Polymorphism Analysis of FecB and BMP15 Genes in Kenguri and Kenguri × NARI Suwarna Sheep

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Received:- 12 September 2025/ Revised:- 17 September 2025/ Accepted:- 23 September 2025/ Published: 30-09-2025

Abstract— *Sheep production in India is constrained by low reproductive efficiency, with litter size being a major limiting factor in improving productivity. Prolificacy in sheep is largely regulated by fecundity genes such as* ***Booroola fecundity (FecB/BMPR1B)*** *and* ***BMP15****, which directly influence ovulation rate and litter size. The present investigation was carried out to detect polymorphisms in these two genes in* ***Kenguri*** *and* ***Kenguri × NARI Suwarna (F1)*** *sheep maintained at the Livestock Research and Information Centre, Veterinary College, Bidar. Blood samples were collected from 30 Kenguri and 30 crossbred sheep. Genomic DNA was isolated using the phenol–chloroform method and evaluated for purity and concentration. PCR amplification was performed using specific primers to amplify a 190 bp fragment of FecB and a 434 bp fragment of BMP15. The PCR products were subjected to* ***restriction fragment length polymorphism (RFLP)*** *using* ***AvaII*** *and* ***StuI*** *restriction enzymes, respectively, and separated by agarose gel electrophoresis. In Kenguri sheep, the FecB locus was monomorphic, with all animals showing the wild-type (++) genotype. However, in the Kenguri × NARI Suwarna (F1) crossbred population, three genotypes were identified:* ***BB (0.16), B+ (0.50), and ++ (0.34)****. The corresponding allele frequencies were* ***B = 0.41*** *and* ***+ = 0.59****. A Chi-square test for Hardy–Weinberg equilibrium revealed significant deviation (*$\chi^2 = 7.82, p < 0.05$*) in the crossbred group, indicating possible selection pressure, genetic drift, or non-random mating. At the BMP15 locus, no polymorphism was detected, and all animals across both populations were found to be monomorphic for the wild-type allele. The results clearly demonstrate the successful* ***introgression of the FecB allele*** *through crossbreeding with the NARI Suwarna strain, thereby enhancing the genetic potential for higher prolificacy in Kenguri sheep. These findings provide molecular evidence supporting the role of targeted crossbreeding in improving reproductive traits. The absence of variability at the BMP15 locus suggests that FecB plays a more prominent role in this population.*

Keywords— Kenguri sheep; NARI Suwarna; FecB polymorphism; BMP15; PCR-RFLP; Hardy–Weinberg equilibrium; Prolificacy.

I. INTRODUCTION

The livestock sector is an important sub-sector of Indian agriculture, contributing significantly to rural livelihoods and national GDP. Although the share of agriculture in the national GDP has been declining, the livestock sector has shown steady growth, contributing about 3.9% to total GDP and nearly 24% to agricultural GDP). Among livestock, sheep occupy an important position, contributing meat, wool, and skin. India has more than 65 million sheep, yet per-animal productivity remains low, mainly due to poor reproductive efficiency.

Prolificacy, or the ability of ewes to produce multiple offspring per lambing, is a key trait influencing mutton production. However, most indigenous Indian breeds are characterized by low twinning rates, thereby limiting productivity (Jain *et al.,* 2006a). The discovery of fecundity genes has opened new opportunities for improving reproductive traits through **marker-assisted selection (MAS)**. Among these, ***FecB (BMPR1B)*** and ***BMP15*** are the most studied. Mutations in these genes are known to increase ovulation rate and litter size in several prolific breeds such as Booroola Merino, Garole, and Hu sheep (Mulsant *et al.,* 2001; Wilson *et al.,* 2001; Davis, 2005).

Kenguri is a medium-sized sheep breed of northern Karnataka, valued for mutton production but known to have very low prolificacy. By contrast, NARI Suwarna, a crossbred strain developed by introgression of the FecB mutation from Garole sheep

into Deccani sheep, has demonstrated a 33% increase in productivity due to higher litter size (Appannavar *et al*., 2010). Crossbreeding of Kenguri with NARI Suwarna offers potential to introduce the FecB mutation into the Kenguri population.

Therefore, the present study was designed with the objective of detecting polymorphisms in ***FecB*** and ***BMP15*** genes in Kenguri and Kenguri × NARI Suwarna (F1) sheep using PCR-RFLP, and to estimate genotypic and allelic frequencies along with Hardy–Weinberg equilibrium.

