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Genetic Polymorphism FecB and BMP15 Genes and Its Association with Litter Size 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. This study was carried out to evaluate the genetic polymorphisms in BMP15 and FecB in Iranian Sangsari sheep breed. Blood samples were taken from 150 Sangsari sheep (140 ewes, 10 rams) from sheep in Damghan genetic modification center and the genomic DNA was extracted using salting out method. 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. Using 2 pairs of specific primers, 2 DNA fragments were amplified from exon 2 of BMP15 (141 bp) and FecB (190 bp) genes. The resulted PCR products were digested using HinfI and AvaII restriction enzymes for BMP15 and FecB genes, respectively. Genotypes of each individual were detected by agarose gel electrophoresis. Restriction digested of PCR products for BMP15 locus with Hinf I enzyme showed a C to T transition. BMP15 and FecB luci were not polymorphic. Further studied required to evaluate the relationship of different genotypes with litter size and ovulation rate.

Key words: PCR, polymorphism, BMP15, FecB, sangsari sheep, genetic polymorphism

INTRODUCTION

Genetic variation in ovulation rate in sheep has been widely documented and the evidence shows substantial differences among breeds and in a number of cases exceptional variation within breeds/strains. Ovulation rate is determined by a complex exchange of endocrine signals between the pituitary gland and the ovary. Three related oocyte-derived members of the Transforming Growth Factor-β (TGF-β) superfamily, namely Growth Differentiation Factor 9 (GDF-9), 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 2 growth factor genes (BMP15 and GDF9 and a related receptor (ALK6) that are expressed in oocytes. Five different Single Nucleotide Polymorphisms (SNP) have been identified in the BMP15 gene (Galloway *et al*., 2000; Hanrahan *et al*., 2004; Bodin *et al*., 2003; Liu *et al*., 2003), 8 SNP_s in GDF9 (Hanrahan *et al*., 2004) and one SNP in ALK6 namely FecB (Wilson *et al*., 2001; Mulsant *et al*., 2001; Souza *et al*., 2001; Davis *et al*., 2002, 2001). In sheep animals heterozygous for these mutaions or heterozygous for 2 of these mutations or homozygouse 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 (Hanrahan *et al*., 2004). Animals homozygouse for BMP15 or GDF9 mutations are sterile due to arrested follicular development from the primary stage of growth. 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 GDF9

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and BMP15 are essential for ovarian follicular development and normal ovulation and/or corpus luteum formation in sheep. Moreover, it is evident that GDF9 (Mc Pherone and Lee, 1993; McGrath *et al*., 1995; Dong *et al*., 1996; Laitinen *et al*., 1998; Hayashi *et al*., 1999; Hsueh *et al*., 2000; Vitt *et al*., 2000; Juengel *et al*., 2004; Hanrahan *et al*., 2004; Sadighi *et al*., 1998), BMP15 (Galloway *et al*., 2000; Hanrahan *et al*., 2004; Bodin *et al*., 2003) and FecB (Wilson *et al*., 2001; Mulsant *et al*., 2001; Souza *et al*., 2001; Davis *et al*., 2002, 2001; Wang *et al*., 2005) have major effects in regulating ovulation rate. 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, 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 (BMP15; also known as GDF9B). Four different alleles of BMP15 (FecXI , $FecX^{H}$, $FecX^{G}$, $FecX^{B}$) all causing the same phenotype have been identified in Romney, Belclare and Cambridge sheep (Galloway *et al*., 2000; Hanrahan *et al*., 2004). 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 (Galloway *et al*., 1996). The inverdale gene was mapped to a 10 cM region at the centre of the sheep X-chromosome (Galloway *et al*., 1996). FecB gene: The Booroola Merino strain carries a single autosomal locus named Fecundity Booroola (FecB) (Piper and Bindon, 1982). Bone Morphogenetic Protein 1B (BMP-IB) receptor (also known as ALK-6), which binds to members of the Transforming Growth Factor-β_(TGF-β) superfamily, is located in the region containing the FecB locus (Lanneluc *et al*., 1994). Booroola sheep have a mutation (Q249R) in the highly conserved intracellular kinase signaling domain of the BMP-IB receptor. The effect on litter size is semi-dominant because embryonic losses cause partial failure of multiple pregnancies (Piper *et al*., 1985). Linkage to the FecB locus was first detected with an anonymous microsatellite marker also linked to Secreted Phosphor-Protein 1 (SPP1) (Montgomery *et al*., 1993). The critical region for the FecB locus lies between the genes for SPP1 and alcohol dehydrogenase. The most striking physiological effects of the FecB locus are on ovulation rate and the size and number of ovulatory follicles in the ovary. Follicles mature and ovulate at significantly smaller diameters in homozygous (BB) and heterozygous (B+) carrier ewes compared with non-carrier or wild-type (++) ewes (Montgomery *et al*., 1993; Baird and Campbell, 1998). The increased number of ovulatory follicles offsets the reduced number of granulosa cells in individual follicles. Consequently, both the total number of granulosa cells from all ovulatory follicles and total oestradiol production from the ovaries of B+/ BB ewes are similar to those of ++ ewes (Montgomery *et al*., 1993; Souza *et al*., 2001).

