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Booroolo allele segregation in Garole Maluro and GM sheep

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ABSTRACT : Garole Sheep of west Bengal (India) is known to carry mutation in an autosomal gent *FecB* (Boraola allel or $FecB^{+}$) on ovine chromosome 6. The mutation is known to affect Ovulation rate and in turn litter size in Garole sheep.The mutated allel is hypothesized to be the original genotype of the breed and by this virtue the sheep produces twins, triplets and quadruplets. To incorporate the character of higher prolificacy in mutation type non-prolific Malpura sheep of semi-arid region of Rajasthan, Garole sheep was used as sire breed in *FecB* introgression programme started in 1997 at Central sheep and wool Research Institute, Avikanagar. Presence of *FecB* allel was detected in Garole and GM sheep. The genotype frequencies of homozygous carriers ($FecB^{B+}$) were 0.41 and 0.11 in Garole and GM, respectively. The corresponding Figures for heterozygous ($Fecb^{B+}$) were 0.48 and 0.60 in Garole and GM, respectively.

KEY WORDS : Fecundity gene *FeeB*, Garole, Gene frequency, Genotypic frequency, Sheep

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INTRODUCTION

Booroola gene is known for its large impact on prolificacy (Visscher *et al.*, 2000). Almost all Indian sheep breeds produce one lamb at each lambing except Garole, a highly prolific breed from hot and humid coastal region of West Bengal will mean litter size 1.74 in the native tract and 1.87 mean liter size in the flocks adapted in the semiarid region of Rajasthan (Bose *et al.*, 1999 and Sharma *et al*., 2001). The hyper prolificacy phenotype of Booroola ewes is due to the presence of the $FecB^{B}$ allele at the *FecB* locus, recently identified as a single amino acid substitution (Q249R) in the coding sequence of bone morphogenetic protein (BMP) type- 1B receptor (BMPR-1B) (Fabre *et al*., 2003). At nucleotide level the $FecB^{B}$ allele is identical to the wild type $FecB^{+}$ except for a transition A to G at position 746 in BMPR-1B gene (Mulsant *et al.*,2001). The mutation was found to segregate in sheep derived from the Boorola Merino, Garole and Javense sheep (Wilson *et al.*, 2001) but could not be detected in other unrelated sheep breeds (Davis *et al.*, 2002). The mutated allele ($FecB^{B}$) is found in very high frequency in Garole sheep and hypothesized to be the original genotype of the Garole sheep (Davis *et al.*, 2002). The other prolific breeds by virtue of $FecB^{B}$ allele, Javanese and Booroola Merino, may have acquired the booroola gene from the Garole sheep of India. The discovery of the *FecB* mutation in the Chinese Hu and Han breeds might be because the mutation arose spontaneously in 2 separate events in the Garole and

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Hu breeds or because these breeds share a common ancestor (Davis *et al.*,2006). The *FecB*^{B} allele has additive effect and increases 1.6 corpora lutea/ ovulations per cycle or 1 to 2 extra lambs. But in Javanese sheep the ovulation rate increase is estimated to be 0.8 and may be due to lower prolificacy potential of background genotype. The effects of *FecB* gene on reproductive characters are well documented (Elsen *et al.*,1990 and Montromery *et al.*,1992) Willingham *et at.* (2002) reported that the *FecB* gene exists in one of the 3 forms within crossbreds, *viz.*, *FecB*^{BB} (homozygous carriers), *Fec B*$^{B+}$(heterozygous carriers) and *FecB*$^{++}$ (non-carriers). The present study discusses gene and genotypic frequencies of fecundity gene (*FecB*) in Garole, Malpura and GM crossbreds and segregation in different generations of GM crossbreds.

MATERIAL AND METHODS

The study was conducted in the Division of Animal Genetics and Breeding and Biotechnology Section of Central Sheep and Wool Research Institute, Avikanagar, Rajasthan (India) Garole sheep were procured from the native tract *i.e.* hot humid region of West Bengal. Malpura native mutton type breed was used as dam breed and Garole was used sire breed in developing Garole x Malpura half-breeds that supposed to segregate fecundity gene in crosses.

