RESEARCH ARTICLE

Study on Genetic Polymorphism in Genes Related to Prolificacy and Foetal Growth using PCR-RFLP in Surti Goats

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Abstract

The Surti is a dual-purpose goat breed of Gujarat. The Insulin-like Growth Factors Binding Protein 3 (IGFBP3), POU Class 1 Homeobox 1 (POU1F1), G-protein coupled Receptor 54 (GPR54), and Fecundity Gene Boorla (FecB) are playing a role in ovulation, prolificacy, and fetal growth. Mutation in Exon-2 Partial Intron-2 with the base size 316 bp, Exon-4 with the base size 508 bp, Exon-5 with the base size 250 bp, and Exon-6 with base size 190 bp region of IGFBP3, POU1F1, GPR54, and FecB genes, respectively, is reported to be related with increased prolificacy and fetal growth. Based on the known mutation information in goat and sheep, PCR primers were designed to screen polymorphism in a total of 50 Surti goats. During the PCR-RFLP study, no polymorphic sites were found for the region abovementioned IGFBP3, POU1F1, GPR54, and FecB genes in Surti goats.

Keywords: Prolificacy gene, Polymorphism, PCR-RFLP, Surti goat

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Introduction

The Surti breed of goat is a good dairy and meat type breed of Gujarat, especially suited for maintenance under complete confinement and stall-feeding (Bayan *et al*., 2018). The landless farmers rear it for income from the sale of milk and animals. Identifying and using genes associated with the litter size for future selection procedures is very important for the genetic improvement and fecundity of goats (Dangar *et al*., 2019). Studies on the inheritance pattern of ovulation rate and litter size in prolific sheep led to identifying a major gene responsible for prolificacy. Marker-assisted selection can be a useful approach for early age selection of goats with high genetic merit. Studies about genetic variability in growth parameters controlling genes can help identify marker genes for growth traits (Pandya *et al*., 2020). Endocrine regulatory mediator governs the litter size and ovulation rate in mammals. In reproductive biology, precisely controlled litter size is critical. The birth of twins and triplets in goat and sheep is widespread. Many researchers have been working to identify the genes that have a crucial role in ovulation, prolificacy, and fetal weight, such as the Insulin-like Growth Factors Binding Protein 3 (IGFBP3), POU Class 1 Homeobox 1 (POU1F1), G-Protein coupled Receptor 54 (GPR54), and Fecundity Boorla (FecB) genes. This study aimed to identify polymorphic sites, if any, in Exon-2 Partial Intron-2, Exon-4, Exon-5, and Exon-6 region of IGFBP3, POU1F1, GPR54, and FecB genes, respectively, in Surti goats.

Materials and Methods

Total 50 Surti goats were selected to study the polymorphism. Blood samples (5 mL) from each of these goats were collected from jugular vein in sterilized BD vacutainers and were transported to the laboratory at 4°C. The DNA was extracted from each goat's blood individually 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 IGFBP3, POU1F1, GPR54 and FecB gene-specific primers given in Table 1.

PCR reaction was performed in 20 µL reaction mixture consisting of 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 given in Table 2 was carried out to amplify a specific region of the gene. After amplification, the PCR product

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was again checked using 2% agarose gel to amplify IGFBP3, POU1F1, GPR54, and FecB gene-specific region and confirm the amplified region's size.

Amplified PCR product was digested using restriction enzyme (RE) (Table 1). The digestion mixture was prepared and used as per Table 3 and subjected to run on 2% agarose gel electrophoresis at 80 volts for 2 hours, and the image was captured using gel doc system.

Results and Discussion

The findings of the present work on the identification of the genetic structure of four genes - IGFBP3, POU1F1, GPR54, and FecB associated with prolificacy and fetal weight traits in Surti goats are presented in Figures 1 to 8.

IGFBP3

The PCR amplification of the IGFBP-3 gene using primers produced a 316 bp fragment (Fig. 1). The restriction digestion of the 316 bp fragment with the enzyme *HaeIII* yielded single fragments of 316 bp in all the samples of Surti goat (Fig. 2).

However, restriction fragment length polymorphism has been observed in the corresponding fragment of IGFBP-3 gene in Chinese goats (Lan *et al.,* 2007[a]) and Nilagiri and Mecheri breeds (Sudhakar, 2009). Similarly, *HaeIII* PCR-RFLP of the fragment (654 bp) of IGFBP-3 gene has shown monomorphism in Marwari, Mandya, Madras Red, Muzzafarnagri, and Bannur breeds of sheep (Kumar *et al.,* 2006).

