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Original Research

Screening of Marwadi, Patanwadi and Dumba Sheep Breeds for Presence of Fecundity Gene Polymorphism by PCR – RFLP and Sequencing

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Abstract

Mutation detection in major fecundity genes has been explored from the migratory flock of Marwadi, Patanwadi and Dumba sheep of Gujarat. This study was conducted to assess whether any mutations in BMP15 were exhibited in three different sheep breeds. The polymorphism of BMP15 was detected by RFLP and SSCP. The FecX^B locus amplified product of the BMP15 gene was digested using HinfI restriction enzymes. The FecX^L locus of the BMP15 gene was found to be polymorphic by SSCP followed by sequencing of variant pattern. All the animals in the present study were found similar restriction fragment pattern, among three genotypes-wild type (X++, 1.0) at FecX^B locus. Hinf I site was not polymorphic in the present sheep subpopulation. The nucleotide sequence of BMP15 gene, at FecX^L locus in Marwadi, Patanwadi & Dumba sheep was also searched for polymorphisms by PCR-SSCP and investigated for variants using cloning-sequencing. The nucleotide sequences were subjected to alignment analysis and it was observed that among three, two nucleotides change in the bases were found, one at position 53, C-T base change and the second at position 182, C-T base change compared to the reference sequences. Out of total 60 sheep screened for mutation for BMP15 gene, all animals were found homozygous wild type for BMP15 gene for PCR-RFLP. None of the animals were found homozygous mutant or heterozygous (carrier) type for BMP15 gene. SSCP-sequencing results showed that the two nucleotide changes might be related with the fecundity of sheep. The present findings indicate that the three sheep populations are not carriers for BMP15 gene mutation.

Key words: BMP15 gene, Dumba Sheep, Marwadi, Patanwadi, PCR-RFLP, SSCP

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Introduction

Sheep is an important species of livestock for India and sheep husbandry is an integral part of rural economy, particularly in north-west and southern part of the country. India has rich diversity of sheep genetic resources, with about 65.06 million sheep (Livestock census, 2012) and 42 distinct breeds distributed in the different agro-climatic regions of the country, however, a large proportion of sheep are of nondescript or mixed breeds. They play an important role in the livelihood of a large proportion of small and marginal farmers and landless labourers. Litter size is one of the most important traits determining the profitability of sheep production. Major source of income to the [i]sheep is the sale lambs for mutton purpose and secondarily wool. However, the productivity of Indian sheep is considerably low. Major reasons include the poor genetic potential of native stock, inadequate feed resources, nutritional deficiency, heat stress, poor health monitoring, and inadequate marketing and credit support to sheep owners (Goyal *et al.*, 2013). Most of the breeds of sheep in India have evolved naturally through adaptation to agro-ecological conditions; to a limited extent, there has been artificial selection for specific needs e.g. meat production or apparel wool. Most of the breeds of sheep are very well adapted to the harsh climate, long migration and lack of vegetation and drinking water. Gujarat is having two well-known breeds of sheep (Patanwadi and Marwadi) and a lesser known uncharacterized breed (Dumba).

Major genes for production traits provide opportunities for rapid increases in the efficiency of sheep production. The effect of a major gene on mean litter size is likely to be influenced by breed, environmental conditions, maternal nutrition and other factors (Asadpour *et al.*, 2012). Two likely candidates for the same phenotypes are the growth differentiation factor 9 (GDF9) and bone morphogenic protein 15 (BMP15) genes. Both genes are members of transforming growth factor beta (TGFbeta) superfamily, coding for distinct proteins, the expression of which in ovarian tissue is exclusively in the oocyte of the developing follicle. GDF9 is expressed in oocytes from the primary stage of follicular development until ovulation (Laitinen *et al.*, 1998). Growth differentiation factor 9 (GDF9) is seen in chromosome 5 in sheep. They are expressed in oocytes and play an important role in ovarian folliculogenesis (Abraham and Thomas, 2012). However, BMP15 is essential for folliculogenesis in sheep (Galloway *et al.*, 2000). In sheep, it is also clear that heterozygotes carrying an inactivating mutation in only one copy of BMP15 have an increased ovulation rate (Galloway *et al.*, 2000). Mutations that increase ovulation rate have been discovered in the BMPR-1B also known as Booroola fecundity gene (FecB), BMP15 and GDF9 genes, and others are known to exist from the expressed inheritance patterns although the mutations have not yet been located (Sudhakar *et al.*, 2013). Naturally occurring mutation in the BMP15 gene in the high prolific Lacaune sheep breed is already reported. The identified variant is a C53Y missense non-conservative substitution leading to the amino acid change of a cysteine with a tyrosine in the mature peptide of the protein. This mutation is associated with an increased ovulation rate and sterility in heterozygous and homozygous animals, respectively. In vitro studies showed that the C53Y mutation was responsible for the impairment of the maturation

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process of the BMP15 protein, resulting in a defective secretion of both the precursor and mature peptide (Bodin *et al.*, 2007). In sheep, six different point mutations viz., FecX^I (Inverdale), FecX^H (Hanna), FecX^L (Lacaune), FecX^G (Cambridge) and FecX^B (Belclare), and FecX (Rasa Aragenosa) have been identified in the BMP15 gene, each having a major effect on ovulation rate (Sithimarjitha *et al.*, 2015). Recently two additional mutations (FecXGr and FecX^o) have been identified in prolific ewes in which homozygous FecXGr /FecXGrGrivette and homozygous FecX^o/FecX^{o}Olkuska ewes are hyper prolific in striking contrast with the sterility exhibited by all other known homozygous BMP15 mutations (Demars *et al.*, 2013).

