Slovak J. Anim. Sci., 45, 2012 (2): 63-66

ISSN 1337-9984

Short communication

DETECTION OF POLYMORPHISM IN BOOROOLA GENE (*FecB*) AND ITS ASSOCIATION WITH LITTER SIZE IN ZEL SHEEP BREED IN IRAN

R. ASADPOUR[1]*, R. JAFARI-JOOZANI[1], S. ALIJANI[2], H. MAHMODI[1]

[1]Department of Clinical Science, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
[2]Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

ABSTRACT

Sixty eight adult ewes of Zel sheep breed were screened with forced PCR RFLP method for the detection of the *FecB* gene and its effects on litter size. Genomic DNA was extracted from the blood of 68 Zel matured ewes with litter size varied from 1 to 2 lambs per ewe lambing. Digestion of the *FecB* gene (190 base pairs) with *AvaII* restriction enzyme resulted in none carrier 190 bp band wild type in 67 samples, which revealed the presence of this mutation in the tested Zel sheep breeds. Results showed that the polymorphism frequencies of *FecB* gene were significantly imbalanced in this breed. Zel sheep breed had two different Booroola genotypes (B+ and ++). The *FecB* mutation was found in one sample, whereas the sample of 67 Zel sheep included two genotypes. In the Zel sheep, the genotype frequencies of BB, B+ and ++ were 0 %, 1.47 % and 98.53 % respectively. Regarding the litter size trait within the different genotype of Zel sheep it was shown that the mean litter size of ewes with the genotype B+ were 2, whereas ewes with the genotype ++ had lower mean (1.73) with one and two litter size. The results of the present study revealed polymorphism of the *FecB* gene in Zel breed.

Key words: Zel sheep; *FecB* gene; litter size; PCR-RFLP

INTRODUCTION

Indigenous farm animal breeds are valuable gene pools for adaptive and economic traits, providing diversified genetic pool, which can help meeting future challenges. The earliest evidence about sheep domestication seems to be found in the areas of Iraq, Iran and the Taurus mountains of Turkey. There are approximately 2.5 million animals of Zel sheep breed. This breed is sexually active all year round with an optimal estrous activity in summer and autumn and is generally single ovulator. Low efficiency is common in all the sheep production systems in Iran mainly due to the low reproductive performance of the ewe (Esmailizadeh *et al.*, 2009). It has been discussed that ovulation rate and the subsequent litter size are the main factors for improving reproductive rate in sheep (Bradford ,1972). Many studies have indicated that the ovulation rate and litter size can be genetically regulated by a set of different genes, collectively named as fecundity (Fec) genes (Davis *et al.*, 1982). The bone morphogenetic protein receptor type IB (BMPRIB) or activin-like kinase 6 or *FecB* on chromosome 6 (Souza *et al*., 2001), identified in the Australian Booroola Merino strain (Piper *et al.*, 1985), is the first major gene to be described that affects ovulation rate and prolificacy in sheep.

Molecular genetics can overcome these limitation offering new opportunities to the improvement of

***Correspondence:** E-mail: r_asadpour@tabrizu.ac.ir
R. Asadpour, Department of Clinical Science, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
Tel.: ++984113392353 Fax: +984113357834

Received: January 28, 2012
Accepted: April 19, 2012

reproductive traits, as it supplies tools to analyse genetic variability directly at the DNA level with the possibility of detecting the individual genes influencing the reproductive characteristics. The identification of polymorphism and DNA markers associated with reproductive traits can lead to genetic improvement through the implementation of marker assisted selection (MAS) by breeder to increase litter size and reproduction efficiency. A previous study of eight prolific breeds (Thoka, Woodlands, Olkuska, Garole, Javanese, Lacaune, Belclare and Cambridge) revealed that *FecB* was present in the Garole sheep of India and the Javanese sheep of Indonesia (Davis *et al.*, 2002). Due to the importance of fingerprinting of economic genes in the Iraninan local sheep breeds, the present study was undertaken to find out the polymorphism of the FecB gene in Iranian Zel sheep breed.

MATERIAL AND METHODS

Animals and sampling

The present study was conducted on a total of 68 animals belonging to Zel Sheep breed. Zel sheep were selected in this study based on previous history of at least two lambing records of multiple births. Approximately 10 ml venous blood collected from each animal was placed into 0.5 ml of 2.7 % EDTA (used as an anticoagulant), and transferred to the laboratory freezer (-20 °C).

DNA isolation

Genomic DNA from blood samples was extracted according to Chomezynski extraction method. DNA concentration was measured at 260 and 280 nm using Biophotometer plus (Eppendorf, Germany). Electrophoresis of each DNA sample on 2 % agarose gel in 1x TBE buffer was undertaken to check the integrity of DNA. An aliquot of total DNA was isolated from each sample and stored at -20 °C until analysis.