MATERIALS AND METHODS

A total of 3445 phenotypic records (representative 350 ewes and 50 rams) collected between 1994 and 2007 in Sangsari breeding centre were used. DNA extraction: Venous jugular blood samples (5 mL ewe^{-1}) were taken from 150 Sangsari sheep (140 ewes, 10 rams) lambed in 2007. Genomic DNA was extracted using soulting out method (Miller *et al*., 1988). Genomic DNA was dissolved in TE buffer and kept at -20°C.

PCR: In a total volume of 25 μL which template PCR reaction contained: 2.5 μL PCR buffer 10-X, 2.5 μL Mgcl2,10 pm of each primers, 0.2 μL Taq DNA polymerase, 0.2 μL dNTPs and 0.8 μL template DNA. BMP15: The amplification was carried out using 35 cycles at 94°C for 5 min, 94°C for 45 sec, 62°C for 40 sec and 72°C for 45 sec, followed by 72°C for 5 min. FecB: The amplification was carried out using 35 cycles at 94°C for 5 min, 94°C for 15 sec, 60°C for 30 sec and 70°C for 30 sec followed by 72°C for 5 min and 99°C for 15 min. Digestion: Restriction enzyme used for BMP15 is Hinf I and FecB is AvaII. Digestion reaction contain 5 μL of PCR product, 5 U appropriate enzyme, 2 μL buffer 10× in 20 μL final volume incubated for 3-6 h at 37°C.

Primers		
BMP15	B2-F:	CAC TGT CTT CTT GTT ACT GTA TTT CAA GAG AC
	B2-R:	GAT GCA ATA CTG CCT GCT TG
FecB	TestF2:	CCA GAG GAC AAT AGC AAA GCA AA
	TestR15:	CAA GAT GTT TTC ATG CCT CAT CAA CAC GGT C

Statistical analysis: 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 (Henderson, 1987).

$$y_i = X_i b_i + Z_i a_i + W_i p_e + e_i$$

where:

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

RESULTS AND DISCUSSION

A DNA fragment with the size of 141 bp was amplifid from exon 2 of BMP15 (Fig. 1) and 190 bp from FecB (Fig. 2) genes succefully.

The resulted PCR products were digested with Hinf I for BMP15 and AvaII for FecB luci and genotypes of each individual were detected by electrophoresis. Restriction digested of PCR products with for BMP15 with HinfI restriction enzyme showed a mutation where, the c nucleotide has changed to T at this locus (C-T). The wild type allele of this gene (B+) with one restriction site resulted DNA fragments with 30 and 111 were not ditected for BMP15 hemozygote and hetrozygote shapes (Fig. 3). But restriction digested of PCR products with for FecB with AvaII restriction enzyme was not showed a mutation where, the A nucleotide has changed to G at this locus. And result with digestion was only one fragment 190 bp. BMP15 and FecB luci were monomorphin studied individuals (Fig. 3 and 4).

