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Genetic polymorphism of bone morphogenetic protein receptor type–1 gene in Black Bengal goat and its association with litter size

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ABSTRACT

The genetic polymorphism of the bone morphogenetic protein receptor type–1 gene (BMPR1B) was studied in 100 does of Black Bengal goats. The study revealed 2 genetic variants, A (0.25) and G (0.75) and 2 genotypes, AG (0.5) and GG (0.5). Genotypic and allelic frequencies at BMPR1B locus revealed the abundance of mutant type (G) nucleotide in Black Bengal goats. All animals, were found to carry the mutant allele. A positive correlation was observed between the litter size and mutant type genotype. Heterozygous carrier and homozygous carrier does had litter size of 1.6± 0.26 and 2.07 ±0.28 respectively. The results showed that BMPR1 B polymorphism may be targeted for upgrading low prolific breeds of Indian goat by introgression of this mutation in non prolific breeds by crossing.

Key words: Association, Black Bengal goat, BMPR1B gene, Litter size

Prolificacy, the ability of a female animal to produce large number of young ones in their life span through high ovulation rate and high embryo survival, is affected by many genes in small ruminants. These include bone morphogenetic protein receptor type–1 gene (BMPR1B), growth differentiation factor -9 (GDF9), bone morphogenetic factor (BMP15) gene, estrogen receptor gene, Kisspeptin gene and others. Candidate gene approach of these prolificacy genes provides an alternate tool for early breeding that can accelerate the improvement of goat (Zhang *et al.* 2009). Inheritance of twinning and triplet is similar in both sheep and goats (Hua *et al.* 2008). These genes have been extensively studied in sheep unlike goat. Studies on relationship between ovulation rate and litter size reported that a set of genes were regulating this complex phenomenon known as fecundity (*Fec*) genes (Davis *et al*.1982, Baird *et al*.1998). These belong to the transforming growth factor beta (TGF-ß) super family. Limited studies have been attempted on prolificacy of goats. Therefore, the genetic basis of caprine prolificacy needs to be explored.

Indian goat breed, Black Bengal, is an important breed for mutton production and most prolific. Twinning is more frequent (56.32%) and quadruplet is least frequent (2.11%) (Hassan *et al.* 2007). It shares the breeding tract of Garrole sheep, which has been extensively studied for prolificacy. Bihar contributes about 7.63% of India's total goat population. Village goat is mostly of Bengal breed. However, crosses with other breeds like Jamunapari, Barbari, Sirohi and Jakharana are also available. This breed is highly prolific and twining percentage has been recorded as 45 (De *et al.* 2007). There is an urgent need to investigate the genes linked to prolificay, which could enable us to formulate the breeding strategies to introduce the prolificacy into local breeds and further genetic improvement. Polley *et al.* (2009) studied major genes responsible for prolificacy in Black Bengal from West Bengal and found BMPR 1B also known as fecundity b (*Fec B*) gene as polymorphic. The present study is in continuation of study by Polley *et al.* (2009) and aims at further studying the polymorphism of BMPR1B gene in Black Bengal goats from Bihar in India and to investigate its linkage with increased prolificacy.

MATERIALS AND METHODS

Experimental flock and sampling: Blood samples of 100 Black Bengal does were randomly collected from does maintained at institute farm and different villages of Bihar. Approximately 5 ml blood sample was collected from jugular vein in EDTA tube and was transferred to –20°C freezer. The history of litter size in respective kidding for every doe was collected from the farmers.

DNA isolation: DNA was extracted from white blood

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cells using a standard phenol/chloroform/isoamyl alcohol extraction protocol (Sambrook and Russel 2001).

PCR amplification and gel electrophoresis: Allele specific amplification of the BMPR1B gene was achieved as per Polley *et al.* (2009). Two sets of primers were used for allele specific amplification of BMPR1B gene. Forward: GCT GGT TCC GAG AGA CAG AAA TAT ATCA, Reverse: CCC CGT CCC TTT GAT ATC TGC AGC AATG was used for targeting 1100 bp for the identification of wild allele (A) of BMPR1B gene.

Forward: GTC GCT ATG GGG AAG TTT GGA TGG GAA, Reverse: ATG TTT TCA TGC CTC ATC AAC ACC GTCC was used for targeting 136bp for the identification of mutant allele (G) of BMPR1B gene. Both PCR reactions were performed in a 25 µl mixture containing 10 pmol primers, 200 µM dNTP (deoxyribonucleotide triphosphate), 25 mM $MgCl_2$, 1 unit of Taq polymerase and 50 ng genomic DNA as template. The PCR cycling parameters were optimized separately for detecting each of the allele specific. Touch-down PCR method was used to optimize the reaction accuracy: 94°C for 5 min, 35 cycles of 95°C for 30 sec, touchdown annealing from Touchdown from 60°C to 52°C then at 52°C (30 cycles for 40 sec) for the wild allele A and Touchdown from 70°C to 65°C then at 65°C (30 cycles for 45 sec) for the mutant allele G, 72°C for 45 sec, and a final extension at 72°C for 7 min. PCR products were electrophoretically separated on 2% agarose gel (5 V/cm) and stained with ethidium bromide and photographed using a molecular imager. Allele discrimination was based on size differentiation (bp) of respective alleles in BMPR1B gene.

