Indian Journal of
Small Ruminants

POLYMORPHISM OF GROWTH HORMONE BMP-15 GENE IN MARWARI GOAT

Aditya Godara, G.C. Gahlot*, Mohammad Ashraf and S.K. Ghorui[1]
Department of Animal Breeding and Genetics, College of Veterinary and Animal Science
Rajasthan University of Veterinary and Animal Sciences, Bikaner- 334 001, Rajasthan
[1]National Research Center on Camel, Bikaner- 334 001, Rajasthan
**E-mail address: gahlotgcbkn@rediffmail.com*

Manuscript received on 18.04.2011, accepted on 22.06.2011

ABSTRACT

A study was undertaken to investigate polymorphism in BMP-15 (*Fec X^B* and *Fec X^G* in exon-2) gene in Marwari goats by RFLP technique. Samples were collected from 60 unrelated goats of Marwari breed from different locations in Bikaner district. Genomic DNA was extracted by phenol:chloroform method and amplified using exon-2 *Fec X^B* and *Fec X^G* specific primers. The amplification was obtained at between 153 bp and 141 bp. The purified amplicons were digested with five base cutter restriction enzymes: *DdeI* and *HinfI* (5'GANTCA 3'CTNAGT). All samples showed the absence of polymorphism at the *Fec X^B* and *Fec X^G* loci of BMP-15 gene. Hence, BMP-15 gene cannot be regarded as the major gene associated with the fecundity of goats. Further investigation may be directed at other loci of BMP-15 gene or other genes, using larger sample size. **(Indian Journal of Small Ruminants 2012, 18(1): 32-36).**

Key words: BMP-15 gene, Goat, Marwari, PCR- RFLP

The Marwari breed is one of the popular goat breeds for meat production in India, particularly in Rajasthan. It possesses high growth rate and fertility and has good adaptation to various farming systems. Conventional breeding programmes are continuing in a number of districts of Rajasthan for genetic improvement of this breed, focusing on growth and meat production. Accuracy of selection programmes and rate of genetic gain in farm animal may be further enhanced using marker assisted selection (MAS). However, identifying useful genetic markers is the first and most critical step in MAS (Spelman and Bovenhuis, 1998). Bone morphogenetic protein (BMP) is present in bone and has capacity to repair and regenerate bone. BMPs were originally identified on the basis of their ability to produce ectopic bone formation when implanted within soft tissue *in vivo*. They also play roles in embryonic development, homeostasis, repairing of various tissue patterning, cell differentiation and apoptosis (Wozney et al., 1988). The BMP family of proteins is the largest subgroup of the transforming growth factor-ß (TGF-ß) superfamily (Rueger, 2002). TGF-ßs act as signaling molecules in tissue and organ development and have a wide range of functions in connective tissue, brain, kidney, and muscle. Currently, there are approximately 30 members of the BMP family. BMP-15 is the one of the members of this family which influences growth and reproduction in animals. The BMP-15 regulates granulosa cell proliferation and differentiation by promoting mitosis, suppressing follicle-stimulating hormone receptor expression and stimulating kit legend expression. It plays a pivotal role in female fertility in mammals (Juengel et al., 2002; Moore et al., 2003). Polymorphism of the BMP-15 gene was associated with both increased ovulation rate and litter size in heterozygous carriers and sterility in homozygous carriers in sheep and goats (Malan, 2000; Juengel et al. 2003; Chu et al., 2004). In recent years, five different mutations in the BMP-15 gene (*FecX^G*, *FecX^B*, *FecX^I*, *FecX^H*, *FecX^L*), and one in the BMPR-1B gene (*Fec B*) were identified. These mutations had great association to the ovulation rate of different sheep breeds, especially the *Fec B* mutation

(Booroola breed) and *FecX*^{G} (Inverdale breed). In the present study, these genes were selected to detect the probable effects on goat prolificacy. Therefore, the objective of this study was to detect polymorphism of the *FecX*^{B} and *FecX*^{G} loci in BMP-15 gene in the Marwari goat using PCR-Restriction Fragment Length Polymorphism (RFLP).

