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ORIGINAL ARTICLE **OPEN ACCESS**

Association of *fshβ* gene polymorphism with litter size in indigenous goat breeds Sirohi and Barbari

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ABSTRACT
Present investigation reveals the association between polymorphism in follicle stimulating hormone beta (fshβ) subunit gene and litter size in Sirohi and Barbari indigenous goat breeds using PCR-RFLP method. The association of fshβ genotypes with litter size performance was analyzed in both the breeds. The 427bp (partial intron 2 and complete exon 2, exon 3) region of fshβ gene was amplified using PCR and subjected to PCR-RFLP which reveals three genotypes AB, BB and AA after digestion with restriction enzyme Mnl I. All three genotypes AB, BB and AA were detected in Sirohi goat and two genotypes AB and AA were observed in Barbari goat breeds. Allele A has the maximum gene frequency 59.62% for Sirohi and 62.96% for Barbari goat breeds. AB genotype showed maximum genotype frequency in both goat breeds viz., 69.23% in Sirohi and 74.07% in Barbari. Genotype AB favoured higher litter size in Sirohi and AA genotypes favoured higher litter size in Barbari goats. Association between genotype and mean litter size between both the breeds was found significant (P≤0.05). These results suggest that the fshβ gene may be a candidate gene that affects prolificacy and can be used for marker assisted selection for prolificacy in goats.
Keywords- *fsh β gene, genotype, PCR-RFLP, polymorphism and prolificacy.*

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INTRODUCTION

Goats are known as poor man's cow. Goat farming is comparatively easy and economically profitable for marginal and landless farmers as goats are well adapted to extreme and diverse geographical conditions. In commercial farming of goats, reproductive traits are of much importance. In mammals, multiple litter size is due to multiple ovulations. Though in goat singlet and twinning are common but quadruplet, quintuplet and sextuplet kids are occurred less frequently. Improvement of reproductive traits using traditional selective breeding will be a slow process for increasing the reproductive performance of low prolific goat breeds [1]. In goats, due to the low heritability and long reproductive cycle the enhancement of reproductive traits has proved to be difficult [2]. However, with the enhancement of new molecular approaches and studies on candidate gene approach will decrease the generation interval and increase the accuracy of selection for selective breeding of goats with high litter size which will increase the net profit and nutritional security. Genetic polymorphism studies on candidate genes associated with productive and reproductive traits will help in selection and propagation of elite goat population. There are many studies on candidates' genes related with litter size in different species like major genes affecting litter size have been successfully identified in sheep breeds, such as *BMPRIB* [3], *BMP-15* [4] and *GDF*9 [5-6], *fshβ* gene [7]. An and co-workers reported, the *fshβ* DNA polymorphism and its association with litter size in Chinese goat breeds [8]. Till now no work has been reported so far in Indian goat breeds related with *fshβ* gene polymorphism and its association with litter size.

Follicle stimulating hormone is a pituitary gonadotropin and acts as one of the most important hormone in mammalian reproduction, development of gonads and their maturation at puberty [9-10]. *fsh* has two subunits, alpha subunit which is common in all and hormone-specific beta subunit with specific biological effect which consists of 3 exons and 2 introns [11]. The *fshβ* is crucial for the proliferation and survivability of follicular somatocytes and cyclic recruitment of ovarian follicles during the development from the early antral stage through maturation to ovulation [12]. *fshβ* gene is chiefly responsible for

follicular development and could act as a potential candidate gene for mammalian reproduction related with prolificacy.

Therefore, by keeping in view the importance of *fshβ gene* in prolificacy and reproductive traits, this study was planned with the objectives to study the polymorphism of *fshβ* gene by PCR-RFLP technique in two indigenous goat breeds Sirohi and Barbari and investigate the association of *fshβ* genotype with litter size in two goat breeds Sirohi and Barbari.

MATERIAL AND METHODS

Blood samples and litter size data were collected from 52 Sirohi and 30 Barbari goat breeds from Livestock farm, Amanala, NDVSU, Jabalpur, Madhya Pradesh. Genomic DNA was extracted from blood samples according to the procedure of John *et al*. [13]. Concentration of genomic DNA samples were checked by Nanodrop ND-2000 spectrophotometer (Thermoscientific, USA) and integrity was checked by using 0.7 per cent agarose gel. The 427 bp (complete exon 2, exon 3 and partial intronic 2) region of *fshβ* gene was amplified by PCR using published primer by Zhang *et al.* [14] shown in Table 1.

