

## 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<sup>L</sup> 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  $\mu$ L 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<sup>TM</sup> thermocycler (Biometra, Germany) and the PCR Master Kit (Cinna Gen Inc., Iran). The kit master mix consisted of 0.04 U/ $\mu$ L of Taq DNA polymerase, 10X PCR buffer, 3 mM MgCl<sub>2</sub> and 0.04 mM dNTPs (each). Each reaction mixture consisted of 12.5  $\mu$ L of the master mix, 1  $\mu$ L of the DNA solution (50 to 100 ng/ $\mu$ L), 1  $\mu$ L of each primer (5 pmol/ $\mu$ L) and some deionized water making up a final volume of 25  $\mu$ L.

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<sup>L</sup>- F (5'-CATGATGGGCCTGAAAGTAAC-3')

FecX<sup>L</sup>- 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, 8 $\mu$ L of each amplification product was added to 10  $\mu$ L 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  $i^{\text{th}}$  banding patterns ( $i=1, \dots, 3$ ).

$P_j$ : is the fixed effect of the  $j^{\text{th}}$  parity number ( $j=1, \dots, 3$ ); class 3 included ewes in third and over parities.

$C_k$ : is the fixed effect of the  $k^{\text{th}}$  year.

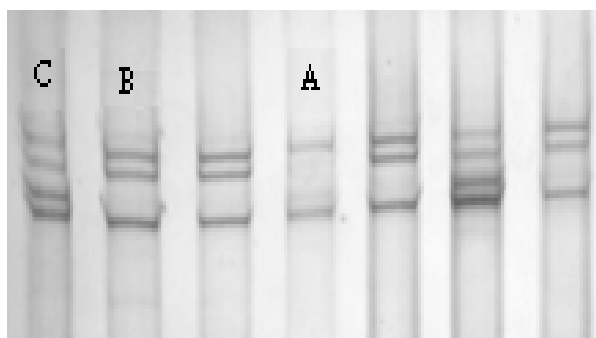
$e_{ijkl}$ : is the random residual error.

## RESULTS AND DISCUSSION

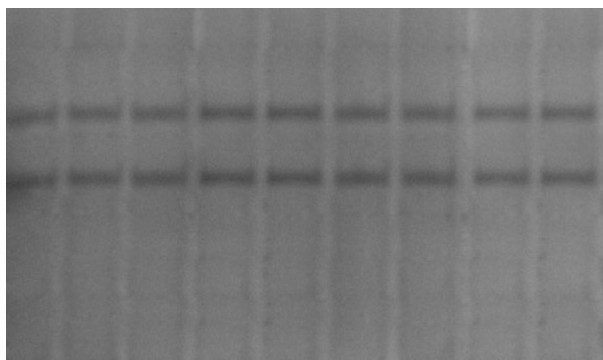
### Gene and genetic frequencies

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 $\pm$ SE of lambing rate in different GH banding patterns

Banding pattern	Lambing rate
A	1.1 $\pm$ 0.04
B	1.12 $\pm$ 0.04
C	1.09 $\pm$ 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 exist-

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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## REFERENCES

