

Genetic Polymorphism in *GDF9* and *FecB* Genes in Dalagh Sheep Breed of Iran

Rasoul Khatam Nejhad and Alireza Khan Ahmadi
Department of Animal Science, Gonbad University, Gonbad, Iran

Abstract: This study was conducted to identify polymorphisms in *GDF9* and *FecB* genes in Dalagh sheep breed. About 100 mature ewes from three flocks in Golestan province were genotyped for the *GDF9* ligand (*FecGH*) and *fecB* (*BMPRIIB*) receptor. Using two pairs of specific primers, DNA fragments with the size of 139 and 190 bp were amplified by using polymerase chain reaction. The PCR products were digested using *DdeI* and *AvaII* restriction enzymes for *GDF9* and *FecB* loci, respectively. The results showed no differences in the band patterns of digested products only the wild type alleles were detected and all animals for these two loci were monomorphic.

Key words: Sheep, Dalagh, *FecB*, *GDF9*, monomorph, Iran

INTRODUCTION

Sheep is a highly diverse species with >900 different breeds that substantially differs at economical production and genetic traits point of view (McNatty *et al.*, 2004). Iran has 27 sheep population and Dalagh is a fat tailed breed which is considered to be an important animal for meat, wool and milk production (Tavakkolian, 2000). Low efficiency is common in sheep due to the low reproductive rates in sheep because of sex-limited nature and low heritability of the trait (5-10%) (Javanmard *et al.*, 2011). Molecular genetic put out new paradigm to conquer this limitation as it relates the traits to genome.

Now-a-days mutations with major effects on Ovulation Rate (OR) and Litter Size (LS) were identified in TGF β superfamily ligands and receptors. The molecules contain over 35 members and many of which play a pivotal role in regulating fertility (Juengel *et al.*, 2004).

GDF9 is a growth factor and a member of TGF β super family is secreted by oocytes in growing ovarian follicles (Laitinen *et al.*, 1998) and is a potent stimulator of granulosa cells proliferation and differentiation. It is critical for progression of the earliest stages of folliculogenesis and in late follicular development (Dong *et al.*, 1996; Galloway *et al.*, 2000). The *GDF* gene has been mapped to sheep chromosome 5 and it spans approximately 2.5 kb and contain two exons and one intron. It has been described that *GDF9* has an autosomal over-dominant inheritance pattern (Davis *et al.*, 1982) for bringing about a rise in OR and LS in heterozygous and infertility in homozygous females (Wilson *et al.*, 2001).

The Booroola phenotype has been documented in Booroola merino is a mutation occurring in *BMPRIIB* receptor. It has been mapped to chromosome six in sheep

(Souza *et al.*, 2001; Wilson *et al.*, 2001). *BMPRIIB* is a potent receptor for various BMP ligands such as BMP15, BMP-2, -4, -6 and BMP-7 that some of which have a multifunctional roles in physiology and specifically in reproduction.

Based on segregation of OR, the mutant allele shows an additive inheritance effects on OR that stimulates the increasing of OR in heterozygous and homozygous carriers by 3-4 and >5 per cycle, respectively (Wilson *et al.*, 2001).

MATERIALS AND METHODS

Genomic DNA was extracted by using salting out method. Quantity and quality of the DNA extracted was assessed by spectrophotometry and electrophoresis. DNA used PCR amplification was modulated in 50 ng μL^{-1} .

The *FecB* and *GDF9* genes were amplified using polymerase chain reactions by specific primers: *FecB* F: 5'-CCAGAGGACAATAGCAAAGCAAA-3', R: 5'-CCAGATGTTTCATGCCTCATCAACAGGTC-3' and *GDF9* G8F: 5'-ATGGATGTTCTGCACCATGGT GTG AACCTGA-3', G8 R: 5'-CTT TAG TCA GCTGAAGTG GGA CAAC-3'.

The PCR was performed in 25 μL of reaction solution using Master kit (Sinaclone company) in a icycler (BioRAD company). The PCR mixture contained 2.5 mM of 10 \times PCR buffer, 2.5 mM MgCl_2 , 200 μM dNTPS, 3 μL mix of PRIMERS, 1U taq and 11 μL ddH $_2$ O. A total of 35 cycles were adapted for denaturation at 95°C 1 min, annealing at 61°C/30, 72°C/1 min also 95°C/1 min, 61°C/30, 72°C/1 min for *FecB* and *GDF9* genes, respectively. Restriction enzymes used for *GDF9* and *FecB* were *DdeI* and *AvaII*. The PCR products of 5 μL were digested

separately with 10U of the two aforementioned enzymes overnight at 37°C in a 20 µL reaction mixture and the resulting products were separated by 6% Acryle Amide gel and visualized by silver nitrate. The *GDF9* gene was cleaved with DdeI by banding pattern 110 and 31 bp. The mutant *FecB* gene was cleaved with AvaII by 130 and 160 band patterns.

RESULTS AND DISCUSSION

RFLP pattern of *GDF9* and *FecB* genes have indicated that all ewes were wild type and no mutations observed in aforementioned population. The fecundity gene was first found in Booroola merino strain (Davis *et al.*, 1982; Piper and Bindon, 1982). Subsequently three groups of researchers discovered sheep carrying booroola gene which has a mutation in BMPRII receptor (Mulsant *et al.*, 2001; Souza *et al.*, 2001; Wilson *et al.*, 2001). The effect of mutation on OR and LS is additive (1.65 OR per each copy of gene) and semi-dominant (1.0 and 1.5 unit increase in litter size), respectively (Piper and Bindon, 1982) (Fig. 1 and 2).

