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Genetic polymorphism of BMPR1B and BMP15 genes and their association with reproductive and growth traits in Barbari, Sirohi and black Bengal goats

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Abstract
The present research has been planned to investigate the genetic polymorphism of BMPR1B and BMP15 genes and their association with the reproductive and growth traits in 30 each of Barbari, Sirohi and Black Bengal goats. The genotyping was carried out by PCR-RFLP technique using restriction enzymes *AvaII* and *HinfI*, respectively for BMPR1B and BMP15 gene. The amplified PCR products of size 190 bp and 141 bp were obtained for BMPR1B and BMP15 gene, respectively. PCR-RFLP analysis of both these genes showed monomorphic pattern in all the three breeds of goat, which revealed only one type of uncut banding pattern of 190bp for BMPR1B and 141bp for BMP15 gene. Therefore, the association study with different economic traits could not be performed at BMPR1B/*AvaII* and BMP15/*HinfI* genes loci. The body weights from 15 to 90 days and 3 to 6 months of age were significantly (p<0.05) higher in Sirohi goats followed by Barbari and Black Bengal. Minimum age at sexual maturity (days) was recorded in Black Bengal goats followed by Sirohi and Barbari goats. Significantly (P<0.05) lower age at first kidding was recorded in Black Bengal followed by Barbari and Sirohi goats. The mean litter size at first and second kidding (Nos.) was significantly (P<0.05) higher in Black Bengal as compared to Barbari and Sirohi goats. Monomorphism was observed in the BMPR1B/*AvaII* and BMP15/*HinfI* genes in screened populations of Barbari, Sirohi and Blank Bengal goats.

Keywords: BMPR1B, BMP15, PCR-RFLP, Barbari, Black Bengal, Sirohi

Introduction
India is a rich repository of indigenous goat genetic resources having 34 well recognized goat breeds, which farms the backbone of rural livelihood security. Apart from this, majority of lesser-known populations of goat represent not only the life line of the rural people but also signify a vast gene pool with untapped potential for future commercial purposes [12]. Goat breeds vary in their physiological characteristic, including ovulation rate and prolificacy. The ovulation rate is determined mainly by the number of ova shed in each oestrus cycle [11]. The heritability of fertility traits is low because environment plays a major role controlling the traits and supposed to be controlled by number of genes. Major genes have potential impact to increase prolificacy in goat as these genes are being manipulated for improving the production performance in selection programme. Booroola gene is the first gene discovered in sheep having an important role in increasing prolificacy of the Merino due to the segregation of FecB gene and the additive effect of mutation for ovulation rate with an increase of 1.65 for each copy [5].

The genetic diversity based on fecundity genes is an essential step towards the future exploitation of the available goat genetic resources in research and breeding programs. Any associated SNP could be used to accelerate the improvement of goat reproductive traits by identifying high prolific animals at an early stage of life. Single nucleotide polymorphisms (SNPs) are the most abundant form of DNA polymorphism which can be used as simple genetic markers for many breeding applications as well as for population studies [4]. The bone morphogenetic protein receptor-type-1B (BMPR1B) gene and bone morphogenetic protein-15 (BMP15) gene have been reported to be the major candidate genes for prolificacy in sheep. However, there is a paucity of information regarding genetic correlation of fecundity genes with economic traits in Barbari, Sirohi and Black Bengal breeds of goats.