MATERIALS AND METHODS

A total of 60 blood samples from Marwari goats were collected randomly from different locations in Bikaner district (Deshnokhe, Kalyansar, Raisar and Daiya villages). The samples were kept in deep freeze at - 20°C till the isolation of genomic DNA. Genomic DNA was extracted from whole blood by phenol-chloroform extraction method as given by Sambrook et al. (1989) with slight modification. The extracted genomic DNA was checked for quality, purity and concentration. Horizontal submarine agarose gel electrophoresis was carried out to check the quality of genomic DNA using 0.8 % w/v agarose. Only the DNA samples of good quality were used for further analysis. *Fec X*^{B} and *Fec X*^{G} alleles in exon 2 BMP-15 gene were amplified using specific primers (Hanrahan et al., 2004) (Table 1). The amplification was obtained at 153 bp and 141 bp, respectively. The amplified product was run on 1.2% agarose gel electrophoresis and the band patterns were obtained with gel documentation system. The amplicons (exon-2 *Fec X*^{B} and *Fec X*^{G}) were purified with Phenol:Chloroform:Isoamyl alcohol extraction method to exclude the possibility of impurity such as Taq DNA polymerase, DNTPs and Taq Buffer.

Table 1. Primers and PCR amplification parameters used to amplify BMP-15 gene

Gene	Primers (5' to 3')	Amplicon size (bp)	Annealing temperature (°C)	Enzyme
FecX^{B}	F-GCCTTCCTGTGTCCCTTATAAGTATGTTCCCCTTA			
	R-TTCTTGGGAAACCTGAGCTAGC	153	57.5	*Dde*I
FecX^{G}	F-CACTGTCTTCTTGTTACTGTATTTCAATGAGAC			
	R-GATGCAATACTGCCTGCTTG	141	63	*Hinf*I

F-Forward primer; R-Reverse primer

The PCR was carried out with denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45 sec, annealing at different temperatures and different times by the primer, extension at 72 °C for 60 sec, with a final extension at 72 °C for 10 min on PX2 Thermal Cycler (Thermo Electron Corporation, USA). For storage, the PCR products were heated to 95 °C for 5 min and immediately placed on ice to prevent DNA strands from re-annealing. Restriction endonuclease (RE) digestion of purified PCR product (amplicon) was done with the *Dde*I and *Hinf*I (5'GANTCA 3'CTNAGT) enzymes. *Dde*I (1.0 µl) and *Hinf*I (5.0 µl) in a total volume of 50 µl at the temperature recommended by the manufacturer was kept for about 3-6 h. The digested product was analyzed by 1.2% agarose gel electrophoresis.

RESULTS AND DISCUSSION

The genomic DNA was successfully isolated from blood samples. The gel electrophoresis pattern of different samples is depicted in Plate1.

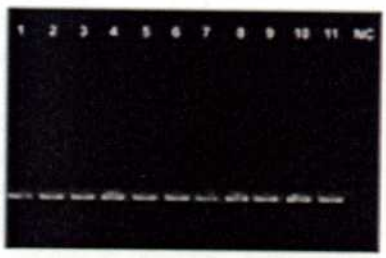

Plate 1. Genomic DNA isolated from Marwari goats (Lane 1-11: Genomic DNA, Lane 12: Negative control)

The amplification was observed at 153 and 141 bp, respectively. The amplified (BMP-15; exon-2 *Fec* X^B and *Fec* X^G) product was run on 1.2% agarose gel electrophoresis and the band pattern has been presented in Plate 2-3. The amplicons (exon-2 *Fec* X^B and *Fec* X^G) were purified with Phenol: Chloroform: Isoamyl alcohol extraction method and digested with five base cutter restriction enzymes *DdeI* (5'CTNAG 3'GANTC) and *HinfI* (5'GANTCA 3'CTNAGT), respectively. The digested fragment has been shown in Plate 4-5. All DNA samples of Marwari goat showed the absence of polymorphism at the *Fec* X^B and *Fec* X^G loci of BMP-15 gene. The findings of the present study are in line with those of Polley et al. (2009) in Indian Black Bengal goat who reported that all known point mutations of BMP15 and GDF9 genes were monomorphic in the animals tested.

