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Application of PCR-RFLP Technique to Determine BMP 15 Gene Polymorphism in Sangsari Sheep Breed of Iran

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Abstract: Phenotypic evaluation and culling of candidate animals for traits by applying traditional animal breeding are usually costly tasks, which require considerable time to be carried out. Molecular genetic as an alternative method, enables animal breeders to select eligible animals for the desirable trait (s) at their earlier ages. Selection based upon markers could result in increasing accuracy as well as selection response of animals. BMP15 gene is known as a candidate because it is responsible for high ovulation and multiple births in sheep. In this study, using for identification $FecX^{I}$ mutation in two exon of BMP15 gene, having taken blood samples of 150 sheep (140 ewes, 10 rams) from sheep in Damghan genetic modification center, using venojects treated with the anti-clot substance (EDTA), the DNAs were salted out and extracted. After the extraction and quantitative and qualitative tests (80% spectrophotometer and gel agarose 8%) the required amounts for each Polymerase Chain Reaction (PCR) were specified. After the polymerase chain reactions, using the relevant primer, the related part was reproduced (154) and then the PCR products were cut by XbaI enzymes for this gene, were two 30 and 124 bp is produced for target sites. In the sample with specified genotype in this study, only wild alleles (+) were observed and all samples had (++) genotypes.

Key words: Sangsari sheep, PCR-RFLP, BMP15, $FecX^{I}$, animals

INTRODUCTION

Sangsari sheep is a light weight and fat tail Iranian breed from, which meat production is considered to be of major economic importance (Fig. 1). Genetic variation in an ovulation rate in sheep has been widely documented and the evidence shows substantial differences between breeds and in a number of cases exceptional variation within breeds/strains (Baird and Campbell, 1998). Ovulation rate is determined by a complex exchange of endocrine signals between the pituitary gland and the ovary (Davis *et al*., 1982; Piper and Bindon, 1982). Subsequently, putative major genes were invoked to explain the increased litter size and/or ovulation rate in a variety of breeds/strains, including inverdale (Davis *et al*., 1991), Cambridge (Hanrahan and Owen, 1985), Thoka (Jonmundsson and 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*., 1992, 2001) sheep. Bone Morphogenic Protein 15 (BMP15) and bone morphogenic protein-IB have been shown to be essential for ovulation rate and follicular growth. From examination of inherited patterns of ovulation rate in sheep, several breeds have been identified with point mutations in two growth factor genes (BMP15 and GDF9) and a related receptor (ALK6) that are expressed in oocytes. Five different Single

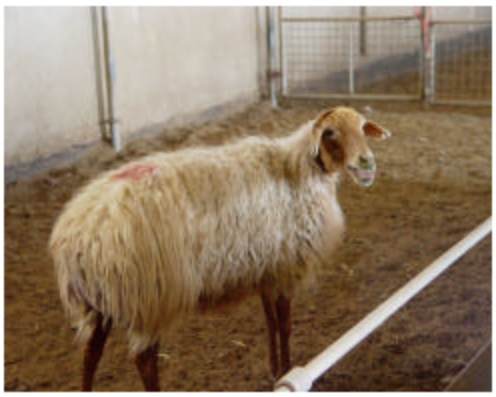

Fig. 1: A female Sangsari sheep breed

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Nucleotide Polymorphisms (SNP) have been identified in the BMP15 gene (Galloway *et al*., 2000; Hanrahan *et al*., 2004; Guan *et al*., 2006). In sheep animals, heterozygous for these mutations or heterozygous for two of these mutations or homozygous for the ALK6 mutation had higher ovulation rate than their wild-type contemporaries, of course from BMP15 mutations, only B2 ($FecX^G$) and B4 ($FecX^B$) and from GDF9 mutations and only G8 ($FecG^H$) had high ovulation rate and fertility. Animals homozygous for BMP15 or GDF9 mutations are sterile due to arrested follicular development from the primary stage of growth (Hanrahan *et al*., 2004).

