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The Polymorphism of FecX^{G} Region Exon 2 of *BMP15* Gene in Hisari Sheep

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Abstract

Booroola gene is one of the major genes in the enhancement of ovulation rate and could be an attractive candidate gene for ovulation rate in sheep. Molecular technologies have been widely used for discovery of mutations in animal species. These mutations have major effects on the important economic traits of sheep and goats. This study was conducted to identify the mutation in FecX^{G} region of exon 2 of BMP15 gene which is associated with twining using polymerase chain reaction-restriction fragment length polymorphism method in Hisari Sheep. For this purpose, blood samples were obtained from 110 sheep. DNA was extracted using the salting out method and quantity and quality properties of DNA were determined using electrophoresis gel. Afterward, a 141 bp fragment, which contains the given polymorphic site of BMP15 gene, was amplified by the specific primers. Restriction enzyme (HinfI G/ANTC) cuts the "wild-type" allele and as a result of digestion, 112 and 29 bp fragments produced. Mutant type allele is not cut because of the removal of the intended cutting site. All the samples showed the similar monomorphic of genotype (+/+) (wild genotype). Results of this study indicate that the investigated polymorphism is not present in the Hisari sheep.

Keywords: Polymorphism; *BMP15*gene; Hisari sheep; Twining; PCR-RFLP

Introduction

Sheep breeding is one of the major activities in the Tajiki animal production and has an important impact on the rural economy. One of the most important traits that influences the profitability of sheep production is litter size. Litter size of almost all Tajiki sheep breeds is low, except the Chios breed.

Hisari sheep is known as a lightweight fat-tailed Tajik breed and also because of its meat, has high economic importance. Variation of the genetic profile in ovulation rate (number of mature oocytes released in a reproductive cycle) has been extensively investigated in sheep. In this regards, substantial differences exist among breeds and in addition, some cases have shown exceptional variations within breeds/strains (Galloway *et al*., 2000).

In recent years, ability to improve the reproductive traits of livestock species has captured increasing attention. Traditional methods of genetic improvement in reproductive traits suffer from limitations such as restricted employment of quantitative genetic approaches and limited gain. Furthermore, improvement of molecular genetics suggests that reproduction associated genes can be utilized in breeding approaches and marker-assisted selection (MAS). In MAS, primary targets often are reproductive traits since the trait can be measured only in one sex and has low heritability. In mammals, ovulation rate is determined through a localized exchange in

ovarian follicle hormones between the oocyte and adjacent somatic cells and via an exchange in hormone signals between the pituitary gland and the ovary (Bahrami & Chekaniazar, 2013; Eppig, 2001; Galloway *et al*., 2000).

Ovulation rate of many mammals is one or sometimes two including primates, possums, cattle, goats and, deer, whereas in other mammals ovulation rate differs between four and 15 including hamsters, rats, mice, dogs, cats and pigs (McNatty *et al*., 2005). Extensive studies on the genetic variation of ovulation rate in sheep show substantial differences among breeds and sometimes exceptional variations within breeds/strains, as noted previously (Bindon *et al*., 1996). Explanation of these exceptional variations is that segregation of a major gene may cause these situations with a large effect on ovarian function. It is postulated that this phenomenon provides an explanation for reported high prolificacy of Booroola sheep (Davis *et al*., 1982; Piper & Bindon, 1982). In addition, the major genes were identified to provide an explanation for increased ovulation rate and/or litter size in various sheep breeds/strains, including Inverdale (Davis *et al*., 1991), Cambridge (Hanrahan *et al*., 2004), Thoka (Jonmundsson & Adalsteinsson, 1985), Javanese (Bradford *et al*., 1986), Olkuska (Radomska *et al*., 1988), Belclare (Hanrahan 1991), Lacaune (Bodin *et al*., 1998) and Woodlands (Davis *et al*., 2001).

