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Booroola Gene (*Fec B*) Polymorphism and its Liaison with Litter Size in Indigenous Sheep Breeds of Telangana, India

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ABSTRACT

The present study was aimed to find out the presence of *Fec B* gene in Deccani and Nellore with its association to litter size. From forty ewes of each Nellore and Deccani breed, having variation in litter size, blood was collected and genomic DNA extraction was done. Animals were screened by PCR-RFLP method for the presence of *Fec B* gene. Digestion of the *Fec B* gene 190 base pair with Ava II restriction enzyme resulted in separation of non-carrier (++) 190 bp band (wild type) and heterozygous carrier (B+) 160+190bp indigenous sheep. Frequency of *Fec B* gene polymorphism distributions was different among these breeds. Among the forty Nellore sheep blood samples, there was only one ewe with (BB) homozygous (160bp) genotype, five ewes with (B/+) heterozygous (160+190bp) genotype and thirty four ewes with (+/+) homozygous (190bp) genotype in *Fec B* loci. There was only five ewes with (B/+) heterozygous (160+190bp) genotype and thirty five ewes with (+/+) homozygous (190bp) genotype in *Fec B* loci and none of the individuals carried homozygous genotype for the Fec B gene in Deccani sheep. The average litter size of ewes with the homozygous *Fec B* (BB) gene was 2.0, heterozygous *Fec B* (Bb) gene was 1.4 and 1.2, respectively in Nellore and Deccani breeds and others were 1.0 in respective genotypes of both breeds. The study revealed the presence of *Fec B* gene in indigenous sheep breeds of Telangana, India having good association with litter size in both the breeds.

Keywords: Nellore, Deccani, litter size, prolific, PCR-RFLP and *Fec B* gene

Sheep are the major small ruminants reared in arid and semi-arid tract of Telangana where Deccani and Nellore breeds are most popular (Pankaj *et al.,* 2013). In sheep, major economic trait which affects the annual meat production from a herd is litter size as higher numbers of lambs give an opportunity to produce more meat at the same time frame (Abdoli *et al.,* 2013; Roy *et al.,* 2011). This characteristic has been revealed in some sheep breeds of India too (Nimbkar *et al.,* 1998). This trait is genetically regulated by the action of single autosomal locus named fecundity gene (Davis, 2005; Tang *et al.,* 2012; Chinmoy, 2014) with a certain effect on ovulation rate, consequently higher litter size (Barzegari *et al.,* 2010; Scaramuzzi *et al.,* 2011). Three types of fecundity genes have been learnt in sheep, they are bone morphogenetic protein receptor IB (BMPRIB) also called as Booroola fecundity gene (Fec B) (Nejhad and Ahmadi, 2012; Mulsant *et al.,* 2001; Souza *et al.,* 2001; Wilson *et al.,* 2001), growth differentiation factor 9 (GDF9) also called as Fec G (Nejhad and Ahmadi, 2012; Moradband *et al.,* 2011; Hanrahan *et al.,* 2004) and bone morpho-genetic protein 15 (BMP15 or GDF9b) also called as Fec X (Barzegari *et al.,* 2010; Moradband *et al.,* 2011; Hanrahan *et al.,* 2004; Polley *et al.,* 2010; Demars *et al.,* 2013; Galloway *et al.,* 2000). Booroola allele (Fec BB) is additive for ovulation rate (Barzegari *et al.,* 2010; Scaramuzzi *et al.,* 2011) and each copy of the allele appreciates the ovulation rate by about 160% resulting into extra lambs at each lambing (Piper *et al.,* 1985; Piper and Nindon, 1996; Chu *et al.,* 2012a; Chu *et al.,* 2012b). Study on presence of *Fec B* genes in Nellore and Deccani sheep breeds, most prevalent in Telangana and Andhra Pradash, is missing. If association of such genes with multiple lamb birth is stabilized, this will nourish our breed improvement programmes in India.

In India, the Fec B mutation has been reported in dwarf Garole sheep (Davis, 2005) and its segregation has been noticed in some crossbreds developed like Nari Suvarna from Garole blood (Nimbkar *et al.,* 2003; Kumar *et al.,* 2008). Twins (62.8%) and triplets (2.3%) also have been noticed in Kendrapada sheep of Orissa, however, the extent role played by these fecundity genes are unknown. Nelllore and Deccani are non-prolific sheep breeds and usually produce single lamb in each lambing, but occasionally produces twins. Thus, if presence of such genes and its relationship with twinning or triplet events is marked, the study will open new area of research for nurturing our own indigenous breeding programs in India. The present study was undertaken to indicate presence of Booroola gene (*Fec b*) polymorphism and its relation with litter size in Deccani and Nellore sheep breeds of Southern India.