Primer synthesis and PCR-RFLP reactions

Primers were synthesized by Bioneer Co. Ltd basing on the sequences described by Davis *et al.* (2002). The primer TestR15 has been engineered to introduce a point mutation so that PCR products from the BMPR-1B gene with the Booroola mutation contained an *AvaII* (Fermentas. Ltd, China) restriction site (G|GACC) whereas products from non-carriers of the mutation lacked this site. Genomic DNA (50-100 ng) was used in a 20 µL of reaction volume. The primers were designed as follows: CCA GAG GAC AAT AGC AAA GCA AA, TestF2, and CAA GAT GTT TTC ATG CCT CAT CAA CAC GGT C (TestR15). The amplification was carried out using 35 cycles at 94 ºC for 15 s, 60 ºC for 30 s and 72 ºC for 30 s, followed by 72 ºC for 5 min and 99 ºC for 15 min. The 190 bp product was then digested using *AvaII*. The resulting products were separated by electrophoresis on a 3.5 % agarose gel and visualised with ethidium bromide. The size of the alleles was determined basing on a DNA molecular weight marker (Bioneer, 25/100 bp Mixed DNA ladder).

Genotyping

The genotype at the *FecB* locus was validated by the PCR-RFLP method (Davis *et al.,* 2002). The forced PCR of the *FecB* gene produced a 190 base pair (bp) band. After digestion with *AvaII* (Fermentas), the *FecB* gene homozygous carriers had a 160 bp band (BB), the non carrier had a 190 bp band (++), whereas heterozygotes had both 160 and 190 bp bands (B+).

Statistical analysis

Genotype and allele frequencies were calculated using the computer software package Pop Gene version 1.3 (Yeh *et al.* 1997). None of the individuals carried homozygous genotype for the *FecB* gene in this breeds but there was only one ewe with (-/+) genotype and 67 ewes with (+/+) genotypes in *FecB* loci. These genotype classes were removed from the dataset for final association analysis. For the studying environmental effects (Parity and age of lambing) on litter size trait Fisher exact test was applied (SAS Institute, 1999).

RESULTS AND DISCUSSION

Results of PCR-RFLP electrophoresis

Results of the *FecB* gene PCR products after *AvaII* digestion can be seen on Fig.1. Thus this PCR-RFLP technique can be used to detect and genotype *FecB* gene clearly. It is clear from the *AvaII* RFLP pattern represented on the figure 1, that digestion of the FecB gene 190 base pair with *Ava II* restriction enzyme resulted in non carrier (++) 190 bp band (wild type) in sixty seven the animals belonging to the Zel sheep breed . Only one sheep had the genotype *FecBB/FecB+*. Illegible or inconclusive results were repeated until the genotyping was clear, or they were excluded from the data analysis.

Allelic and Genotypic Frequencies

Allelic and genotypic frequencies of the *FecB* mutation of the BMPR-IB gene are presented in Table 1. A total of 68 individuals from Zel breed the forced PCR-RFLP approach. The results (Table 1) showed that the frequency of polymorphism distributions of the *FecB* gene was most imbalanced in this breed. Zel sheep breed had two different Booroola genotypes (*B+* and ++). The *FecB* genotypes in the Zel sheep included *FecBB/ FecBB* (n = 0), B+ or *FecBB/ FecB+* (n = 1) and ++ or *Fec B+/ FecB+* (n = 67), and their genotype frequencies

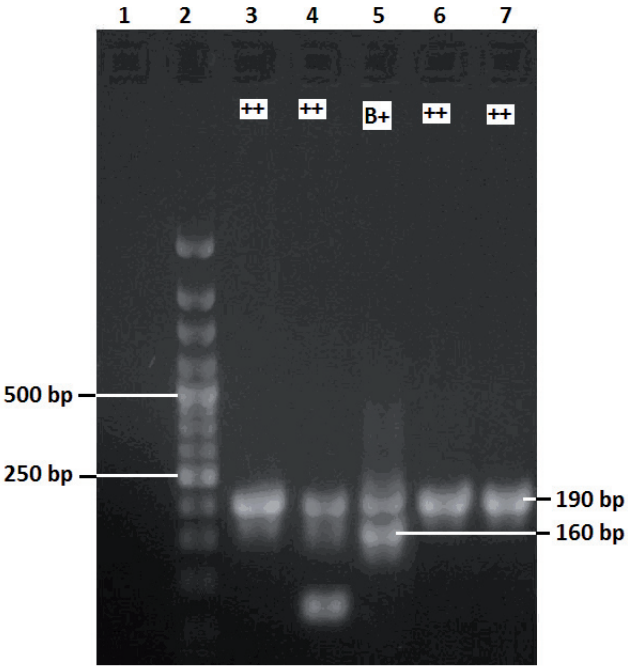

Fig. 1: Image of PCR product of the *FecB* mutation of the BMPR-IB gene digested with Ava II. The wild-type allele (++) is 190 bp, and the mutant allele (B) is 160 bp.; Lane 1: Non Template Control (NTC) Lane 2: DNA molecular weight marker (Bioneer, 25/100 bp Mixed DNA ladder); lanes 3, 4, 6 & 7: ++ genotype (Amplification of wild-type); Lane 5 = B+ genotype (heterozygote)

