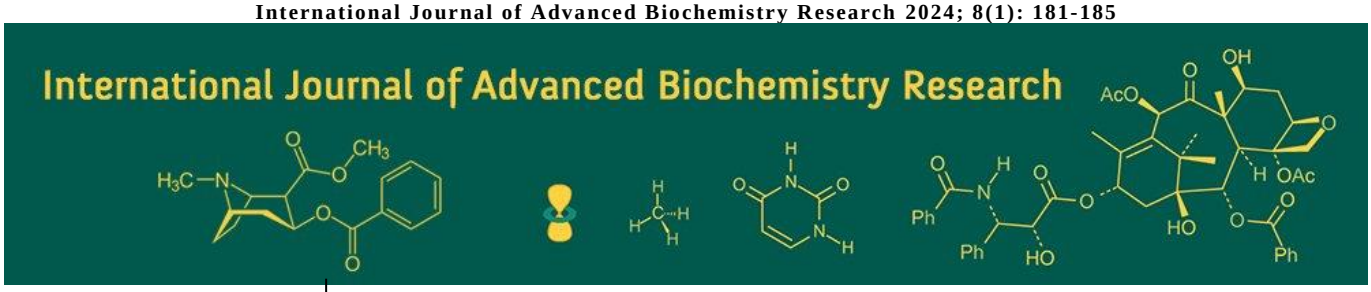

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Introgression FEC B gene in small farmer's sheep flocks of Tamil Nadu for increasing lamb production

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Abstract
A study was conducted in small farmer sheep blocks in Tamil Nadu to check for the presence of the fecundity gene, Fec B. Using the PCR RFLP method, a total of 144 genomic DNA samples were screened for the Fec B gene mutation. The test results indicated that the sheep flocks owned by the farmers contained a sizable percentage of FecB mutations. The frequency of the wild, FecB homozygote (BB) and heterozygote (B+) genotypes in NARI Swarna introgressed with Mecheri and Madras Red sheep was 50.00 and 38.70; 48.70 and 56.40 and 01.30 and 04.83 percent, respectively. The frequencies of the wild FecB heterozygote (B+), homozygote (BB), and homozygote (B) genotypes in Sandyno sheep were 88.14, 11.86, and 0.00 percent, respectively. In contrast, the FecB gene was not detected in any of the screened samples from Mecheri and Tiruchy Black sheep in Coimbatore, and all of the animals had the uncut 140 bp band wild type (++) genotype.

Keywords: Fec B, fecundity gene, PCR RFLP, Mecheri, Maras red

Introduction
For farmers and laborers without access to land, raising sheep is a crucial source of income. India ranked third in the world for sheep population, making up around 6% of the total. While the sheep population in Tamil Nadu has decreased over the decades from 8.02 million in 1951 to 4.50 million in 2019, with a sharp decline of -40.10 percent observed between 2007 and 2012, the sheep population in India has increased from 39.10 million in 1951 to 74.26 million in 2019. The majority of sheep breeds found in Tamil Nadu are producing singles and very infrequently twins as a result of their genetic make-up and the scarcity of nutrition. Because it is taboo to grow twins and because it is difficult to supplement feed externally, sheep farmers also prefer raising singles. A rise in prolificacy boosts the farmer's net profit. Selection for both qualities is possible since "prolificacy" and "growth rate" have a generally favorable genetic association. Low (<0.1) heritability of litter size means that there will be little response to selection. Introgression of the "Fec B" gene, a single autosomal gene known to have a significant impact on "prolificacy in sheep," is another method of increasing prolificacy in sheep. A growing number of progressive farmers of days favor twins. Therefore, the goal of this study was to determine whether the native sheep breeds of Mecheri, Madras Red, Niligiri, and Sandyno in Tamil Nadu that have been introduced with NARI Swarna had the fecundity (Fec B) gene in their flocks of farmers' sheep. In sheep, the mutated gene FecB (Booroola), which is recognized for its fecundity, causes an increase in ovulation frequency and litter size (Davis, 2005; Gootwine *et al.*, 2006) [2]. Sheep with FecB mutations ovulate more frequently, and gene differences cause the sheep to twine around 1.6 times more frequently than they would normally (Piper and Bindon, 1996) [13].