MATERIALS AND METHODS

Experimental design

A total of 80 adult ewes from two Southern Indian breeds namely Deccani (n= 40), and Nellore (n=40) have been selected which includes some of the animals with previous history of at least two lambing records of multiple births from Small Ruminant Research Station, Hayathnagar Research Farm, ICAR-Central Research Institute for Dryland Agriculture (17°27′N latitude and 78°35′E longitude and about 515 m above sea level), Hyderabad, India. Blood sample from each ewe was collected from jugular vein into 10 mL vacationer tubes containing the anticoagulant, Ethylenediaminetetra-Acetic Acid (0.5ml of 2.7% EDTA), and transferred to the laboratory freezer (-20 °C).

DNA isolation: Genomic DNA was extracted from blood samples as per phenol-chloroform extraction method. The DNA pellets were re-suspended in 50 µl of sterile distilled water and stored at -20˚C till further usage. The purity and concentration of DNA samples was appraised using UV-visible range spectrophotometer. Before PCR amplification, DNA concentration was adjusted to 50ng/µl. All DNA samples had the 260/280 OD ratios in the range of 1.8-2, indicative of high purity. Integrity of DNA was checked by electrophoresis of each DNA sample on 2 % agarose gel in 1x TBE buffer and later on visualization of band under gel documentation system.

Primer synthesis and PCR-RFLP reactions

The Booroola (BMPR-IB) gene is positioned on ovine chromosome 6 having a coding sequence of 1509 bp, which could be separated into 10 exons and coded 502 amino acids (Souza *et al.,* 2001). It was intended a pair of particular primers based on the A746G mutation of ovine BMPR-IB gene, and ultimately the amplification product size was 190 bp. Primers were synthesized by Xcelris Labs Ltd., Ahmedabad established on the sequences as described (Davis *et al.,* 2002). Genomic DNA (50-100 ng) was used in a 20 µL of reaction volume. The primer sequences were 5'- CCA GAG GAC AAT AGC AAA GCA AA -3' as forward and 5'- CAA GAT GTT TTC ATG CCT CAT CAA CAC GGT C-3' as reverse.

The polymerase chain reactions (PCRs) were distrained in a volume of 25µl, containing approximately 10×PCR buffer (50 mmol/l KCl, 10 mmol/l Tris–HCl (pH 8.0), 0.1% Triton X-100) 2.5 µl, 1.5 mmol/l MgCl2 2.5 µl, 250 µmol/l each deoxynucleoside triphosphate (dNTP) 2.5 µl, 50 ng each primer, 100 ng ovine genomic DNA, and 1.5U Taq DNA polymerase. PCR conditions maintained were: denaturation at 94 ˚C for 5 min, followed by 35 cycles of denaturation at 94˚C for 30 s, annealing at 60 ˚C for 30 s, extension at 72˚C for 30 s, with a final extension at 72˚C for 5 min. The products were kept at 4 ˚C. The PCR products were digested with *AvaII* (which contain 5 µL of PCR product, 5U suitable enzyme and 2 µL buffer 10×) in 20 µL final volume incubated for 3-6 h at 37˚C. The resultants were separated by electrophoresis on a 3% agarose gel and visualized with Ethidium bromide under gel documentation system. The size of the alleles were determined based on a DNA molecular weight marker (GeNei^{TM}, 100 bp DNA ladder).

Genotyping: The genotype at the Fec B locus was further validated by the PCR-RFLP method (Davis *et al.,* 2002). The PCR of the Fec B gene produced a 190 base pair (bp) band. After digestion with *AvaII* (Thermo Fischer), the Fec B 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

Data were expressed as % and numbers. Statistical calculations were performed with the SPSS 13 computer program (SPSS Inc., Chicago, Illinois, USA). Data were subjected to analysis of variance as per standard procedure (Wilkinson *et al.,* 1996).

RESULTS AND DISCUSSION

Results of the Fec B gene PCR products after *AvaII* digestion were displayed at Fig.1 and 2, which indicates that PCR-RFLP technique can be helpful to detect genotype Fec B gene undoubtedly. From the fig 1 and 2, it is obvious that digestion of Fec B gene 190 base pair with *Ava II* restriction enzyme resulted in non-carrier (++) 190 bp band (wild type) and heterozygous carrier (B+) 160 + 190bp animals identification (Kasiriyan *et al.,* 2009). Genotypic frequencies of Fec B gene of Nellore and Deccani sheep are exhibited at Table 1. The frequencies of polymorphic distributions of the Fec B genes were different among these breeds.

