RESEARCH ARTICLE

Association Study of Fecundity Gene BMP 15 with Prolificacy in Surti Goats under Farm and Field Condition of South Gujarat Region

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Abstract

The Surti is a dual-purpose goat breed of Gujarat. The bone morphogenetic protein 15 (BMP15), a gene of transforming growth factor-beta (TGF-β) superfamily ligands, plays a role in ovulation and litter size. Mutation in the Exon-2 region of BMP15 gene with base size 575 bp increases litter size. Based on the known mutation information in goat and sheep PCR primers were designed to screen polymorphism in total 100 Surti goats, 50 Surti goats from University farm and 50 Surti goats from field units of the Southern part of Gujarat. Two polymorphic sites were found during the study, one at 500 bp and another at 400 bp. Based on this polymorphism, three genotypes were denoted as AA, AB, and AC for a wild or non-polymorphic region of 575 bp, polymorphic region at 500 bp, and polymorphic region at 400 bp, respectively. The AA, AB, and AC genotypic frequency was 0.58, 0.29, and 0.13 with average kidding rate 3.01 ± 0.09, 4.65 ± 0.32 and 2.84 ± 0.24, respectively. Polymorphic region of BMP15 gene at 500 bp (AB genotype) might be used as marker genotype for higher kidding rate at early age selection of female Surti goat.

Keywords: BMP15, Fecundity gene, Polymorphism, Prolificacy, Surti goat.

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Introduction

The Surti breed of goat is known to be a good dairy and meat-type breed of Gujarat, especially suited for maintenance under complete confinement and stall-feeding (Bayan *et al.,* 2018). The breeding tract of this breed is mainly Vadodara, Bharuch, Surat, Navsari, and Valsad districts of south Gujarat. Surti goats are of pure white body coat color reared by the landless farmers for their source of income by selling milk and animals for meat. Identifying and using genes associated with the litter size for future selection procedures are very much important for genetic improvement and the fecundity of goats (Dangar *et al.,* 2019). Studies on the inheritance pattern of ovulation rate and litter size in prolific sheep led to the identification of a major gene responsible for prolificacy. Marker-assisted selection can be useful for early age selection of goats with high genetic merit. Studies pertaining to genetic variability in growth parameters controlling genes can help in the identification of marker genes for growth traits (Pandya *et al.,* 2020).

In reproductive biology, precisely controlled litter size is critical and important. Endocrine regulatory mediator governs the litter size and ovulation rate in all mammals. The birth of twins and triplets in goat and sheep is very common. Many researchers have worked and many are working to identify the genes that have crucial role in prolificacy (Dangar *et al.,* 2021). Identification and implementing genes related to the litter size for future selection procedures are very important for genetic improvement to increase litter size and, consequently, goat milk and meat. Like sheep, identifying the genes responsible for the prolificacy in goats, known as fecundity genes, is important to goat farming (Sharma *et al.,* 2016). Bone morphogenetic protein 15 (BMP15) gene, also

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known as FecX, located on chromosome X, plays a major role in increasing ovulation rate in heterozygous conditions. This BMP15 (FecX) gene belongs to the transforming growth factor-beta (TGF- β) superfamily (Fabre *et al.,* 2006). Therefore, this study aimed to find out the association of this gene with prolificacy in Surti goats under farm and field conditions.

Materials and Methods

Animals and Sample Collection

A total of 100 Surti goats were selected for this study. Records related to kidding and kids born per kidding were recorded and collected from the farm register in the case of 50 goats of LRS, NAU, Navsari, and by field survey in the case of 50 fields Surti goats. Blood samples (5 mL) from all 100 goats were collected from the jugular vein in sterilized BD vacutainers and were transported to the laboratory at 4°C.

DNA Extraction and PCR-RFLP

The DNA was extracted from blood as per the standard phenol-chloroform DNA extraction procedure described by Sambrook and Russell (2001). The quality of extracted DNA was checked using 0.8% agarose gel at 80 volts for 2 hours. The quantity of DNA was measured using Nano-Drop Spectrophotometer.

Amplification of DNA by PCR was done by using BMP15 gene specific primers given in Table.1. PCR reaction was performed in final reaction mixture of 20 µL consisted 10.0 µL master mix, 0.8 µL forward primer, 0.8 µL reverse primer, 0.3 µL DNA and 8.1 µL Mili-Q-water. PCR protocol to amplify specific region of BMP15 gene was started with initial denaturation at 94°C for 1 min for 1 cycle, followed by 94°C for 45 sec., annealing at 57°C for 1 min and 72°C for 1 min for 35 cycles and final extension at 72°C for 1 min After amplification, the PCR product was again checked using 2% agarose gel for amplification of BMP15 gene specific region and size of the amplified region.