It is reported that there are three prolificacy loci in ruminant. FecB (Booroola) gene is one of the major genes in the enhancement of ovulation rate (Souza *et al*., 2001). GDF-9 (growth and differentiation factor 9) and BMP-15 (Bone Morphogenetic Protein 15) genes make their sources in oocytes (Bahrami *et al*., 2014; Eckery *et al*., 2002; Pennetier *et al*., 2004). BMP15 gene which is located on chromosome X contains 2 exons (Galloway *et al*., 2000). Six mutations have been detected within this gene including $FecX^{R}$ (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). $FecX^{L}$ corresponds to a T/A transition at position 896 in the cDNA coding for the BMP-15. Homozygous females have small non-functional ovaries and are infertile. The second mutation in the BMP15 is $FecX^{H}$ mutation. Ovulation rate and litter sizes in heterozygous carriers and homozygous females are identical in owner Inverdale ($FecX^{I)}$ and Hanna ($FecX^{H}$) families. Hanna allele ($FecX^{H}$) corresponds to a C/T transition at position 871. The Hanna mutation introduces a premature stop at the amino acid position 291 of the unprocessed peptide (amino acid 23 in mature protein, Q23stop) leading likely to a loss of bioactivity of the BMP-15 protein produced by the $FecX^{H}$ allele. The other two mutations in BMP-15 are $FecX^{G}$ (Galway) and $FecX^{B}$ (Belclare) mutations. Galway allele corresponds to a C/T transition at nucleotide 718. $FecX^{G}$ mutation leads up to a premature stop codon at amino acid 239 of unprocessed protein thus no mature protein is produced. Belclare allele corresponds to a G/T transition at nucleotide 1100. Serine to isoleucine change occurs at amino acid 99 of the mature protein (S99I) in $FecX^{B}$ mutation (Bodin *et al*., 2007; Davis *et al*., 2001; Galloway *et al*., 2000; Hanrahan *et al*., 2004). BMP-15 is essential for female sheep fertility and folliculogenesis. In addition, BMP-15 gene has an important role in mammalian fertility because it expresses in the oocyte of developing follicle as a transforming growth factor beta (TGFβ). So, BMP-15 gene acts as granulose cell proliferation and differentiation regulator via follicle-stimulating hormone receptor expression and promotion of granulose cell mitosis. Aside from its essential role in follicular development in sheep and collaboration in granulose cells function regulation, biological functions of BMP15 gene is not exactly appreciated or understood (Bodin *et al*., 2007; McNatty *et al*., 2005). In regard to its role in sheep folliculogenesis, two copies of naturally occurring inactivating BMP15 mutations in the same gene cause infertility and blockage of the follicular development at the primary stage. It is postulated that BMP15 role in fertility of small ruminants is based on the non-covalently bond heterodimers and homodimers of the BMP15 proteins (Hanrahan *et al*., 2004). The aim of this research is determining $FecX^{G}$ polymorphism in exon 2 of gene *BMP15* by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique in Hisari sheep which is an

endogenous Tajiki breed, with high production potential.

Material and Methods

For this study, 110 blood samples (34 female and 56 male animals varying from 6 months to 2 years of age) were randomly obtained from 320 Hisari breed sheep reared at Dushanbe. Then, blood samples were obtained from the jugular vein using 10 ml vacuum tube containing EDTA. Samples were transported on ice to the Laboratory of Biotechnology Yashour located in Dushanbe. Afterward, DNA extraction was performed on white blood cells (WBC) using extracting salt and DNA extraction method of Miller *et al*., (1988). To determine the quality and quantity of DNA, the extracted DNA were analyzed by electrophoresis on a 0.7 % agarose gel. Polymerase chain reaction was carried out by using following sequences for amplification of the $FecX^G$ region exon 2 of BMP-15 gene in Hisari sheep (Table 1). The total volume of PCR reaction was 25 µl.