Materials and Methods

Total number of 90 goats comprised of 30 each of Barbari, Sirohi and Black Bengal goats were randomly selected in the study from the flocks of three genetic groups maintained at Goat Breeding Farm, Amanala, N.D.V.S.U., Jabalpur (M.P.).About 3 ml blood from each animal was used for extraction of genomic DNA using the standard procedure [9]. The concentration, purity and quality of DNA were checked by UV spectrophotometer and agarose gel electrophoresis (0.8%). The published primers [7] were used for the amplification of BMPR1B (190 bp) gene (Forward: 5'-CCAGAGGACAATAGCAAAGCAAA-3'; Reverse: 5'-CAAGATGTTTTCATGCCTCATCAACAGGTC- 3') and BMP15 (141 bp) (Forward: 5'-CACTGTCTTCTTGTTA-3'; Reverse: 5'-CTGTATTTCAATGAGACGATGCAATACTGCCTGCTTG-3') gene. PCR amplification of each gene was carried out in a final reaction volume of 25 µl. Each PCR tube contains 2X PCR Master mix (Fermentas) 12.5 µl, forward and reverse primers 1.0 µl each, genomic DNA 3.0 µl (30 ng/µl) and DNAase free water 7.0 µl. The PCR tubes were kept in a thermo cycler (Veriti 96 well thermal cycler, Eppendorf) programmed for 35 cycles with initial denaturation at 95^0C/5 min, denaturation at 94^0C/45 sec, annealing at 60.0^0C/45 sec for BMPR1B gene and 63.0ºC/45 sec for BMP15gene, extension at 72^0C/45 sec and final extension at 72^0C for 10 min. PCR product was analyzed on 2.0 % agarose gel. 5 µl of amplified PCR product mixed with 1 µl of 6x gel loading dye (bromophenol blue) was loaded along with 100 bp DNA ladder as a molecular size marker in a separate lane. The electrophoresis was conducted at constant voltage of 80 volt for 90 min at 37^0C using 10X TBE buffer. The amplified products in the gel were visualized by UV transilluminator and photographed using Gel documentation system (Gel-Doc, Bio-Rad, USA).The restriction enzymes *AvaII* and *HinfI* were used to digest the amplified PCR products of BMPR1Band BMP15 gene, respectively. Restriction digestion of the PCR product was performed in a total 30µl reaction mixture having 10X Buffer Tango 2 µl, PCR reaction mixture 10 µl, restriction enzyme (10 units/µl) 1 µl and 17 µl nuclease free water. The reaction mixture was incubated at 37^0 C for overnight digestion in water bath and then electrophoresed on 3 % agarose gel containing 1% ethidium bromide @ 5 µl/100 ml at constant voltage of 80 V for 1 hour, using 0.5 X TBE buffer and 6X Gel loading dye (Bromophenol blue). 5µl of RE digested PCR product was mixed with 1µl of 6x gel loading dye (Bromophenol blue) and loaded into wells along with 100 bp DNA ladder (Fermentas Life Science, Range 100-1000bp) as a molecular size marker in a separate lane. The PCR-RFLP bands were visualized under UV light and documented by Gel documentation system (Gel-Doc, Bio-Rad, USA) and recorded after comparing with band size with 100 bp DNA ladder. Genotyping of gene at each locus (viz., BMPR1B/*AvaII* and BMP15/*Hinf*) was carried out according to the band pattern of respective genotypes.

The various growth (i.e. body weight at birth and then after fortnightly up to 3 months of age, monthly up to 6 months of age and weaning weight) and reproductive (i.e. age at sexual maturity, age at first kidding, Litter size per kidding) traits of Barbari, Sirohi and Black Bengal breeds of goats were recorded to find out association of polymorphic variants atBMPR1B and BMP15 gene loci.

Gene and genotype frequencies at different loci were estimated using Popgene 32 software [15]. The chi-square test [14] was used to test the population of Barbari, Sirohi and Black Bengal breeds of goat for Hardy Weinberg equilibrium. The least squares means and standard error of different economic traits were estimated by using computer statistical software (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.).

Results and Discussions

The amplified fragments of BMPR1B (190 bp) and BMP15 (141 bp) genes showed a size of 190 bp (Figure 1) and 141 bp (Figure 3), respectively, indicating the primer specific amplification.

The PCR product of similar size using same set of primers was also reported in different breeds of goat [3, 7, 13]. The PCR product of similar size was also reported by Polley *et al*., 2009[13] in Black Bengal goat and Islam *et al*., 2019[7] in Beetal and Teddy goats.

PCR-RFLP Analysis of BMPR1B and BMP15 gene

PCR-RFLP analysis of BMPR1B/*AvaII* and BMP15/*HinfI* gene showed monomorphic pattern in all the three breeds (viz. Barbari, Sirohi and Black Bengal) of goats under study. It revealed only one type of banding pattern of 190 bp (Figure 2) and 141bp (Figure 4) for BMPR1Band BMP15 gene, respectively in all the screened population of three breeds of goat. Only AA genotype was found in all the screened samples of three goat breeds. We could not identify any goat with AB and BB genotypes in the tested population of Barbari, Sirohi and Black Bengal breeds of goat. This indicated the absence of restriction site of *AvaII* restriction enzyme in 190 bp amplicon of BMPR1B gene and *HinfI* in 141 bp amplicon of BMP15 gene in the tested population of goats.