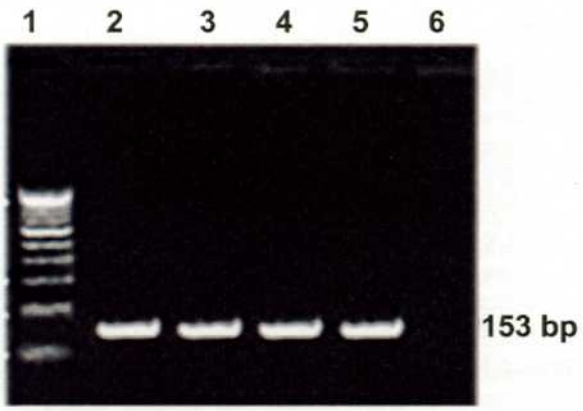

Plate 2. Amplification of BMP-15 (exon Fec^B) gene by PCR (Lane 1: 100 bp ladder, lane 2-5: Amplicons (BMP-15, exon Fec^B gene); lane 6: Negative control)

The results of present investigation are also in agreement with those of Deldar-Tajangookeh et al. (2009) in Iranian goats who observed no evidence of mutation in $FecX^B$ and $FecX^G$ in these goats using PCR-RFLP technique and all were monomorphic for exon 2 BMP-15 gene. Also, none of the polymorphisms were found in the coding region of BMP- 15 mature peptide of Markhoz goats of Iran with *Dde I* digestion (Arefnezhad, 2007). Hua et al. (2008) in Chinese goats also reported absence of the polymorphism in ovine fecundity major genes *FecB* and *FecX*. The absence of mutation in BMP-15 of six breeds of Chinese goats was reported by He et al. (2006).

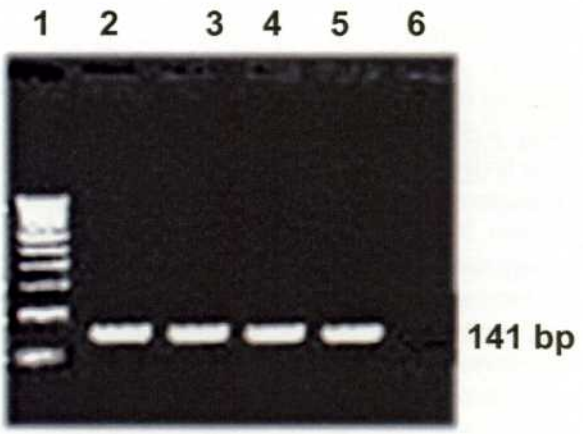

Plate 3. Amplification of BMP-15 (exon Fec^G) gene by PCR (Lane 1: 100 bp ladder, lane 2-5: Amplicons (BMP-15, exon Fec^G gene); lane 6: Negative control)

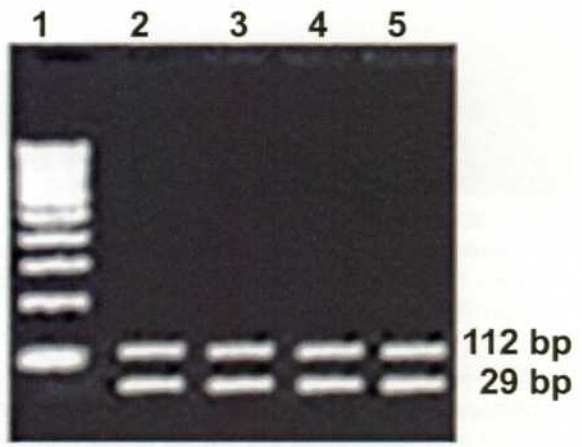

Plate 4. Monomorphism in BMP-15 (exon Fec^G) gene of Marwari goat digested with *Hinf I* restriction enzyme (Lane 1: 100 bp ladder, lane 2-5: Digested amplicons)

Mutations at five different points in exon 2 of BMP-15 gene are associated with prolificacy in some breeds of sheep (Montgomery et al., 2001). Mutations in fecundity genes GDF-9 and BMP-15 have important economic values in sheep breeding and probably ruminant reproduction (Galloway et al., 2000; Hanrahan et al., 2004; McNatty et al., 2005). Action of a single major gene responsible for high ovulation rate is reported in the Booroola Merino, Inverdale, Belclare and Cambridge sheep but there is no evidence of major gene for litter size in other prolific sheep such as Finish Landrace and Romanov and these findings indicate that at least two genetic control mechanisms for high prolificacy operates in sheep (Gordon, 2004). The biological effect of the mutations in mammals varies among species (Yan et al., 2001). Furthermore, Hashimoto et al. (2005) recently suggested that species-specific differences in BMP-15 processing may be associated with the differences in ovulation rate amongst species.