POU1F1

The amplification with primers of POU1F1 gene produced a 508 bp fragment consisting of exon 4 (Fig. 3). The restriction digestion by the endo-nuclease, *EcoRI* also yielded two fragments of sizes 284 and 224 bp (Fig. 4) in Surti goats. These results indicated the monomorphic nature of the gene in Surti goat breed.

Table 1: The primer pairs, expected product size and restriction enzyme of the IGFBP3, POU1F1, GPR54 and FecB gene used

Candidate Gene	*Region of Gene*	*Primers*	*Product Size (bp)*	*Restriction Enzyme*
IGFBP3	Exon 2 Partial Intron 2	F-5'- GAAATGGCAGTGAGTCGG -3' R-5'- TGGGCTCTTGAGTAATGGTG -3'	316	*HaeIII*
POU1F1	Exon 4	F-5'- ATACCAGGCAATTCTACACTG – 3' R-5'- GGCCTTGCTTTTCTTTATAG - 3'	508	*EcoRI*
GPR54	Exon 5	F-5'- GGCAATGCAGCCCTTGTC – 3' R-5'- ACCGATGTAGAAGTTGGTCAC - 3'	250	*Hinf1*
FecB	Exon 6	F-5'- CCAGAGGACAATAGCAAAGCAAA -3' R-5'- CAAGATGTTTTCATGCCTCATCAACAGGTC-3'	190	*AvaII*

Table 2: PCR Protocol of IGFBP3, POU1F1, GPR54 and FecB genes used

IGFBP3			*POU1F1*			*GPR54*			*FecB*		
Temp (°C)	*Time*	*Cycles*	*Temp (°C)*	*Time*	*Cycles*	*Temp (°C)*	*Time*	*Cycles*	*Temp (°C)*	*Time*	*Cycles*
94	10 min	1	94	10 min	1	94	10 min	1	94	10 min	1
94	30 sec	35	94	45 sec	35	94	30 sec	35	94	1 min	35
59	30 sec		58	45 sec		57.8	30 sec		60	1 min	
72	30 sec		72	1 min		72	30 sec		72	2 min	
72	5 min	1	72	10 min	1	72	5 min	1	72	10 min	1

Table 3: Gene-specific restriction enzyme with specific digestion protocol

IGFBP3			*POU1F1*		
Restriction Enzyme	*Digestion Mixture*	*Temp(°C) Duration*	*Restriction Enzyme*	*Digestion Mixture*	*Temp(°C) Duration*
HaeIII	1 µL RE 5 µL PCR product 5 µL buffer 14 µL mili-Q water	37°C for 15 minutes 80°C for 20 minutes	*EcoRI*	2 µL RE 3 µL PCR product 5 µL buffer 15 µL mili-Q water	37°C for 3 hrs. 65°C for 20 minutes
GPR54			*FecB*		
Restriction Enzyme	*Digestion Mixture*	*Temp(°C) Duration*	*Restriction Enzyme*	*Digestion Mixture*	*Temp(°C) Duration*
Hinf1	1 µL RE 1 µL PCR product 2 µL buffer 21 µL mili-Q water	37°C for 15 minutes	*AvaII*	1 µL RE 5 µL PCR product 5 µL buffer 14 µL mili-Q water	60°C 15 Minutes

Polymorphism of the gene has been elucidated by sequencing and SSCP in different breeds of sheep (Bastos *et al.,* 2006). The polymorphism of POU1F1 gene for different markers has been reported earlier in goats (Lan *et al.,* 2007[a]).

GPR54

The amplification of GPR54 gene with primers produced a 250 bp fragment consisting of exon 5 (Fig. 5). The restriction digestion by the endonuclease, *Hinf1,* also yielded single fragments of sizes 250 bp (Fig. 6) in Surti goats. These results indicated the monomorphic nature of this gene in the Surti goat breed.

G-Protein Coupled Receptor 54 gene [GPR54] is otherwise termed as KiSS1R. The reproductive role of GPR54 gene was first established by Seminara *et al.* (2003), and a study on mice and humans concluded that mutations in GPR54 gene caused autosomal recessive idiopathic hypogonadotropic hypogonadism in humans and mice. A homozygous single nucleotide variant (443T>C) in exon 3 of GPR54 gene was reported by Seminara *et al.* (2003), which substituted a serine for normal leucine at position 148 (L148S). They further stated that this variant did not appear to be a polymorphism since it occurred only in reproductively affected individuals. Further reports by Popa *et al.* (2005), Aparicio (2005) and Li *et al.* (2008) confirmed the reproductive role of this gene.

