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Genetic polymorphism of follicle stimulating hormone beta (*FSHβ*) gene and its association with body weight, scrotal measurements and seminal quality traits in indigenous goats

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In goat farming, buck fertility is important because most farms typically retain one buck for every few dams. Follicle stimulating hormone is essential for regulating fertility and varies in its beta subunit (*FSHβ*), which provides specificity. To explore the possibilities of using *FSHβ* gene as candidate marker, this study was aimed to investigate polymorphism at *FSHβ* gene by PCR-RFLP assay and association of polymorphic variants of *FSHβ* with body weights, scrotal and seminal attributes in indigenous goats. The amplicons of 313 bp was obtained for *FSHβ* gene from the DNA of 35 Barbari, 26 Black Bengal, and 31 Sirohi bucks, respectively. *FSHβ* was found to be monomorphic in all three breeds using PCR-RFLP analysis. Using *PstI* and *HinfI* restriction enzymes, respectively, it revealed a single type of uncut banding pattern of 313 bp and single fragment of 157 bp. Sequence analysis also confirmed absence of *PstI* recognition site, revealing an A>G substitution at nucleotide position 4531. Frequency of AA genotype and A allele was found to be maximum. All traits, with the exception of mass motility, showed a significant breed effect. Based on the findings, it was concluded that targeted region of *FSHβ* gene was fixed goat population under investigation.

Keywords: Barbari, Black Bengal, Buck fertility, Livestock, Polymorphism, Sirohi

The livestock sector is an important component of the Indian economy[1]. It contributes to an extent of 6.17 and 30.87% to the total national and the total agriculture gross value added, respectively. Among livestock, goats are an important constituent of food security and of human livelihood of landless and marginal farmers in India. India has the second largest goat population in the world[2]. Barbari and Sirohi are dual-purpose breed of goat while Black Bengal is mainly kept for chevon production. Sirohi goats are mainly reared by pastoralists in arid and semiarid regions of central and southern Rajasthan, whereas, Barbari is mainly reared in western districts of Uttar Pradesh and its adjoining regions of Rajasthan and Black Bengal is primarily found in West Bengal and its nearby states[3].

Buck fertility have a paramount importance in goat farming, since only one buck is kept over few dams[4-6]. Due to its polygenic inheritance, interactions with other traits, and significant environmental influence, its estimation is extremely complex[7-9]. The low heritability of traits and the time required for traditional breeding methods have made grading goats for reproductive traits difficult[10,11]. Molecular biology tools allow the detection of candidate genes which governs reproductive traits in animals[12-14]. The expected phenotypic variation caused by polymorphisms at their locus can be used as selection criteria for the genetic improvement of different reproductive traits[15,16].

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are members of gonadotropin hormones family and integral parts of the neural and endocrine interchange among the hypothalamus, pituitary and gonads[17-18]. They control steroid hormone synthesis and gamete production and also play important role for the genetic improvement of

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reproductive traits[19-21]. Alpha (FSHα) and beta (*FSHβ*), the two subunits of FSH, are encoded by two different genes. However, the beta subunit exhibits variability and offers biologic specificity[22,23]. The study of genetic polymorphism of *FSHβ* gene and its effects on semen quality traits explore the possibilities of *FSHβ* gene being used as candidate marker gene for these traits. Here, we investigated the genetic polymorphism of *FSHβ* gene by PCR-RFLP assay in bucks of Sirohi, Barbari and Black Bengal breeds and its association with semen quality traits.

Materials and Methods

Experimental animals

The study was conducted on total 92 adult bucks of Barbari (n=35), Black Bengal (n=26) and Sirohi (n=31) breeds of indigenous goat. The bucks were maintained at Goat Breeding Farm of the NDVSU at Amanala, Jabalpur, Madhya Pradesh, India, located within the longitude 21°6ˈN to 26°30ˈN and latitude 74°9ˈE to 82°48ˈE. Jabalpur has a subtropical climate with three different seasons. The annual ambient temperature in this region ranges from 20°C to 45°C with a relative humidity between 10% and 70%, and an average annual rainfall of 1160 mm[24]. These bucks were housed under semi-intensive system and reared on routine feeding and management practices of the farm. They were allowed for browsing for one hour twice in a day at 8.30 a.m. and 3.00 p.m. In their shed, concentrate mixture was fed to them on a rate of 10 g per kg of body weight. Fresh and clean drinking water was provided to them at *ad lib*. They were routinely dewormed with ivermectin at 6 months interval and vaccinated against FMD, HS, BQ and PPR.