II. MATERIALS AND METHODS

2.1 Experimental Animals:

The study was conducted on a total of 60 sheep comprising 30 purebred Kenguri and 30 Kenguri × NARI Suwarna (F1) animals. The animals were maintained at the Livestock Research and Information Centre, Veterinary College, Bidar, Karnataka. The Kenguri × NARI Suwarna (F1) animals had approximately 10–15% Garole inheritance through the NARI Suwarna strain.

2.2 Blood Collection and DNA Extraction:

Blood samples (10 ml) were collected aseptically from the jugular vein into sterile vacutainers containing EDTA as anticoagulant. Samples were transported on ice and stored at 4 °C until DNA extraction. Genomic DNA was extracted using the phenol–chloroform method (Andersson *et al*., 1986). DNA quality was assessed on 0.8% agarose gel electrophoresis, and concentration and purity were determined spectrophotometrically (OD260/OD280 ratio).

2.3 PCR Amplification:

A 190 bp fragment of the *FecB (BMPR1B)* gene was amplified using primers reported by Davis *et al.* (2002). For *BMP15*, primers were designed to amplify a 434 bp fragment of exon 2, based on the *Ovis aries* sequence (NCBI accession NC–019484.2). PCR reactions were carried out in 25 µl volumes containing 50 ng genomic DNA, 200 µM dNTPs, 1.5–3.0 mM $MgCl_2$, 10 pmol of each primer, 1 U of Taq DNA polymerase, and 1× buffer. Thermal cycling involved initial denaturation (95 °C, 5 min), 35 cycles of denaturation (95 °C, 30 s–1 min), annealing (61 °C for *BMP15* and 67 °C for *FecB (BMPR1B)*, 30–60 s), extension (72 °C, 30–45 s), and a final extension at 72 °C for 10 min. The details of primers for both *FecB (BMPR1B)* and *BMP15* genes are presented in table 1.

TABLE 1
PRIMER SEQUENCES OF *FECB (BMPR1B)* AND *BMP15* GENES

Candidate gene	5'-Primer sequence-3'		Length (bp)	Product Size (bp)
FecB	Forward	CCAGAGGACAATAGCAAAGCAAA	23	190
	Reverse	CAAGATGTTTTCATGCCTCATCAACACGGTC	31	
BMP15	Forward	AGAGCCACTGTGGTTTACCG	20	434
	Reverse	GATGCAATACTGCCTGCTTG	20	

2.4 RFLP Analysis:

PCR products were subjected to restriction digestion. *AvaII* enzyme was used to detect the FecB mutation in the BMPR1B gene, while *StuI* enzyme was used for the BMP15 amplicon. Digestion products were resolved on 3% agarose gel containing ethidium bromide and visualized under UV light using a Gel Doc system.

2.5 Statistical Analysis:

Genotype and allele frequencies were estimated following standard formulae. Hardy–Weinberg equilibrium was tested using the chi-square (χ^2) test (Falconer and Mackay, 1996).

III. RESULTS AND DISCUSSION

High-quality genomic DNA was extracted from all 60 animals, with concentrations ranging from 220.4 to 637.5 µg/ml and OD260/OD280 ratios between 1.7 and 1.9, indicating that the samples were suitable for PCR analysis. Amplification of the FecB and BMP15 loci produced single, sharp fragments of 190 bp and 434 bp, respectively, confirming primer specificity and template integrity (Figure 1). RFLP analysis of the *FecB* gene showed a marked contrast between the two populations studied. All Kenguri sheep were monomorphic for the wild-type genotype (++), confirming the absence of the *FecB* mutation in this breed. In contrast, the Kenguri × NARI Suwarna crossbred animals displayed clear polymorphism, with three genotypes detected: BB (16.6%), B+ (50.0%), and ++ (33.4%). The corresponding allele frequencies were B = 0.41 and + = 0.59 (Table 2). The genotype frequencies are also illustrated in a bar diagram (*Figure* 2), which highlights the predominance of the heterozygous B+ genotype in the crossbred population. The digestion pattern obtained with *AvaII* enzyme clearly differentiated the genotypes, with BB showing 160 bp and 30 bp bands, ++ showing an undigested 190 bp fragment, and B+ displaying all three fragments (Figure 3).