The inverdale effect is due to mutations in an oocyte-derived growth factor gene BMP15 (also known as GDF9B) (Galloway *et al*., 2002). Two different independent point mutations in BMP15 have been identified (called Inverdale ($FecX^I$) and Hanna ($FecX^H$)) (Davis *et al*., 2001). The BMP15 and GDF9 mutations are thought to result in reduced levels of mature protein or altered binding to cell-surface receptors (McNatty *et al*., 2005). From examination of phenotypes of these mutations and subsequent physiological studies, it is clear the BMP15 is essential for ovarian follicular development and normal ovulation and/or corpus luteum formation in sheep. Moreover, it is evident that BMP15 (Hanrahan *et al*., 2004; Galloway *et al*., 2002; Chu *et al*., 2007). BMP15 gene an X-linked gene that increased ovulation rate by about 1.0, but caused sterility in homozygous carrier females was first described in Romney sheep and named the inverdale gene (FecX) (Davis *et al*., 1991; Davis *et al*., 1992). The infertile ewes have small undeveloped streak ovaries, which never ovulate. Discovered that inverdale sheep carried a mutation in an oocyte-derived growth factor gene, bone morphogenetic protein 15. Four different alleles of BMP15 ($FecX^I$, $FecX^H$, $FecX^G$ and $FecX^B$) all causing the same phenotypes have been identified in Romney, Belclare and Cambridge sheep (Hanrahan *et al*., 2004; Galloway *et al*., 2002). The gene is well suited to sheep farming systems in which specialist flocks of prolific ewes are mated to meat breed sires and all offspring of both sexes are slaughtered. The specialist ewe flock, which all carry the BMP15 mutation and have a litter size about 0.6 higher than non-carrier ewes is maintained by mating other non-inverdale ewes with carrier inverdale rams and retaining the daughters. The Inverdale gene was mapped to a 10 centiMorgans (cM) region at the center of the sheep X-chromosome (Galloway *et al*., 1996).

The main objective of the present research was to apply PCR-RFLP technique for determining BMP15 gene polymorphism in Sangsari sheep breed of Iran.

MATERIALS AND METHODS

A total of 3445 phenotypic records (representative 350 ewes and 50 rams) collected between 1994 and 2007 in Sangsari breeding center were used. A multiple traits animal model was utilized to predict breeding value (based upon BLUP statistical method) of individual animals for the traits of the number of lambs born per lambing and mating. DFREML algorithm was applied to estimate genetic and environmental variance and covariance components.

$$y_i = X_i b_i + Z_i a_i + W_i p_{ei} + e_i$$

Where:

y_i = A matrix of observations for the ith trait
b_i = A matrix of fixed factors for the ith trait
a_i = A matrix of random additive genetic effect of sheep for the ith trait
p_{ei} = A matrix of random effect of permanent environment of sheep for the ith trait
e_i = A matrix of random residual effect for the ith trait
X_i, Z_i, W_i = Design matrices for fixed and random effects in the model

$$E(y_i) = X_i b_i, E(a_i) = E(P_{ei}) = E(ei) = 0$$

$$Var(y_i) = Z_i A Z_i' \sigma_{gi}^2 + W_i I W_i' \sigma_{pei}^2 + I\sigma_i^2$$

$$Cov(y_i, y_j) = Z_i A Z_j' \sigma_{gij} + W_i I W_j' \sigma_{Peij} + I\sigma_{eij}$$

$$Var\begin{bmatrix} a \\ p_e \\ e \end{bmatrix} = \begin{bmatrix} A \otimes G & \cdots & \cdots \\ \cdots & I_{pe} \otimes P_e & \cdots \\ \cdots & \cdots & I_n \otimes R \end{bmatrix}$$

$$G = \begin{bmatrix} \sigma_{g1}^2 & \sigma_{g12} & \cdots & \sigma_{g1n} \\ \sigma_{g21} & \sigma_{g2}^2 & \cdots & \sigma_{g2n} \\ \cdots & \cdots & \cdots & \cdots \\ \sigma_{gn1} & \cdots & \cdots & \sigma_{gn}^2 \end{bmatrix}$$

$$P_e = \begin{bmatrix} \sigma_{pe1}^2 & \sigma_{pe12} & \cdots & \sigma_{pe1n} \\ \sigma_{pe21} & \sigma_{pe2}^2 & \cdots & \sigma_{pe2n} \\ \cdots & \cdots & \cdots & \cdots \\ \sigma_{pen1} & \cdots & \cdots & \sigma_{pen}^2 \end{bmatrix}$$

$$R = \begin{bmatrix} \sigma_{e1}^2 & \sigma_{e12} & \cdots & \sigma_{e1n} \\ \sigma_{e21} & \sigma_{e2}^2 & \cdots & \sigma_{e2n} \\ \cdots & \cdots & \cdots & \cdots \\ \sigma_{en1} & \cdots & \cdots & \sigma_{en}^2 \end{bmatrix}$$