The findings of *FecB* locus in present study was corresponded to that of shal (Ghaffari *et al.*, 2007), Thoka, Lacaune, Suffolk and Dorset breeds (Davis *et al.*, 2002). *FecB* mutation has been reported in some prolific sheep breeds such as javanes, Garol (Davis *et al.*, 2002) hu and small tail Han sheep (Davis *et al.*, 2002). Kumar *et al.* (2006) in their studies on garol x malpura crosses found that mean litter size in BB and B+ ewes were significantly higher than those of the wild type ewes by 0.7 and 1.14

extra lamb, respectively. Also ewe's reproduction efficiency of BB and B+ were significantly higher than compared to ++ ewes.

Naturally occurring heterozygous mutation in *GDF9* exon2 would lead to hyperfertility phenotype (*FecGH*) in Cambridge and Belclair ewes while homozygous carriers were sterile (Hanrahan *et al.*, 2004). The results were in agreement with Ghaffari *et al.* (2007). Also Based on PCR-SSCP in small tail Han sheep Chu *et al.* (2005) indicated that the polymorphism was detected in *FecGH* had no significant effect on litter size. Akbarpour *et al.* (2008) using SSCP analysis and sequencing observed no evidence of *FecGH* existence in ghezel sheep breed.

CONCLUSION

The results of the present studies showed that there was no polymorphism both in *FecGH* and *FecB* loci. Base on these results we can proposed that next studies should focused on other loci relevant to fecundity genes and fertility in this breed.

REFERENCES

- Akbarpour, M., M. Houshmand, A. Ghorashi and H. Hayatgheybi, 2008. Screening for *FecGH* mutation of growth differentiation factor 9 gene in Iranian ghezel sheep population. *Int. J. Fertility Sterility*, 2: 139-144.
- Chu, M.X., L.H. Sang, J.Y. Wang and S.C. Ye, 2005. Study on BMP15 AND *GDF9* as candidate genes for prolificacy of small tailed han sheep. *Acta Genetica Sinica*, 32: 38-45.
- Davis, G.H., G.W. Montgomery, A.J. Allison, R.W. Kelly and M.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., S.M. Galloway, I.K. Ross, S.M. Grogan and J. Ward *et al.*, 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (*FecB*) mutation. *Biol. Reprod.*, 66: 1869-1874.
- Dong, J.W., D.F. Albertini, K. Nishimori, T.R. Kumar, N. Lu and M.M. Matzuka, 1996. Growth differentiation factor 9 is required during early ovarian folliculogenesis. *Nature*, 383: 531-535.
- Galloway, S.M., K.P. McNatty, L.M. Cambridge, M.P. Laitinen and J.L. Juengel *et al.*, 2000. Mutations in an oocyte-derived growth factor gene (*BMP15*) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nature Genet.*, 25: 279-283.



Fig. 1: PBR anlysis of 190 bp PCR products of *FecB* gene in Dalagh sheep



Fig. 2: PBR anlysis of 130 bp PCR products of *GDF9* gene in Dalagh sheep

- Ghaffari, M., A. Nejati-Javaremi and G. Rahimi, 2007. Detection of Polymorphism in Oocyte Derived Growth Factor (GDF-9) gene associated with twinning in shal sheep breed. Proceedings of the 5th National Biotechnology Congress of Iran, October 27-30, 2007, Tehran, Iran, pp: 475.
- Hanrahan, J.P., S.M. Gregan, 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.
- Javanmard, A., N. Azadzadeh and A. Esmailizadeh, 2011. Mutations in bone morphogenetic protein 15 and growth differentiation factor 9 genes are associated with increased litter size in fat-tailed sheep breeds. *Vet. Res. Commun.*, 35: 157-167.
- Juengel, J.L., K.J. Bodensteiner, D.A. Heath, N.L. Hudson and C.L. Moeller *et al.*, 2004. Physiology of *gdf9* and *bmp15* signalling molecules. *Anim. Reprod. Sci.*, 82-83: 447-460.
- Kumar, S., A.P. Kolte, A.K. Mishra, A.L. Arora and V.K. Singh, 2006. Identification of the *FecB* mutation in Garole x Malpura sheep and its effect on litter size. *Small Rumin. Res.*, 64: 305-310.
- Laitinen, M., K. Vuojolainen, R. Jaatinen, I. Ketola and J. Aaltonen *et al.*, 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.
- McNatty, K.P., L.G. Moore, N.L. Hudson, L.D. Quirke and S.B. Lawrence *et al.*, 2004. The oocyte and its role in regulating ovulation rate: A new paradigm in reproductive biology. *Reproduction*, 128: 379-386.
- Mulsant, P., F. Lecerf, S. Fabre, L. Schibler and P. Monget *et al.*, 2001. Mutation in bone morphogenetic protein receptor-1B is associated with increased ovulation rate in Booroola Merino ewes. *Proc. Nat. Acad. Sci. USA.*, 98: 5104-5109.
- Piper, L.R. and B.M. Bindon, 1982. Genetic segregation for fecundity in booroola merino sheep. Proceedings of the World Congress on Sheep and Beef Cattle Breeding, (SBCB'1982), The Dunmore Press Ltd. New Zealand, pp: 395-400.
- Souza, C.J., C. MacDougall, C. MacDougall, B.K. Campbell, A.S. McNeilly and D.T. Baird, 2001. The Booroola (*FecB*) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (*BMPR 1B*) gene. *J. Endocrinol.*, 169: R1-R6.
- Tavakkolian, J., 2000. An Introduction to Genetic Resources of Native Farm Animals in Iran. Animal Science Research Institute, Iran.
- Wilson, T., X.Y. Wu, J.L. Juengel, I.K. Ross and J.M. Lumsden *et al.*, 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein 1b receptor (alk-6) that is expressed in both oocytes and granulosa cells. *Biol. Reprod.*, 64: 1225-1235.