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 AvII 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 HinfI enzyme showed a C to T transition. BMP15 and FecB luci were not polymorphic. Further studies 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; Hanahan et al., 2004; Bodin et al., 2003; Liu et al., 2003), 8 SNP in GDF9 (Hanahan 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 mutations or homozygous for 2 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 (FecX5) and B4 (FecX6) and from GDF9 mutations and only G8 (FecG8) had high ovulation rate and fertility (Hanahan et al., 2004). Animals homozygous 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
and BMP15 are essential for ovarian follicular development and normal ovulation and/or corpus luteum formation in sheep. Moreover, it is evident that GDF9 (McPherrone and Lee, 1993; McOath et al., 1995; Dong et al., 1996; Lattinen et al., 1998; Hayashi et al., 1999; Hsu 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. Discoverered 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 (FecX1, FecX2, FecX3, FecX4) all causing the same phenotype have been identified in Romney, Beelacare 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 Bondon, 1982). Bone Morphogenetic Protein 1B (BMP-1B) 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 (Lannecu et al., 1994). Booroola sheep have a mutation (Q249R) in the highly conserved intracellular kinase signaling domain of the BMP-1B 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 homoygous (BB) and heterozygous (Bb) 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 Bb 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 Sargsari breeding centre were used. DNA extraction: Venous jugular blood samples (5 mL ewe−1) were taken from 150 Sargsari sheep (140 ewes, 10 rams) lamed in 2007. Genomic DNA was extracted using soultin 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 10X, 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 HinI and FecB is AvaII. Digestion reaction contain 5 μL of PCR product, 5 U appropriate enzyme, 2 μL buffer 10X in 20 μL final volume incubated for 3-6 h at 37°C.

<table>
<thead>
<tr>
<th>Primers</th>
<th>BMP15</th>
<th>B2-F:</th>
<th>CAG TGT CTT GTF ACT GTA TIT CAA GAG AAC</th>
<th>B2-R:</th>
<th>CAA CAT GTC CCT GCT GCT TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>FecB</td>
<td>TestF2:</td>
<td>CCA GAG GAC AAT AGC AAA GCA AA</td>
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<tr>
<td>TestR15</td>
<td>CAA GAT GTC ATT ATG CCT CAT CAA CAC GTC</td>
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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).
\[ y_i = \beta_0 + \beta_1 x_i + \epsilon_i \]

where:
- \( y_i \) = A vector of observations for the \( i \)th trait
- \( \beta_0 \) = A vector of fixed factors for the \( i \)th trait
- \( \beta_1 \) = A vector of random additive genetic effect of sheep for the \( i \)th trait
- \( \epsilon_i \) = A vector of random residual effect for the \( i \)th trait
- \( X_i, Z, \) 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 amplified from exon 2 of BMP15 (Fig. 1) and 190 bp from FecB (Fig. 2) genes successfully.

The resulted PCR products were digested with Hinfl for BMP15 and AvaI for FecB luci and genotypes of each individual were detected by electrophoresis. Restriction digested of PCR products with for BMP15 with Hinfl 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 detected for BMP15 homozygote and heterozygote genotypes (Fig. 3). But restriction digested of PCR products with for FecB with AvaI 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 monomorphic studied individuals (Fig. 3 and 4).

**BMP15:** Result digest in Sangarei sheep showed only fragments 111, 30 bp. BMP15 locus was monomorphic in our studied which was not same result obtain by Hanrahan et al. (2004), Chae 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 Ha (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 Sangarei sheep (Iran). The results found in the present research are in accordance with those obtained for Madras Red,
Deccani and Bunur breeds (Davis et al., 2006, 2002; Wilson et al., 2001; Baird and Campbell, 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 Bunur and Deccani crossbreds following executing breeding programs (Davis et al., 2006, 2002; McPherson 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 routes. 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 Tocka, Woodlens, Oolusa, Iacan, Belkhir 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 high prolificacy of these 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; Hsu 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 Campbell, 1998). On the other hand, twinning 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; Sazua 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; Sazua 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 routes 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 twinning 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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