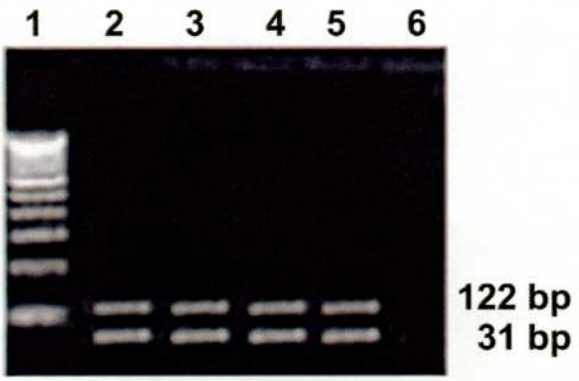

Plate 5. Monomorphism in BMP-15 (exon Fec^{B}) gene of Marwari goat digested with *Dde I* restriction enzyme (Lane 1: 100 bp ladder, lane 2-5: Digested amplicons, lane 6: Negative control)

GDF-9, BMP-15 and BMPR-1B genes enhanced the primary and prenatal follicular growth *in vitro* and *in vivo* and expressed in all the stages of the normal ovarian follicle development (Hayashi et al., 1999; Hanrahan et al., 2004). These genes were detected increasing ovulation in sheep breeds, especially *Fec G^{H}* mutation of GDF-9 gene in Belcare and Cambridge breeds, *Fec X^{G}*, *Fec X^{B}*, *Fec X^{I}*, *Fec X^{H}* and *Fec X^{L}* mutation of BMP-15 gene in several sheep breeds and Fec B mutation in BMPR-1B gene (Davis, 2005). In the present study, the results indicated that none of these mutations can be detected in Marwari goats including the high fecundity and low fecundity goats, these mutations had no obvious effect on the difference of prolificacy in goats. There was no genetic polymorphism of *FecX^{B}* and *FecX^{G}* loci of BMP-15 gene in Marwari goat. Hence, BMP-15 gene cannot be regarded as the major gene associated with the fecundity of goats. Further investigation should be directed at other loci of BMP-15 gene or other genes, using larger sample size.

ACKNOWLEDGMENT

The authors are thankful to the Director, National Research Centre on Camel, Bikaner for providing facilities.

REFERENCES

Arefnezhad, B. 2007. Molecular analysis of BMP-15 (Bone Morphogenetic Protein-15) gene in Markhoz goats. M.Sc. thesis, Department of Animal Science, Shiraz University, Shiraz, Iran. p. 133.

Chu, M.X., Li, B.X., Wang, S.C.Ye. and Fang, L. 2004. Association between PCR-SSCP of growth differentiation factor 9 gene and high prolificacy in small tail Han sheep. Animal Biotechnology 15: 111-120.

Davis, G.H. 2005. Major genes affecting ovulation rate in sheep. Genetics Selection Evolution 37: S11-S24.

Deldar-Tajangookeh Hamid, Shahneh Ahmad Zare, Zamiri Mohammad Javad, Kohram Hamid and Nejati-Javaremi Ardeshir. 2009. Study of BMP-15 gene polymorphism in Iranian goats. African Journal of Biotechnology 8: 2929-2932.

Galloway, S.M., McNatty, K.P., Cambridge, L.M., Laitinen, M.P.E. Juengel, J.L., Jokiranta, T.S., McLaren, R.J., Luiro, K., Dodds K.G., Montgomery, G.W., Beattie, A.E., Davis, G.H. and Rit, O. 2000. Mutations in an oocyte-derived growth factor gene (BMP-15) cause increased ovulation rate and infertility in a dosage-sensitive manner. Nature Genetics 25: 279-283.