Table 1. Primer sequences used for $FecX^G$ Region Exon 2 of BMP15 gene (Feraye *et al.,* 2011)

Primer	Sequence (5′→3′)	Product length (bp)
Forward (F)	CACTGTCTTCTTGTTACTGTATTTCAATGAGAC	141
Reverse (R)	GATGCAATACTGCCTGCTTG	

In this study, the amplification reaction conditions were carried out using 35 cycles. The thermal cycling conditions included at the first step initial denaturing temperature in 94°C for 4 min, 35 cycles of denaturation at 94°C for 45 seconds, annealing temperature 63°C for 30 seconds, multiply temperature of 72°C for 30 seconds and final extension temperature was 72°C for 4 min. PCR products placed on 12% polyacrylamide gel electrophoresis and in marker size was confirmed accuracy of desired fragment. 141bp desired fragment after the amplification were introduced under restriction enzyme treatment (G↓ANTC) *Hinf*I. Afterward, products of electrophoresis were demonstrated on 12% polyacrylamide gel (and 2% buffer TBE). Then, photos were prepared using Gel documentation system.

Results

DNA extraction from Hisari sheep blood samples was performed using Miller et al (1988) with a few changes, as noted in previous section (Miller *et al.*, 1988). Determination of concentration and quality of DNA was done in each sample compared with standard concentration of lambda phage DNA that cut with *Eco*RI and *Hind*III shear enzymes. Extracted DNA did not generate any abnormalities samples on the agarose gel that it indicates that DNA molecules are not broken. Also, in this experiment, the contamination effects were found by salt in the extracted solution that signifies on the purity of the extracted DNA. It should be noted that ammonium chloride in this method has a role in cell lysis.

After the optimization of reaction conditions of PCR for the amplification of $FecX^G$ locus in exon2 of BMP15 gene of sheep breed, for observation banding patterns, the PCR product were electrophoresed in the polyacrylamide gel (Fig. 1). As expected, 141 bp fragment of exon 2 of this gene in the studied breed was proliferated without any non-specific band and unwanted products and it was used to confirm the veracity of fragment size obtained from the 100 bp ladders. It should be noted that PCR products of all samples were similar and there was no difference in terms of the length of the proliferated string. it confirms only a target fragment for the used primers on the DNA strand.

The 112 bp fragment was resulted from the amplification of BMP-15 gene by the restriction endonucleases of *Hinf*I G/ANTC enzyme, and the digestion products in the presence of the size markers were electrophoresed on 12% polyacrylamide gels (like the electrophoresis of PCR products on BMP-15genes). Restriction enzyme cuts the "wild type" allele and as a result of digestion, 112 and 29 bp fragments produced. In figure2, 29 bp fragment is not observable due to small size and mutant type allele is not cut because of the removal of the intended cutting

site. After the electrophoresis of digested products, all samples showed the same genotype (+/+) i.e. wild genotype. The data obtained from this trial indicate that the BMP-15 gene in Hesari sheep breed is monomorphic or wild (Fig. 2).

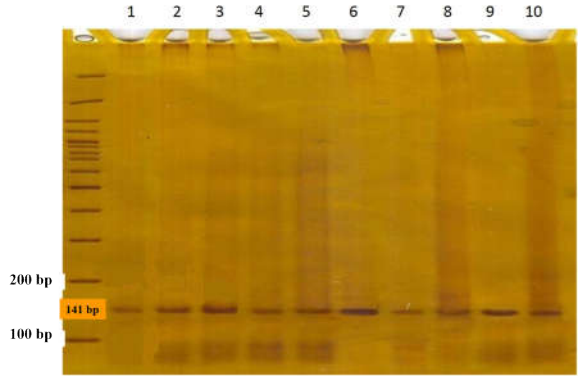

Fig. 1. Pattern of amplification of exon 2 of *BMP15* gene in polyacrylamide gel electrophoresis (before digestion) of different samples (columns 1-10)

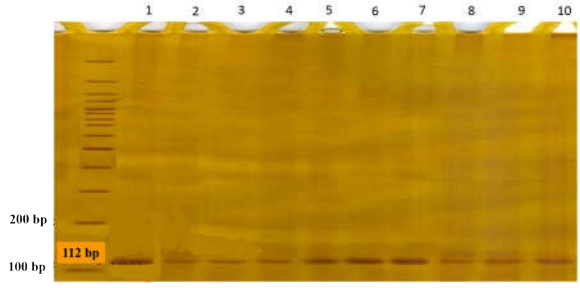

Fig. 2. Results of enzymatic digestion of exon 2 of *BMP15* gene in polyacrylamide gel electrophoresis.