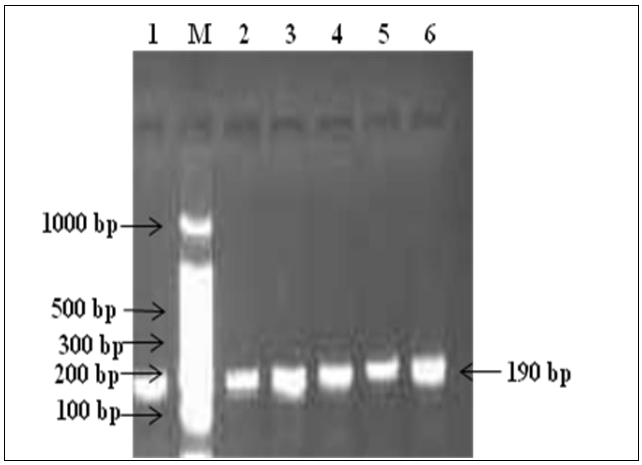

Fig 1: Amplified PCR product of BMPR1B gene on 2% agarose gel. Lane 1-2 (Barbari), 3-4 (Sirohi), 5-6 (Black Bengal), M (100 bp DNA ladder)

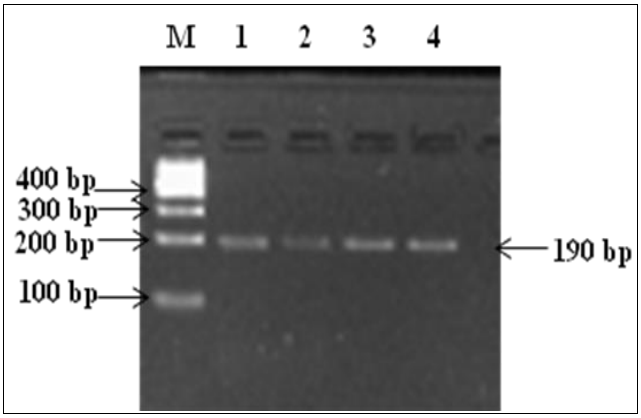

Fig 2: PCR-RFLP pattern of BMPR1B gene on 3% agarose gel Lane 1 (Barbari), 2(Sirohi), 3-4 (Black Bengal), M (100 bp DNA ladder) Lane 1-4 AA Genotype (190 bp)

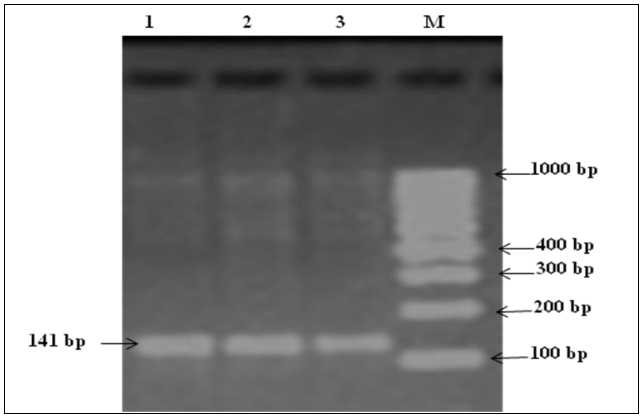

Fig 3: Amplified PCR product of BMP15 gene on 2% agarose gel Lane 1 (Barbari), 2(Sirohi), 3(Black Bengal), M (100 bp DNA ladder)

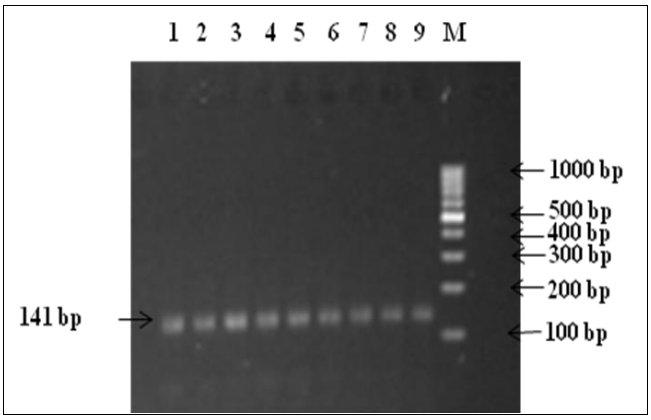

Fig 4: PCR-RFLP pattern of BMP15 gene on 3% agarose gel Lane 1-3 (Barbari), 4-6 (Sirohi), 7-9 (Black Bengal), M(100 bp DNA ladder) Lane 1-9 AA Genotype (141 bp)

