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

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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 genotype for the GDF9 ligand (FecGH) and fecB (BMPRIB) 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 monomorph.

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 TGFβ surerfamily 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 TGFβ super family is secreted by oocytes in growing ovarian follicles (Laitinen *et al*., 1998) and is a potent stimulator of granullosa cells proliferation and differentiation. It is critical for progression of the earliest stages of folliculgensis 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 BMPRIB receptor. It has been mapped to chromosome six in sheep (Souza *et al*., 2001; Wilson *et al*., 2001). BMPIB 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 a 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'-CCAGAT GTTTTCATGCCTCATCAACAGGTC-3' and GDF9 G8 F: 5'-ATGGATGTTCTGCACCATGGT GTG AACCTGA-3', G8 R: 5'-CTT TAG TCA GCTGAAGTG GGA CAAC-3'.

The PCR was preformed 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×PCR buffer, 2.5 mM $MgCl_2$, 200 μm dNTPS, 3 μL mix of PRIMERS, 1U taq and 11 μL ddH_2O. 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

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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 BMPRIB 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, Safolk 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.

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

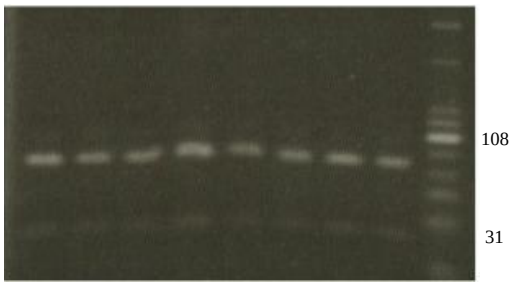

Fig. 2: PBR anlysis of 130 bp PCR products of *GDF9* gene in Dalagh sheep

Naturally occurring heterozygous mutation in GDF 9 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 existance 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.

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