BMP15: Result digest in Sangsari sheep showed only fargments 111, 30 bp. BMP15 lucus was monomorph in our studied which was not same result obtain by Hanrahan *et al*. (2004), Chue *et al*. (2007), Guan *et al*. (2006) and Davis *et al*. (2006).

FecB: The FecB mutation is present in Booroola Merino (Australia; Mulsant *et al*., 2001; Souza *et al*., 2001; Wilson *et al*., 2001), Garole (India; Davis *et al*., 2002), Javanese (Indonesia; Davis *et al*., 2002), Small Tailed Han (Yan *et al*., 2005; Wang *et al*., 2003a; Jia *et al*., 2005; Davis *et al*., 2006) and Hu (China) sheep (Wang *et al*., 2003b; Yan *et al*., 2005; Davis *et al*., 2006; Guan *et al*., 2006; Chu *et al*., 2004, 2007). But this study showed that the FecB mutation is not present in Sangsari sheep (Iran). The results found in the present research are In accordance with those obtained for Madras Red,

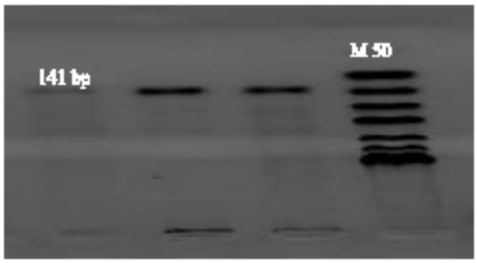

Fig. 1: PCR product BMP15 gene. Fragment of size marcer:501,489-404-331-242-190-147-111,110-67-34

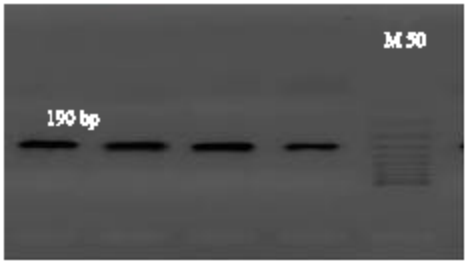

Fig. 2: PCR productFecB gene. Fragment of size marcer: 500-450-400-350-300-250-200-150-100-50

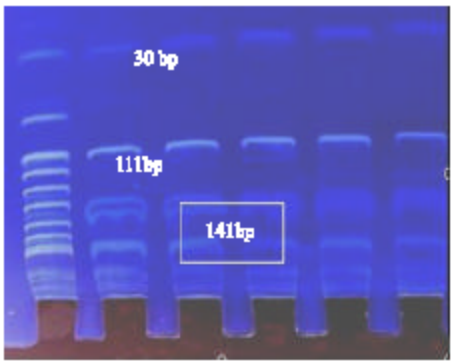

Fig. 3: Digest product BMP15 gene. Fragment of size marcer:501,489-404-331-242-190-147-111,110-67-34

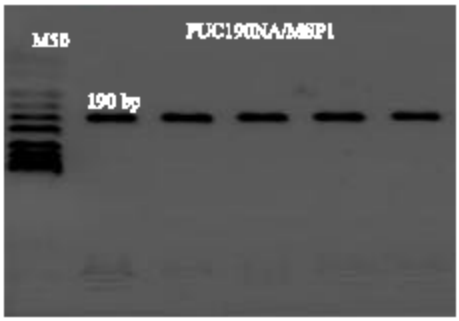

Fig. 4: Digest product FecB gene. Fragment of size marcer: 500-450-400-350-300-250-200-150-100-50

Deccani and Bunnur breeds (Davis *et al*., 2006, 2002; Wilson *et al*., 2001; Baird and Compbell, 1998; Gootwine, 1986). Research in India has shown that Garol breed was the only breed carrying FecB allele (India; Davis *et al*., 2002, 2005).