TABLE 2
GENOTYPIC AND ALLELIC FREQUENCIES AT *FECB* LOCUS IN *KENGURI* AND CROSSBRED SHEEP

Breed/Group	Sample size (n)	Genotype frequencies (%)	Allele frequencies
		BB	B+
Kenguri	30	0	0
Crossbred	30	16.6	50

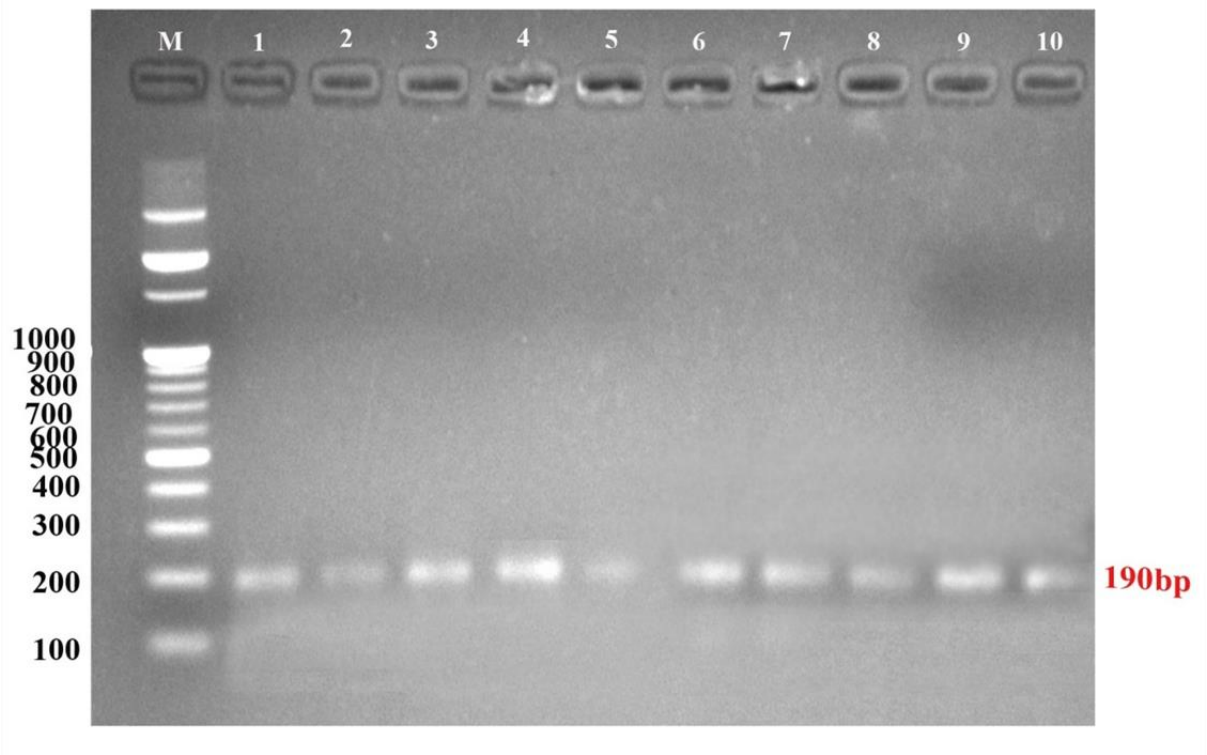

FIGURE 1: PCR amplification of *FecB* gene

A chi-square test revealed a significant deviation from Hardy–Weinberg equilibrium ($\chi^2 = 7.82$, $p < 0.05$) in the crossbred group (Table 3), suggesting that the population may be under selection pressure, genetic drift, or non-random mating. The excess of heterozygotes (50%) supports the view that the population is at an early stage of allele introgression, where crossbreeding with NARI Suwarna has contributed the mutant allele. Similar deviations from Hardy–Weinberg equilibrium have been reported in prolific breeds undergoing selection for litter size (Davis *et al.,* 2002).