Where:

G = Apparent and positive matrix
P_c = Genetic relationship matrix
I_{po} I_a = Unit matrix
σ^2_g = Additive genetic variance for the i th trait
σ^2_{po} = Perennial environment variance for the i th trait
σ^2_a = Residual variance for the i th trait
σ_{gi} = Additive genetic covariance for the i and jth trait
σ_{poi} = Perennial environment covariance for the i and jth trait
σ_{ai} = Residual covariance for the i and jth trait

In the next stage, blood samples were taken from 150 sheeps (including 140 ewes and 10 rams). Each sample consisted of 5 mL blood collected in venoject tube contained EDTA. The DNA contents of the samples were extracted by salting out method. PCR technique was performed in a total volume of 25 μL including 1.5 μL DNA, 1 unit of Taq polymerase (plam-cycler (Corbete research version 2.2)) 0.2 μL dNTP, 2.5 μL buffer 10× PCR, 0.25 μL of each forward and reverse primers, 0.75 μL $MgCl_2$ (50 $\mu g\ \mu L^{-1}$) and distilled water (14). The following primers were subsequently used:

Forward : 5'-GAA GTA ACC AGT GTT CCC TCC ACC CTT TTC T-3'
Reverse : 5'-CAT GAT TGG GAG AAT TGA GAC C-3'

Polymerase chain reaction was repeated 35 cycles by thermocycler instrument as follows: 5 min at 94°C, 30 sec at 94°C, 40 sec at 62°C, 30 sec at 70°C for 35°C cycles and final stage in clued 4 min at 72°C and 15 min at 99°C. An aliquot of each PCR products were subjected to electrophoresis 1.5% agarose gel and be side a size marker and staining with ethidium bromide 0.5 $\mu g\ mL^{-1}$.

Size bands were found to be about 154 bp (base pairs) and remaining PCR products were treated with narrow spectrum XbaI enzyme. Final products were subjected to electrophoresis in 3% agarose gel and stained with ethidium bromide. Animals carrying BMP15 mutation produce segments of 30 and 124 bp, while animals are of wild genotype shows 154 bp segments.

RESULTS AND DISCUSSION

According to the results found in the present study, it was revealed that the average number of lambs were 0.94 and 0.90 per mated and pregnant ewe respectively and several breeding value calculator for this trait two, respectively were 0.73 and 0.69 obtained for sheep selection. In present study, performed experimental for existence mutation $FecX^I$ in BMP15 gene that result performed polymerase chain reaction for $FecX^I$. Also that expected fragment 154 bp of this gene propagation and used set grace accuracy products of marker size and then PCR product digested with enzyme XbaI for this gene. In the sample with specified in this study, only wild allele (+) were observed and all samples had (++) genotypes (Fig. 2).

In study that performed upon toward multiple birth sheeps 8 country India, New Zealand, Philippine, Indonesia, Holland, Island, France and Ireland result homogeneous obtained and none of garol, Woodlands, Olkuska Cambridge ewes that too none of 1acaun bread rams not carried $FecX^I$ mutation that with obtained result in this experimental accordance (Davis *et al*., 2001). Also, study future that upon toward breed 20th one and sheeps type with upside productivity performed testimony upon existence $FecX^I$ gene in sheep in stance experimental no accent (Davis *et al*., 2001, 2002, 2006) and indicate sheep that carried FecB mutation, no carried $FecX^I$ mutation and this indicated truly that however, breed sangsari sheep no carried this mutation and performed experimental accuracy this sentence grace. Yet possible genes carried whit other major effect upon towards reproduction and birth multiple. However, no conditional polytrophic effects brooroola and inverdal gene upon towards body weight and of face Sangsari sheep in soft Caracas. This hypothesis that reproduction with body weight has negation correlation no rejection. Because however, texal 1amps carried Brooroola genes, but have heavy carcass and plume fat (Davis *et al*., 2001, 2002, 2006).

On the other hand, twining is under control of genetic and environmental factors and that natural environment is against of this trait. Therefore, nutrition could greatly influence the expression of major gene effect affecting on reproductive performance of the animals (Davis *et al*., 2001).

