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Absence of Polymorphism in Booroola (*FecB*) Gene in Indian Muzzafarnagari Sheep Breed

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ABSTRACT

Booroola (*FecB*) gene is also known as Bone morphogenetic protein receptor type 1B (*BMPR1B*) gene, expressed in oocytes and granulosa cells. *BMPR1B* gene has been characterized by a point mutation at 746th position (A to G) leading to a non-synonymous substitution of Glutamine with an Arginine at 249th position (Q249R) produced 'hyperprolific' Booroola sheep. This mutation was associated with prolificacy in sheep with increase ovulation rate. In the present study, DNA was isolated from blood samples collected from the Muzzafarnagari sheep breeds (n = 200) maintained at LFC (Livestock farm complex) of DUVASU, Mathura, U.P. The *Ava*II/PCR-RFLP assay of 190 bp amplified product of *FecB* gene revealed only ++ genotype (190 bp uncut, 100%) with + allele (1.0) in screened sheep population. The studied region of the *FecB* gene showed monomorphic pattern revealed that *FecB* A (wild type; +) allele seems to be fixed in screened sheep population. Consequently, we could not perform association analysis with reproduction trait. Therefore, it would be suggested to further screening the status of this SNP along with other fecundity genes in large diversified population to exploit it under marker assisted selection.

HIGHLIGHTS

- The *Ava*II/PCR-RFLP assay of 190 bp *FecB* amplified product revealed only AA (++) genotype (100%) with A (+) allele (1.0).
- *FecB* gene showed monomorphic pattern revealed that *FecB* A (wild type; +) allele seems to be fixed in screened sheep population.

Keywords: Booroola, *FecB*, *BMPR1B*, PCR- RFLP, Muzzafarnagari

The Bone morphogenetic protein receptor type 1B (*BMPR1B*) gene is the member of transforming growth factor β (*TGF-β*) superfamily, which has a great influence on cumulus cell expansion, ovulation cycle and skeletal system development. The *BMPR1B* gene is mapped at the *FecB* locus present between the Osteopontin (*OPN*) and epidermal growth factor (*EGF*) genes on sheep chromosome 6 with a coding sequence of 1509 bp, comprising of 10 exons that code for 502 amino acids. A single nucleotide substitution "A"→"G" at nucleotide position 746 of *BMPR1B* cDNA which results in the non-synonymous substitution from glutamine (Glu) to arginine (Arg) at position 249 (Q249R) produced 'hyperprolific' Booroola sheep (Souza *et al.*, 2001). The mutation in Booroola (*Fec B*) allele is associated with the additive effect on ovulation rate and increase in litter size such that one copy of the *FecB* allele may increase the ovulation number by 160% and the litter size by 100%, resulting into extra lambs at each lambing (Kumar *et al.*, 2008).

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FecB mutation has been reported to be associated with prolificacy traits such as litter size, ovulation rate in different exotic sheep (Asadpour *et al.*, 2012; Shafieiyan *et al.*, 2013; Al-Barjinji and Othmann, 2013) and few Indian sheep breeds (Roy *et al.*, 2011; Debnath and Singh, 2014; Praveena *et al.*, 2017; Dash *et al.*, 2018; Mishra *et al.*, 2018). However, this polymorphism study is missing in Indian Muzzafarnagari sheep breed, which are mainly distributed in Muzaffarnagar and adjoining districts of Western Uttar Pradesh (India). This breed, highly adaptable to semi-arid region, is known for faster growth rate and mainly meant for mutton purpose but can also produce sizeable quantity of coarse wool. Therefore, the present study was undertaken to investigate the status of *A>G* polymorphism in *BMPR1B* gene in Indian Muzzafarnagri sheep breed.