RESULTS AND DISCUSSION

BMPR1B gene polymorphism: Allele specific amplification in Black Bengal goats revealed that BMPR1B gene is polymorphic in nature. Two alleles A (wild type nucleotide-non carrier) and G (*Fec B* mutant nucleotide-carrier) were identified at this locus. Two different genotypes AG (1100 bp and 136bp) and GG (136bp) were detected (Fig. 1). The presence of the 'A' nucleotide in wild type animals codes for glutamine amino acid but presence of 'G' replaces this amino acid with arginine. The genotypic and allelic frequencies at BMPR1B gene in Black Bengal goats were calculated and presented in Table 1.

The frequencies of AG and GG genotypes existed in equal frequency in the population studied. The allelic frequencies for A and G alleles were 0.25 and 0.75 respectively. It indicated the abundance of mutant type (G) nucleotide in Black Bengal goats. Similar alleles A and G were reported by Polley *et al*. (2009). Three genotypes AA,

Table 1. Allelic and genotype frequencies of Black Bengal goat for BMPR1B gene

Genotype	Genotype frequency	Allele frequency
AG (50)	0.5	G=0.75
GG (50)	0.5	A=0.25

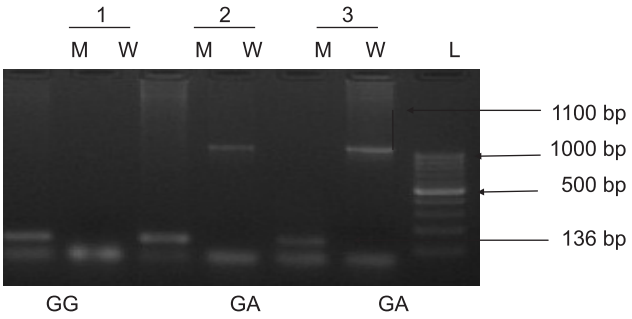

Fig. 1. Agarose gel electrophoresis (2%) of allele specific *Fec B* (BMPR1B) PCR product. Lanes 1–3 amplification of mutant (M, 136 bp) or wild type (W, 1100 bp) and both in Black Bengal goat genomic DNA. L, DNA molecular weight marker. Lane 1 represents homozygous mutant having presence of only mutant allele. Lanes 2 and 3 represent heterozygous mutant having presence of both mutant and wild allele.

AG and GG were detected with the frequencies 0.11, 0.64 and 0.25 respectively. The allele frequency for A and G allele were 0.43 and 0.57 respectively. Unlike the findings of Polley *et al.* (2009) no homozygous wild type genotype was detected in our study. All animals were found to be carrier for mutant allele. The absence of homozygous wild type genotype may be due to uneven distribution of genotypes in the field or may be some natural selection may exist, such that more prolific goats have more chances of survival. This also presents a situation where the wild type genotype is endangered and so, conservation programme is required for the same. Our findings contradicted the findings of Ahlawat *et al.* (2011) in Black Bengal who reported monomorphic for BMPR1B gene. Prolificacy of Raigarh goat from Odisha was also independent of BMPR1 B (Fec B) gene polymorphism (Palai *et al.* 2013).

Effect of BMPR1B gene polymorphism on litter size: Number of mutation in BMPR1B gene is directly proportional to litter size and ovulation rate. This increase in ovulation rate of *Fec B* carriers is associated with a precocious maturation of many of antral follicles that may undergo ovulation with a smaller size (Fabre *et al.* 2006). The *Fec B* mutation has so far been identified in the Booroola Merino (Souza *et al.* 2001), Garole, Javenese (Davis *et al.* 2002) and Small Tailed Han, Hu sheep (Davis *et al.* 2006). In the present study, positive correlation was observed between litter size and mutant genotype. Heterozygous carrier and homozygous mutant does had litter size of 1.6± 0.26 and 2.07 ±0.28 respectively (significant difference at 5% level). Earlier study reported that heterozygous carrier and homozygous mutant does had 3.04 and 3.1 respectively (Polley *et al.* 2009). The results showed that these goats have high litter size as compared to Jamunapari, Sirohi, Jakhrana and Barbari. Role of *Fec B* mutation as an important tool for making breeding decisions in Indian sheep was assessed by Pardeshi *et al.* (2005). Similarly, this *Fec B* mutation can be used as a marker tool for selecting prolific goats and for improving less prolific goat breeds.

Further validation studies are required to confirm the link with increased prolificacy in Black Bengal goats and to investigate association of mutant genotype with survivability. This will be fruitful in formulating breeding strategies to maximize the benefits of increased prolificacy in these breeds and introgression of this mutation in non prolific breeds by crossing.

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