Table 1: Genotype frequencies of the Fec B mutation of the BMPR-IB gene and litter size in Nellore and Deccani breeds

Genotype	Nellore (n=40)			Deccani (n=40)		
	BB	B+	++	BB	B+	++
Genotype frequency (%)	2.5	12.5	85.0	0	12.5	87.5
Number of animals	1	5	34	0	5	35
Average litter size	2.0	1.4	1.0	—	1.2	1.0

Among 40 Deccani sheep breed blood samples, there was only five ewes with (B/+) heterozygous (160+190bp) genotype and thirty five ewes with (+/+) homozygous (190bp) genotype at Fec B loci (Fig. 1). None of the samples carried homozygous genotype for the Fec B gene in Deccani sheep. Out of 40 Nellore sheep breed blood samples, there were only one ewe with (BB) homozygous (160bp) genotype, five ewes with (B/+) heterozygous (160+190bp) genotype and thirty four ewes with (+/+) homozygous (190bp) genotype at Fec B loci (Fig. 2).

Among the various genotypes of Nellore breed, average litter size of ewes having homozygous Fec B (BB) gene was 2.0, with heterozygous Fec B (Bb) gene was 1.4 and others were 1.03 (Table 1). Similarly, the average litter size of Deccani ewes having heterozygous Fec B (Bb) gene was 1.2 and others were 1.0. A similar trend has been reported elsewhere in Zel sheep breed of Iran (Asadpour *et al*., 2012).

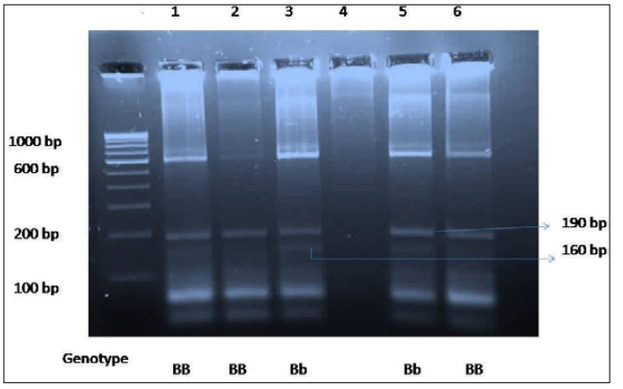

Fig. 1: Fec b gene treated with restriction enzyme Ava II in Deccani sheep

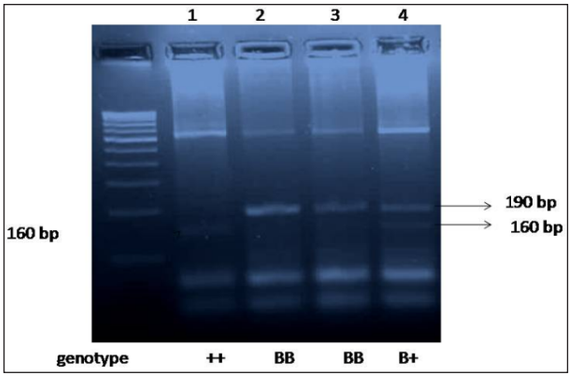

Fig. 2: Fec b gene treated with restriction enzyme Ava II in Nellore sheep

Ovulation rate and subsequently the litter size in sheep augments with the number of mutations in BMPR IB gene (Barzegari *et al.,* 2010; Moradband *et al.,* 2011; Fabre *et al.,* 2006) and the similar trend was observed in the present study also. Nellore or Deccani breed had low litter size in most of the cases due to presence of non-carrier 190bp band pattern (wild type). The disparity in litter size among the breeds and animals within breeds with heterozygous carrier (B+) Fec B gene may be attributed to the age, nutrition status of animals and environmental factors or combination of few or all of these factors and also non-exhibition of Fec B in carriers (Davis *et al.,*

2002; Mishraa *et al.,* 2009). The study demonstrated the presence of Fec B gene segregation in some of the blood samples of sheep which may be responsible for higher litter size. Hence, exhaustive study is indispensable to examine the segregation of Fec B gene in local breeds and identification of animals with homozygous Fec B (BB) gene for escalating the prolificacy in sheep through molecular breeding programs.

CONCLUSION

The study validated the presence of *Fec B* gene in indigenous sheep breeds namely Deccani and Nellore of southern India and its pronounced association with litter size in both the breeds as exhibited from differential genotypic frequencies having dissimilar prolificacy.

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