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Evaluation of fecundity (*FecB*) mutation in Muzaffarnagari sheep of India

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Abstract
The present study was aimed to evaluate (BMPR-1B) *FecB* mutation in Muzaffarnagari sheep breed of India. A total of about 160 blood samples were collected from Muzaffarnagari flock maintained at Central Institute for Research on Goats, Makhdoom, Mathura, and Uttar Pradesh. The Muzaffarnagari sheep was genotyped for the presence of *FecB* mutation by using forced polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Results revealed the absence of *FecB* mutation in this flock. Among analysed records (N=4525), 83.7% were singles and 16.3% were twins. The increased twinning rate in this flock may be due to some other prolificacy genes. Further investigation is needed to rule out the cause for increased prolificacy in this flock of sheep.

Keywords: *FecB*, Muzaffarnagari sheep, absence of polymorphism,

Introduction
Sheep farming has been an important source of income for farmers of arid and semi-arid regions of India since it provides a valuable and persistent source of income throughout the year. The Muzaffarnagari is one of the heaviest and largest mutton breeds of India and is widely distributed in the semi-arid region of western Uttar Pradesh, near Meerut, Muzaffarnagar, Saharanpur, Bijnor and in some parts of Delhi and Haryana. With a population of about 0.18 million, it holds about 0.30% of India's total sheep population. The breed has a better potential for meat and carpet wool production than other Indian sheep breeds. Fertility is one of the important parameters controlling the biological efficiency of sheep in regard to meat, milk and wool production (Notter *et al.* 2000) [10]. Mutations with major effects on ovulation rate and litter size have been identified in genes of the TGF-β super family and a TGF-receptor, namely BMP-15, GDF9 and BMPR-IB (ALK6), (Galloway *et al.* 2000; Hanrahan *et al.* 2004; Souza *et al.* 2001) [5, 6, 12] and some other genes have also been identified. The *FecB* mutation was first identified in Booroola Merino sheep originating from Australia but recent DNA marker technology has revealed that its origin trace back to either the Bengal or Cape sheep (Garole) imported to Australia in the late 18th century (Turner 1982) [15]. *FecB* mutation (c.746A>G) was found in chromosome number 6 (Souza *et al.* 2001) [12] which is syntenic to the human chromosome 4 (Montgomery *et al.* 1993) [9]. The *FecB* mutation has been identified in other prolific breeds of sheep *viz.* dwarf Garole (Davis *et al.* 2002) [3], Kendrapada (Kumar *et al.* 2008) [7] and Nilagiri sheep (Sudhakar *et al.* 2013) [13] from India, Javanese from Indonesia (Davis *et al.* 2002) [3], Hu and small tailed Han from China (Davis *et al.* 2006) [2]. Being heaviest breed, twinning is very rare and almost nill (NBAGR) in this sheep, but Muzaffarnagari flock of CIRG, makhdoom have twinning rate of 16.3%. Present investigation was aimed at evaluating this flock of sheep for *FecB* gene which might be the reason for this increase in prolificacy.

Materials and Methods
Blood Collection and DNA extraction
About 5 ml of blood was collected from 160 animals randomly from the Muzaffarnagari flock maintained at CIRG, Makhdoom, Uttar Pradesh in a 5 ml sterile anticoagulant (EDTA) coated

tube and cold chain maintained till the samples reaches laboratory. Genomic DNA was isolated from the blood samples by the standard Phenol-Chloroform-Isoamyl alcohol extraction method (Sambrook and Russel 2001) [11].

Quality, Purity and Concentration of DNA

The quality of DNA was checked using horizontal submarine agarose gel electrophoresis with 1% w/v agarose carried out at 60V for 1hour and visualized with 0.5µg/ml of ethidium bromide (10mg/ml) under UV transilluminator and documented by using gel documentation system (Sambrook and Russel 2001) [11]. Samples with good quality DNA alone were carried over for further analysis. Purity and concentration of samples were checked by A260/A280 spectrophotometer. Further DNA samples were diluted with NFW based on concentration, vortexed and stored at 4°c overnight and if needed diluted again after checking for concentration once again in 1% agarose gel electrophoresis.