Genomic DNA extraction

About 5 mL of the blood was collected from the jugular vein of each buck into sterile 10 mL vacutainer containing 0.5M EDTA as anti-coagulant and stored at 4ºC till further processing. Genomic DNA was extracted from whole blood samples using the standard phenol-chloroform extraction method in the Molecular Genetics Laboratory of Department of Animal Genetics and Breeding, CVSc & AH, Jabalpur, as per the protocol of John *et al.*[25]. The DNA samples were dissolved in Tris-EDTA (TE) buffer which was made from 10 mM Tris HCl (pH 7.5) and 1 mM EDTA (pH 8.0) and were stored at −20°C for use. Purity and DNA concentration was checked using Nanodrop spectrophotometer (ND-2000c) at optical density (OD) 260 nm and 280 nm. The quality of genomic DNA was checked by 0.8% agarose gel electrophoresis at 80 V for 60 min. The samples were diluted in autoclaved milliQ water in 1.5 mL tube (Eppendorf) to make the final concentration of DNA in each sample up to 50ng/µL. The diluted DNA samples were used for PCR amplification of *FSHβ* gene[26].

Polymerase chain reaction (PCR)

FSH beta subunit gene specific primer (313 bp) was custom synthesized and used for amplification of gene[27] (Table 1). PCR was performed in a total reaction volume of 25 µL consisting of 12.5 µL of 2X PCR master mix (Gotaq® Green Master mix), 2.0 µL (about 100 ng) of diluted genomic DNA, 1.0 µL (10 pmole) of each primer (forward and reverse) and 8.5 µL of nuclease free water (NFW).The cycling protocol was standardized as an initial denaturation of 5 min at 95°C, followed 35 cycles of denaturation at 95°C for 45 s, annealing temperature at 56°C for 45 s, extension at 72°C for 1 min and final extension at 72°C for 10 min[26].

The amplified PCR products were visualized by electrophoresis in 2% agarose gel, containing 500 ng/µL of ethidium bromide in 1X TBE buffer. Five microlitre (5 µL) of amplified PCR product was loaded in each lane. As a molecular size marker, 100 bp DNA ladder (Fermentas Life Science, Range 100-1000 bp) was loaded in a separate lane. The electrophoresis was conducted at constant voltage of 100 volt for 60 min at 37°C using 1X TBE buffer. The amplified products in the gel were visualized by UV transilluminator and photographed using Gel documentation system (Gel-Doc, Bio-Rad, USA)[26].

PCR – RFLP assay

The restriction enzyme *PstI* and *HinfI* were procured from Promega Corporation and used to digest the amplified PCR products of FSH beta gene separately in a total 30 µL reaction mixture having 2 µL of 10X Buffer H (Promega), 15 µL of PCR product, restriction enzyme 1 µL (*PstI or HinfI*) and 12 µL of NFW. The reaction mixture was spanned for

Table 1 — Primer sequence and PCR product size for FSH beta gene

Gene	Nucleotide sequence	Region	Product Size
FSHβ	F: 5'-CTTCCAGACTACTGTAACTCATC-3' (R: 5'-GTAGGCAGCTCAAAGCATCCG-3'	Part of intron-2 and completing coding region of exon-3	313 bp

Table 2 — Protocol for digestion of PCR products by restriction enzymes

Gene	Restriction Enzyme	Restriction site	Incubation (1 h)	Inactivation (10 min)
FSHβ	*PstI*	5'…CTGCA↓G…3' 3'…G↑ACGTC…5'	37ºC	80°C
	HinfI	5'…G↓ANTC…3' 3'…CTNA↑G…5'	37ºC	80°C

few seconds for uniform mixing and then incubated at 37°C for 1 h digestion in water bath and then inactivated at 80°C for 10 min[26] (Table 2).

Digested products were electrophoresed on 3% agarose gel containing 1% ethidium bromide @ 5 µL/100 mL at constant voltage of 100 V for 1 h, using 1X TBE buffer. About 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 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 the band size with 100 bp DNA ladder. Genotyping of gene locus (viz., *FSHβ*) was carried out according to the band pattern of respective genotypes[26].