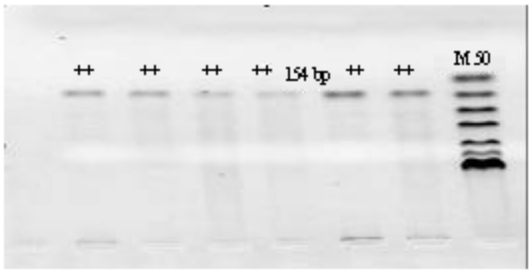

Fig. 2: PCR product BMP15 gene. Fragment of size marker:501,489-404-331-242-190-147-111,110-67-34

There is a report indicating that ovulation rate in Javanese breed carrying FecB is half of that of Merinos breed due to inappropriate environmental conditions such as low quality of feeds (Bradford *et al*., 1986; Davis *et al*., 2006; Hanrahan *et al*., 2004). Although, there is the same mutant allele for Booroola and Garol breeds, ovulation and also lambing rates for Booroola is higher than that of Garol breed. This could be due to differences of breeds, nutrition and any other genetic factors such as modifier genes (Davis *et al*., 2002, 2006).

In connection to Sangsari breed of Iran, low rate of lambing may be explained by the harsh mountain environment for rearing of this breed. This could lead to a low rate of lambs per individual ewes. With respect to the phenotypic observations and also molecular experiments, it seems that the occurrence of spontaneous mutation phenomenon for this breed to be rejected. If this may not be the case, because there has not been any planned selection program and also there has been natural selection against this trait, the mutant allele has been removed from the population gradually. Moreover, particular mountain environmental condition for this breed has caused a selection trend against this trait.

In addition, the import of the mutant allele from exotic breeds to the Sangsari breed is unexpected due to a closed environment and inaccessible commercial routs to the region of the breed rearing. On the other hand, due to small size of the sample studied in this research, there is a probability that the mutant allele was not available in the sample. Therefore, there is a need to undertake a further research on a relatively larger size sample for the population. A number of other mutant genes affecting lambing, rates have been also detected for which the Sangsari breed may be studied.

CONCLUSION

With respect to the positive effect of increased lambing rate on meat production and decreased the number of breeder ewes on the pasture, finding major genes affecting on twining traits is of great importance from the economic point of view.

Therefore, establishing a well-planned program to import the mutant alleles into the local Iranian sheep breeds could result in a significant increase of production level leading to a higher level of breeder's income.

REFERENCES

Baird, D.T. and B.K. Campbell, 1998. Follicle selection in sheep with breed differences in ovulation rate. Mol. Cell. Endocrinol., 145: 89-95.

Bodin, L., J.M. Elsen, J.P. Poivey, M.San Cristobal-Gaudy, J.P. Belloc and F. Eychenne, 1998. Hyper-prolificacy in the French Lacaune sheep breed: A possible major gene. Proceeding of the 6th World Congress on Genetics Applied to Livestock Producrion, 27: 11-14.

Bradford, G.E., J.F. Quirke, P. Sitorius, I. Inounu, B. Tiesnamurti, F.L. Bell, I.C. Fletcher and D.T. Torell, 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, M.X., Z.H. Liu, C.L., Giao, Y.Q. He, L. Fang, S.C. Ye, G.H. Chen and G.Y. Wang, 2007. Mutations in BMPR-IB and BMP15 genes are associated with litter size in small Tailed Han sheep (*Ovis aries*). J. Anim. Sci., 85: 324-330.

Davis, G.H., G.W. Montgomery, A.J. Allison, R.W. Kelly and A.R. Bray, 1982. Segregation of a major gene influencing fecundity in progeny of Booroola sheep. N.Z. J. Agric. Res., 25: 525-529.

Davis, G.H., J.C. McEwan, P.F. Fennessy, K.G. Dodds and P.A. Farquhar, 1991. Evidence for the presence of a major gene influencing ovulation rate on the X-chromosome of sheep. Biol. Reprod., 44: 620-624.

Davis, G.H., K.G. Dodds, R. Wheeler and N.P. Jay, 2001. Evidence that an imprinted gene on the X-chromosome increases ovulation rate in sheep. Biol. Reprod., 64: 216-221.

Davis, G.H., J.C. McEwan, P.F. Fennessy, K.G. Dodds, K.P. McNatty and O. WaiSum, 1992. Infertility due to bilateral ovarian hypoplasia in sheep homozygous ($FecX^I$ $FecX^I$) for the Inverdale prolificacy gene located on the X-chromosome. Biol. Reprod., 46: 636-640.