Genotyping for *FecB* gene

Genotyping for *FecB* gene was proceeded by forced PCR RFLP technique with forward (5'-GTCGCTATGGGGAAGTTTGGATG-3') and reverse (5'-CAAGATGTTTTCATGCCTCATCAACACGGTC-3').
primers to introduce a point mutation in the amplified product that creates *AvaII* restriction site (G↓GACC) and (C↓TCAG) in the DNA of *FecB* carrier animals. PCR reaction components and programme were given in the table 1 and 2. The amplified product was checked in submarine horizontal 2% w/v ultrapure metaphor agarose gel electrophoresis at 65 V for 1 hour and visualized with ethidium bromide in transilluminator and documented with gel documentation system.

Restriction enzyme digestion

The 140 bp product was then digested with *AvaII* enzyme and the details of reaction were given in the table 3. The resultant products were run along with 50 bp ladder and molecular marker in a horizontal submarine 3.5% ultrapure metaphor agarose w/v gel electrophoresis at 45 V for 5 minutes followed by 65 V for 2 hours and visualized with ethidium bromide and documented using gel documentation system.
In the presence of *FecB* gene, amplified product would have been digested and cleaved at the restriction site and in case of its absence, restriction enzyme will leave the product uncleaved. Homozygous carriers ($FecB^{BB}$) will have two fragments (110bp and 30bp), heterozygous animals ($FecB^{BB+}$) will have three segments (140bp, 110bp and 30bp) and non-carriers ($FecB^{++}$) will have uncut 140 bp fragment.

Result and Discussion

Our present study found that all the 160 samples showed intact single band (140bp) under gel doc indicating that all the animals were non-carriers for $FecB^{++}$ gene (Figure 1) and the frequency of non-carriers were found to be 100%. Our results were in agreement with the findings of some authors who reported *FecB* absence among various sheep breeds. Debnath and Singh RV (2014) [4] in Balangir and Bonpala sheeps, Sudhakar *et al*. (2014) [14] in Mecheri sheep, Yatoo *et al*. (2015) [16] in Dorper sheep of Jammu and Mishra SK *et al*. (2018) [8] in Kajali sheep among Indian sheep breeds. Amr *et al*. (2009) [1] in five Egyptian sheep breeds.
Among analysed records (N=4525), 83.7% were singles and 16.3% were twins. This increased twinning rate in this flock may be due to the action of other prolificacy genes like *FecX*, *FecG* etc. which will also bring about increase in prolificacy.

Table 1: Components of PCR reaction for BMPR-1B gene

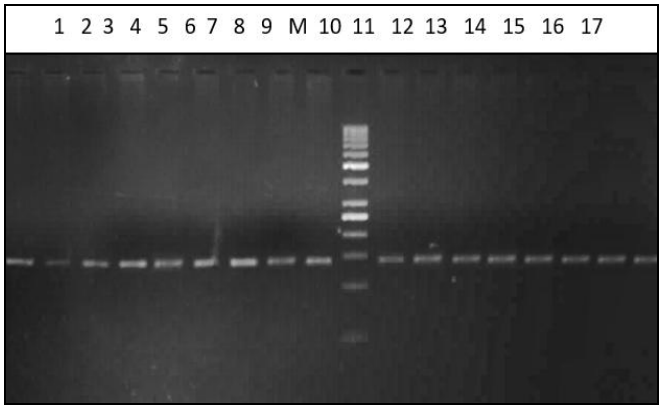
S. No	PCR-reaction components	Amount (µl)
1	Nuclease free water	10.5 µl
2	Forward primer (10pm/µl)	0.5 µl
3	Reverse primer (10pm/µl)	0.5 µl
4	2X Green taq PCR MM	12.5 µl
5	Genomic DNA (100mg/µl)	1.0 µl
	Total	25 µl

Table 2: PCR programme for *FecB* gene

S. No	Step	Temperature	Time
1	Initial denaturation	94 °C	5 min
2	Denaturation	94 °C	1 min
3	Annealing	66 °C	1 min
4	Extension	72 °C	1 min
	30 cycles of step 2 to 4		
5	Final extension	72 °C	10 min

Table 3: Components of restriction enzyme digestion reaction for BMPR-1B gene

S. No	Reaction components	Amount
1	Restriction enzyme (10µg/µl)	0.2 µl
2	10 X assay buffer	1.0 µl
3	Autoclaved distilled water	3.8 µl
4	PCR product	15.0 µl
	Total volume	20 µl



Lane M: 50bp ladder
Lane 1-17: Homozygous non-carrier ($FecB^{++}$)

Fig 1: Genotypic pattern of 140bp amplicon gene of *FecB* gene/*AvaII* forced PCR-RFLP from Muzaffarnagari sheep

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Conflict of Interest

Not available

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Not available

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