Amplified PCR product was digested using restriction enzyme (RE) given in Table 1. Total of 15 µL of the digestion mixture was prepared using 0.3 µL RE, 5 µL PCR product, 1.5 µL buffer, 8.2 µL Mili-Q-water. The digestion mixture was digested at 60°C for 15 minutes and subjected to run on 2% agarose gel electrophoresis at 80 volts for 2 hours, and image was captured using gel doc system.

Association and Statistical Analysis

Association of prolificacy data and genotyping of Surti goats was done by classifying goats according to their respective genotype and total kids born to date. Specific genotype-wise average kids born and their association was analyzed using R software version 3.3.2. Duncan's new multiple range test was performed to test the significance level of each genotype at $p < 0.05$.

Results and Discussion

Prolificacy-related data of Surti goats collected during the present study are shown in Table 2. Surti goats with first parity are less prone to produce twins as only 10% twining rate was observed in the present study. In comparison, during second and third parity, the twining percentage was shown very high as 62.5% and 76.8%, respectively. This is the normal tendency found in Surti goats that in first parity always produce single kid and twining starts after second parity and continues for subsequent parity. In the present study, less (10%) twining rate found in first parity may be due to a limited number of animals in this parity. Overall, a lesser twinning rate (57%) as compared to the present study was reported by Kuralkar *et al.* (2013) in Berari goats, while a higher twinning rate (69.50% and 64.81%) was observed by Sharma *et al.* (2016) and Panhale *et al.* (2018) in the same breed.

Genotyping of Surti goats was done by collecting blood samples from each goat under study, and DNA was extracted. Extracted DNA was checked for quality (Fig. 1) and quantity. As per references, the PCR product size of BMP15 gene was found as 575 bp in the present study.

Restriction digestion of BMP15 gene PCR product was done using *BssSI* restriction enzyme, and two points polymorphism were found in Surti goats (Fig. 2). The restriction digestion patterns found were an undigested or intact band of exon-2 region of BMP15 gene with 575 bp size, polymorphism at 500 bp produced two bands of 500 bp and 75 bp, and polymorphism at 400 bp produced

Table 1: The primer pairs, expected product size, and restriction enzyme of the BMP15 gene

Candidate gene	*Region of gene*	*Primers (5' – 3')*	*Product size*	*Restriction enzyme*
BMP15	Exon 2	F-TCCCTAAAGGCCTGAAAGAGT R-GCTGAAGGCAAGGAATAGAATC	575 bp	*BssSI*

Table 2: Parity wise classification, total kids born up to specific parity and twining percentage in Surti Goat

	Parity and kids born					
Total No. of animals	*Parity 1*	*Kids*	*Up to Parity 2*	*Kids*	*Up to Parity 3*	*Kids*
50 from LRS, NAU	8	9	7	24	35	152
50 from field	2	2	1	3	47	157
Total 100	10	11	8	27	82	309
Twining rate	10%		62.5%		76.8%	

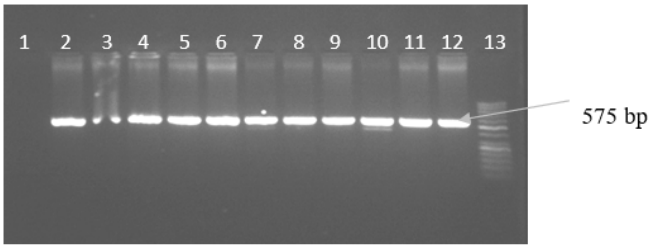

Fig. 1: Agarose gel electrophoresis (2%) of allele specific BMP15 gene for PCR product. Lane 1: PCR without genomic DNA (Template negative control). Lanes 2-12: PCR amplified product of BMP15 gene (size 575 bp). Lane 13: DNA molecular weight marker (50 bp DNA Ladder)

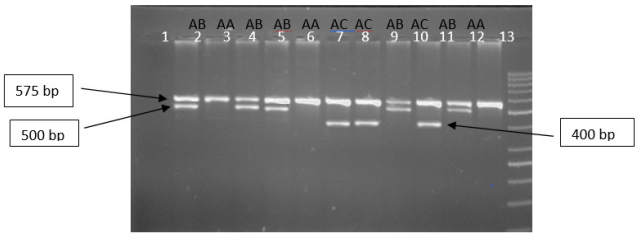

Fig. 2: Restriction pattern of PCR-RFLP product for BMP15 gene. Lane 1: PCR without genomic DNA (Template negative control). Lanes 2-12: Digested PCR product of BMP15 gene. Lane 13: DNA molecular weight marker (50 bp DNA Ladder)