Sequence analysis

Gene sequencing of representative amplicons of all the three breeds was done. Sanger's dideoxy chain termination sequencing method was applied to sequence the amplicons, in automatic ABI Prism DNA sequencer (EurofinPvt. Ltd., Bangalore). Bovine *FSHβ* gene sequence, accession no. M83753 was retrieved from NCBI database and aligned with sequences of *FSHβ* gene of the present goats' population by ClustalW method using BioEdit version 7.2 software[28].

Estimation of genotypic and allelic frequency

The genotypic frequencies and allelic frequency were estimated. Chi square test was employed to test whether the population of goats was in Hardy Weinberg Equilibrium. The above statistical analysis was done using POPGENE software, version 1.32[29].

Body weight and scrotal measurements

The body weight (kg) of each animal was recorded using a digital weighing scale in the morning. For scrotal measurements, testicular length, width and thickness of each testis was recorded in millimetres using Vernier calliper along with scrotal circumference, which was recorded with a metric tape in the widest region of the scrotum after maximum ventrocaudal traction of the gonads. Each measurement was recoded thrice and the mean values were used for statistical analysis. Testicular volume (TV) and testicular weight (TW) was calculated using the following formula[30]:

Testicular volume (TV) (mL) = 0.5236 × (L)(W)2;

Testicular weight (TW) (g) = 0.5533 × (L)(W)2

where L = testicular length (mm), W = testicular width (mm)

Semen collection and evaluation

The semen samples were collected from each buck in the morning between 8:00 and 8:30 a.m. by artificial vagina (AV). Total three ejaculates were collected from each buck on a regular interval of three days. Prior to collection, to receive the heterosexual stimulation, bucks were exposed to does which were already in estrus. Semen was kept at 34ºC in water bath after collection and evaluated for ejaculate volume, mass motility, sperm concentration and abnormal spermatozoa rate (%). Volume of each ejaculate was directly recorded in millilitres (mL) by observing volume graduations of collection tube attached with AV set. A drop of freshly collected neat semen of each sample was examined on a clean, dry glass slide without the cover slip, at 37ºC under a low power microscope for mass motility assessment. A swarming mass of waves and eddies was observed in the microscopic field depending upon the degree of movement of all viable sperms in the semen and graded from 0 to +4. For abnormal spermatozoa rate (ASR) of each sample was expressed in terms of percentage by counting abnormal spermatozoa out of 200 after staining the semen with eosin-Nigrosin stain on a clean, dry and grease free glass slide. The number of spermatozoa present in each ejaculate was estimated by haemocytometer, then concentration of spermatozoa in per mL of semen was counted and expressed as million/mL by using the formula given below[31]:

No. of sperms/mL = (N × 400/80) × 10 × 200 × 1000

where, N = number of sperms in 80 small squares.

Statistical analysis

The data generated was subjected to multivariate analysis of variance (MANOVA) using computer statistical software (IBM SPSS, 28.0.1.0)[32]. The significant differences among genotypes and breeds, for the mean values of various parameters, were estimated by Duncan's multiple range test (DMRT). Following statistical model was used to find out the association of various polymorphic variants of *FSHβ* gene with semen quality traits:

$$Y_{ijk} = \mu + B_i + G_j + (BG)_{ij} + e_{ijk}$$

where, Y_{ijk} is the observation of the parameter of k^{th} individual of the i^{th} breed for j^{th} genotype, μ is the population mean, B_i is the set of fixed effect due to breeds, G_j is the set of random cross classified effects due to genotypes, $(GB)_{ij}$ is the interaction of i^{th}breed with j^{th} genotype and e^{ijk} is the random residual error of each observation

Results

DNA Extraction and PCR amplification of FHSβ Gene

Most of the DNA samples were lying in the range of 1.65-1.92 (OD260/280) indicating that DNA samples were highly pure with very low contamination in the form of proteins or other impurities. Reprocessing was done using phenol-chloroform extraction for samples whose OD ratio fell outside of this range. The concentration of isolated DNA samples was found within the range of 120.8 ng/µL to 680 ng/µL. The extracted genomic DNA samples were found to be of good quality in 0.8% agarose gel electrophoresis (Fig. 1). On PCR amplification of part of intron-2 and complete coding region of exon-3 of *FSHβ* gene, amplicons of 313 bp size were observed in all the breeds (Fig. 2), indicating the primer specific amplification.