Table 1: Primer sequences and information of the goat *fshβ* gene.

Primers	Sequences (5'-3')	Product size (bp)	Amplified region	Annealing temp
***fshβ* F1**	5'GTATTCAATCCCTGTCTCA 3'	**427**	Partial intronic 2 and complete exon 2, exon 3	53° C
***fshβ* R1**	5'GTAGGGTCTTCTGTGGTG 3'			

F-forward, R-reverse primers

The PCR reactions were performed in a final reaction volume of 25µl having 2X Master mixture (Fermentas), 1µM of forward and reverse primer and 1µl genomic DNA (50ng/µl). The following PCR amplification condition was used to optimize the reaction accuracy performed in Thermal cycler (ABI, USA): 94°C for 5 min, 35 cycles of 94°C for 30 sec, annealing temperature 53°C (Table 1) for 30 sec, 72°C for 30 sec, and a final extension at 72°C for 10 min. The different genotypes of *fshβ* gene were identified by the PCR-RFLP method using fast digest restriction endonuclease *Mnl* I. The following reaction mixture were subjected for PCR-RFLP, 10µl of PCR product, 0.5U restriction enzyme (*Mnl I*) and 10X assay buffer in the final volume of 25µl. Reaction mixtures were incubated at 37°C for 15 min followed by deactivation at 65°C for 20 minutes. Reaction products were detected by 2 per cent agarose gel and were visualized under UV light and documented by gel documentation system. The genotyping was done by the presence and absence of specific size band patterns.

Statistical analysis

The gene and genotype frequencies were calculated using Microsoft Excel as per Hardy-Weinberg law. The association between genotype and litter size was analyzed statistically by one-way ANOVA program. The significance of the difference was determined by F-test at the significant level of 0.05.

Ethical approval: Ethical approval was not needed for abovementioned study.

RESULTS AND DISCUSSION

PCR-RFLP: The PCR amplification of *fshβ* gene with specific primer yields 427bp band size (partial intronic 2 and complete exon 2, exon 3) from *caprine* genomic DNA as expected (Figure 1). Restriction digestion with enzyme *Mnl* I showed three genotypes AB (427, 278 and 149bp) BB (278 and 149bp) and AA (427bp) of *fshβ* gene with specific band patterns and sizes. In Sirohi goat breed all the three genotypes were observed whereas in Barbari goat breed two genotypes AA and AB were detected (Figure 2 and Figure 3).

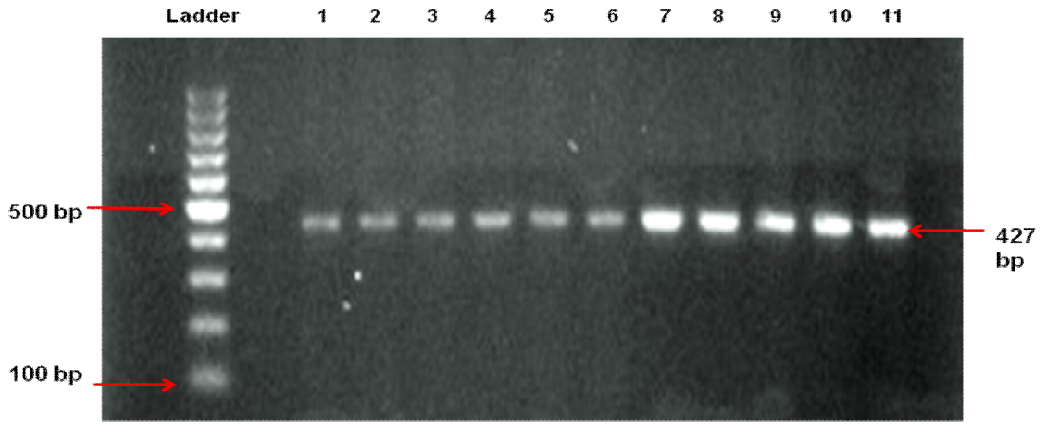

Figure 1: PCR amplified product of *fshβ* gene (partial intronic 2 and complete exon2, exon3) 427bp in Sirohi and Barbari goat breed. L1-6: amplicon of Sirohi, L7-11: amplicon of Barbari.