Table 3: Genotype wise average kids born in Surti goats

		Genotype		
Particulars		*AA*	*AB*	*AC*
50 farm goats (LRS)	Parity 1	2	4	2
	Kids	2	5	2
	Parity 2	2	5	0
	Kids	4	20	0
	Parity 3	12	18	5
	Kids	40	95	17
	Total animals	16	27	7
	Total parity	42	68	17
	Total kids born	46	120	19
	Avg. kids born/Parity	1.12 ± 0.04^{b}	1.71 ± 0.05^{a}	1.11 ± 0.08^{b}
		$p < 0.001$		
	Avg. kids born/Animal	2.87 ± 0.21^{b}	4.44 ± 0.31^{a}	2.71 ± 0.47^{b}
		$p < 0.01$		
50 Field goats	Parity 1	2	0	0
	Kids	2	0	0
	Parity 2	1	0	0
	Kids	3	0	0
	Parity 3	39	2	6
	Kids	124	15	18
	Total animals	42	2	6
	Total parity	121	6	18
	Total kids born	129	15	18
	Avg. kids born/Parity	1.06 ± 0.01^{b}	2.5 ± 0.08^{a}	1.0 ± 0.00^{b}
		$p < 0.001$		
	Avg. kids born/Animal	3.07 ± 0.11^{b}	7.5 ± 0.50^{a}	3.0 ± 0.00^{b}
		$p < 0.001$		
Overall: Total animals		58	29	13
Total parity		163	74	35
Total kids born		175	135	37
Avg. kids born/Parity		1.08 ± 0.02^{b}	1.78 ± 0.04^{a}	1.06 ± 0.04^{b}
		$p < 0.001$		
Avg. kids born/Animal		3.01 ± 0.09^{b}	4.65 ± 0.32^{a}	2.84 ± 0.24^{b}
		$p < 0.001$		

Means with at least one common superscript within the row do not differ significantly ($p > 0.05$)

two bands of 400 bp and 175 bp. Intact band of 575 bp was assigned AA genotype, band pattern of 575 bp with 500 bp and 75 bp together was assigned AB genotype and band pattern of 575 bp with 400 bp and 175 bp together was assigned AC genotype. Panhale *et al.* (2018) reported a monomorphic pattern, where the fragment of 153 bp of gene digested with *DdeI* restriction enzyme yielded only one type of restriction pattern (122 bp and 31 bp) in all the Berari goats. Godara *et al.* (2011) also reported a monomorphic pattern for the same gene in Marwari goats. The role of this gene in folliculogenesis and ovigenesis was described by Hua *et al.* (2008) and expressed that identification of new polymorphism or markers could be used as candidate genes for fecundity in Boer, Haimen, Huanghai, Nubi and Matou goat breeds of China. The monomorphic pattern was also observed for the gene in Cele Black sheep (Shi *et al.,* 2010), Bonpala sheep of Sikkim (Roy *et al.,* 2011), Barbarian sheep (Jemmali *et al.,* 2012), Markhoz goats (Shokrollahi, 2015) and Iranian Arabic sheep breed (Mohammadi, 2016).

Out of 100 Surti goats, 58 goats were found with AA genotype, while 29 and 13 goats were found with AB and AC genotypes, respectively. The results show that AB genotype produced significantly higher ($p<0.001$) average kids (4.65±0.32) up to their three parity followed by AA genotype containing goats producing average 3.01 kids and goats with AC genotype produced average 2.84 kids (Table 3). The results revealed that AB genotype containing goats produced significantly ($p < 0.001$) higher (1.78) kids per parity followed by genotype AA (1.08) and AC (1.06) kids per parity.

The mechanism of increased ovulation was found in BMP15 gene heterozygous mutant females; the altered proteins result in increased sensitivity of granulosa cells to FSH, which would lead to accelerated follicular development and precocious ovulation of small follicles (Moore *et al.,* 2004; Moore and Shimasaki, 2005). Ovulation rates in BMP15 mutants are high in the heterozygotes, while the homozygous mutants show a primary ovarian failure resulting in complete sterility (Monteagudo *et al.,* 2009). Ewes with simultaneous mutations in BMP15 had higher ovulation rates than those with either mutation separately (Arnyasi *et al.,* 2004).

The BMP15 is located on the X chromosome, and five point mutations and one deletion increase the prolificacy in various sheep breeds. These mutations include non-synonymous amino acid substitution (FecXI, FecXB, FecXL), premature stop codons (FecXG, FecXH), and a 17 bp deletion (Monteagudo *et al.,* 2009) of the reading frame of the functional gene. The increased prolificacy in six goat breeds of China (Boer, Haimen, Huanghuai, Nubi, Matou, and Jining Grey) was not associated with any known point mutation in the BMP15 gene (Chu *et al.,* 2007; Hua *et al.,* 2008). On the other hand, a goat with low fecundity (Yunling Black goat) was not related to any point mutations in this gene (Cui *et al.,* 2009).

Conclusion

BMP15 gene was found polymorphic in female Surti goat. Two different polymorphisms were present in the BMP15 gene, one at 500 bp and another at 400 bp in female Surti goats. A polymorphic region at 500 bp (AB genotype) plays a highly significant role in the higher prolificacy of Surti goat as compared to base size 575 bp (AA genotype) and polymorphic site 400 bp (AC genotype). Polymorphic region AB may be used as a marker genotype for early age selection of female Surti goat.

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