Materials and Methods
Blood samples were collected from NARI Swarna introgressed with Mecheri and Madras Red sheep in the farmers sheep flocks in and around Tamil Nadu and the animals were selected randomly based on history of introgression (Table 1). A total of 144 blood samples were collected and the genomic DNA was extracted according to Sambrook *et al*. (1989) [14]. Forced PCR and RFLP method was used to screen the 140 bp of BMPR-1B region using F-5'TCGCTATGGGGAAGTTTGGATG3' and R 5' CAAGATGTTTTCATGCCTCATCAACACGGTC 3' primers from Bioserve (Wilson *et al*. 2001) [16].

The reverse primer consciously introduced by a point mutation would create a restriction site in mutated strand, wild type ewes lacking the sites. About 100 ng of template DNA was put in 25µl reaction volume from Amplicon Taq DNA 2x Master Mix in PCR with the following amplification conditions; initial denaturation at 94 °C for 1 min, followed by 94 °C for 15 s, 60 °C for 30 s, 72 °C for 30 s for 35 cycle and final extension at 72 °C for 5 min. The products were tested in 3% agarose gel and then 10 µl PCR products was digested with *AvaII* enzyme from Thermo Scientific with recognition sequence (G/GACC) mixture which was prepared according to the pamphlet at 37^0C for 4 h and loaded in 3.0% agarose gel with 5 µl DNA molecular size marker (50 base pair ladder ready-to-use) from Bio-Rad. To confirm the nucleotide variation in the PCR RFLP pattern, amplified *FecB* 140 bp gene PCR products was carried out. The 140 bp fragments were sequenced to ascertain the presence of *FecB* mutation.

Results and Discussion

The frequency distribution of *Fec*B mutation in NARI Swarna Introgressed Mecheri and Madras Red breeds are given in Table 1. The resultant 140-base pair (bp) PCR product digested with *Ava*II restriction enzyme produced three different genotypic pattern *viz*., BB homozygote with 110 bp band, B+ heterozygote showed 140 and 110 bp bands and the wild ++ homozygote revealed uncut 140 bp band (Figure 1).

In total, 144 animals were genotyped from farmers flock *viz*., 82 Sheep of Mecheri cross with NARI Swarna and 62 sheep of Madras Red cross with NARI Swarna. The 82 animals of Mecheri cross with NARI Swarna shown the genotype frequency of 50.00% (0.500) in ++ Homozygous wild type, 48.70% (0.487) in B+ Heterozygous for Fec B mutation and 01.30% (0.013) in BB Homozygous for Fec B mutation (Table 1) and Madras Red cross with NARI Swarna shown the genotype frequency of 38.70% (0.387) in ++ Homozygous wild type, 56.40% (0.564) in B+ Heterozygous for Fec B mutation and 4.83% (0.049) in BB Homozygous for Fec B mutation (Table 2). The overall genotype frequency of 45.13% (0.160 and 0.292) in ++ Homozygous wild type, 52.09% (0.229 and 0.292) in B+ Heterozygous for Fec B mutation and 2.78% (0.007 and 0.021) in BB Homozygous for Fec B mutation for male and female respectively (Table 1&2 and Figure 2). The frequency of B gene was 0.743 and 0.699; + gene was 0.257 and 0.331 in Mecheri cross with NARI Swarna and Madras Red cross with NARI Swarna respectively (Table 2 and Figure 3). The overall frequency of B and + gene was 0.712 and 0.288 respectively (Table 2).