PCR-RFLP Assay of FHSβ Gene

PCR-RFLP analysis of part of intron-2 and complete coding region of exon-3 of *FSHβ* gene revealed absence of recognition site for *PstI*restriction enzyme and only one type of uncut banding pattern of 313 bp size was observed (Fig. 3A). Whereas recognition site for *HinfI* restriction enzyme was found to be present, however a single band of 157 bp size was observed in all the samples (Fig. 3B). Absence of recognition site for *PstI*was also confirmed by multiple sequence alignment of goats' sequences with bovine *FSHβ* gene sequence (M83753). In bovine *FSHβ* gene, recognition site for *PstI* (CTGCA↓G) was found to be present from nucleotide position 4527 to 4532 but in all the goats' sequences adenine was found to be substituted with guanine (A>G) at nucleotide position 4531 (Fig. 4). Therefore, part of intron-2 and complete coding region of exon-3 of *FSHβ* gene was found to be monomorphic in all the three breeds.

Genotype and allelic frequency at *FSHβ* gene locus

The frequency of genotype AA and allele A were observed 1.0 each in tested population of Sirohi, Barbari and Black Bengal bucks (Fig. 5). We could not identify any animal with AB and BB genotypes therefore chi-square test for correspondence between observed and expected genotypic frequencies could not be done. Based on the present results it was hypothesized that the populations of these breeds of goats under study were in Hardy-Weinberg equilibrium at part of intron-2 and complete coding region of exon-3 of *FSHβ* gene.

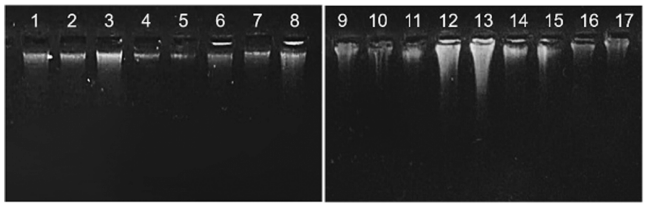

Fig. 1 — Genomic DNA of goats electrophoresed on 0.8% agarose gel. [Lanes 1-6 Sirohi, 7-12 Barbari and 13-17 Black Bengal]

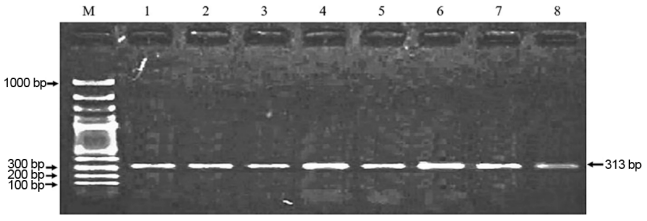

Fig. 2 — Amplified PCR product of *FSHβ* gene in bucks on 2% agarose. [M: 100 bp DNA Ladder. Lanes Amplicons (313 bp). 1-3 Sirohi, 4-7 Barbari and 8-9 Black Bengal]