MATERIALS AND METHODS

Animals and DNA isolation

A total of 200 adult females of 2-5 years age Muzzafarnagari sheep were utilized in the present investigation which were maintained at Livestock Farm Complex (LFC), DUVASU, Mathura (U.P.), under Muzaffarnagari sheep project entitled "Conservation and genetic improvement of Muzzafarnagari sheep for multiplication of superior germplasm" under the sponsorship of Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Government of India. The flock were being reared under proper managemental practice in semi-intensive system having 27% twining rate. Genomic DNA was extracted from venous blood using the standard protocol of Sambrook and Russel (2001). The primers (F: 5′- CCAGAGGACAATAGCAAAGCAAA-3′ and 'R: 5′- CAAGATGTTTTCATGCCTCATCAACAGGTC -3′) used for amplification of *BMPR1B* gene were as described by El-Hanafy and El-Saadani (2009).

*Ava*II PCR-RFLP Assay

Each 25 µl PCR reaction contained 2 µl (50 ng/µl) template DNA, 0.5µl (10 pmol/µl) of forward and reverse primers, 2.5 µl of 10 × PCR buffer (10 mM Tris–HCl, pH 8.8 at 25ºC, 50 mM KCl), 2.5 mM of $MgCl_2$, 2.5 mM of each dNTPs, and one unit of *Taq* DNA polymerase (Fermentas, USA). The PCR conditions for amplification of *FecB* gene were initial denaturation at 94°C for 5 min, followed by 94°C for 15 sec, annealing at 60°C for 30 sec, 72°C for 30 s for 30 cycle and final extension at 72°C for 5 min. The PCR product was checked by agarose gel (1.0%) electrophoresis. The restriction digestion was carried out at 37ºC with *Ava*II restriction enzyme (New England Biolab, USA) for overnight in a total volume of 15µl containing 5.0 µl of PCR product, 1.5 µl of 10X RE buffer and 10 units of (1.0 µl) of restriction enzyme. For restriction fragment analysis, digested products were checked on 1.5% agarose gel electrophoresis.

Statistical Analysis

The data was generated by estimating the frequency of different *FecB* genotypes. The allelic and genotypic frequencies were estimated by standard procedure (Falconer and Mackay, 1996).

RESULTS AND DISCUSSION

The results revealed that restriction digestion of 190 bp amplified product of the *BMPR1B* gene showed absence of polymorphism by performing 1.5% agarose gel electrophoresis. The *Ava*II/PCR-RFLP assay revealed monomorphic pattern (uncut genotypes; ++ (wild; AA) genotype; 190 bp) (Fig. 1).

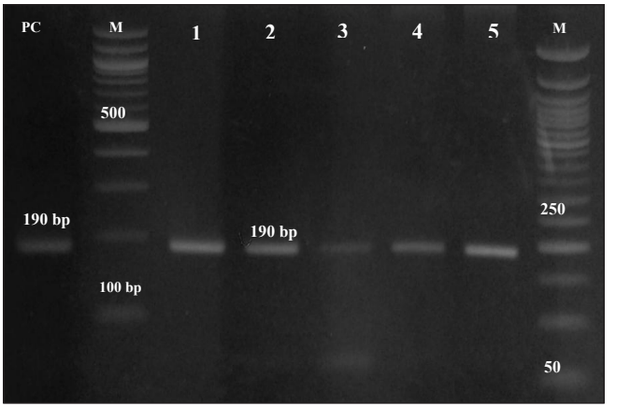

Fig. 1. *FecB*/*Ava*II PCR-RFLP assay in 1.5% agarose gel showing monomorphic pattern in Muzaffarnagri Sheep. Lane M1: Marker (100 bp DNAladder, New England Biolabs, Cat No. N3231S), M2: Marker (50 bp DNAladder, New England Biolabs, Cat No. N3231S), Lane 1-5: RFLP product of 190 bp

Table 1: Genotypic frequencies of *BMPR1B* **(***FecB***)**/*Ava*II gene in different sheep breeds as observed by various authors