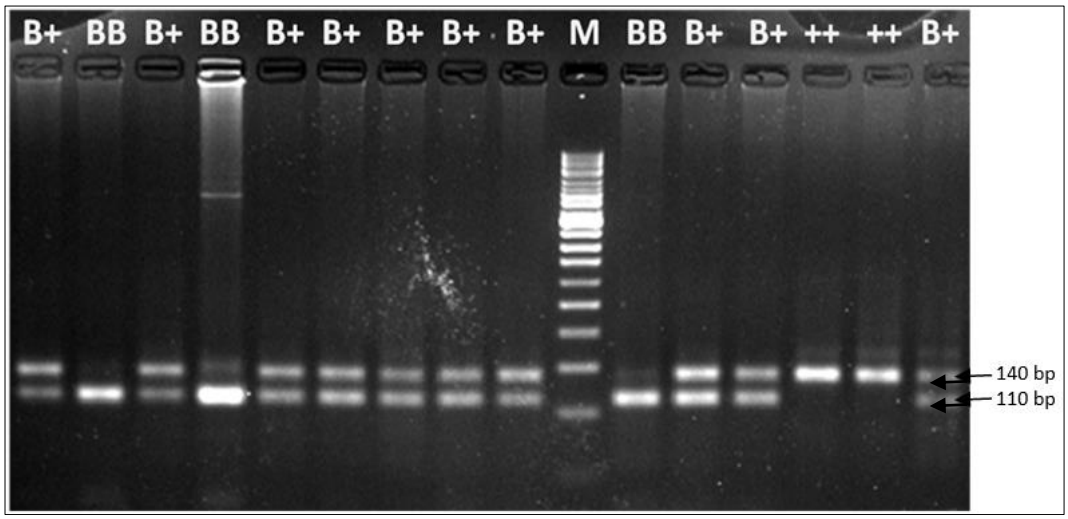
The Adenine (A) to Guanine (G) nucleotide at 110 bp levels and the observed sequence pattern hosted in the DNA Data Bank of Jaban (DDBJ) with GenBnak Accession Nos. LC152969.1, LC152970.1, and LC152971.1 (Figure 4) confirmed the nucleotide sequencing of PCR products of the FecB region (140 bp). The presence of both natural (Adenine) and mutant (Guanine) genotypic patterns in the Madras Red sheep population and the Mecheri sheep population was validated by the nucleotide variation.

The FecB genotyping was done on two native Tamil Nadu breeds. Mecheri and Madras Red Sheep were introduced. Davis *et al.* (2002) [3] verified that the FecB allele has been fixed in the Garole population by using homozygote Garole rams for introgression purposes. The findings indicated that a portion of the Mecheri and Madras Red Lambs were devoid of the FecB mutation. Asadpour *et al.* (2012) [1] found a similar outcome in sixty-seven Zel sheep breed animals with a non-carrier (++) 190 bp band (wild type) in the AvaII RFLP pattern. The Deccani sheep breed was also found to have non-carrier FecB mutations, according to publications (Nimbkar *et al.*, 2003; Pardeshi *et al*., 2005; Kumar *et al.*, 2008) [11, 12, 7]. Additionally, the F1 generation of Mecheri X Garole crossbred individuals showed 100% carrier status for the FecB allele in their genotypes, and the findings verified that the Garloe homozygote (BB) rams contributed only one FecB allele. The homozygote person received alleles from both the ewe and the ram, while the carrier heterozygote (B+) individual received only one allele from the Garloe ram. This suggests that the heterozygote Mecheri ewes utilized for breeding were also present in the Mecheri population, confirming the prevalence of FecB alleles.

According to López-Ramírez *et al.* (2014) [8], high prolificacy is a primary trait of hairy breeds. They analyzed the nucleotide sequence of the cDNA of the BMPR1B and GDF9 genes of 20 Black belly ewes for the presence of FecB mutations, which are responsible for high prolificacy. The hilly areas of Tamil Nadu's Nilagiris district are home to the beautiful wool-producing Nilagiri sheep breed. According to López-Ramírez *et al.* (2014) [8], it may also be investigated if a gene mutation that causes high prolificacy in Mecheri sheep is the cause. The FecB allele is present in the Mecheri and Madras Red sheep breeds because this abundant gene was introduced via the Booroola Merino's crossbreeding with the Garole.

To increase the reproduction rate at desired performance levels and for other traits, Booroola Garole has been crossed with a number of breeds in various nations (Southey *et al.*, 2002; Meyer *et al.*, 1994) [15, 9]. The Madras Red sheep breed has a higher rate of twin births and the highest concentration of FecB mutations when compared to the other two types. One possible explanation for the lack of homozygote FecB mutation in the Mechri sheep breed is the culling of ewes that give birth to twins. These two kinds of sheep have a higher incidence of twins than any other breed in Tamil Nadu. This could be because they carry the FecB gene, which increases ovulation. Therefore, the Mecheri and Madras Red ewes' FecB mutation will have an additive effect on the ovulation rate and increased litter size.

The productive and lam survival rates are better in Mecheri and Madras Red ewes. On the other hand, the phenotypic of the lambs in the crossbred F1 Generation of Mecheri x Garole and Madras Red x Garole crossings differed greatly from the parent breeds and were smaller than the typical weight of Mecheri and Madras Red lambs. This could be the cause of the phenotypic crossing of smaller Garole rams. Furthermore, breeds with altered phenotypes are less valuable on the market than their original counterparts.