The present study's results were contrary to the findings of Feng *et al.* (2010), who found polymorphism in exon 1 and 5 of GPR54 gene of high litter size containing Jining Grey goats. Possible associations between variations in KiSS1 and GPR54 genes with litter size and sexual precocity in goats were reported (Feng *et al.,* 2010). Maitra *et al.* (2014) identified nine SNPs in these breeds, of which 4 were novel, and none of them was found to be associated with reproductive traits, but the difference in litter size and age of sexual maturity was reported for different genotypes.

FecB

A 190-bp fragment from FecB gene of Surti goat amplified using PCR produced a 190 bp fragment (Fig. 7). The digestion process of these fragments by AvaII restriction enzyme revealed the absence of the restriction site (G^GACC) at position 160^161 in tested animals, yielding uncut fragments at 190-bp. The result showed that all tested Surti goats had the same homozygous non-carrier genotype (++) (Fig. 8). Prolificacy and fetal growth are two essential reproduction parameters with economic importance in small breeding programs. Souza et al. (2002) reported genetic polymorphism in BMPR-IB gene and its association with the FecB gene and the high prolificacy in Booroola Merino sheep using PCR-RFLP technique. The genetic polymorphism in FecB gene and its--association with some economically important growth parameters was identified by Guan *et al.* (2007) in Hu sheep (homozygous BB carriers) and Merino prolific meat breed, with three genotypes (BB, B+, and ++) of different frequencies. The animals with genotypes BB and B+ had larger mean litter sizes, heart girth, and chest width than those with genotype ++ ewes. Some workers reported the association between FecB gene and some reproduction and

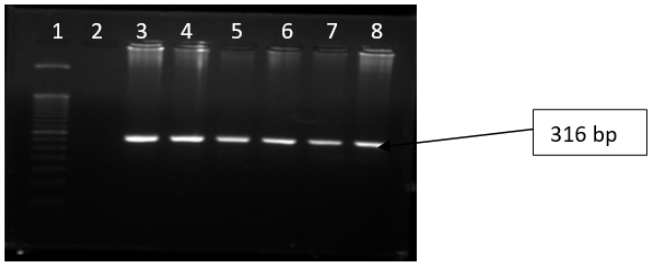

Fig. 1: Agarose gel electrophoresis (2%) of Exon 2 and partial Intron 2 region of IGFBP3 gene. Lane 1: DNA molecular weight marker (100bp DNA Ladder) Lane 2: PCR without genomic DNA (Template negative control). Lanes 3 to 8: PCR amplified products of IGFBP3 gene (size 316 bp).

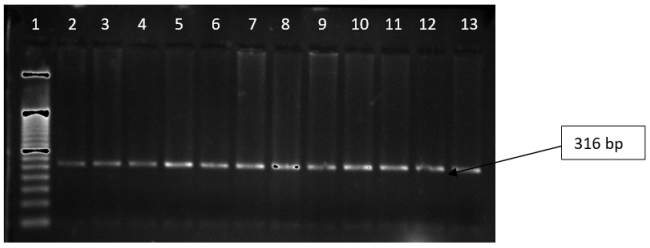

Fig. 2: Restriction pattern of PCR-RFLP product for IGFBP3 gene. Lane 1: DNA molecular weight marker (50bp DNA Ladder). Lanes 2-13: Digested PCR product of IGFBP3 gene.

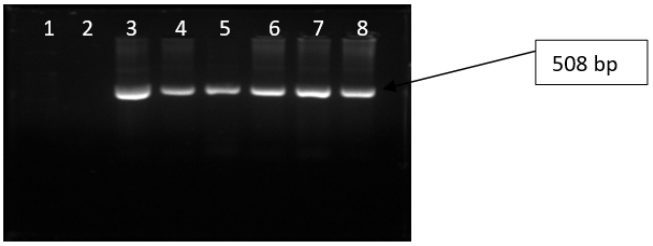

Fig. 3: Agarose gel electrophoresis (2%) of Exon 4 region of POU1F1 gene. Lane 1: DNA molecular weight marker (100bp DNA Ladder) Lane 2: PCR without genomic DNA (Template negative control). Lanes 3 to 8: PCR amplified products of POU1F1 gene (size 508 bp).