Genotype frequency and gene frequency at BMPR1B and BMP15 gene locus

In the present study, all the screened populations of Barbari (30), Sirohi (30) and Black Bengal (30) breeds of goat were found to be monomorphic at both the gene loci. The present finding revealed only one type of restriction pattern (AA genotype); which was of 190 bp for BMPR1B and 141 bp for BMP15 gene. AB and BB genotypes were not observed in the tested populations of three breeds of goat at both gene loci. The genotypic frequency AA was 1.00 at both gene loci in all the screened breeds of goat under present investigation. The allelic frequency for allele A was 1.00 and for allele B was 0.00 in present investigation. Chi-square values for testing correspondence between observed and expected genotypic frequencies at this locus were found to be non-significant (P>0.05) in Barbari, Sirohi and Black Bengal goat, indicating that the populations of these three breeds of goat under study were in Hardy-Weinberg equilibrium (HWE) at these gene loci.

Similar findings of HWE were reported by Ahlawat *et al*., 2014 [2] in the promoter and exonic regions of BMPR1B gene in 8 indigenous goat breeds (Barbari, Beetal, Black Bengal, Malabari, Jakhrana, Osmanabadi, Sirohi and Ganjam). They also reported absence of any polymorphism in exonic regions of FecB gene in of all the investigated breeds of Indian goat.

The present results are in accordance with findings of the investigations reported by Kumar *et al.*, 2008 [10] in Malpura and Kendrapada sheep, He *et al.*, 2010 [6] in Chinese Goats and Aditya *et al.*, 2011[1] in Marwari goat. They reported absence of mutations at BMPR1B gene locus in goat. The present investigation at BMPR1B and BMP15 genes loci did not find any mutation due to absence of restriction site for *AvaII* and *HinfI* restriction enzyme, respectively. The absence of B allele in goat population taken under the present study may be due to relatively small sample sizes or there could be to negative effects of B allele on individual performance. Therefore, individuals with missing genotype BB may have been eliminated from the population during breeding process.

Association of polymorphic variants ofBMPR1B and BMP15 genes with economic traits

All the screened goats of Barbari, Sirohi and Black Bengal breeds were found monomorphic at BMPR1B/*AvaII* and BMP15/*HinfI* genes loci. Therefore, the association study of these two genes with different economic traits could not be performed. However, the comparative mean of different economic traits *viz.* body weight at birth, body weight at fortnightly interval from 15 day to 90 days of age, body weight at monthly interval from 4 months to 6 months of age, body weight at weaning, age at sexual maturity, age at first kidding, litter size at first and second kidding have been discussed below (Table 2 and 3).The result of least squares analysis of variance for above traits have showed significant (p<0.01) differences among breeds (Table 1).

Table 1: Least squares analysis of variance

S. No.	Traits	Mean Sum of Squares		F-value
		Breed (2)	Error (87)	
1.	Birth weight (0 day)	2.884	0.069	41.652**
2.	Body weight (15 days)	19.821	0.104	190.321**
3.	Body weight (30 days)	52.637	0.167	315.165**
4.	Body weight (45 days)	96.405	0.212	455.027**
5.	Body weight (60 days)	144.194	0.730	197.469**
6.	Body weight (75 days)	200.351	0.842	238.053**
7.	Body weight (90 days)	273.939	1.006	272.229**
8.	Body weight (4 months)	313.315	1.090	287.448**
9.	Body weight (5 months)	369.273	1.119	329.958**
10.	Body weight (6 months)	416.705	1.112	374.799**
11.	Body Weight at weaning (kg)	236.121	0.753	313.373**
12.	Age at sexual maturity (days)	250867.378	1812.832	138.384**
13.	Age at first kidding (days)	267594.844	1425.194	187.760**
14.	Litter size at first kidding (Number)	2.311	0.230	10.053**
15.	Litter size at seconds kidding (Number)	2.100	0.238	8.826**

**Significant (p<0.01), MS-mean sum of squares, Figures in parenthesis are degree of freedom

Body weight at birth

The effect of breed was found significant (P<0.01) for body weight at birth (Table 2).The least squares means for birth weight (kg) was found to be 1.80±0.038, 1.93±0.049 and 1.34±0.431 in Barbari, Sirohi and Black Bengal goats, respectively. The average birth weights in Sirohi and Barbari goats were significantly (p<0.05) higher than Black Bengal goats (Table 2).

