Original Research

Genetic Polymorphism Study of *FecX*^{G} Mutation in Exon 2 Region of Bone Morphogenic Protein 15 (*BMP15*) Gene in Indian Muzzafarnagari Sheep Breed

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Abstract

Bone morphogenetic protein 15 (BMP15), member of TGF-β family, expressed in oocyte is essential for ovulation. The BMP15 gene of sheep links to the X chromosome so it is also known as FecX gene. The FecX^{G} mutation in BMP15 gene is characterized by an C>T substitution (Q239Ter) at coding position 718 in exon 2 region that results in the insertion of a premature stopping point in the transcription of the protein, associated with increase in ovulation in sheep. 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 141 bp amplified fragments of the of BMP15 gene were digested with HinfI restriction enzyme. The HinfI/PCR-RFLP assay of BMP15 gene revealed only GG genotype (141 bp uncut, 100%) with G allele (1.0) in screened sheep population. The studied region of the BMP15 gene showed monomorphic pattern. It revealed that FecX G allele seems to be fixed in screened sheep population.

Key words: *BMP15*, Exon 2, *Hinf*I, Muzzafarnagari, PCR-RFLP, Ovulation

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Introduction

Bone morphogenetic protein 15 (*BMP15*), also known as *FecX* is a member of the TGF-β superfamily. It expresses in oocytes and has potent role in prolificacy of sheep that affect the ovulation rate. The ovine *BMP15* gene links to the X chromosome (*FecX* locus) and consists a coding 1179 nucleotide sequence in

two exons, separated by a 5.4 kb intron, encoding a 393 amino acid residue precursor and a 125 amino acid mature peptide (Galloway *et al*., 2000). *BMP15* regulates granulosa cell proliferation and differentiation by promoting granulosa cell mitosis, suppressing follicle-stimulating hormone receptor expression and stimulating kit ligand expression, all of which play a pivotal role in female fertility in mammals (Juengel *et al*., 2002; Moore *et al*., 2004; Chu *et al*., 2007). Five different *BMP15* mutated alleles, called *FecX* alleles led to increased ovulation rates in heterozygous ewes and sterility in homozygous ewes (Hanrahan *et al*., 2004). In previous studies, heterozygous ewes with $FecX^{I}$ (Inverdale), $FecX^{H}$ (Hanna), $FecX^{B}$ (Belclare), $FecX^{G}$ (Galway) or $FecX^{L}$ (Lacaune) mutations exhibited one to two additional ovulations compared with non-carriers, whereas homozygous ewes were sterile (McNatty *et al*., 2005). This finding indicates the critical impact of *BMP15* or *FecX* on ovarian function in mammals (Bodin *et al*., 2007). Among them, $FecX^{G}$ mutation is characterized by the C to T transition at nucleotide 718 position in exon 2 region. This introduces a premature stop codon in the place of glutamic acid at amino acid residue 239 (Q239Ter) of the unprocessed protein, which presumably resulted in complete loss of *BMP15* function. The $FecX^{G}$ mutation was associated with increased ovulation rate and sterility in Cambridge and Belclare sheep (Hanrahan *et al*., 2004).

SNP *C>T* has been reported in exon 2 of the *BMP15* gene and associated with prolificacy in several exotic (Chu *et al*., 2007; Javanmard *et al*., 2011; Moradband *et al*., 2011 and Elkorshy *et al*., 2013) and few Indian sheep breeds (Kumar *et al*., 2008). However, scanty information is available on $FecX^{G}$ polymorphism in Indian sheep breeds including Muzzafarnagari breed. Therefore, the present study was undertaken to investigate the status of $FecX^{G}$ (*C>T*) polymorphism in exon 2 region of *BMP15* gene in Indian Muzzafarnagari sheep breed.

Materials and Methods

Animals Source, DNA Extraction and HinfI/PCR-RFLP

A total of 200 adult females (2-5 years of age) of Muzzafarnagari sheep maintained at Livestock Farm Complex (LFC), DUVASU, Mathura (U.P.), were utilized in the present investigation. Genomic DNA was extracted from venous blood using the standard protocol of Sambrook and Russel (2001). The PCR cycle conditions and primers (F: 5′- CACTGTCTTCTTGTTACTGTATTTCAATGAGAC -3′ and 'R: 5′- GATGCAATACTGCCTGCTTG -3′) were used for amplification of *BMP15* exon 2 region as per method described by Moradband *et al*. (2011). The PCR-RFLP was carried out overnight at 37°C with *Hinf*I restriction enzyme in a total volume of 15µl containing 5.0 µl of PCR product, 1.5 µl of 10X RE buffer and 10 units (1.0 µl) of enzyme.

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Statistical Analysis

The data was generated by estimating the frequency of different *BMP15* genotypes. The allelic and genotypic frequencies were estimated by standard procedure (Falconer and Mackay, 1996).

Results and Discussion

The amplified fragments of the *BMP15* exon 2 region revealed 141 bp product (Fig. 1). The results revealed that all the screened samples of Muzzafarnagari sheep were found monomorphic for *FecX*^{G} gene. The *Hinf*I PCR-RFLP assay revealed only one type of banding pattern GG genotype, which was of 141 bp (uncut) in all the screened samples.