Breed	Genotypic frequency			Allelic frequency		References
	BB (%)	B+ (%)	++ (%)	B	+	
Garole × Malpura	9.30	60.50	30.20	0.40	0.60	Kumar *et al.*, 2008
Prolific Garole	23.00	77.00	0.00	0.61	0.39	Polley *et al.*, 2010
Indian Bonpala	75.00	23.00	2.00	0.87	0.13	Roy *et al.*, 2011
Zel	0.00	1.47	98.53	0.74	0.26	Asadpour *et al.*, 2012
Shahabadi	0.0	76.00	24.00	0.37	0.63	Debnath and Singh, 2014
Lori	0.00	5.20	94.80	0.026	0.974	Nanekarani *et al.*, 2016
Chotanagpuri	60.80	34.30	4.80	0.413	0.587	Oraon *et al.*, 2016
Indonesian Fat Tailed	6.00	26.80	67.20	0.193	0.807	Maskur *et al.*, 2016
Nellore	2.50	12.50	85.00	0.09	0.91	Praveena *et al.*, 2017
Deccani	0.0	12.50	87.50	0.065	0.935	
Indian Kendrapada	35.30	49.40	15.30	0.60	0.40	Dash *et al.*, 2017
Kenguri × NARI Swarna	0.00	76.60	23.30	0.383	0.616	Asharani *et al.*, 2018
Muzzafarnagari	0.00	0.00	100.0	0.0	1.0	Present study

BB (mutant homozygote; GG) and +B (carrier; AG) genoptype was absent in the screened population. This revealed that all the screened sheep population used in the present study were monomorphic in nature with only + allele (1.0) with ++ genotype (100%). In the present investigation, the genotypic frequency of B+ and BB genotypes was 0.0% while that of ++ genotype was 100% in all the screened animals. Similar reports were given by El-Hanafy and El-saadaani (2009) in the Egyptian sheep breed; Jamshidi *et al.* (2013) in Sangsari sheep breed of Iran; Somarney *et al.* (2013) in Malin and Dorper sheep breed; Debnath and Singh (2014) in Balangir and Bonpala sheep; Yatoo *et al.* (2015) in Dorper sheep breed; Mishra *et al.* (2018) in Kajali sheep breed and Asharani *et al.* (2018) in Kenguri sheep breed. However, several authors observed polymorphic pattern in these genotypes with different genotypic frequencies (Table 1).

In the present work, the allelic frequency of allele + (A) and B (G) was of 1.0 and 0.0, respectively in all the screened Muzzafarnagari sheep. Similar results were reported by El-Hanafy and El-saadaani (2009) in the Egyptian sheep breed; Jamshidi *et al.* (2013) in Sangsari sheep breed of Iran; Al-Thabhawee *et al.* (2014) in Local sheep breed; Mishra *et al.* (2018) in Kajali sheep breed and Asharani *et al.* (2018) in Kenguri sheep breed. In contrast, several authors reported the presence of mutant allele B along with wild allele + with varying degree of frequencies (Table 1).

In the present study, *Fec*B mutaion was absent in the screened population despite of flock having 27% twinning rate. Similar reports of high litter size with absence of *Fec*B mutation was also reported previously (Yatoo *et al.*, 2015; Mishra *et al.*, 2018). It seems to be *Fec*B A allele was (+; wild type) fixed in the screened flock. Twinning present in our flock may be due to environmental effect and association with some other genes related to fertility.

CONCLUSION

In the present study, the *Ava*II/PCR-RFLP assay of 190 bp *FecB* amplified product revealed only AA (++) genotype (190 bp uncut, 100%) with A (+) allele (1.0) in screened sheep population. The studied region of the *FecB* gene showed monomorphic pattern revealed that *FecB* A allele seems to be fixed in screened sheep population despite the fact that the sampled population had 27% twinning rate. Consequently, we could not perform association analysis with reproduction trait. Therefore it would be suggested to further screening the status of this SNP along with other fecundity genes in large diversified population to exploit it under marker assisted selection.

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