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Assessing the role of *FecB* mutation in productivity of Indian sheep

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DNA samples from four Indian sheep breeds, viz. Garole, Deccani, Bannur and Madras Red were screened by PCR–RFLP to determine the presence of *FecB* mutation in these breeds. The Garole was the only breed, which carried the *FecB* mutation. The *FecB* mutation was introgressed from Garole sheep into Deccani sheep and Bannur sheep, and the performance of the crossbred sheep is being monitored in subsequent generations. Approximately half of the first backcross ewes (progeny of *FecB* heterozygote F1 rams) was found to carry one copy of *FecB* mutation, as expected. The *FecB* PCR–RFLP test was found to be fast, accurate and useful as a tool for making breeding decisions.

Keywords: *FecB* (Booroola) gene, Garole sheep, PCR–RFLP test, prolificacy.

SHEEP occupy a special niche in the Indian agricultural production system and are important for the rural economy. They are efficient converters of otherwise unutilized poor-quality grass and crop residues into meat and skin. Profitable and sustainable sheep production is a high priority for India and genetic improvement of sheep for meat production is one of the important developmental priorities. Enhancing reproductive rate is a logical approach to improving economic efficiency of meat production. However, the litter size of almost all Indian sheep breeds, except the Garole, is low and is thus a major constraint to sheep-meat production. Introduction of a prolificacy gene into non-prolific sheep breeds having other desired traits may effectively increase the reproductive performance of local breeds. The concept of breeding for increased fecundity has been accepted and adapted in ruminant breeding[1]. However, the conventional method of selective breeding to increase the reproductive performance of local sheep breeds would be a slow process, since the heritability of litter size is typically low and there is not much variation to exploit in the non-prolific breeds.

The highly prolific 'Booroola', a strain of Merino sheep, developed in Australia was selected solely on the lambing performance of ewes[2]. Ovulation rate analysis of this breed further provided strong evidence for single gene control of the trait[3]. The Booroola fecundity gene, *FecB*, is a single autosomal mutation that shows additive effect for ovulation rate and partial dominance for litter size[4,5]. In early 2001, Wilson *et. al.*[6] identified the mutation that causes super prolificacy of Booroola, which was confirmed by groups in France and the UK[7,8]. The mutation was found in the bone morphogenetic receptor type 1B (*BMPR1B*) gene. It is hypothesized that this mutation might be reducing the signalling through the receptors of granulosa cells. The *BMPR1B* gene in humans is located in a region of chromosome 4 that contains homologues of genes that have been shown to be linked with the Booroola mutation[8]. Recently, it has been shown that the Garole possesses the same mutation as the Booroola[9], supporting the theory put forth by Turner[2] that the Bengal sheep that arrived in Australia in 1792 might be the probable source of *FecB* mutation in Booroola.

The Garole is the only known prolific sheep breed in India and is a native of Sunderban, the swampy delta region of the Ganga river in West Bengal[10]. This breed is mainly reared for meat production. However, it has low growth rate and body weight and poor survival in harsh semi-arid environment[11]. Therefore, three Indian sheep breeds, viz. Deccani, Bannur and Madras Red with more desirable

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characteristics for meat production were screened for *FecB* mutation using the PCR–RFLP test developed by Wilson *et al.*[6]. The Deccani, the main sheep breed of Maharashtra, is a coarse-wool sheep reared for lamb production and is one of the largest contributors to meat production in India. The reproductive performance of the Deccani is low, with an average litter size of 1.04. The Madras Red is a hair sheep breed from northeastern Tamil Nadu, South India, reared mainly for meat. The Bannur is another hair sheep breed from Karnataka, South India and is considered to have the best body conformation for meat production.

To perform the PCR–RFLP test, blood samples of the Lonand, Kolhapuri, Sangamneri, Sangola and Madgyal subtypes of the Deccani breed, Bannur and Madras Red were collected from main representative areas of the respective states. Care was taken to include only unrelated animals from distant flocks. Crossbreeding between the Garole (G) and the Deccani (D)/Bannur (B) was carried out at Nimbkar Agricultural Research Institute (NARI), Phaltan in order to determine whether a composite sheep having medium–high prolificacy and which can survive in the semi arid environment of the Deccan plateau in Maharashtra could be developed[12]. Ten F1 rams (G × D/B) were produced by crossing Deccani/Bannur ewes with nine Garole (G) rams. Each of the ten F1 rams was then single-sire mated to Deccani (D), Bannur (B) and Deccani × Bannur (D × B) or Bannur × Deccani (B × D) ewes to produce 25% Garole {G × D/B × D or G × D/B × (D × B)} or G × D/B × (B × D) progeny (12 to 27 daughters per sire representing ten progeny groups). Two hundred and twenty seven females resulting from this backcrossing were included in the analysis for *FecB* mutation. Ninety of these 25% Garole ewes were further inseminated with Deccani ram semen and the resulting progeny were analysed for the same mutation. Furthermore, the test was extended to three hundred and forty samples collected from shepherds' flocks where homozygous $FecB^{B}/FecB^{B}$ crossbred rams had been introduced by NARI. These experiments were performed to study the extent of management of increased prolificacy of the crossbred animals under local environment of sheep rearing in Maharashtra.