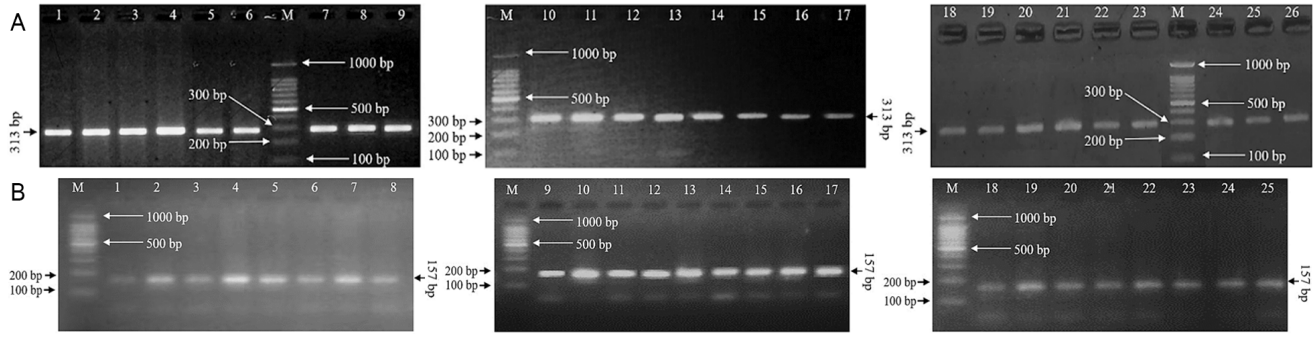

Fig. 3 — PCR-RFLP pattern of *FSHβ* gene digested with (A) *PstI*; and (B) *HinfI*. [Lanes: M: 100 bp DNA Ladder. (A) AA Genotype (313 bp); 1-9 Sirohi, 10-17 Barbari and 18-26 Black Bengal; and (B) AA Genotype (313 bp); 1-8 Sirohi, 9-17 Barbari and 18-25 Black Bengal]

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                    ....|....| ....|....| ....|....| ....|....| ....|....|
                            4510       4520       4530       4540       4550
M83753.1            ACGTACCCAG TAGCCACTGA ATGTCACTGC AGCAAGTGCG ACAGCGACAG
Barbari FSH Beta    ACGTACCCAG TAGCCACTGA ATGTCACTGC GGCAAGTGTG ACCGCGACAG
Black Bengal FSH Beta ACGTACCCAG TAGCCACTGA ATGTCACTGC GGCAAGTGTG ACCGCGACAG
Sirohi FSH Beta     ACGTACCCAG TAGCCACTGA ATGTCACTGC GGCAAGTGTG ACCGCGACAG
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Fig. 4 — Sequences alignment of the *FSHβ* gene amplicons. [Numbers above the sequences denotes nucleotide positions]

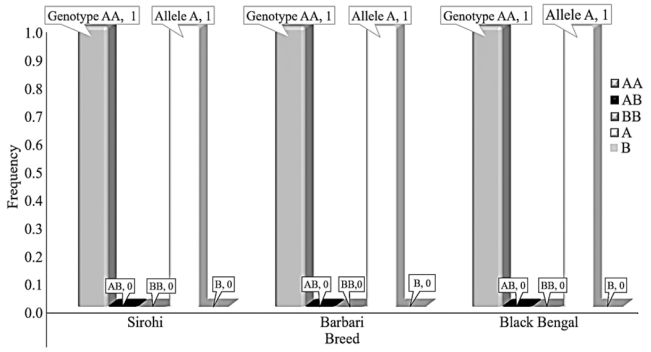

Fig. 5 — Distribution genotypic frequency and allelic frequency at *FSHβ* gene locus in Sirohi, Barbari and Black Bengal Goats

Association of polymorphic variants of *FSHβ* gene with body weights, scrotal measurements and semen quality traits

Since only AA genotype was detected in all the bucks, therefore effects of genotype and breed × genotype interaction on the all the traits were nullified. However, breed effect was found to be significant (P <0.05) for all the traits except mass motility. Body wights were recorded significantly (P <0.05) higher for Sirohi bucks, followed Barbari and Black Bengal. Similarly, scrotal circumferences were recorded significantly higher for significantly (P <0.05) higher for Sirohi bucks, followed Barbari and Black Bengal. Likewise, all the scrotal parameters *viz.* length, width and thickness of both the testis were recorded significantly (P <0.05) higher in Sirohi bucks, except left testicular thicknesses, which was significantly higher and statistically (P <0.05) similar in Sirohi and Barbari bucks. Similarly, testicular volumes and testicular weights were also recorded significantly higher and statistically (P <0.05) similar in Sirohi and Barbari bucks. Significantly (P <0.05) higher semen volumes and sperm concentrations were recorded for Sirohi and Black Bengal bucks respectively, whereas, significantly (P <0.05) lower abnormal spermatozoa rates were recorded for Black Bengal bucks. However, higher mass motility was recorded in semen samples of Black Bengal bucks but effect of breed was found to be non-significant (P >0.05). Least squares means and standard errors for all the traits have been shown in Table 3.