Discussion

According to the phenotypic observations and molecular analysis, it seems that the existence of the examined mutations in this breed is rejected. If the mutation has happened spontaneously, since there is no selective planning, mutation removal can be possible. Also, due to the closure of the breeding environment of this breed and being away from the global and regional trading routes, the possibility of the transfer of these mutations from the foreign breeds to these breeds and their proliferation is far from expectation. The other reason is the environmental breeding conditions of this breed which is different from mutation carrying breeds because Tajikistan Hisari sheep breed grows in a mountainous region and bad weather conditions and the environmental conditions always act a barrier against high fertility and twinning. Furthermore, natural selection has also prevented the occurrence of such traits in this sheep. On the other hand, since this study has been conducted in a small population, the intended mutations may not exist in selected blood samples. Since other mutations have also been identified that are associated with twinning, so it is recommended to study the existence of them in this breed. One of the most important applications of molecular genetics in animal science is to identify animals with high fertility. The BMP-15 gene is very important in fertility and efficiency of the breeding of sheep; ovulation rate increases in the sheep with one copy of the BMP-15 gene. For the first time Hanrahan *et al*., (2004) have reported some mutations in BMP-15 gene by molecular analysis of this gene in Belclare and Cambridge breed of sheep that was associated with twining of mentioned breeds (Hanrahan *et al*., 2004). After that, other studies were conducted to find these mutations in other breeds (Chu *et al*., 2004) and in goat (Hua *et al*., 2007). Following these issues, our study showed infertility phenotype in the homozygous state.

A study was conducted in order to evaluate the polymorphism of BMP-15 and its impact on twinning rate and growth traits from birth to 15 months in Sanjabi sheep breed. According to their study, 80% of animals showed MM genotype and 20 % showed the NN genotype. Also, it was shown that BMP-15 gene had a significant effect on twinning. Fixed effects of season and parous (different parities) had no significant effect on twinning. The average of twinning of NN genotype was more than MM genotype. Infertility was not seen in Sanjabi sheep breed since the observed genotype in this breed was only in the form of homozygous, so, the hypothesis of infertility in homozygous animals with BMP-15 gene is rejected (Soleimani *et al*., 2011).

According to phenotypic studies in our herd and its comparison with the famous twin-bearing sheep, lambing rate of this sheep is lower than the studied twin-bearing sheep (not stated), probably due to adverse environmental conditions and breeding area of Hisari sheep breed. Furthermore, obtained results from the

analysis of Hisari sheep breed is in contrast with the results of the Sanjabi sheep breed.

Since the environment acts against fertility genes, a suitable environment should be provided to improve reproduction and fertility conditions, because Tajikistan sheep do not have the fertility genes that possess BMP-15 effect in polymorphic form. In order to specific determination of the mutations, sequencing test should be performed. Finally, it can be stated that this gene is monomorphic in Tajikistan breeds and its different phenotypes probably cause infertility. Considering the effect of increased number of born lambs or twinning on the amount of produced meat per head of ewe per year, reduction in the number of breeding ewes on pastures and prevention of the degradation of pastures, conducting studies seems to be necessary for finding genes that have major twinning effect on various breeds in Tajikistan. In addition, by entering the twining genes and planning for proliferation and stabilization of mutations associated with fertility genes, a substantial contribution can be made to increase production and income of dairy farmers of the country. Results showed no polymorphic BMP-15 gene, so no relationship of genotypes with the percentage of twining can also be examined. The frequencies of wild genotype (+/+) for exon 2 of BMP-15 gene is equal to one and it is zero for heterozygous and mutant genotypes because all the animals had the same genotype. The frequency of wild allele was one and the mutant allele for both positions was zero. Thus, no relationship can be examined in this context.