- Aaltonen J., Laitinen M.P., Vuojolainen K., Jaatinen R., Horelli-Kuitunen N., Seppa L., Louhio H., Tuuri T., Sjoberg J., Butzow R., Hovatta O., Dale L. and Ritvos O. (1999). Human growth differentiation factor 9 (GDF-9) and its novel homolog GDF-9B are expressed in oocytes during early folliculogenesis. *J. Clin. Endocrinol. Metabolism*. **84**, 2744-2750.
- Ahani Azari M., Yousefi S. and Dehnavi E. (2011). Evaluation of  $\kappa$ -casein and growth hormone genes polymorphism in native Dalagh sheep. *Slovak J. Anim. Sci.* **44(4)**, 129-133.
- Baldi A. (1999). Manipulation of milk production and quality by use of somatotropin in dairy ruminants other than cow. *Domest. Anim. Endocrinol.* **17**, 131-137.
- Barracosa H. 1996. Estudo de polimorfismos genetic osedasuaasociação com capacidades de produç ãoleiteiraem caprinos de raçaAlgarvia e ovinos de raça Serra da Estrela. Dissertação de Mestrado, Univ. do Algarve, Faro, Portugal.
- Bastos E., Cravador A., Azevedo J. and Guedes-Pinto H. (2001). Single strand conformation polymorphism (SSCP) detection in six genes in Portuguese indigenous sheep breed "Churra da Terra Quente". *Biotechnol. Agron. Soc. Environ.* **5**, 7-15.
- Bauman D.E. (1999). Somatotropin mechanism in lactating cows: from basic science to commercial application. *Domest. Anim. Endocrinol.* **17**, 101-116.
- Bodin L., Di Pasquale E., Fabre S., Bontoux M., Monget P., Ersani L. and Mulsant P. (2007). A novel mutation in the bone morphogenetic protein 15 gene causing defective protein secretion is associated with both increased ovulation rate and sterility in Lacaune sheep. *J. Endocrinol.* **148**, 393-400.
- Bodin L., San Cristobal M., Lecerf F., Mulsant P., Bibe B., Lajous D., Belloc J.P., Eychenne F., Amigues Y. and Elsen J.M. (2002). Segregation of a major gene influencing ovulation in progeny of Lacaune meat sheep. *J. Genet. Selec. Evol.* **34**, 447-464.
- Breier B.H. (1999). Regulation of protein and energy metabolism by the somatotropic axis. *Domest. Anim. Endocrinol.* **17**, 209-218.
- Dube J.L., Wang P., Elvin J., Lyons K.M., Celeste A.J. and Matzuk M.M. (1998). The bone morphogenetic protein 15 gene is X-linked and expressed in oocytes. *Mol. Endocrinol.* **12**, 1809-1817.
- Folch J., Ramón J.P., Cocero M.J., Alabart J.L. and Beckers J.F. (2001). Exogenous growth hormone improves the number of transferable embryos in superovulated ewes. *Theriogenology*. **55(9)**, 1777-1785.
- Galloway S.M., McNatty K.P., Cambridge L.M., Laitinen, M.P.E.J., Juengel L.T., Jokiranta S.R., McLaren J., Luuro K., Dodds K.G., Montgomery W., Beattie A.E., Davis G.H. and Ritvos O. (2000). Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *J. Nat. Genet.* **25**, 279-283.
- Gluckman P.D., Mueller P.L., Kaplan S.L., Rudolph A.M. and Grumbach M.M. (1979). Hormone ontogeny in the ovine fetus. I. Circulating growth hormone in mid and late gestation. *Endocrinology*. **104**, 162-168.
- Gootwine E., Ofir R. and Yossefi S. (1996). Characterization of *PvuII* polymorphisms between the ovine growth hormone GH2-N and GH2-Z gene copies. *Anim. Biotechnol.* **7**, 135-143.
- Gootwine E., Suttie J.M., McEwan J.C., Veenvliet B.A., Littlejohn R.P., Fennessy P.F. and Montgomery G.W. (1998). The physiological effects of natural variation in growth hormone gene copy number in ram lambs. *Domest. Anim. Endocrinol.* **14**, 381-390.
- Hanrahan J.P., Gregan S.M., Mulsant P., Mullen M., Davis G.H., Powell R. and Galloway S.M. (2004). Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovisaries*). *J. Biol. Reprod.* **70**, 900-909.
- Hazout A., Junca A.M., Ménézo Y., Demouzon J. and Cohen-Bacrie P. (2009). Effect of growth hormone on oocyte competence in patients with multiple IVF failures. *Reprod. Biomed. Online*. **18(5)**, 664-670.
- Jaatinen R., Bondestam J., Raivio T., Hilden K., Dunkel L., Groome N. and Ritvos O. (2002). Activation of the bone morphogenetic protein signaling pathway induces inhibin beta (B)-subunit mRNA and secreted inhibin B levels in cultured human granulosa luteal cells. *J. Clin. Endocrinol. Metab.* **87**, 1254-1261.
- Koch J.M., Wilmoth T.A. and Wilson M.E. (2010). Periconceptional growth hormone treatment alters fetal growth and development in lambs. *J. Anim. Sci.* **88**, 1619-1625.
- Laitinen M., Vuojolainen K., Jaatinen R., Ketola I., Aaltonen J., Lehtonen E., Heikinheimo M. and Ritvos O. (1998). A novel growth differentiation factor-9 (GDF-9) related factor is co-expressed with GDF-9 in mouse oocytes during folliculogenesis. *Mech. Dev.* **78**, 135-140.
- Marques M.R., Santos I.C., Belo C.C., Cravador A., (2001). Associations between SSCPs in the GH gene and milk traits in "Serra da Estrela" ewes. Pp. 57-82 in Proc. Int. Conf. Farm Anim. Endocrinol. BASE, Gembloux, Belgium.
- Monteagudo L.V., Ponz R., Tejedor M.T., Lavina A. and Sierra I. (2009). A 17 bp deletion in the bone morphogenetic protein 15 (BMP15) gene is associated to increased prolificacy. *Anim. Reprod. Sci.* **110**, 139-146.
- Nejati Javaremi A., Rahimi G. and Amiri S. (2007). Detection of polymorphism in FecX<sup>L</sup> gene associated with twinning in Louri Bakhtiary sheep using PCR-SSCP. Pp. 55-63 in Prpc. 5<sup>th</sup> Nation. Biotechnol. Cong. Tehran. Iran.
- Polly S., De S., Brahma B., Mukherjee A., Vinesh P.V., Batabyal S., Arora J.S., Pan S., Kumar Samanta A., Kumar Datta T. and Lal Goswami S. (2010). Polymorphism of BMP1B, BMP15 and GDF9 fecundity genes in prolific Garolesheep. *J. Trop. Anim. Health. Prod.* **42**, 985-993.