Body weight at fortnightly intervals (from 15 day to 90 days)

The effect of breed was found significant (P<0.01) for body weight at fortnightly interval from 15 day to 90 days (Table 1). The mean body weights (kg) at 15, 30, 45, 60, 75 and 90 days of age were found to be 3.12±0.063, 3.56±0.048 and 1.98±0.494; 4.33±0.090, 5.18±0.048 and 2.58±0.0612; 5.54±0.121, 6.65±0.048 and 3.14±0.510; 6.62±0.121, 8.05±0.046 and 3.74±0.516; 7.60±0.279, 9.47±0.046 and 4.36±0.514; 8.98±0.306, 10.92±0.046 and 4.99±0.556, respectively in Barbari, Sirohi and Black Bengal breeds of goat. The body weight at fortnightly interval (from 15 day to 90 days) between Barbari, Sirohi and Black Bengal goats showed significant difference (P<0.05). The body weights from 15 to 90 days of age were significantly (p<0.05) higher in Sirohi goats followed by Barbari and Black Bengal (Table 2).

Table 2: Least squares means and standard errors for body weights (kg) from birth to 90 days of age

Breeds	Genotype	Traits						
		BW(0D)	BW(15D)	BW(30D)	BW(45D)	BW(60D)	BW(75D)	BW(90D)
Barbari	AA(30)	1.80±0.038	3.12±0.063	4.33±0.090	5.54±0.121	6.62±0.121	7.60±0.279	8.98±0.306
	AB (0)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	BB (0)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Mean (30)	1.80^{A}±0.038	3.12^{B}±0.063	4.33^{B}±0.090	5.54^{B}±0.121	6.62^{B}±0.121	7.60^{B}±0.279	8.98^{B}±0.306
Sirohi	AA(30)	1.93±0.049	3.56±0.048	5.18±0.048	6.65±0.048	8.05±0.046	9.47±0.046	10.92±0.046
	AB (0)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	BB (0)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Mean (30)	1.93^{A}±0.049	3.56^{A}±0.048	5.18^{A}±0.048	6.65^{A}±0.048	8.05^{A}±0.046	9.47^{A}±0.046	10.92^{A}±0.046
Black Bengal	AA(30)	1.34±0.431	1.98±0.494	2.58±0.612	3.14±0.510	3.74±0.516	4.36±0.514	4.99±0.556
	AB (0)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	BB (0)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Mean (30)	1.34^{B}±0.431	1.98^{C}±0.494	2.58^{C}±0.612	3.14^{C}±0.510	3.74^{C}±0.516	4.36^{C}±0.514	4.99^{C}±0.556

Figures in parenthesis shows number of observations, values with different superscript in columns differ significantly (p<0.05), BW- Body weight, 0- at birth, D- days.

Table 3: Least squares means and standard errors for body weights (4 to 6 months in kg), ASM, AFK, LS-I and LS-II

Breed	Genotype	Traits							
		BW (4M)	BW (5M)	BW (6M)	WW	ASM (days)	AFK (days)	LS-I (Numbers)	LS-II (Numbers)
Barbari	AA(30)	9.97±0.297	10.82±0.301	11.64±0.298	8.68±0.230	456.27±11.440	565.40±9.290	1.27±0.082	1.20±0.074
	AB (0)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	BB (0)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Mean (30)	9.97^{B}±0.297	10.82^{B}±0.301	11.64^{B}±0.298	8.68^{B}±0.230	456.27^{A}±11.440	565.40^{A}±9.290	1.27^{B}±0.082	1.20^{B}±0.074
Sirohi	AA(30)	12.04±0.046	13.46±0.046	14.68±0.046	10.61±0.129	411.33±6.453	570.07±5.020	1.13±0.063	1.10±0.056
	AB (0)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	BB (0)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Mean (30)	12.04^{A}±0.046	13.46^{A}±0.046	14.68^{A}±0.046	10.61^{A}±0.129	411.33^{B}±6.453	570.07^{A}±5.020	1.13^{B}±0.063	1.10^{B}±0.056
Black Bengal	AA(30)	5.70±1.064	6.51±1.081	7.26±1.108	5.08±0.586	280.27±22.954	404.20±43.146	1.67±0.858	1.60±0.954
	AB (0)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

	BB (0)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Mean (30)	5.70^{C}±1.064	6.51^{C}±1.081	7.26^{C}±1.108	5.08^{C}±0.586	280.27^{C}±22.954	404.20^{B}±43.146	1.67^{A}±0.858	1.60^{A}±0.954

Figures in parenthesis shows number of observations, values with different superscript in columns differ significantly (p<0.05). BW- Body weight, M- month, WW- weaning weight, ASM-Age at sexual maturity, AFK-Age at first kidding, LS-litter size.