Discussion

In animals, FSH gene is an important regulator of fertility[33,34]. It is mainly expressed in gonadotrope where its expression is controlled primarily by activin and inhibin with additional regulation by gonadotrophin-releasing hormone (GnRH)[35]. FSH supports the function of sustentacular cells which in turn supports sperm cell differentiation and maturation at various stages of spermatogenesis[36]. Follicle-Stimulating Hormone (FSH) is essential for mammals' normal gametogenesis. It is vital for ovarian development and follicle maturation in females whereas, it determines Sertoli cell number and is required for quantitatively and qualitatively normal spermatogenesis in males. The G-protein coupled receptor (FSHR), which is only expressed on granulosa and Sertoli cells, mediates the action of FSH[37,38].

Absence of polymorphism in part of intron-2 and complete coding region of exon-3 of *FSHβ* gene was also reported in Bali cattle[39] using restriction enzymes *PstI*. In Baluchi, Iran Black and Arman breeds of sheep also, polymorphism in exon-2 of *FSHβ* gene could not be detected using both *Acc*Iand *Hinf*Irestriction enzyme[40]. The present study similarly indicated that the three breeds under consideration are monomorphic at the *FSHβ* gene locus. The recognition site is found to be absent for *Pst*Irestriction enzyme in *FSHβ* gene. However, recognition site was found to be present for *Hinf*Irestriction enzymes but banding pattern was uniform in all the samples (Fig. 3B). On contrarily to the findings of present study, three different banding patterns in part of intron-2 and complete coding region of exon-3 of *FSHβ* gene by using *Pstl* restriction enzymes were reported by Ishak *et al.*[39] in Brahman, Holstein Friesian (HF), Simental and Limosin cattle, which were AA (202 bp and 99 bp), AB (313 bp, 202 bp and 99 bp) and BB (313 bp) genotypes, respectively. In exon-10 of *FSHβ* gene, three different banding patterns, which were BB (304 bp), AA (214 bp and 90 bp) and AB (304 bp, 214 bp and 90 bp) genotypes, were observed by

Table 3 — Least squares means and standard errors for body weights, scrotal measurements and semen quality traits at *FSHβ* gene locus in Barbari, Black Bengal and Sirohi bucks

Breed	Barbari (35)			Black Bengal (26)			Sirohi (31)		
Genotype	AA(35)	AB (0)	BB (0)	AA (26)	AB (0)	BB (0)	AA (31)	AB (0)	BB (0)
Body weight	34.80^{B}±0.421	–	–	30.1^{C}±0.437	–	–	41.86^{A}±0.362	–	–
Scrotal circumference (cm)	23.45^{B}±0.420	–	–	19.77^{C}±0.415	–	–	24.42^{A}±0.442	–	–
Right testicular length (mm)	106.29^{B}±1.191	–	–	84.43^{C}±0.924	–	–	110.84^{A}±1.222	–	–
Right testicular width (mm)	42.89^{B}±0.623	–	–	35.68^{C}±0.659	–	–	49.007^{A}±1.098	–	–
Right testicular thickness (mm)	46.3^{B}±0.748	–	–	36.26^{C}±0.681	–	–	43.97^{A}±1.319	–	–
Left testicular length (mm)	107.98^{B}±1.416	–	–	83.3^{C}±0.994	–	–	111.02^{A}±1.219	–	–
Left testicular width (mm)	42.25^{B}±0.625	–	–	35.27^{C}±0.708	–	–	49.72^{A}±0.930	–	–
Left testicular thickness (mm)	46.35^{A}±0.718	–	–	36.37^{B}±0.905	–	–	45.77^{A}±0.678	–	–
Testicular weight (g)	129.44^{A}±5.040	–	–	62.5^{B}±3.198	–	–	126.83^{A}±6.427	–	–
Testicular volume (mL)	122.49^{A}±4.770	–	–	59.14^{B}±3.026	–	–	120.02^{A}±6.082	–	–
Semen volume (mL)	0.53^{B}±0.008	–	–	0.49^{C}±0.006	–	–	0.62^{A}±0.009	–	–
Mass motility (0 to +4)	2.97±0.108	–	–	3.07±0.140	–	–	3.06±0.115	–	–
Sperm concentration (10^6/mL)	2631.61^{B}±038.584	–	–	2800.99^{A}±035.190	–	–	2670.81^{B}±40.750	–	–
Abnormal spermatozoa rate (%)	$8.09A^{B}$±0.236	–	–	7.73^{B}±0.336	–	–	8.52^{A}±0.170	–	–

[Figures in the parenthesis are the no. of observations; Mean values bearing different superscript differ significantly (P<0.05)]

Nazifi *et al.*[40] in Baluchi, Iran-Black and Arman breeds of sheep and by Hatif *et al.*[41] in Iraqi goats.