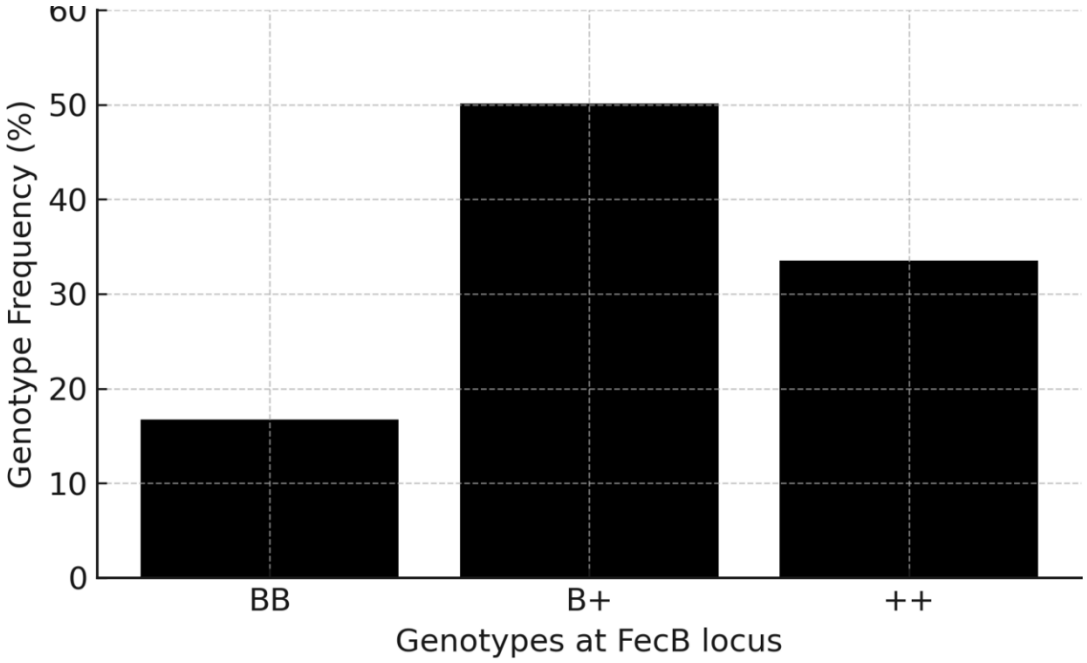

FIGURE 2: Genotype frequencies at *FecB* locus

TABLE 3
HARDY–WEINBERG EQUILIBRIUM TEST FOR *FECB* LOCUS IN CROSSBRED SHEEP

Genotype	Observed (O)	Expected (E)	$(O–E)^2/E$
BB	5	7.56	0.87
B+	15	14.28	0.03
++	10	8.16	0.41
Total $\chi^2 = 7.82$ ($p < 0.05$)			

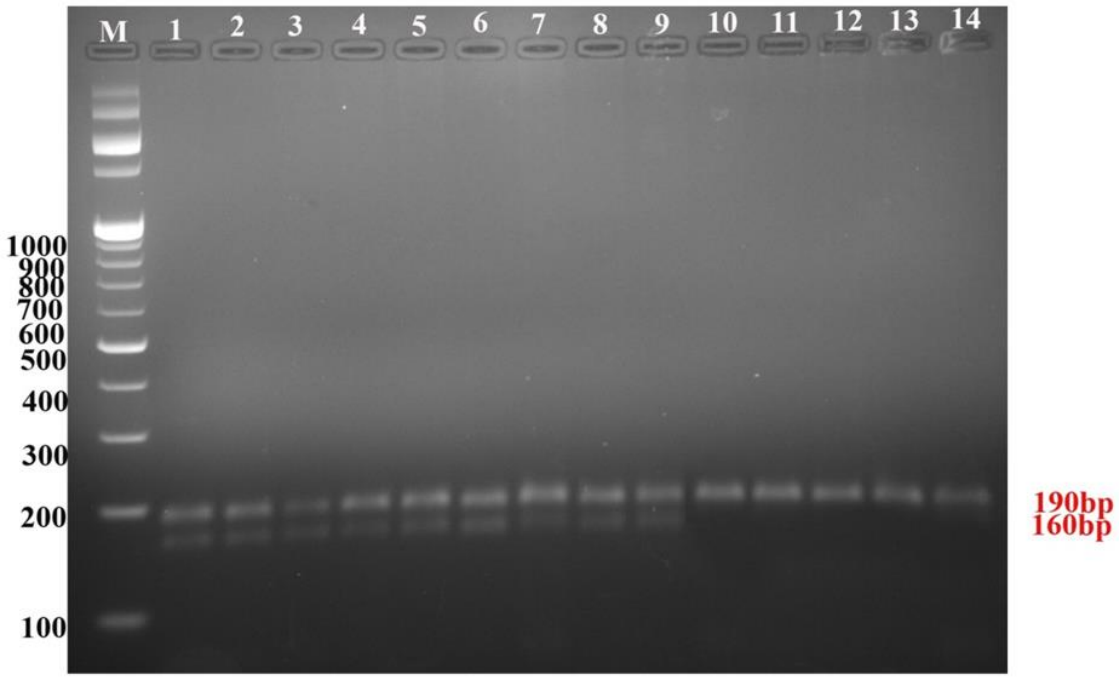

FIGURE 3: *AvaII* enzyme Digestion of *FecB* gene

In the case of the *BMP15* gene, no polymorphism was observed in either Kenguri or crossbred populations. All animals displayed the wild-type allele, with a single undigested 434 bp band observed following *StuI* digestion (Figure 4). This finding is consistent with earlier reports in Indian sheep such as Nilagiri and Kendrapada (Sudhakar *et al.,* 2013; Roy *et al.,* 2011), which were also monomorphic at the *BMP15* locus. By contrast, prolific breeds such as Inverdale and Hanna carry *BMP15* mutations that significantly increase ovulation rates but result in sterility when present in homozygous state (Galloway *et al.,* 2000; Hanrahan *et al.,* 2004).