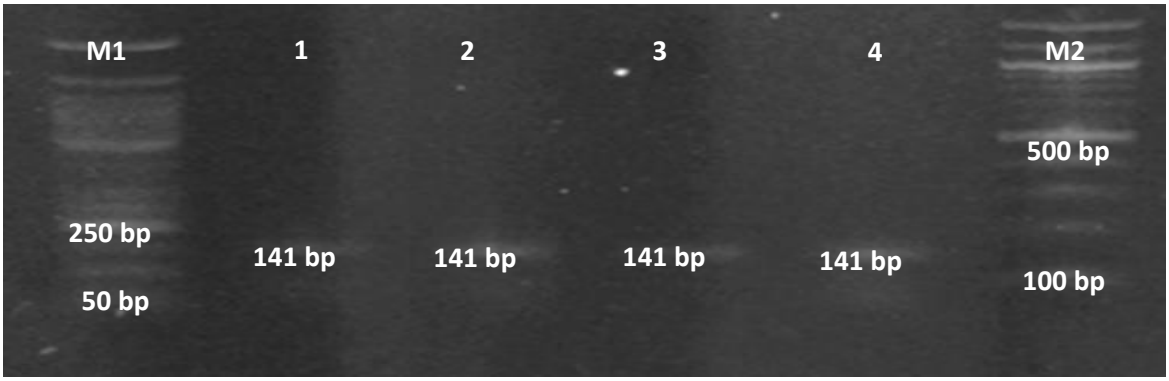

Fig. 1: *Hinf*I/PCR-RFLP assay of *BMP15* (*FecX*) gene revealed by 1.5% agarose gel electrophoresis in Muzaffarnagri sheep, M1: Marker (100 bp DNA ladder, New England Biolabs, Cat No. N3231S), Lane 1: 141 bp PCR product Lane 2-4: RFLP product of 141bp showing uncut 141 bp; GG genotype.

The genotypic frequencies of G+, ++ and GG genotypes were 0.0%, 0.0% and 100%, respectively in all the screened animals and the allelic frequency of allele + and G was observed as 0.0 and 1.0, respectively. These findings were similar to the report of Kasiriyan *et al*. (2011) in Sangsari sheep breed while were in contrast with the findings of Polley *et al*. (2010) and Kumar *et al*. (2015) in Garole. Dincel *et al*. (2018) in Chios and Pineda *et al*. (2018) in Colombian Creole sheep breeds wherein ++ (wild type) genotype was observed with fixed wild allele (+). However, the genotypic frequencies of ++, G+ and GG genotype as observed by Elkorshy *et al*. (2013) in Barki, Ossimi, and Rahmani sheep breeds, Chu *et al*. (2014) in Small Tailed Han sheep breed and Barakat *et al*. (2017) in Barki, Ossimi, and Rahmani sheep breeds were not in accordance with the findings of the present study as they have observed polymorphic pattern in these genotypes with varying degree of frequency of mutant allele G (Table 1).

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Table 1: Genotypic frequencies of *BMP15* (*FecX*)/*Hinf*I gene in different sheep breeds as observed by other authors

Breed	Genotypic Frequency			Allelic Frequency		References
	GG (%)	G+ (%)	++ (%)	G	+	
Garole	0	0	100	0	1	Polley *et al*., 2010
Sangsari	100	0	0	1	0	Kasiriyan *et al*., 2011
Barki	0	68	32	0.66	0.34	Elkorshy *et al*., 2013
Ossimi	0	70	30	0.65	0.35	
Rahmani	0	71	29	0.64	0.36	
Small Tailed Han	0	60	40	0.3	0.7	Chu *et al*., 2014
Garole	0	0	100	0	1	Kumar *et al*., 2015
Mehraban	25.9	74.1	0	62.9	37.1	Nadri *et al*., 2016
Lori	54.1	45.9	0	22.9	77.1	
Barki	0	68	32	0.34	0.66	
Ossimi	0	72	28	0.36	0.64	Brakat *et al*., 2017
Rahmani	0	76	24	0.38	0.62	
Kenguri	0	100	0	0.5	0.5	Asharani *et al*., 2018
Kenguri X NARI Swarna	0	96.7	3.3	0.484	0.516	
Chios	0	0	100	0	1	Dincel *et al*., 2018
Colombian Creole	0	0	100	0	1	Pineda *et al*., 2018
Muzzafarnagari	100	0	0	1	0	Present study

Conclusion

In the present study, we observed monomorphic pattern of $FecX^{G}$ mutation in *BMP15* gene in screened Muzzafarnagari breed, consequently we could not perform association study with reproduction trait because in these screened sheep $FecX^{G}$ allele was found fixed. However, all the screened animals were fertile and 27% twinning rate was found in our Muzzafarnagari flock. These results may be due to small sample size and close organized herd from where the samples were taken for study making it imminent to further investigate this SNP along with other fecundity genes on large diversified population for better understanding of functioning of reproduction traits in sheep.

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