- Saadat Noori M. and Siah Mansoor S. (1995). Sheep Husbandary and Management, 5<sup>th</sup> Ed. Afshari Publication, Tehran.
- Santos I.C., Marques M.R., Belo C.C. and Cravador A. (2004). Polymorphism analysis at the growth hormone gene in Merino da Beira Baixa ewes. *Biotechnol. Agron. Soc. Environ.* **8**, 40-41.
- SAS Institute. (1996). SAS<sup>®</sup>/STAT Software, Release 6.11. SAS Institute, Inc., Cary, NC.
- Scaramuzzi R.J., Murray J.F., Downing J.A. and Campbell B.K. (1999). The effects of exogenous growth hormone on follicular steroid secretion and ovulation rate in sheep. *Domest. Anim. Endocrinol.* **17**, 269-277.
- Shiri S.A.K., Saghi D.A., Nasiri M.R., Emrani H., Montazer Torbati F. and Mohammadzade M. (2006). Survey of genetic diversity growth hormone and growth hormone receptor genes in Iranian indigenous sheep breed (kordian sheep) using a non-radioactive SSCP. Pp. 21-28 in Proc. 57<sup>th</sup> Ann. Meet. Europ. Asso. Anim. Prod. Antalya, Turkey.
- Souza C.J.H., Mac Dougall C., Campbell B.K., Mc Neilly A.S. and Baird D.T. (2001). The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1B (BMPR-1B) gene. *J. Endocrinol.* **169**, 1-6.
- Tahmorespoor M., Ansary M., Taheri A. and Gholami H. (2011). PCR-SSCP variation of GH and STAT5A genes and their association with estimated breeding values of growth traits in Baluchi sheep. *Anim. Biotechnol.* **22**, 37-43.
- Veldhuis J.D., Anderson S.M., Shah N., Bray M., Vick T., Gentili A., Mulligan T., Johnson M.L., Weltman A., Evans W.S. and Iranmanesh A. (2001). Neuro physiological regulation and target-tissue impact of the pulsatile mode of growth hormone secretion in the human. *Growth Horm. IGF Res.* **11**, 25-37.
- Zare Y., Nejati Javaremi A. and Rahimi G. (2007). Detection of polymorphisms in two points of the gene associated with twinning (BMP15) in Shalsh sheep. Pp. 72-81 in Proc. 5<sup>th</sup> National Biotechnol. Cong. Iran. Thehran, Iran.
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