Blood sampling and estimation of gene and genotypic frequencies :

Blood samples of 31 Garole and 71 Garole x Malpura and 55 Malpura sheep were collected randomly from sheep flocks by venipuncture in ACD. The DNA was isolated by standard proteinase K digestion, phenol-cholroform extraction and ethanol precipitation.

The mutation Q249R was assayed using Forced RFLP-PCR with PCR primers F-12 (5'- GTCGCTAT GGGGAAGTTTGGATG-3') and R-15 (5'CAAGATGTT TTCATGCCTCATCA ACACGGTC-3') as described by Wilson *et al.* (2001). These primers amplified a140 bp region of the booroola allele. The R-15 primer introduces a point mutation in PCR product amplified from the BMPR-1B gene and the copies having A to G transition at position 746 thus, gains Ava II restriction site, whereas PCR product from non-carrier (*FecB*$^{+}$) does not complete restriction site and fails to cut by Ava II. The *FecB*^{BB} (homozygous carrier) individual showed single 110 bp band, *FecB*$^{B+}$ (heterozygous carrier) showed 140 and 110 bp bands and the *FecB*$^{++}$ animals (homozygous non- carrier) reveled a 140 bp band. This data was used to find out gene and genotypic frequencies of carrier and non-carrier animals as per Falconer and Mackay (1996).

RESULTS AND DISCUSSION

The results of the present study as well as relevant discussions have been presented under following sub heads:

Genotype :

The genotype of sheep carrying *Fec*^{BB} in Garole, Malpura and GM. Crosses are presented in Table 1 and forced RFLP of *FecB* gene amplified. The *FecB*^{BB} was detected in 99 per cent Garole animals and 77 per cent GM crosses. Presence of the *FecB* mutant allel could not be detected in Malpura animals. In Garole carriers the *FecB*^{B} allel was present in 13 (41%) and 15 (48%) animals in homozygous and heterozygous state, respectively, out of 31 samples studied Davis *et al.* (2002) also reported that *FecB* is present at a high frequency in Garole sheep. In GM grosses *FecB*^{BB} allel was detected in 71 per cent animals in heterozygous.

Gene and genotypic frequencies :

The results (Table 1) indicated that gene

Table 1 : *FecB* genotype and gene frequency and overall litter size in Garole, Malpura and GM crosses

***	Genotype frequency			Total	Gene frequency	
	BB	B+	++		Allele B	Allele +
Garole	13 (0.41)	15 (0.48)	3 (0.09)	31	0.60	0.33
Malpura	0 (0)	0 (0)	55 (1)	55	-	1
GM	8 (0.11)	42 (0.60)	21 (0.30)	71	0.40	0.61
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frequency for $FecB^{BB}$ allel was high in Garole sheep (0.66) and in GM crosses (0.40) genotype frequencies for homozygous carriers was (0.41) in Garole and (0.11) in GM crosses, whereas genotype frequencies for heterozygous careers were estimated to be (0.48) and (0.60) for Garole and GM crosses, respectively. Davis *et al*. (2002) Suggested that this particularly frequency of mutated allele ($FecB^{B}$) Could be a part of natural genotype of the Garole sheep and the heterozygote's in this population may have emerged from out crossing with other sheep breeds. Piper and Bindon (1982) first proposed the hypotheses that the high prolificacy trait in Booroola sheep is the result of a single major gene. They were able to demonstrate a putative major gene by segregating ewes according to whether or not they had any record of triplet litters later Davis *et al*. (1982) further provided strong evidence for the presence of major gene in Booroola sheep

The detection of *FecB* mutation in Garole sheep strongly supports the theory that the Booroola gene was introduced into Australia through Garole sheep late in 18th (Davis *et al.*, 2002). Knowledge that fecundity gene is present in Garole and Garole x Malpura sheep will allow breeding strategies to be developed that maximize the benefits of *FecB*.

Though above results are based on relatively less number of observations and hence, further work needs to be conducted to reach definite conclusions but it is clearly indication of the fact that Garole x Malpura crosses may be propagated as new crossbred sheep having *FecB* gene towards evolving new prolific strain for enhancing mutton production.

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