Davis, G.H., S.M. Galloway, I.K. Ross, S.M. Gergan, J. Ward, B.V. Nimbkar, P.M. Ghalsasi, C. Nimbkar, G.D. Gray, I. Inounu, B. Tiesnamurti, J.P. Hanrahan and T. Wilson, 2002. DNA Tests in Prolific Sheep from 8 countries provide new evidence on origin of the Booroola (FecB) Mutation. Biol. Reprod., 66: 1869-1874.

Davis, G.H., L. Balakrishnan, I.K. Ross, T. Wilson, M. Galloway, B.M. Lumsden, J.P. Hanrahan, M.X. Mullen, Z. Mao, G.L. Wang, Z.S. Zhao, J.J. Robinson, A.P. Mavrogenis, C. Papachristoforou, C. Peter, R. Baumung, P. Cardyn, I. Boujenane, N.E. Cockett, E. Eythorsdottir, J.J. Arranz and D.R. Notter, 2006. Investigation of the Booroola (FecB) and Inverdale ($FecX^I$) mutations in 21 prolific breeds and strains of sheep sampled in 13 countries. Anim. Reprod. Sci., 92: 87-96.

Galloway, S.M., K.P. McNatty, L.M. Cambridge, M.P.E. Laitinen, J.L. Juengel, T.S. Jokiranta, R.J. McLaren, K. Luiro, K.G. Dodds, G.W. Montgomery, A.E. Beattie, G.H. Davis and O. Ritvos, 2000. Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. Nat. Genet., 25: 279-283.

Galloway, S.M., K.P. McNatty, L.M. Cambridge, M.P.E. Laitinen, J.L. Juengel, T.S. Jokiranta, R.J. McLaren, K. Luiro, K.G. Dodds, G.W. Montgomery, A.E. Beattie, G.H. Davis and O. Ritvos, 2002. Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. Nat. Genet., 25: 279-283.

Galloway, S.M., V. Hanrahan, K.G. Dodds, M.D. Potts, A.M. Crawford and D.F. Hill, 1996. A linkage map of the ovine X-chromosome. Gen. Res., 6: 667-677.

Guan, F., S.R. Liu, G.Q. Shi, J.T. Ai, D.G. Mao and L.G. Yang, 2006. Polymorphism of FecB gene in nine sheep breeds or strains and its effects on litter size, lamb growth and development. Acta Genetica Sinica, 33: 117-124.

Hanrahan, J.P. and J.B. Owen, 1985. Variation and repeatability of ovulation rate in Cambridge ewes. Anim. Prod., 40: 529.

Hanrahan, J.P., 1991. Evidence for Single Gene Effects on Ovulation Rate in the Cambridge and Belclare Breeds. In: Elsen, J.M., L. Bodin and J. Thimonier (Eds.). Major Genes for Reproduction in Sheep. Paris: INRA, pp: 93-102.

Hanrahan, J.P., S.M. Gergan, P. Mulsant, M. Mullen, G.H. Davis, R. Powell and S.M. Galloway, 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 (*Ovis aries*). Biol. Reprod., 70: 900-909.

Jonmundsson, J.V. and S. Adalsteinsson, 1985. Single Genes for Fecundity in Icelandic Sheep. In: Land, R.B. and D.W. Robinson (Eds.). Genetics of Reproduction in Sheep. Butterworths, London, pp: 159-168.

McNatty, K.P., J.L. Jungel, K.L. Reader, S. Lun, S. Myllymaa, S.B. Lawrence, A. Western, M.F. Meerasahib, D.G. Mottershead, N.P. Groome, O. Ritvos and M.P. Laitinen, 2005. Bone Morphogenetic Protein 15 (BMP15) and Growth Differentiation Factor 9 (GDF9) co-operate to regulate granulose cell function in ruminants. Reproduction, 129: 481-487.

Piper, L.R. and B.M. Bindon, 1982. Genetic Segregation for Fecundity in Booroola Merino Sheep. In: Barton, R.A. and W.C. Smith (Eds.). Proceedings of the World Congress on Sheep and Beef Cattle Breeding. Palmerston North, New Zealand, pp: 395-400.

Radomska, M.J., E. Martyniuk, J. Klewiec and A. Knothe, 1988. Inheritance of high prolificacy of the Olkuska sheep (preliminary results). J. Agric. Sci. Finland, 60: 597-598.