DNA was isolated from white blood cell pellets isolated from sheep blood samples[13] and also from blood samples taken on FTA paper as per the manufacturer's instructions (Whatman Biosciences, UK). Sixteen nanogram DNA per sample per reaction was amplified in 4 µl volume containing 1X PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% w/v gelatin), 100 µM each of three unlabelled dNTPs, 2.5 ng (7 µM) of both forward and reverse primers, 0.22 µg Taq start antibody (Clontech, USA), 0.1 U Amplitaq DNA polymerase (Perkin Elmer, USA) and 0.020 µCi ^{33}PdATP (BRIT, India). The reaction consisted of 1 cycle of denaturation (95°C, 2 min 30 s) followed by 29 cycles of denaturation (95°C, 10 s), annealing (62°C, 30 s) and extension (72°C, 30 s) and one final cycle of extension (72°C, 5.0 min) in a DNA thermal cycle (PTC 200, USA). The amplified PCR product was subjected to *Ava*II digestion by adding a restriction enzyme cocktail (8 µl) containing 1X RE buffer M (Amersham, USA), 44 mM $MgCl_2$, acetylated BSA (1.2 µg), water and 0.4 U*Ava*II enzyme (Amersham, USA) and incubating for 3 h at 37°C on the PCR machine. Following digestion, the fragments were electrophoresed on 6% denaturing sequencing gel, exposed to X-ray film for at least 24 h and scored for the mutation depending upon size and number of bands as homozygous mutation $FecB^{B}/FecB^{B}$ (110 bp); heterozygous $FecB^{B}/FecB^{+}$ (110 and 140 bp) and homozygous wild type $FecB^{+}/FecB^{+}$ (140 bp; Figure 1). The PCR–RFLP tests were performed in triplicate. The ovulation rates (ORs) of pure Garole, Deccani and heterozygous and non-carrier Deccani–Garole crosses were determined laparoscopically at NARI[12,14].

The *FecB* mutation was observed to be present only in the prolific sheep breed, Garole and was not found in the Deccani, Madras Red and Bannur breeds (Table 1). The results suggested that probably there were no natural avenues for transfer of the mutation from Garole into the other breeds analysed here. This is not surprising, considering the vast distance (1500–2000 km) between northeastern India where the Garole is found and that of central and South India where the other breeds are found. The twelve samples of Garole showing $FecB^{B}/FecB^{+}$ genotype indicated that either the gene is not fixed in the Garole breed or these animals are crossbred. Among the ten backcross progeny groups with

Figure 1. Detection of *FecB* mutation by PCR–RFLP test. Amplification of genomic DNA from different sheep breeds (lanes 1–26) using forward (F: 5′ GTC GCT ATG GGG AAG TTT GGA TG 3′) and reverse primer (R: 5′ CAA GAT GTT TTC ATG CCT CAT CAA CAC GGT C3′) followed by *Ava*II digestion, revealed a 140 bp band in homozygous wild type animals. Lanes 1–10, 13, 17 ($FecB^{+}/FecB^{+}$), 110 bp band in homozygous mutated type animals Lanes 18–26 ($FecB^{B}/FecB^{B}$) and both 140 bp and 110 bp band in heterozygous animals. Lanes 11, 12, 14, 15, 16 ($FecB^{B}/FecB^{B}$)). Expected sizes of non-carrier (140 bp) and carrier (110 bp) of *FecB* mutation are indicated with arrows.

Table 1. *FecB* genotypes of pure breeds

Breed/strain	$FecB^{B}/FecB^{B}$	$FecB^{B}/FecB^{+}$	$FecB^{+}/FecB^{+}$	Total
Garole	124	12	–	136
Deccani subtypes				
Lonand	–	–	150	150
Sangola	–	–	20	20
Kolhapuri	–	–	20	20
Madgyal	–	–	20	20
Sangamneri	–	–	20	20
Bannur	–	–	26	26
Madras Red	–	–	20	20