Body weight at monthly intervals (from 4 to 6 months of age)

The effect of breed was found significant (P<0.01) for body weight at monthly interval from 4 to 6 months of age (Table 1). The mean body weights (kg) at 4, 5 and 6 months of age were found to be 9.97±0.297, 12.04±0.046 and 5.70±1.064; 10.82±0.301, 13.46±0.046 and 6.51±1.081; 11.64±0.298, 14.68±0.046 and 7.26±1.108, respectively in Barbari, Sirohi and Black Bengal goats. The body weight at monthly interval (from 4 to 6 months) between Barbari, Sirohi and Black Bengal goats showed significant difference (P<0.05). The body weights from 4 to 6 months of age were significantly (p<0.05) higher in Sirohi goats followed by Barbari and Black Bengal (Table 3).

Body weight at weaning

The effect of breed was found significant (P<0.01) for body weight at weaning (Table 1). The mean body weights (kg) at weaning were found to be 8.68±0.230, 10.61±0.129 and 5.08±0.586 in Barbari, Sirohi and Black Bengal goats, respectively. Significantly (p<0.05) higher body weight at weaning was recorded in Sirohi as compared to Barbari and Black Bengal goats (Table 3).

Age at sexual maturity

The effect of breed was found significant (P<0.01) for age at sexual maturity (Table 1). The age at sexual maturity was found to be 456.27±11.440, 411.33±6.453 and 280.27±22.954 in Barbari, Sirohi and Black Bengal goats, respectively. Minimum age at sexual maturity (days) was recorded in Black Bengal goats followed by Sirohi and Barbari goats. There was a significant difference among the three breeds of goat for age at sexual maturity (Table 3).

Age at first kidding

The effect of breed was found significant (P<0.01) for age at first kidding (Table 1). Significantly lower age at first kidding (days) was recorded in Black Bengal (404.20±43.146) followed by Barbari (565.40±9.290) and Sirohi (570.07±5.020) goats. However, the difference in age at first kidding was found non-significant between Barbari and Sirohi breeds of goat (Table 3).

Litter size at first (LS-I) and second kidding (LS-II)

The effect of breed was found significant (P<0.01) for Litter size at first and second kidding (Table 1). The litter size at first kidding (Nos.) was significantly higher in Black Bengal goats (1.67±0.858) than in Barbari (1.27±0.082) and Sirohi (1.13±0.0.63) goats with no significant difference between Barbari and Sirohi goats. Similarly, the least squares means of litter size in second kidding (Nos.) in Barbari, Sirohi and Black Bengal goats were estimated to be 1.20±0.074, 1.10±0.056 and 1.60±0.954, respectively. Significantly higher litter size at second kidding was recorded in Black Bengal as compared to Barbari and Sirohi goats (Table 3).

Available literature did not reveal references on association of BMPR1B and BMP15 genes variants with body weight at birth, body weight at fortnightly interval from 15 day to 90 days of age, body weight at monthly interval from 4 months to 6 months of age, body weight at weaning, age at sexual maturity, age at first kidding, litter size at first and second kidding in Barbari, Sirohi and Black Bengal breeds of goat. Hence, the results obtained in the present study could not be compared.

However, reports are available on association of FecB gene and its association with litter size in goat by various workers [2, 8, 16] but they found tested population monomorphic at this gene locus. Among indigenous breeds, litter size was not reported to be associated in Black Bengal, Jakhrana and Beetal goats [8]. However, significant effect of genotype on litter size in non-descript goat was reported [8]. Islam *et al*., 2019 [7] reported polymorphic variation at BMPR1B and BMP15 genes loci and detected three different genotypes in Beetal and Teddy goat breeds. They noticed significant association of CT genotype with litter size in all parties. The heterozygous (CT) animals of both breeds had the largest litter size in all parties, showing the over dominance of the heterozygotes. In contract to present study, Polly *et al*., 2009[13] reported polymorphic variation at BMPR1B gene locus in Black Bengal goat. They observed the single nucleotide polymorphism in BMPR1B gene in Black Bengal goats and they reported that the heterozygous carrier and homozygous carrier animals had 3.04 and 3.11 kids, respectively, compared with wild-type genotype, which had 2.7 kids/doe.

Conclusion

As we have noticed monomorphism in the BMPR1B/*AvaII* and BMP15/*HinfI* genes in screened populations of Barbari, Sirohi and Blank Bengal goats, therefore, these findings cannot be applied for selection of prolific goats.

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