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Analysis of *BMP4* gene *HaeIII* polymorphism in Assam Hill goat

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Abstract
BMP4 has been studied as a candidate gene for prolificacy in many species. The objective of the present study was to detect incidence of mutation in exonic region of *BMP4* gene of Assam Hill goat. Blood samples pertaining to 80 randomly selected Assam Hill goats having a kidding history of single as well as multiple births maintained at three field units' *viz*., Batabari, Nahira and Tetelia under "All India Coordinated Research Project on Goat Improvement" were utilized. DNA was extracted using modified phenol chloroform extraction method. The quantity and quality of extracted DNA were assessed using spectrophotometry and agarose gel electrophoresis. A 517 bp fragment of *BMP4* gene was amplified by using Polymerase Chain Reaction. The digestion of purified product with *HaeIII* restriction enzyme produced two fragments of 151 bp and 366 bp in agarose gel electrophoresis for all the samples revealing monomorphic banding patterns. Nucleotide sequencing revealed a single nucleotide mutation 159 (A→ T) in samples having a history of multiple birth. However, further research on substantially large number of individuals of Assam Hill goat is needed to validate the finding.

Keywords: Assam Hill goat, *BMP4*, polymorphism, PCR-RFLP

1. Introduction
Kidding percentage is the most important factor affecting profitability in goats. Improvement of reproduction by traditional selective breeding methods has been proved to be difficult due to low heritability [1] and a long reproductive cycle. Thus, there is a need to find out key mutations in the candidate genes and effect of these mutations on fecundity; which will be a rapid and economical method to improve the speed of goat breeding. Candidate gene approach provides a good breeding tool that can enhance the frequency of multiple births early in life, which has been proposed as a direct search for Quantitative Trait Loci (QTL) to improve quantitative traits [2]. The information utility from candidate genes in breeding programs has the potential to substantially enhance the accuracy of selection and increasing selection differential [3]. Detection of genetic markers, along with mutants of the genes associated with economically important traits, could assist the breeders in designing practical animal breeding plans [4].

Bone Morphogenetic Proteins (*BMP*s) are candidate genes belonging to the member of the *TGF-β* (Transforming Growth Factor-beta) super family. *BMP*s play a major role in embryonic development, homeostasis, repairing of various tissue patterning, cell differentiation and apoptosis [5]. *BMP*s are important due to their crucial role in follicular growth and differentiation, cumulus expansion and ovulation [6]. So far, more than 30 members have been identified in *BMP* family of which *BMP4* is the most important one. *BMP4* can inhibit progesterone production by granulosa cells and decrease basal granulosa cells progesterone secretion and totally abolish FSH-stimulating action both in cattle [7, 8] and sheep [9, 10]. Therefore, *BMP4* could have implications for reproductive function in mammals.

Assam Hill goat is an important goat germplasm found in Assam. It is a meat type animal with high prolificacy, which is characterized by small body size, shorter generation interval with a higher percentage of multiple births. Till now, no study has been carried out regarding the identification of *BMP4* gene polymorphism in Assam Hill goat. Therefore, the present study was carried out to detect the presence of polymorphism in the *BMP4* gene of Assam Hill goat.

2. Materials and Methods

2.1 Collection of blood and extraction of DNA

A total of 80 blood samples from randomly selected Assam Hill goats having a kidding history of single as well as multiple births, maintained at three field units *viz*., Batabari, Nahira and Tetelia under 'AICRP on Goat Improvement', Goat Research Station, Burnihat were utilized in the present study. Out of these, 10 samples from animals with history of single birth were taken as control. Five ml of blood was collected aseptically from the jugular vein in a vacutainer tube containing 2.7% EDTA as an anticoagulant. The samples were brought to the laboratory in double walled ice-boxes containing ice packs and stored at -20 °C until the genomic DNA was extracted. Genomic DNA was extracted using phenol chloroform extraction method [11] with slight modifications by using DNA zol reagent instead of SDS and Proteinase K. The purity of genomic DNA was assessed by UV spectrophotometer (Nanodrop Spectrophotometer, Model: UV/VIS 916) and Optical Density (OD) values were measured at 260 and 280 nm with TE buffer as blank. The concentration of genomic DNA was estimated spectrophotometrically by taking OD value at 260 nm. Quality of isolated genomic DNA samples was checked by using agarose gel electrophoresis which was visualized under gel documentation system (Kodak 100).