In fact, due to a long geographic interval between rearing environments for these three breeds, the mutant allele might be spontaneous occurred and subsequently has been imported to Bunnur and Deccani crossbreds following executing breeding programs (Davis *et al*., 2006, 2002; McPherone and Lee, 1993; McGrath *et al*., 1995; Wang *et al*., 2003b, 2005). Geographically, Sangsari natural breeding environment is located between Mazandaran and Isfahan provinces indicating that this allele could not be imported to the local breeds through commercial routs. In addition, this breed is actually of high level of resistance against harsh environment and diseases leading to importance failure of the mutant allele into Sangsari flocks over the time.

The mutant allele (FecB) was not found in Tooka, Woodlens, Olcusa, lacan, Belklir and Cambridge breeds reared in 8 countries (New Zealand, India, Philippine, Indonesia, Poland, Island, France and Ireland) while, the gene was reported to be available for Garol (Bangladesh) and Javanez (in Indonesia) breeds (Indonesia; Davis *et al*., 2002).

A number of researchers believe that Javanez sheep was originated from a region located between India and Bangladesh while some other researchers believe that Javanez sheep received the Booroola gene from Australian merinos rams in 1860's at that time they were used to improve local breeds for meat and wool purposes.

The fact is that no association was really found between those breeds suggesting that other major gene(s) effects except of FecB gene may be responsible for high prolificacy of those groups of sheep breeds (Davis *et al*., 2006; 2002; Mulsant *et al*., 2001; Liao *et al*., 2004; Wilkie *et al*., 1999; Vitt *et al*., 2000; Rohrer, 1999; Piper *et al*., 1985; Gllowary *et al*., 1996).

A study undertaken on 21 sheep breeds of high prolificacy in 13 different countries detected FecB gene in HU and Han breeds of China indicating that the mutant gene was stabilized in the Garol and HU but segregated in Javanez, Booroola merinos and Han breeds (Davis *et al*., 2006). Recent findings confirm previous theories regarding the mutant gene transmission from the Garol to Australian (Booroola) and Indonesian (Javanez) sheep populations (Davis *et al*., 2002, 2006).

Garol (from India) and Hu (from China) sheep breeds have been stocked in the way of commercial silk worm through which the mutant allele may be transferred by transit merchants (Davis *et al*., 2002, 2006; Wang *et al*., 2003a; Yan *et al*., 2005; Jia *et al*., 2005; Guan *et al*., 2006; Juengel *et al*., 2004; Laitinen *et al*., 1998; Hsueh *et al*., 2000; Hayashi *et al*., 1999; Dong *et al*., 1996). Although Iran has also been along the way of the silk worm, there was, in fact, little chance to receive the FecB allele due to the fact that Sangsari breed rearing environment has been much more far away from that way. Furthermore, at phenotypic level there is no resemblance between Sangsari and mutant-carrier breeds suggesting that no identical descend could be available for these breeds. Hu and Garol breeds may be possibly derived from the same descend.

This mutant gene has been transmitted to the other breeds via crossbreeding in a number of countries. Awassi breed, for example, is of low are of lambing to which the Booroola gene has been transmitted and fixed since 1986 (Davis *et al*., 2002, 2006; Wang *et al*., 2003a; Yan *et al*., 2005; Jia *et al*., 2005; Guan *et al*., 2006). The results of the experiments indicated that fertility rate of this breed increased from 1.2-2 lambs per lambing without significant decrease of milk production (Baird and Compbell, 1998). 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.

There is a report indicating that ovulation rate in Javanez breed carrying FecB is half of that of Merinos breed due to inappropriate environmental conditions such as low quality of feeds (Davis *et al*., 2002, 2006; Wilson *et al*., 2001; Sauza *et al*., 2001; Mulsant *et al*., 2001). 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*., 2006; Davis *et al*., 2002; Suuza *et al*., 2001; Mulsant *et al*., 2001).

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 rate 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 trait 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.

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