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Short Communication

SCREENING FOR BOOROOLA (*FecB*) MUTATION IN MUNJAL, NALI AND NALI CROSSES

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SUMMARY

Considering the importance of Booroola fecundity gene in enhancing reproductive performance of sheep, 10 Nali and 20 Munjal and 20 Nali crosses were used to detect the *FecB* gene polymorphism through PCR-RFLP technique. After isolating genomic DNA from blood samples, PCR protocol for amplification of 140 bp and 198 bp fragments corresponding to exon 8 and exon 9 region of *FecB* gene was standardized on digestion with *Ava*II and *Ssp*I and no restriction site was observed in exon 8 and exon 9, respectively in all animals under study suggesting absence of mutated form of *Fec*B gene.

Key words: Booroola, fecundity, PCR-RFLP, Nali, Munjal

Most of the Indian sheep breeds have single litter size except Garole sheep of West Bengal with either size of 2.27 (Nimbkar *et al.*, 1998), renowned for high reproductive efficiency and prolificacy which is due to presence of Booroola fecundity (*FecB*) gene mutation. The *FecB* is a single autosomal gene which increases ovulation rate and litter size in sheep being co-dominant for ovulation rate and partially dominant for litter size (Piper *et al.*, 1985, Montgomery *et al.*, 1992). The *FecB* locus is situated on the region of ovine chromosome 6, which is syntenic to human chromosome 4 (Montgomery *et al.*, 1993). Piper *et al.* (1985) and Piper and Bindon (1996) found that the effect of *FecB* mutation is additive for ovulation rate and each copy increases ovulation rate by about 1.6 and approximately one to two extra lambs in Booroola Merinos. Recently, Davis (2004) reported that one copy of the *FecB* gene increases ovulation rate in Booroola Merino by about 1.5 and two copies by 3.0.

It is believed that Garole sheep might have contributed to the prolificacy gene (*FecB*) in Booroola merino sheep of Australia. The *FecB* gene can be introgressed in other Indian breeds to improve fecundity, so as to increase productivity of sheep enterprises. Assumption that a major gene for prolificacy is also present in other sheep breeds need to be tested. Considering the importance of *FecB* gene in prolificacy, possibility of polymorphism of *FecB* gene was studied in Nali, Munjal and Nali crosses.

The study was conducted on 10 Nali, 20 Munjal and 20 Nali crosses of sheep maintained at animal farm of the university and 5 Garole sheep samples were collected from Rajasthan. Approximately 10 ml of venous blood was collected in 15 ml sterile polypropylene centrifuge tube containing 0.5 ml of 0.5 M EDTA. Genomic DNA was isolated as described by Sambrook and Russel (2001) with minor modifications. Exon 8 region (140 bp) of *FecB* gene was amplified by PCR using the primer pairs as described by Wilson *et al.* (2001). Exon 9 region of *FecB* gene was amplified by PCR using the primer pair F-II (5'TATCAAAGGGACGGGGTCCTGG3') and R-II (5'TCGATGGGCAATTGCTGGTTTGC3') which was designed on mRNA sequence of *FecB* gene of sheep (GenBank Acc. No. AF357007) using fast PCR software. For RFLP analysis, 5 µl of the PCR product of exon 8 and exon 9 were digested with five units of restriction enzymes *Ava*II and *Ssp*I, respectively in a final volume 20 µl at 37°C for 2 h. After the digestion, heat denaturation of the enzyme was done at 70° C for 10 min for *Ava*II, whereas at 65° C for 20 min for *Ssp*I. The restriction fragments were resolved on 3% agarose gel electrophoresis at 80 V for 90 min in 1X

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TAE buffer and photographed using a gel documentation system.

In all animals under study only one DNA band of 140 bp size was obtained after digesting PCR product of exon 8 region of *FecB* gene with *Ava*II restriction enzyme. The absence of restriction enzyme site indicated that there was no mutated form of *FecB* gene in Munjal, Nali crosses and Nali breed of sheep. Thus, all the animals are homozygous for wild type. However, five sheep of Garole breed were also screened for this point mutation as a control and *Ava*II restriction site was present in two animals out of five resulting in a restriction pattern of 110 bp alone indicating homozygous mutated (*FecB*^{BB}), 110 bp and 140 bp together indicating heterozygous carrier (*FecB*$^{B+}$) and 140 bp alone indicating homozygous wild type (*FecB*$^{++}$) (Fig). The *FecB* gene polymorphism has so far been reported only in Indian Garole sheep breed (Pardeshi *et al.*, 2005) and in some other highly prolific sheep breed of the world, like Australian Booroola Merino (Montgomery *et al.*, 2001) and Hu sheep breed of China (Guan *et al.*, 2007). A 140 bp product harbouring point mutation at 830 bp position (A to G transition) in exon 8 region (GenBank Acc. No. AF312016) is responsible for polymorphism.

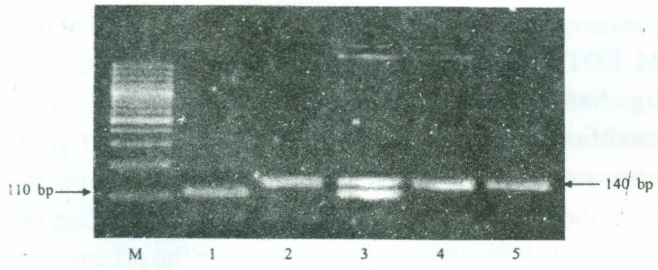

Fig. Electrophoretic mobility of RE fragment obtained by digestion of Garole *Fec*B exon 8 (140 bp) PCR product with *Ava*II in 3% agarose gel.
Lane M: 100 bp ladder, Lane 1: 110 bp RE product (*Fec*B^{BB} genotype), Lane 2, 4, 5: Uncut RE product (*Fec*B^{++} genotype), Lane 3: 110 bp and 140 bp (*Fec*B^{B+} genotype).

In order to screen all animals under study, for the presence or absence of *FecB* gene polymorphism, a 198 bp product harbouring another point mutation at 1113 bp position (C to A transversion) in exon 9 region (GenBank Acc. No. AF357007) was digested with *Ssp*I. In all the animals, only one DNA band of 198 bp size was obtained after digestion with *Ssp*I restriction enzyme. The absence of restriction site indicated that absence of mutated form of exon 9 of *FecB* gene in Munjal, Nali and Nali crosses of sheep.

Above findings of this study indicate that *FecB* gene polymorphism for exon 8 region is present only in Garole breed of sheep whereas absent in Munjal, Nali and Nali crosses which indicates that there was no point mutation at 830 bp position of exon 8 as described by Wilson *et al.* (2001). Similar results have been reported by Pardeshi *et al.* (2005) in the Deccani, Bannur and Madras Red breeds who suggested that probably there was no natural avenue for transfer of the mutation from Garole into the other breeds of their study. This is not surprising, considering the vast distance (1500-2000 km) between north-east region where Garole sheep is found and that of central and south India where the other breeds are found. The *FecB* gene polymorphism for exon 9 region is absent in Munjal, Nali and Nali crosses indicated that there was no point mutation at 1113 bp position of exon 9 as described by Souza *et al.* (2001). Because of small sample size in the present study, results need to be tested on large sample population.

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