Therefore, in the case of the Tajik sheep may be argued that nature acts in favor of the competence of animal for survival. And in regard to the fact that Tajikistan is located in mountainous geographical conditions and pastures are in relatively poor conditions and traditional sheep farming, probably, nature also acts against the twinning gene. So, mutations that affect the reproduction have not remained or they have been removed over the time. Twin-bearing animal needs better nutrition as compared to monotocous animal and on the other hand, Tajik sheep produce lesser milk. Therefore the absence of this gene in Hisari sheep breed is a big disadvantage because the maximum efficiency of this animal can be exploited with this gene and also this gene can be transferred to other livestock that lacks this gene. Of course, all these issues do not mean that in Tajik sheep there is no gene with a major effect on twining, but there may be a gene or genes that have not been identified yet.

Conclusion

Although absence of this gene in Hisari sheep breed is a big disadvantage, but considering the fact that sheep farming in Tajikistan is moving towards industrialization, animals with low breeding rate would not be cost effective. So we can increase the number of born lambs using different methods of hybridization with foreign breeds, gene transfer, etc., in order to make it affordable in intensive and industrial system of sheep farming and to preserve the genetic diversity.

References

Bahrami Y, Chekaniazar V. 2013. Effectiveness of calpastatin and calpain genes on meat quality and tenderness in Iranian livestock breeds. *J Appl Env Biol Sci* 3(8):13-19.

Bahrami Y, Mohammadi S, Chekani-Azar HR, Mousavizadeh SA. 2014. The polymorphism of GDF-9 gene in hisari sheep. *Bio Inter J* 6(2): 46-52.

Bindon BM, Piper LR, Hilliard MA. 1996. Reproductive physiology and endocrinology of *Prolific sheep.* Wallingford: CAB International, 453-470.

Bodin L, Di Pasquale E, Fabre S, Bontoux M, Monget P, Er-sani L, 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 Endo* 148: 393-400.

Bodin L, Elsen JM, Poivey JP, San Cristobal-Gaudy M, Belloc JP, Eychenne F. 1998. Hyperprolificacy in the French Lacaune sheep breed; a possible major gene. *Procedure 6th World Congress Genetics Applied to Livestock Production; Armidale, Australia* 11-14.

Bodin L, Lecerf F, Pisselet C, SanCristobal M, Bibe B, Mulsant P. 2003. How many mutations are associated with increased ovulation rate and litter size in progeny of Lacunae meat sheep. *In: Proceedings of the International orkshop on Major Genes and QTL in Sheep and Goats*, *Toulouse, France, CDROM Communication* 2-11.

Bradford GE, Quirke JF, Sitorius P, Inounu I, Tiesnamurti B, Bell FL, Fletcher IC, Torell DT. 1986. Reproduction in Javanese sheep: evidence for a gene with large effect on ovulation rate and litter size. *J Anim Sci* 63: 418-431.

Chu MX, Li BX, Wang JY, Ye SC, Fang L. 2004. Association between PCR-SSCP of growth differentiation factor 9 genes and high prolificacy in small tail Han sheep. *Anim Biotech* 15: 111-120.

Davis GH, Dodds KG, Wheeler R, Jay NP. 2001. Evidence that an imprinted gene on the X chromosome increases ovulation rate in sheep. *Bio Rep* 64: 216-221.

Davis GH, McEwan JC, Fennessy PF, Dodds KG, Farquhar PA. 1991. Evidence for the presence of a major gene influencing ovulation rate on the X-chromosome of sheep. *Bio Rep* 44: 620-624.

Davis GH, McEwan JC, Fennessy PF, Dodds KG, McNatty KP, Wai Sum O. 1992. Infertility due to bilateral ovarian hypoplasia in sheep homozygous ($FecX^{I}$) for the Inverdale prolificacy gene located on the X chromosome. *Bio Rep* 46: 636-640.

Davis GH, Montgomery GW, Allison AJ, Kelly RW, Bray AR. 1982. Segregation of a major gene influencing fecundity in progeny of Booroola sheep. *New Zeal J Agri Res* 25: 525-529.

