Survey of FecXL Locus of BMP15 Gene and Growth Hormone (GH) Gene and Their Effects on Lambing Rate in Zel Sheep

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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 FecXH (Rasa) (Monteagudo et al. 2009), FecXI (Hanna) and FecXL (Inverdale) (Galloway et al. 2000), FecXG (Galway) and FecXB (Belclare) (Hanrahan et al. 2004) have been detected within the BMP15 gene. Bodin et al. (2007) for the first time found FecXL mutation, they described the phenotypic and molecular characterization of a new C53Y mutation identified in the BMP15 gene in the Lacaune sheep, named FecXL. FecXL, 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 FecXL 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 spectrophotometrically 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 mM MgCl₂ and 0.04 mM dNTPs (each). Each reaction mixture consisted of 12.5 μL of the master mix, 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'-GAAACCTCCTTCTCGCC C-3')
GH-R (5'-CCAGGTCTAGGAAGGCACA-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⁺- F (5'-CATGATGGGCTGAAAGTAAC-3')
FecX⁻- 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 mM 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**

Retrospective 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.
- \( \mu \): 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 (\( j=1, . . . , 3 \)).
- \( C_k \): 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 PopGene 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<sup>L</sup> 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.

**Table 1** The banding patterns frequency of GH

<table>
<thead>
<tr>
<th>Gene</th>
<th>Pattern banding</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH</td>
<td>A</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.29</td>
</tr>
</tbody>
</table>

**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

<table>
<thead>
<tr>
<th>Banding pattern</th>
<th>Lambing rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.1±0.04</td>
</tr>
<tr>
<td>B</td>
<td>1.12±0.04</td>
</tr>
<tr>
<td>C</td>
<td>1.09±0.05</td>
</tr>
</tbody>
</table>

**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 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 Baloochi 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<sup>L</sup>**

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<sup>L</sup> 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<sup>L</sup> locus of BMP15 gene in Zel sheep breed. In addition, in Iranian sheep, no mutation in FecX<sup>L</sup> 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<sup>L</sup> mutation in the Indian Garole sheep.

**CONCLUSION**

The results indicated that GH gene is polymorphic but for FecX<sup>L</sup> 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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