2.2 PCR amplification

One pair of PCR primer F: 5'-ACGAAGGTCAGTCCCTACC3' and R: 5'-ACCAAACATTTCCCCAGCGA3' (GenBank accession no. EU104684.1 using PRIMERSELECT program of LASERGENE software DNASTAR Inc., Madison, WI, USA) was designed to amplify the *BMP4* gene. PCR was carried out in 50 µl volume containing 1 µl of 10pmol/µl each primer, 2 µl DNA template, 1 µl $MgCl_2$, 25 µl master mix and 20 µl nuclease free water. Amplification condition for the gene was as follows:

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension
BMP4	94 °C for 5 minutes	94 °C for 45 seconds	57 °C for 45 seconds	72 °C for 45 seconds	72 °C for 10 minutes
		35 cycles			

The obtained PCR products were separated and confirmed by horizontal submarine agarose gel electrophoresis (1.5%) in 1X TAE buffer at 110V using 100 bp DNA ladder.

2.3 Restriction Fragment Length Polymorphism (RFLP) analysis

The PCR products (20 µl) of *BMP4* gene were digested with restriction enzyme *HaeIII* (New England Biolab, UK). The reaction mixture was vortexed for few seconds for uniform mixing and then incubated at 37 °C for overnight. The enzyme digested products were loaded @ 10 µl on 2.5% agarose gel. Electrophoresis was carried out at 110 V for 1 hour and 15 minutes and the bands were visualized and documented using gel documentation system. The bands were analyzed by comparing with 50 bp DNA ladder.

2.4 Sequence analysis

The PCR amplicons of BMP4 gene from goats with history of single as well as multiple births were sequenced at first base DNA sequencing division, Malaysia by automated DNa sequencer following Sanger's dideoxy chain termination method [12]. The sequences were analyzed by using Clustal W method of DNASTAR Software (Lasergene, USA) to generate sequence alignment reports and residue substitution.

3. Results and Discussion

A single band on agarose gel confirmed the extraction of DNA. The yield of DNA extracted from 2 ml of whole blood ranged from 106 ng /µl to 247 ng/µl with a mean of 181 ng/µl which was diluted in 0.5M Tris EDTA (TE) buffer to form a uniform final concentration of 100 ng/ µl. The OD ratio was in the range of 1.7-1.9 indicating purity of extracted DNA.

Amplification of *BMP4* gene with the primers resulted in generation of 517 bp DNA fragment (Fig 1) which is consistent with the expected size as determined from their gene sequence information.

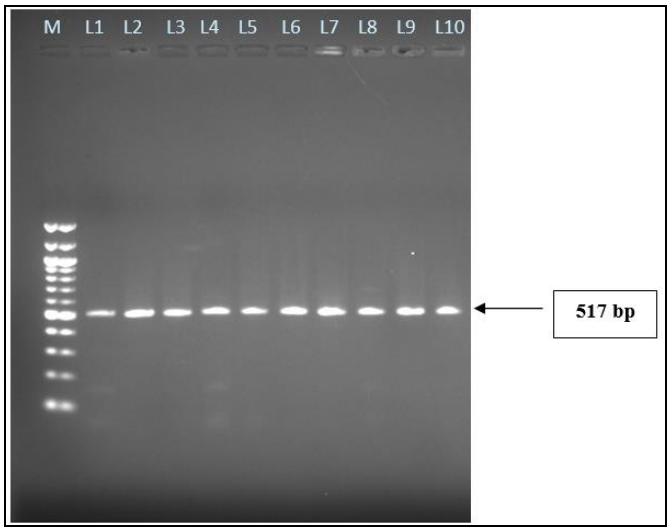

Fig 1: PCR AMPLIFICATION OF *BMP4* GENE (517 bp) L1-L10: PCR amplicons of *BMP4* gene of Assam Hill goat M: Marker 100