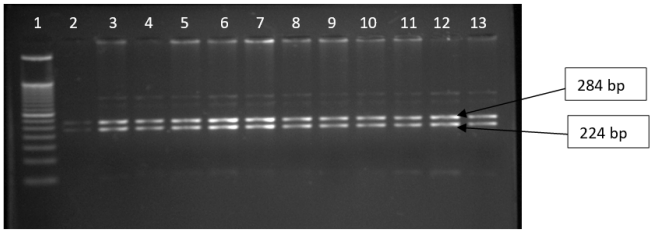

Fig. 4: Restriction pattern of PCR-RFLP product for POU1F1 gene. Lane 1: DNA molecular weight marker (50 bp DNA Ladder). Lanes 2-13: Digested PCR product of POU1F1 gene.

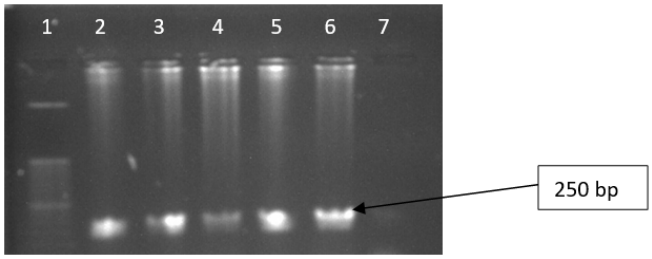

Fig. 5: Agarose gel electrophoresis (2%) of Exon 1 region of GPR54 gene. Lane 1: DNA molecular weight marker (50bp DNA Ladder) Lanes 2 to 6: PCR amplified products of GPR54 gene (size 250 bp) Lane 7 PCR without genomic DNA (Template negative control).

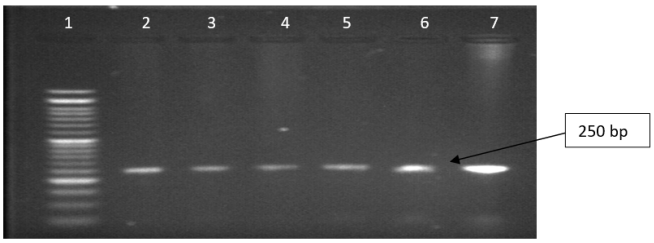

Fig. 6: Restriction pattern of PCR-RFLP product for GPR54 gene. Lane 1: DNA molecular weight marker (50bp DNA Ladder). Lanes 2-6: Digested PCR product of GPR54 gene. Lanes 7: PCR product of GPR54 gene.

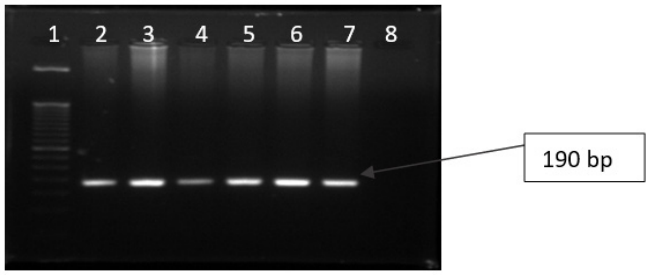

Fig. 7: Agarose gel electrophoresis (2%) of Exon 6 region of FecB gene. Lane 1: DNA molecular weight marker (100bp DNA Ladder) Lanes 2 to 7: PCR amplified products of FecB gene (size 190 bp) Lane 8 PCR without genomic DNA (Template negative control).

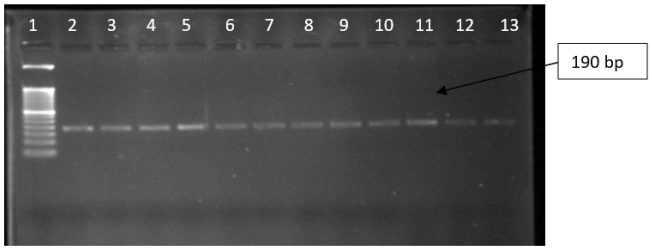

Fig. 8: Restriction pattern of PCR-RFLP product for FecB gene. Lane 1: DNA molecular weight marker (50bp DNA Ladder). Lanes 2-13: Digested PCR product of FecB gene.

fertility parameters (; Smith *et al.,* 1996; Cognie *et al,.* 1998). The FecB gene has positive effects on litter size and ovulation rate and negative effects on fetal growth, development, and body mass (Wang *et al.,* 2003; Liu *et al.,* 2003).

Conclusion

The present study provided information about the reproductive role of IGFBP3, POU1F1, GPR54 and FecB genes in Surti goats and points towards the need for detailed work in this area to screen a large population different regions of IGFBP3, POU1F1, GPR54, and FecB genes.

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