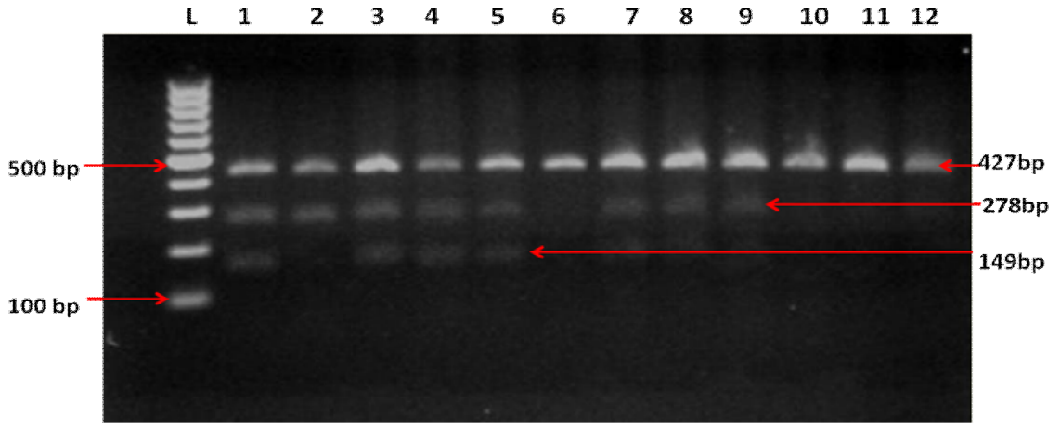

Figure 2: RE digestion (*MnI* I) of *fshβ* gene (partial intronic 2 and complete exon2, exon3) 427bp in Sirohi goat breed. L1-3-5, 7-9: AB genotype, L2: BB genotype, L6, 10-11: AA genotype, L2: undigested PCR product as control.

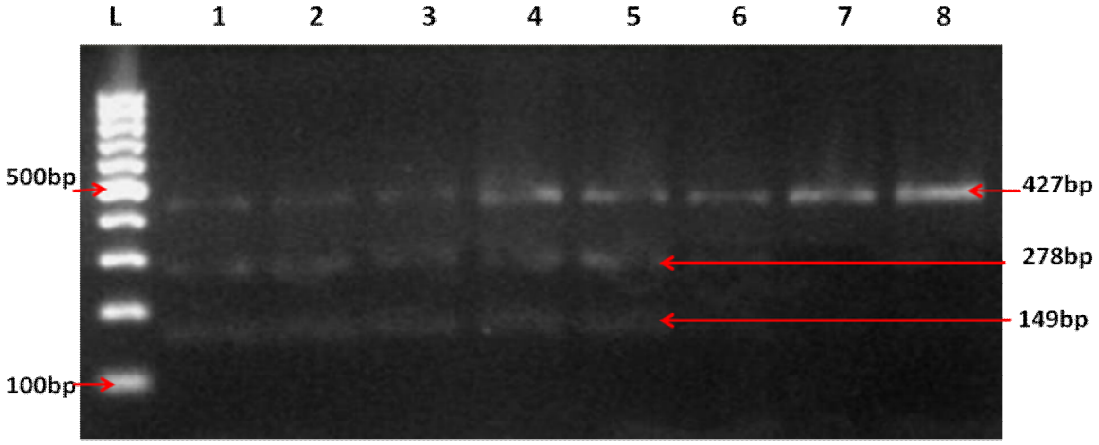

Figure 3: RE digestion (*MnI* I) of *fshβ* gene (partial intronic 2 and complete exon2, exon3) 427bp in Barbari goat breed. L1-6: AB genotype, L7: AA genotype, L8: undigested PCR product as control

Gene and genotypes frequencies: Gene and genotypes frequencies were calculated as shown in table 2 and 3 respectively.

Table 2: Gene frequencies in Serohi and Barbari goat breed.

Gene frequency	Sirohi (%)	Barbari (%)
A	59.62	62.96
B	40.38	37.04

Table 3: Information of genotype frequencies.