25% Garole genes generated at NARI, 37–63% carried one copy of the Booroola allele ($FecB^B/FecB^+$). Considering all the backcrossed ewes together irrespective of their individual group, nearly half (49.53%) of the 25% Garole ewes were heterozygous ($FecB^B/FecB^+$), as is expected for the Mendelian inheritance of a single copy of the mutation present in one parent. Further analysis of 194 progenies with 12.5% Garole genome from *FecB* heterozygous sires revealed the presence of one copy of *FecB* in 90 animals (46.39%) and the wild type in 104 animals (53.60%). Among the 340 progenies of $FecB^B/FecB^B$ rams in local shepherds' flocks, 55% was found to be $FecB^+/FecB^+$ and 45% $FecB^B/FecB^+$. The ORs of the *FecB* homozygote (mostly pure Garole), *FecB* heterozygote (Garole–Deccani crosses) and non-carrier ewes (Deccani, Bannur and their crosses with Garole) as determined at NARI were 3.37, 2.09 and 1.08 respectively[10]. The effect of one copy of the *FecB* gene on litter size (LS) in 25% Garole ewes was 0.52, 0.61 and 1.03 for the first, second and third parities respectively[12]. These studies showed that the introgression of one copy of the Garole gene ($FecB^{BB}$) increased the prolificacy of Deccani sheep by about 0.7 lambs born/ewe lambing and the OR by nearly one corpus luteum per pair of ovaries, which was lower than that estimated by Piper *et al.*[4]. However, similar results have been observed in other crosses involving the Booroola strain, viz. Booroola Merino × Rambouillet and Booroola Merino × Awassi, where prolificacy was increased by 0.65 and 0.6 lambs born/ewe lambing respectively[15,16]. A few other undesirable characteristics such as reduction in body size, decrease in growth rate and increase in embryo mortality were observed in the Garole crossbred animals[14]. In many cases, Booroola crossbred lambs have also been reported to have inferior growth rate and small mature body size[17–20] and a reduction in milk yield[21]. While it is likely that some of these negative traits are due to multiple births/multiple lambs reared per ewe rather than the *FecB* mutation itself, it is desirable to keep the Garole genes to a minimum possible level by selecting other productivity traits and using the PCR–RFLP test to select for increased prolificacy. It is possible that there may be some synergism between the effects of *FecB* and Deccani sheep, since Deccani sheep, have been observed to have higher milk production and consequently better ability to rear lambs than Garole sheep (unpublished data).

The mean OR (5.7) and litter size (2.6) of the Booroola are quite high[22] compared to the Garole, though the same mutation is present in both breeds. This may be due to environmental differences (e.g. plane of nutrition), a breed effect or the presence of other genetic factors such as modifier genes or novel mutations within the *BMPR IB* gene that dampen the expression of the *FecB* trait in the Garole sheep. In West Bengal, which is the natural habitat of the Garole sheep, the mean litter size is 2.23 (based on information collected from farmers)[23], whereas in the semi-arid environment of Maharashtra, the mean litter size[10] is 1.74. Alternatively, the genetic effects enhanced by the environmental factors might be contributing to such suppressed expression of the gene in the Garole sheep.

To date, the prolificacy gene has been introgressed from the Booroola Merino into several breeds in different countries to improve the reproduction rate while maintaining desirable levels of performance for other traits[15,24,25]. However, earlier attempts for developing and establishing new breeds of sheep incorporating the *FecB* gene and having consistent and predictable product prior to the development of DNA tests required a lot of effort and time and could not meet the growing demands of the market. The speed and efficiency of selection was increased by use of molecular markers associated with the *FecB* gene in selecting the desired genotypes in the breeding programmes[16,26,27]. However, indirect DNA tests required prior information about the *FecB* status of at least one parent to develop the pedigree, which needed progeny testing and the analysis of OR data. It was also essential that the animal carrying the *FecB* gene should inherit the whole chromosomal region spanned by the markers, since recombination between the markers hampered detection of the *FecB* status of the animal. Three microsatellite markers Oar-AE101, Oar-HH 55 and BM1329 commonly used for the early identification of *FecB* carriers resulted in 10% error due to recombination between the markers[17]. The discovery of mutation in the BMPRIB (ALK6) receptor developed into PCR–RFLP test was a major breakthrough for the animal production industry. The test was successfully used by Davis *et al.*[9] to screen the *FecB* mutation in prolific sheep breeds from various countries. This test overcomes the disadvantages of the marker test and is easy to perform. Early detection of allelic status of the *FecB* gene and comparison of phenotype data with marker data not necessary, are some additional advantages. A large number of samples can be tested in a short period, which would help animal husbandry researchers to design breeding strategies and to advise shepherds appropriately. In summary, status of the *FecB* mutation was studied in four Indian sheep breeds, and productivity of Indian sheep which have had *FecB* introgression was successfully monitored using PCR–RFLP test.

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