Eckery DC, Whale LJ, Lawrence SB, Wylde KA, Mc-Natty KP, Juengel JL. 2002. Expression of mRNA encoding growth differentiation factor 9 and bone morphogenetic protein 15 during follicular formation and growth in a marsupial, the brushtail possum (*Trichosurus vulpecula*). *Mol Cell Endo* 192: 115-126.

Eppig JJ. 2001, Oocyte control of ovarian follicular development and function in mammals. *Rep* 122: 829-838.

Feraye EG, Iraz A, Hanefi D, Ahmet M, Kemal Ö. 2011. Determination of BMP-15, BMPR-1B and GDF-9 Gene Mutations of the Indigenous Sheep Breeds in Turkey. *Kaf Üniv Vete Fakü Derg* 17: 725-729.

Galloway SM, McNatty KP, Cambridge LM, Laitinen M, Juengel LT, Jokiranta SR, McLaren J, Luiro K, Dodds KG, Montgomery W, Beattie AE, Davis GH, Ritvos O. 2000. Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and in fertility in a dosage- sensitive manner. *Nat Genet* 25: 279-283.

Hanrahan JP. 1991. Evidence for single gene effects on ovulation rate in the Cambridge and Belclare breeds, In: Elsen JM, Bodin L, Thimonier J, (eds). *Major Genes for Reproduction in Sheep.* Paris: INRA 93-102.

Hanrahan JP, Gregan SM, Mulsant P, Mullen M, Davis GH, Powell R, Galloway SM. 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 Bio Rep* 70: 900-909.

Hua GH, Chen SLJ, Ai T, Yang LG. 2007. None of polymorphism of ovine fecundity major genes FecB and FecX was tested in goat. *Anim Rep Sci* 19-24.

Jonmundsson JV, Adalsteinsson S. 1985. Single genes for fecundity in Icelandic sheep. In: Land RB, Robinson Dw, (eds). *Genetics of Reproduction in Sheep.* London, Butterworths, 159-168.

McNatty KP, Juengel JL, Reader KL, Lun S, Myllymaa1 S, Lawrence SB, Western A, Meerasahib MF, Mottershead DG, Groome NP, Ritvos O, Laitinen MPE. 2005. Bone morphogenetic protein 15 and growth differentiation factor 9 cooperate to regulate granulosa cell function in ruminants. *Rep* 129:481-487

Miller SA, Dykes DD, Polesky HF. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nuc Acid Res* 16: 1215-1219.

Monteagudo LV, Ponz R, Tejedor MT, Lavina A, Sierra I. 2009. A 17 bp deletion in the bone morphogenetic protein 15 (BMP15)

gene is associated to increased prolificacy. *Anim Rep Sci* 110: 139-146.

Pennetier S, Uzbekova S, Perreau C, Papillier P, Mermillod P, Dalbiestran R. 2004. Spatiotemporal expression of the germ cell marker genes MATER, ZAR1, GDF9, BMP15, and VASA in adult bovine tissues, oocytes, and preimplantation embryos. *J Bio Rep* 71: 1359-1366.

Piper LR, Bindon BM. 1982. Genetic segregation for fecundity in Booroola Merino sheep. In: Barton RA, Smith, W.C (eds). *Proc. World Congress on Sheep and Beef Cattle Breeding*. Palmerston North: New Zealand. 395-400.

Radomska MJ, Martyniuk E, Klewiec J, Knothe A. 1988. Inheritance of high prolificacy of the Olkuska sheep (preliminary results). *J Agri Sci* 60: 597-598.

Soleimani B, Rahemi-Meyanji G, Charaeian B. 2011. The polymorphism of BMP15 gene in exon 2 of the twinning and weight in sheep sanjabi. *J Bio* l4: 1-7.

Souza C J, MacDougall C, Campbell B K, McNeilly A S, Baird D T. 2001. The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (BMPR1B) gene. *J Endocrinol* 169:1-6.