++: Homozygous wild type; *B*+: Heterozygous for *FecB*; *BB*: Homozygous for *FecB* gene.

Fig 1: The forced PCR-RFLP showing different banding patterns of *Fec*B genotype in 3 per cent agarose gel electrophoresis.

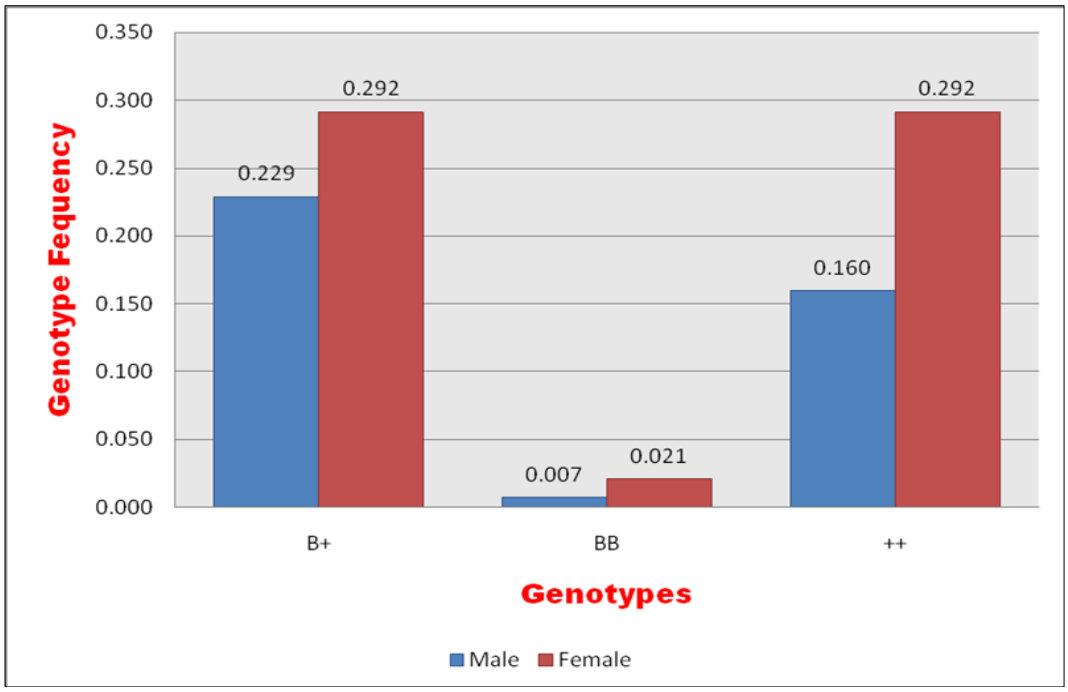


Fig 2: Genotype Frequency among Males and Females

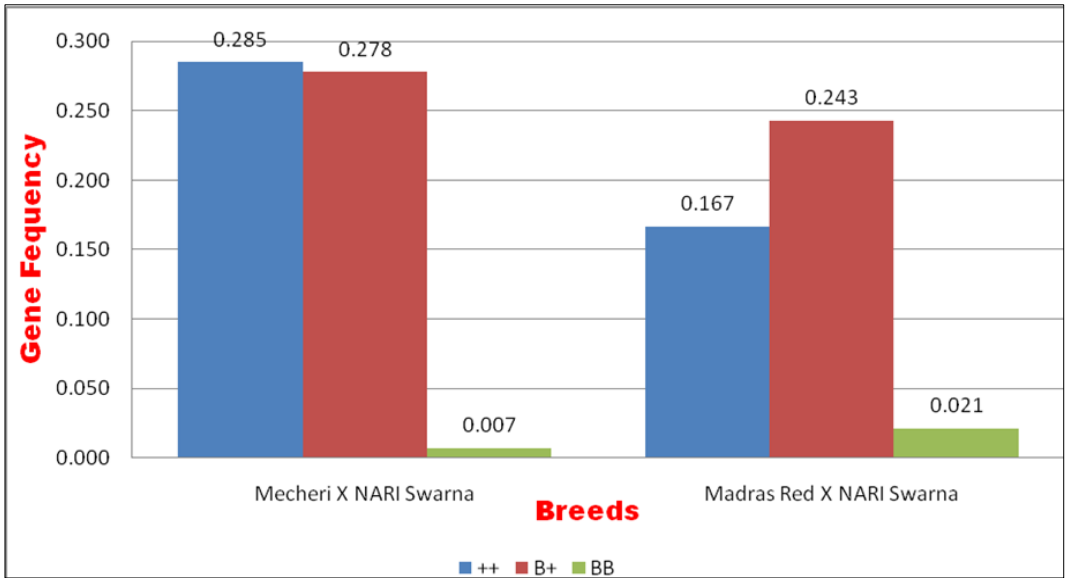


Fig 3: Presence of FecB Gene Frequency among the breeds

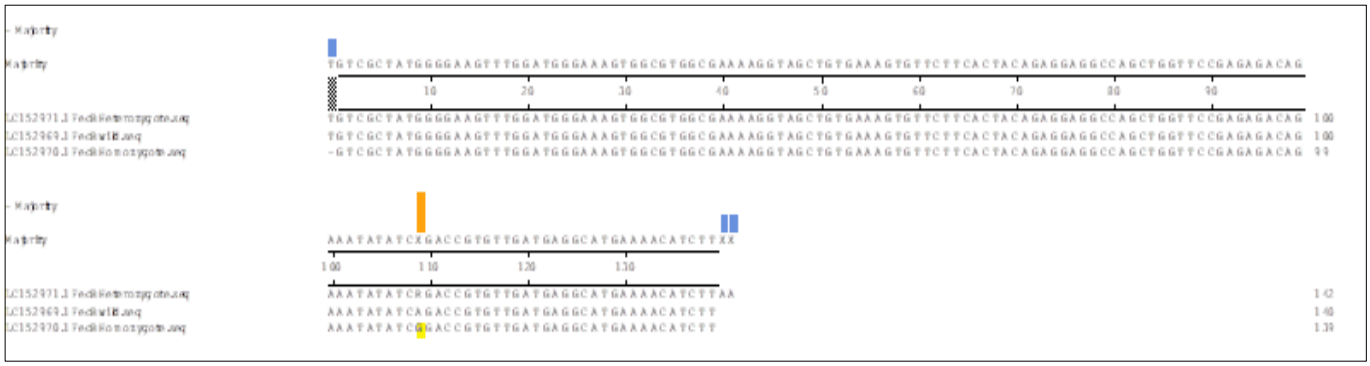


Fig 4: The nucleotide sequencing of *FecB* region in the NARI Swarna Introgressed Sheep

Table 1: Genotype Frequency in Sheep Flocks

Sl. No.	Breeds	No. of Animals Genotyped	Genotype Frequency (%)			Gene Frequency (%)	
			++	B+	BB	+	B
1.	Mecheri X NARI Swarna	82	50.00 (41)	48.70 (40)	01.30 (1)	74.39	25.61
2.	Madras Red X NARI Swarna	62	38.70 (24)	56.40 (35)	4.83 (3)	66.93	33.07
	Overall	144	45.13 (65)	52.09 (75)	2.78 (4)	71.18	28.82

* (Values in the parenthesis indicates the number of animals)
++: Homozygous wild type; *B+*: Heterozygous for *FecB*; *BB*: Homozygous for *FecB* gene.

Table 2: Gene Frequency in Sheep Flocks

Sl. No.	Breeds	No. of Animals Genotyped	Genotype Frequency						Gene Frequency	
			++		B+		BB		+	B
			Male	Female	Male	Female	Male	Female		
1.	Mecheri X NARI Swarna	82	0.110 (09)	0.390 (32)	0.183 (15)	0.304 (25)	0.000 (00)	0.013 (01)	0.743	0.257
2.	Madras Red X NARI Swarna	62	0.225 (14)	0.161 (10)	0.290 (18)	0.274 (17)	0.016 (01)	0.032 (02)	0.669	0.331
	Overall	144	0.160 (23)	0.291 (42)	0.229 (33)	0.292 (42)	0.007 (01)	0.021 (03)	0.712	0.288

Conclusion

The study's findings showed that the Mecheri and Madras Red sheep breeds had the FecB gene discovered. The FecB gene can be quickly screened for mutations using the forced PCR-RFLP approach, which also significantly lowers the sequencing costs associated with FecB genotype mutational screening. It would be a welcome innovation to introduce homozygous FecB (BB) mutant Mecheri and Madras Red rams into the flocks and farms of respective farmers. This would boost profitability without expanding the size of the flocks in local sheep breeds.

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