively. Gels were run at (230-260 V) for (4-6 h), at 4 ˚C. The electrophoresis was carried out in a vertical unit in 1x TBE buffer. The gels were stained with silver nitrate to observe the conformational patterns.

Statistical analysis

Retropsective records for four years (2008 up to spring of 2011) and most of the ewes were in mature age were reviewed to assess rate of parity in ewes. The rate of the parity in this population for most of ewes was twice a year (spring-fall). Allele and genotype frequencies were also calculated using Pop-Gene, 1.31 software.

In order to test the association of different conformational patterns with lambing rate, statistical analysis was performed using General Linear Model (GLM) procedure of the SAS program and least squares means of the banding patterns were compared using the Tukey test (SAS, 1996). The following model was used:

$$y_{ijkL} = \mu + G_i + P_j + C_k + e_{ijkL}$$

Where:
Y_{ijkl}: is the dependent variable.
μ: is the overall mean.
G_i: is the fixed effect of the i^{th} banding patterns (i=1,. . .,3).
P_j: is the fixed effect of the j^{th} parity number (j=1,. . .,3); class 3 included ewes in third and over parities.
C_k: is the fixed effect of the k^{th} year.
e_{ijkl}: is the random residual error.

RESULTS AND DISCUSSION

Gene and genetic frequencies

Allele and genotype frequencies were calculated with Pop-Gene software. The PCR-SSCP was carried out on polyacrylamide gel and three (A, B and C) different conformational patterns with difference frequencies for GH gene were observed (Figure 1) but no genetic polymorphism was

found for $FecX^L$ locus of BMP15 gene and all samples showed the same genotypes (Figure 2). The distribution of banding patterns and their frequency for GH gene are presented in Table 1.

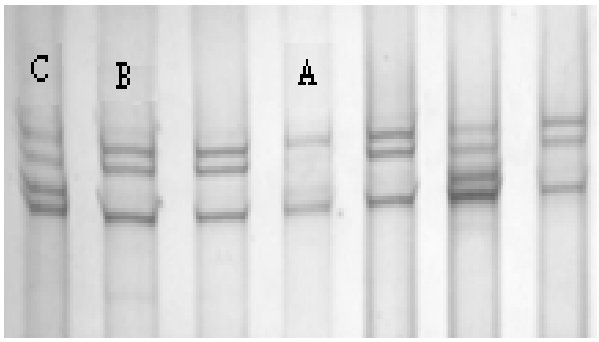

Figure 1 SSCP analysis of the 365 bp fragment of GH gene on 8% polyacrylamide gel

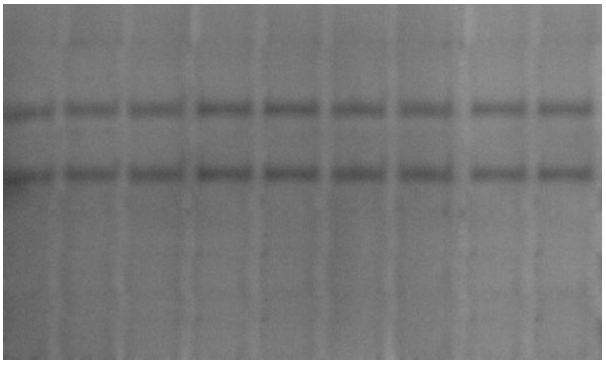

Figure 2 SSCP analysis of the 312 bp Fragment of BMP15 Gene on 12 polyacrylamide gel

Table 1 The banding patterns frequency of GH

Gene	Pattern banding	Frequency
GH	A	0.32
	B	0.39
	C	0.29

Genetic effect on lambing rate

Statistical analysis was performed using General Linear Model (GLM) procedure on records during 2008 up to spring of 2011 and according that, no significant association (P>0.05) was found between GH conformational patterns and lambing rate in present population (Table 2).

Table 2 LS Means±SE of lambing rate in different GH banding patterns

Banding pattern	Lambing rate
A	1.1±0.04
B	1.12±0.04
C	1.09±0.05

GH

Three (A, B and C) different conformational patterns for GH gene were determined. The frequency of pattern B (0.39) was higher than the other patterns. Analysis of the data revealed no significant association (P>0.05) between lambing rate and conformational patterns of GH gene. Current results of GH polymorphism are in agreement with previous studies by by Tahmorespoor *et al*. (2011) and Ahani Azari *et al*. (2011) that detected three conformational patterns using the SSCP method in exon 5 of this gene in Baluochi and Dalagh breeds. Shiri *et al*. (2006) also observed three conformational patterns in exon 4 of gene in Kordian sheep.

However, other researchers such as Bastos *et al*. (2001) identified two conformational patterns using the SSCP analysis of exon 4 of the GH gene. They also observed five different conformational patterns in exon 5 of the GH gene. Marques *et al*. (2001) analyzed five ovine GH exons by PCR-SSCP in 200 Portuguese Serra da Estrela ewes and revealed that all exons except exon 1 are polymorphic. Folch *et al*. (2001) reported that the GH treatment did not significantly effect on the percentage of ewes in estrus and the ovulation rate. Koch *et al*. (2010) showed ewes treated with GH had bigger size at fetal growth and development in lambs. Hazout *et al*. (2009) reported that administration of GH increased pregnancy rate.

$FecX^L$

The BMP15 gene has been found to be closely associated with prolificacy in sheep (Bodin *et al*. 2002; Fabre *et al*. 2006; Galloway *et al*. 2000; Hanrahan *et al*. 2004; Monteagudo *et al*. 2009; Souza *et al*. 2001). In ovine ovaries, like in other mammals, the $FecX^L$ locus of BMP15 gene is exclusively expressed in the developing oocyte from primary follicles to pre-ovulatory follicles (Galloway *et al*. 2000; Dube *et al*. 1998; Aaltonen, 1999; Jaatinen *et al*. 2002; Laitinen *et al*. 1998).

The results of the present study showed that there is no genetic polymorphism for $FecX^L$ locus of BMP15 gene in Zel sheep breed. In addition, In Iranian sheep, no mutation in $FecX^L$ were found in Shal (Zare, 2007), and Lori-Bakhtaran (Nejati Javaremi *et al*. 2007), sheep breeds. Polly *et al*. (2010) did not found the $FecX^L$ mutation in the Indian Garole sheep.

CONCLUSION

The results indicated that GH gene is polymorphic but for $FecX^L$ locus of BMP15 no banding patterns were observed. Statistical analysis has shown that there are no significant associations between conformational patterns of GH gene and lambing rate. Further studies are necessary to confirm the association between these genes and lambing rate with larger number of animals in Zel and other sheep breeds before definitive conclusions can be made. In my opinion, some other statistical procedures to detect the possible exis-

tence of a major gene involved in prolificacy in this breed should be performed before starting an expensive and long study on this subject.

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