Genotype frequency	Sirohi (%)	Barbari (%)
AA	25.00	25.93
AB	69.23	74.07
BB	5.77	0.00

Association of genotypes with litter size: Association of genotypes and litter size in Sirohi and Barbari goat breeds for *fshβ* gene was found to be non-significant within breed (P≥0.05). However, AB genotypes favoured the higher litter size in comparison to AA and BB genotypes in Sirohi. In case of Barbari, AA genotypes favoured the higher litter size in comparison to AB genotypes (Table 4).

Table 4: Mean and standard errors of litter size of two breeds.

Breed	Genotype	Mean and standard error
Barbari	AB (20)	1.60±0.11
	AA (10)	1.72±0.14
Sirohi	AA (13)	1.00±0.00
	AB (36)	1.19±0.28
	BB (3)	1.00±0.00

Figure within parenthesis indicate number of goats under genotype.

Association of genotypes and litter size between Sirohi and Barbari breeds for same genotype: The comparison between Sirohi and Barbari goat breeds was done to find out the association in same genotype and litter size in between two breeds. After one way ANOVA analysis it was found that AB and AA genotypes had significant difference (P≤0.05) for Sirohi and Barbari (Table 5).

Table 5: Association of genotypes and litter size between Sirohi and Barbari breeds.

Breed	genotype	Mean and standard error
Sirohi	AB (36)	1.19±0.28
Barbari	AB (20)	1.65±0.24
Sirohi	AA (13)	1.00±0.00
Barbari	AA (10)	1.28±0.24

Association between genotype and litter size in Sirohi (Sr) and Barbari (Br) goat breeds was significant at P≤0.05. Figure within parenthesis indicate number of goats under genotype

In this study, the *fshβ* gene polymorphism with litter size in *capra hircus* was studied. Frequency distributions of the *fshβ* geneotype were different among two goat populations. Three genotypes AA, AB and BB were observed in Sirohi and AA and AB in Barbari. The polymorphism of *fshβ* gene had a significant effect on goat reproduction traits. The AB genotype was highly associated with larger litter size. The endocrine mechanisms underlying genetic differences in ovulation rate have also been studied extensively in sheep [15]. Liang and co-workers [16] studied the polymorphism of *fshβ* gene and its relationship with litter size in Boer goats, Angora goats, Liaoning Cashmere goats and Jining Grey goats by using PCR-SSCP technique and found three genotypes in Boer goats (AA,CC and AC) and three genotypes in Jining Grey goats (AA, AB and AC) and reported that AA genotype favours the high prolificacy than AB and AC genotypes in Jining Grey goats and suggested that follicle-stimulating hormone beta-subunit (*fshβ*) gene can act as a candidate gene for the prolificacy in goats [17-18]. In our study, AB favours the higher litter size in Sirohi and AA in Barbari goat breed. Zhang *et al*. [14] conducted a study on *fshβ* gene and reported the polymorphism in exon 3 of *fshβ* gene and its association with litter traits and superovulation in four goat breeds including Boer, Matou, Black and Boer-Matou crossbred (BM) by PCR-SSCP and PCR-RFLP and observed three genotypes named AA, AB and BB in all breeds. Association analysis showed AA genotype with higher litter size both in average and in parities from first

to fourth (P≤0.05). In present study on Sirohi and Barbari goats, three genotypes AB, AA, and BB were observed and AB genotype favours the higher litter size in Sirohi and AA genotypes favours the higher litter size in Barbari goat breeds, however it is statistically non-significant within the breed. Association within genotype and mean litter size between Sirohi and Barbari breeds were found significant. Therefore, the results and outcome in this study will be valuable for goat reproduction improvement and selection.

CONCLUSION AND ACKNOWLEDGEMENT

In the present study, it can be concluded that the polymorphism of *fshβ* gene is present in Sirohi and Barbari indigenous goat breeds and it has significant effect on larger litter size. Association within genotype and mean litter size between Sirohi and Barbari breeds were found significant. Therefore, the *fshβ* gene may act as a candidate gene for reproduction traits in goat breeds. The authors wish to thank the entire staff of the Livestock farm Amanala, NDVSU, Jabalpur (Madhya Pradesh) for their support in the samples and data collection and Animal Biotechnology Centre, NDVSU, Jabalpur (M.P.) for the laboratory support.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

COMPETING INTERESTS

No competing in interests.

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