Gordon, I. 2004. Controlled Reproduction in Sheep and Goats. Wallingford, UK, , CAB International, p. 407.

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 GDF-9 and BMP-15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). Biology of Reproduction 70: 900-909.

Hashimoto, O., Moore, R.K. and Shimasaki, S. 2005. Posttranslational processing of mouse and human BMP-15: Potential implication in the determination of ovulation quota. Proceedings of the National Academic of Science, USA 102: 5426-5431.

Hayashi, M., McGee, E.A., Min, G., Klein, C., Rose, U.M., van Duin, M. and Hsueh, A.J. 1999. Recombinant growth differentiation factor-9 (GDF-9) enhances growth and differentiation of cultured early ovarian follicles. Endocrinology 140: 1236-1244.

He, Y.Q., Chu, M.X., Wang, J.Y., Fang, L. and Ye, S.C. 2006. Polymorphism on BMP-15 as a candidate gene for prolificacy in six goat breeds Chinese. Journal of Anhui Agricultural University 33: 61-64.

Hua, G.H., Chen, S.L., Ai, J.T. and Yang, L.G. 2008. None of polymorphism of ovine fecundity major genes FecB and FecX was tested in goat. Animal Reproduction Science 108:279-286.

Juengel, J.L., Hudson, N.L., Heath, D.A., Smith, P., Reader, K.L., Lawrence, S.B., O'Connell, A.R., Laitinen, M.P.E., Cranfield, M., Groome, N.P. Ritvos, O. and McNatty, K.P. 2002. Growth differentiation factor 9 and bone morphogenetic protein 15 are essential for ovarian follicular development in sheep. Biology of Reproduction 67: 1777-1789.

Juengel, J.L., Hudson, N.L., Whiting, L. and McNatty, K.P. 2003. Effects of immunization against bone morphogenetic protein 15 and growth differentiation factor 9 on ovulation rate, fertilization and pregnancy in ewes. Biology of Reproduction 70: 557-561.

Malan, S.W. 2000. The improved Boer goat. Small Ruminant Research 36: 165-170.

McNatty, K.P., Smith, P., Moore, L.G., Reader, K., Lun, S., Hanrahan, J.P., Groome, N.P., Laitinen, M., Ritvos, O. and Juengel, J.L. 2005. Oocyte expressed genes affecting ovulation rate. Molecular and Cellular Endocrinology 234: 57-66.

Montgomery, G.W., Galloway, S.M., George, H., Davis, G.H. and McNatty, K.P. 2001. Genes controlling ovulation rate in sheep. Reproduction 121: 843-852.

Moore, R.K., Otasuka, F. and Shimasaki, S. 2003. Molecular basis of bone morphogenetic protein-15 signaling in granulosa cells. Journal of Biological Chemistry 278: 304-310.

Polley, S., De, S., Batabyal, S., Kaushik, R., Yadav, P., Arora, J.S., Chattopadhyay, S., Pan, S., Brahma, B., Datta, T.K. and Goswami, S.L. 2009. Polymorphism of fecundity genes (BMPR1B, BMP15 and GDF9) in the Indian prolific Black Bengal goat. Small Ruminant Research 85: 122-129.

Rueger, D.C. 2002. Biochemistry of bone morphogenetic proteins. In: S. Vukicevic and T.K. Sampath (Eds.), Bone Morphogenetic Proteins: From Laboratory to Clinical Practice. Cambridge, Mass: Birkhäuser 1-18.

Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.

Spelman, R. and Bovenhuis, H. 1998. Genetic response from marker assisted selection in an outbred population for differing marker bracket sizes and with two identified quantitative trait loci. Genetics 148: 1389-1396.

Wozney, J.M., Rosen, V., Celeste, A.J., Mitsock, L.M., Whitters, M.J., Hewick, R.M. and Wang, E.A. 1988. Novel regulations of bone formation: molecular clones and activities. Science 242: 1528-1534.

Yan, C., Wang, P., DeMayo, J., DeMayo, F.J., Elvin, J.A., Carino, C., Prasad, S.V., Skinner, S.S., Dunbar, B.S., Dube, J.L., Celeste, A.J. and Matzuk, M.M. 2001. Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. Molecular Endocrinology 15: 854-866.