In order to determine the polymorphism, if any in amplified fragment of BMP4 gene, a restriction enzyme *HaeIII* having recognition site (GG/CC) was used to digest 517 bp fragment. The RFLP analysis revealed single type of banding pattern yielding two fragments, each 151 bp and 366 bp (Fig. 2). The monomorphic banding patterns of the digested product revealed that all the animals possessed similar genotype. Though the objective of the present study was to identify *HaeIII* polymorphism within *BMP4* gene in Assam Hill goat, but we could not find any mutation in the restriction site. It has been reported that *BMP4* is one of the best evolutionary conserved growth factors [13] and there were few reports about *BMP4* gene SNPs, so this result is a good authentication for it. This result is also in agreement with the findings for exon 2 of *BMP4* gene in Jining Grey, Inner Mongolia Cashmere goats, Angora and Boer goats [14], and in Xuhuai White, Boer and Haimen goats [15], respectively.

The sequences of the amplified region of different samples were analyzed. These sequences were then aligned and compared using BLAST. The sequence alignment revealed

99-100% similarity in the amplified region of BMP4 among the screened samples. However, a distinct SNP (single nucleotide polymorphism) was observed at position 159 (A→T) in samples with history of multiple birth and single birth (Fig. 3 and 4). Similar to this finding, an SNP (G1534A) was identified in exon 2 and a microsatellite in 3′ flanking region of *BMP4* gene in nine different goat breeds of India *viz.*, Barbari, Beetal, Black Bengal, Malabari, Jhakrana, Osmanabadi, Sangamneri, Sirohi and Ganjam [16]. In a study on Small Tail Han sheep, Chu and his co-workers [17] also reported one single nucleotide mutation C→A at 305 bp of exon 3 of BMP4 gene in genotype BB in comparison with genotype AA and found that genotype BB had 0.61 or 1.01 lambs more than those with genotype AB or AA. However, in the present study, the numbers of samples sequenced were less in number and thus an association study could not be carried out. Nevertheless, further studies using a greater number of individuals are warranted.

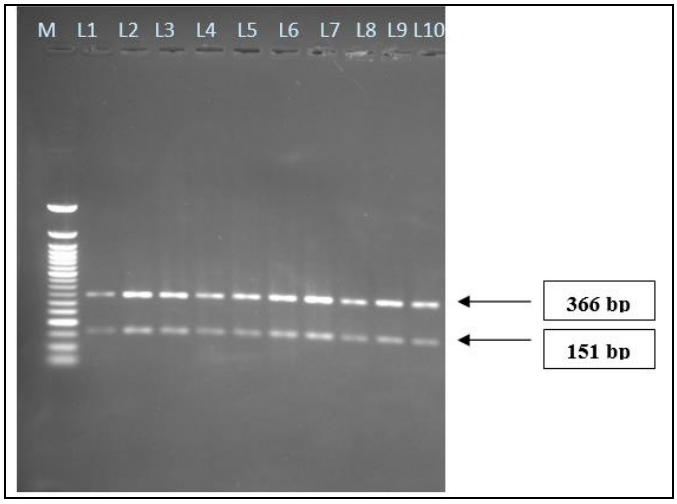

Fig 2: PCR-RFLP OF *BMP4* GENE USING *HaeIII* (151 and 366 bp) L1-L10: Digested products (151 and 366 bp), M: Marker 50 bp

Fig 3: Screenshot Of The Sequence Of *Bmp4* Gene

Fig 4: Clustal Multiple Sequence Alignment of *Bmp4* By Muscle 3.4 Showing Snp At Location 159

4. Conclusion

In the present study, the results showed monomorphic banding pattern in all the studied samples of Assam Hill goat with respect to *HaeIII* restriction site of BMP4 gene.

However, sequencing of the amplified region revealed an SNP at position 159 of the amplified fragment which could be related to prolificacy based on litter size records. This SNP may serve as valuable genetic marker for litter size in Assam Hill goat provided a GWAS (genome wide association study) using a great number of individuals is warranted. Due to the crucial role of *BMP4* in follicular growth and differentiation, cumulus expansion and ovulation, further investigation on other regions of the BMP4 gene for its possible influence on reproductive and growth performance is desirable.

5. Acknowledgement

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