Table 1: Allelic and Genotype frequencies of the *FecB* mutation of the BMPR-IB gene

Genotype	BB	B+	++
Number	0	1	67
Allelic Frequencies (%)	-	0.74	99.26
Genotype frequencies (%)	0	1.47	98.53

Table 2: Comparison of litter size among three genotypes in Zel breed

Genotype	BB	B+	++
Number	0	1	67
Number of sample	0	1	67
Mean litter size	0	2	1.73

were 1.47 % for *FecBB/ FecB*+ and 98.53 % for *FecB*+/*FecB*+. None of the individuals carried homozygous genotype for the Fec B gene in this breeds.

Litter-size distribution

In this context "litter size" is defined as a number of lambs born per ewe lambing. Within this genotype the average litter size of ewes with the BB genotype was 2, whereas ewes with the genotype ++ had lower mean (1.73) with one and two litter size (Table 2). The ewes with the B+ genotype also produced more lambs than ewes with the ++ genotype, although the difference was not statistically significant.

RFLP is a rapid, simple and exact technique for single nucleotide polymorphism (SNP) genotyping. After a forced restriction site was introduced into one of the primers, the PCR product contained a certain restriction enzyme site. This forced PCR-RFLP approach has been used previously to genotype prolific sheep (Souza *et al*., 2001; Davis *et al*., 2002).

Our results showed that the *FecB* mutation as a 160 bp band (Fig. 1; Line 5) may be present in Zel sheep (Iran). This might be due to breeding season, age, nutritional status, management flock and genotype. But the *FecB* mutation has not been fixed in Zel sheep. Also it seems highly unlikely that the gene could become fixed in a population in the absence of ovulation rate records and DNA marker tests. In addition, the important of the mutant allele from exotic breeds to the Zel breed is unexpected due to closed environment and inaccessible commercial routs to the region of the breed rearing. Similar results were also reported by Kasiriyan *et al.* (2009) who found that digestion of the *FecB* gene 190 bp with *AvaII* restrictin enzyme resulted in non carrier wild type in all tested individuals from Iranian Lori- Bakhtiari, Shal and Sangsari sheep breeds, respectively. On the other hand, the *FecB* mutations have been reported in some of the world's most prolific sheep breeds such as Australian Booroola Merino (Souza *et al.*, 2001), Indian Garole (Davis *et al*., 2002), Indonesian Javanese (Davis *et al*., 2002), Small-tailed Han , Hu sheep of China (Davis *et al.*, 2006) as well as the Booroola Merino, but appears to be absent in prolific European breeds (Davis *et al*. 2002, 2006).

It has been reported that the litter size and ovulation rate in sheep increase with number of mutations in BMPR1B gene (Fabre *et al.*, 2006). The presence of non carrier 190 bp band pattern (wild type) in most of the animals belonging to the Zel breed studied could be explained on the basis of low litter size in these breeds, since the presence of ++ wild type is significantly correlated with low litter size. Our result showed that there were positive relationships between mutation of the *FecB* gene and litter size in Zel sheep breed, where the average litter sizes of ewes with the B+ genotype was 2, which was greater than that (1.73) of ewes with the

++ genotype. The results of this study are in agreement with the report of Farquhar *et al.* (2006), who indicated that effect of the B allele in a Romney background, prolificacy of B+ ewes was higher than that of ++ and BB ewes, suggesting an over-dominance effect of the *FecB* gene. The average effects of the *FecB* were originally summarized by Piper *et al.* (1985) as one copy of the *FecB* (B+) increased ovulation rate by +1.0 to +1.5 ova and litter size by +0.8 to +1.2 lambs born across a range of genetic and environmental background. They stated that the effect of a second copy (BB) appeared to be additive for ovulation rate and varied from additive to dominant for litter size depending on the background genotype. This increase in ovulation rate of *FecBB* carriers is associated with a precocious maturation of a large number of antral follicles that ovulate at a smaller size than non-carrier follicles (McNatty *et al.*, 1986). The difference in a litter size within the genotype B+ and ++ in our study could be due to a lower prolificacy potential from the background genotype, age, environmental factors such as the relatively low nutritional value of the tropical forages available to these ewes, or to a combination of these factors. This study suggests that other major gene(s) effect except of the *FecB* gene may be responsible for high prolificacy Zel sheep breed. The effect of a major gene on mean litter size is likely to be influenced by breed, environmental conditions, maternal nutrition and other factors. This study showed that the *FecB* mutation might be present in Zel breed. On the other hand, due to small size of the samples analyzed in this study, there is a probability that mutant allele might be present at higher frequency in the analyzed animals. Therefore, there is a need to undertake a further research on a substantially larger number of the population.