However, different researchers have reported polymorphism in the *FSHβ* gene using the single strand confirmation polymorphism (SSCP) assay. An *et al.*[42] found one polymorphic locus occurring in exon-2 of *FSHβ* gene with three genotypes (EE, EF and FF) in Xinong Saanen dairy goat and Boer goat by PCR-SSCP. Polymorphism in intron-2 and exon-3 of the *FSHβ* gene was also reported by Zhang *et al.*[43] using PCR-SSCP in Boer, Matou, Black, and Boer–Matou crossbred goats with three genotypes (AA, AB and BB). With PCR-SSCP, Mokhtari *et al.*[44] also reported *FSHβ* gene polymorphism in Iran's Mehraban breed of sheep, they discovered three and six electrophoretic patterns associated with exon-2 partial intron-2 and intron-2 exon-3 partial intron-3, respectively.

In the present populations of bucks of all the three breeds, one new SNP (G>A) was detected at 4531 bp position in gene sequencing analysis of *FSHβ* (Fig. 4). Similarly, Nikbin *et al.*[45] found three SNPs viz., 200 A>G, 226 T>C and 237 A>G in exon-3 of caprine *FSHβ* gene in Boer and Boer-crossbred bucks, out of them 200 A>G and 226 T>C were novel. In addition, Niu *et al.*[46] reported three novel SNPs viz., 284 G>T, 2908 G>A and 2963 G>A in exon-1 and exon-2 of *FSHβ* gene in Rex rabbits. Further, Plakkot & Kanakkaparambil[47] detected one SNP T>C, in *FSHβ* coding sequence, at position 413 in Attappady Black and Malabari breeds of goats. SNP at position 4531 was identified in our study in relation to the bovine *FSHβ* gene sequence (M83753), but since it was present in each of the bucks, it could not be used to genotype the bucks.

Furthermore, it was found that Sirohi bucks were better for the majority of the investigated parameters which suggests that body size and testicle size is could be a good indicator for sperm production (Table 3). Similar to our findings, Omari *et al.*[48] also reported significant effect of breed on testicular dimension and seminal attributes in Shami, Mountain Black and Hybrid breeds of goat.

Due to the absence of a *PstI* restriction site and the presence of a *HinfI* restriction site in every experimental animal, the current study at the *FSHβ* gene locus did not find any mutations. In our study, the absence of the B allele in the population of goats could be attributed to two possible causes: first, sampling error could arise from the small sample sizes, as all screened animals had homozygous AA genotypes; second, selection could occur due to the detrimental effects of the B allele on individual performance. Therefore, individuals with missing genotype BB may have been eliminated from the population during evolutionary process.

Conclusion

In the present study, region of *FSHβ* gene 313 bp (part of intron-2 and complete coding region of exon-3) was successfully amplified with gene specific primers. Absence of polymorphism in selected region of *FSHβ* was observed in present population of bucks, indicating a lack of genetic variation in the studied region. A new SNP at position 4531, but it was present in all bucks, making it unsuitable for genotyping. Association of *FSHβ* gene polymorphism with body weights, scrotal measurements, and semen quality traits showed that the effects of genotype on

these traits were nullified, since only one genotype was detected in all bucks. However, significant breed effects were observed, with Sirohi bucks exhibiting higher body weights, scrotal circumferences, and most semen quality traits compared to Barbari and Black Bengal bucks. The study contributes to the understanding of genetic aspects related to reproductive traits in goats, emphasizing the importance of breed differences in influencing these traits. The absence of genetic variation in the studied region of the *FSHβ* gene raises questions about the potential impact of selection pressures or sampling errors on the observed monomorphism. Further research with larger sample size may provide a more comprehensive understanding of the genetic factors influencing reproductive traits in goat breeds.

Ethics approval

The study protocol was approved by Institutional Animal Ethical Committee, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh (No. 001/IAEC/Vety./2021 dated 27/01/2021).

Conflict of Interest

Authors declare no competing interest.

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