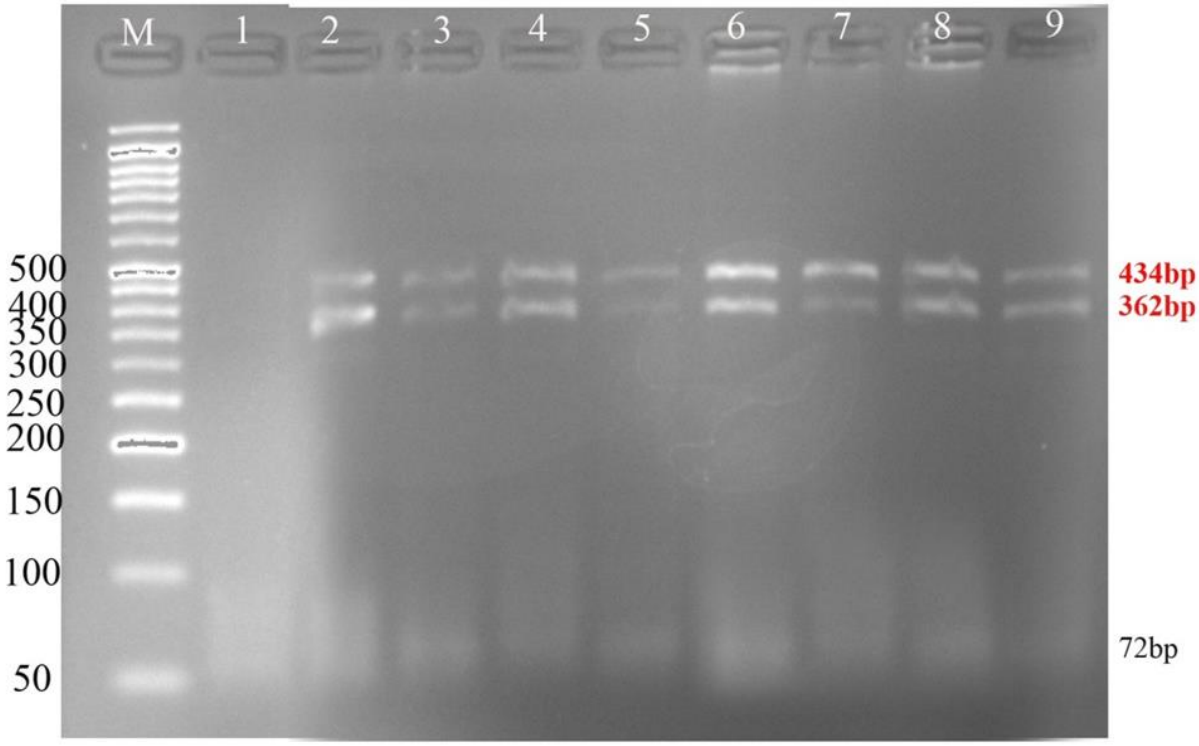

FIGURE 4: *StuI* enzyme digestion of *BMP15* gene

The results of this study clearly establish that the *FecB* mutation has been successfully introgressed into Kenguri sheep through crossbreeding with NARI Suwarna. The presence of polymorphism in the crossbred population but not in pure Kenguri indicates that the observed genetic variation is a direct outcome of the breeding program. The moderate frequency of the B allele (0.41) suggests that with systematic selection, the allele can be fixed or maintained at an optimal frequency to maximize reproductive efficiency. Comparable findings have been reported in Garole, Kendrapada, and Small Tailed Han sheep, where the FecB mutation has been strongly associated with higher litter size (Kumar *et al.,* 2008; Chu *et al.,* 2007).

IV. CONCLUSION

This study confirms the absence of the *FecB* mutation in purebred Kenguri sheep and its successful introgression into Kenguri × NARI Suwarna (F1) sheep. The presence of three genotypes at the *FecB* locus in crossbred animals, along with significant deviation from Hardy–Weinberg equilibrium, suggests ongoing selection pressure. The BMP15 locus was monomorphic in both populations. These findings provide a molecular basis for incorporating **FecB-based marker-assisted selection** in breeding strategies aimed at enhancing reproductive efficiency in Kenguri sheep. Future studies involving larger populations and direct association with reproductive performance traits are essential to validate these findings and ensure sustainable genetic improvement.

CONFLICT OF INTEREST

Author declares no conflict of interest.

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