REFERENCES

BRADFORD, G. E. 1972 Genetic control of litter size in sheep. *J. Reprod. Fertil*. Suppl ., vol. 15, 1972, p. 23-41

DAVIS, G. H. – GALLOWAY, S. M. – ROSS, L. K. – GREGAN, S. M. – WARD, G. – NIMBKAR, B. V. – GHALSASI, P. M. 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (*FecB*) mutation. *Biology of Reproduction*, vol. 66, 2002, p. 1869-1874.

DAVIS, G. H. – BLKRISHNAN, L. – ROSS, I. K.- WILSON, T. – GALLOWAYl, S. M. LUMSDEN, B. M. – HANRAHAN, J. P. – MULLEN, M. – MAO, X. Z. – WANG, G. L. – ZHAO, Z. S. – ZENG, Y. Q. – ROBINSON, J. J. – MAVROGENIS, A. P. – PAPACHRISTOFOROU, C. – PETER, C. – BAUMUNG, R. – CARDYN, BAUJENANE, I. – COCKETT, N. E. – EYTHORSDOTTIR, E. – ARRANZ, J. J. – NOTTER, D. R. 2006. Investigation of the Booroola (*Fec B*) and Inverdale (*Fec X I*) muatation in 21 prolific breeds and strains of sheep samples in 13 countries. *Anim Reprod Sci.*, vol. 92, 2006, p. 87-96.

DAVIS, G. H. – MONTGOMERY, G. W. – ALLISON, A. J. – KELLYl, R. W. – BRAY A. R. 1982. Segregation of major geneinfluencing fecundity in progeny of Booroola sheep. *New Zealand Journal of Agricultural Research.*, vol. 25, 1982, p. 525–529.

ESMAILZADEH, A. K – DAYANI, O. – MOKHTARI M. S. 2009. Lambing season and fertility of fat-tailed ewes under extensive production system are associated with live weight and body condition around mating. *Anim. Prod. Sci.*, 49, 2009, p. 1086–1092

FABRE, S. – PIERRE, A. – MULSANT, P. – BODIN, L. – DIPASQUALEIP, E. – PERSANI, L. – MONGET, P. – MONNIAUX, D. 2006. Regulation of ovulation rate in mammals: contribution of sheep genetic models. *Reproductive Biology and Endocrinology*, vol. 4, 2006, p. 20.

FARQUHAR, P. A.– DODDS, K. G.– DAVIS, G. H. 2006. Introgression of the Booroola mutation (*FecB*) leads to hyper-prolificacy in a Romney sheep flock. *8th World Congress on Genetics Applied to Livestock Production*, Belo Horizonte, MG, Brazil.

KASIRIYAN, M. – HAFEZEYAN, H. – SAYAHZADEH, H. – JAMISHIDI, R. – ASGHARI, S. R. – IRAJEYAN, G. H. – BUESAGH, H. 2009. Genetic polymorphism in *FecB* and BMP15 genes and its association with litter size in Sangsari sheep breed of *Iran. J. Anim. Vet. Adv.*, vol. 8, 2009, p. 1025–1031

McNATTY, K. P. – LUN, S. – HEATH, D. A. – BALL, K. – SMITH, P. – HUDSON, N. L. – McDIAMRMID, J. – GIBB, M. – HENDERSON, K. M. 1986. Differences in ovarian activity between Booroola Merino ewes which were homozygous, heterozygous and non-carriers of a major gene influencing their ovulation rate. *J. Reprod. Fertility*, vol. 77, 1986, p. 193-205.

PIPER, L. R. – BINDON, B. M. – DAVIS, G. H. 1985. The single gene inheritance of the high litter size of the Booroola Merino. In: Land, R.B., Robinson, D.W. (Eds.), *Genetics of Reproduction in Sheep. Butterworths*, London, 1985, p. 115–125.

SAS Institute (1999) *SAS Procedure Guide*. 1999. Version 8. SAS Institute, Inc., Cary, p 1643

SOUZA, C. J. – MacDOUGAL, C. – CAMPBELLB, K. – McNEILLY A.S. – BAIRED D. T. 2001. The Booroola (*FecB*) phenotype is associated with a mutation in the bone morphogenetic receptor type IB (BMPR-IB) gene. *J. Endocrinol.*, vol. 2, 2001, p.-6.

YEH, F. C. – YANG, R. C. – BOYLE T. B. J. – YE, Z. H. – MAO, J. X. 1997. Pop Gene, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada. http://www.ualberta.ca/*fyeh