The aim of the study was to screen Marwadi, Patanwadi and Dumba sheep for point mutations in BMP15 gene viz., FecX^B (Belclare) the mutation corresponding to G to T transition, leading to a substitution of the Serine at position 99 of the mature BMP15 protein with an Isoleucine.

Materials and Methods

The research and laboratory procedures for the sample preservation, DNA extraction, PCR, PCR-RFLP, PCR-SSCP, and its cloning and sequencing were carried out at the Department of Animal Genetics and Breeding and Department of Animal Biotechnology, College of Veterinary Science and A.H., Anand. Samples were collected from the Marwadi (20), Patanwadi (20) and Dumba (20) ewes from the breeding tract. Blood samples from 60 animals were collected from the Jugular in 9 ml capacity vacutainer (EDTA, K3). DNA was extracted from collected blood samples by John's method (John *et al.*, 1990). The DNA concentration was determined by Nanodrop and 3µl (30 ng/ µl) of DNA was used as the template for the PCR reaction.

Single Nucleotide Polymorphism Detection Assays

A specific primer pair has been designed to generate a forced Hinf I mutation (C to T nucleotide change) in the BMP15 gene, whereas products from non-carriers of the mutation lack this site. Genomic DNA was PCR amplified using primer sequences for FecX^B as given in Table 1. The amplification reaction conditions were carried out using 35 cycles at 95°C for 5 min, followed by 30 s, 64°C for 45 s, 72°C for 30 s, followed by 72°C for 10 min. The 153 bp PCR products were digested with HinfI restriction enzyme. The FecX^L genotyping was carried out using SSCP-PCR technique as described by (Bodin *et al.*, 2007) (Table 1). The amplification reaction conditions were carried out using 33 cycles at 95°C for 5 min, followed by 30 s, 58°C for 30 s, 72°C for 1 min, followed by 72°C for 10 min. The amplified products were visualized as a single compact band of expected size under UV light and revealed amplicons of 321 bp size.

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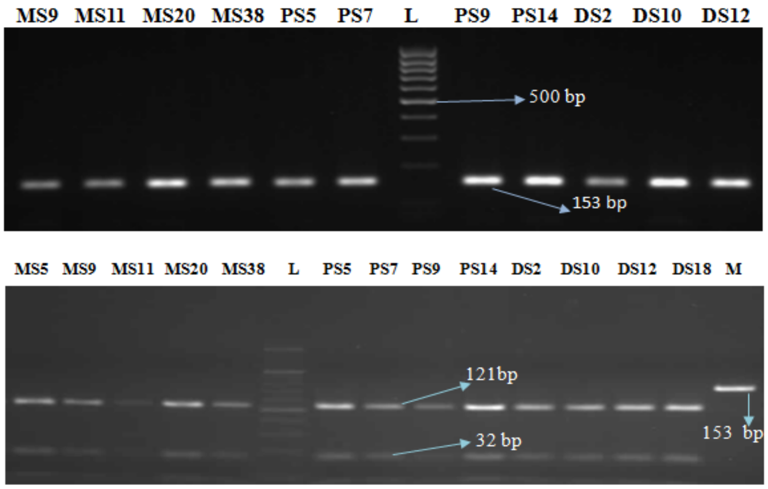
Table 1: List of primers used for BMP15 gene polymorphism identification

S. No.	Gene	Primer Name	Sequence	PCR Product Size
1	FecX^{B}	FecX^{B-}F	GCCTTCCTGTGTCCCTTATAAGTATGTTCCCCTTA	153 bp
2		FecX^{B-}R	TTCTTGGGAAACCTGAGCTAGC	
3	FecX^{L}	FecX^{L}-F	CATGATGGGCCTGAAAGTAAC	321 bp
4		FecX^{L}-R	GGCAATCATACCCTCATACTCC	

Genomic DNA fragments of ovine BMP-15 were amplified by PCR using BMP15 primers. Amplifications were performed as follows: 25 µL of each PCR mixture containing 50-100 ng of total DNA, PCR Master mix (Cat no. K0171, MBI Fermentas) containing 0.05 U/µl Taq DNA polymerase (recombinant) in reaction buffer, MgCl2 (4 mM) and dNTPs (0.4 mM of each).

PCR-RFLP and SSCP Analysis

The polymorphism of BMP15 was detected by RFLP and SSCP. The PCR product was digested in a total of 10 µL reaction containing 1 × buffer L, 4 U Hinf I (Fermentas), 300 ng PCR products at a constant temperature (37°C) for 3 h. The digested products were electrophoresed on agarose gel, all digested PCR products from three marker loci were separated by 3.0% agarose gel and visualized with ethidium bromide staining under gel documentation system (Fig. 1).



*MS= Marwari Sheep, PS= Patanwadi sheep, DS= Dumba sheep

Fig. 1: PCR amplification and agarose gel electrophoresis of BMP15 gene–153 bp and R.E. digestion product of 121bp & 32bp.

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To study point mutation present in BMP15 was analyzed by PCR-SSCP. The PCR product was snap chilled and loaded onto a polyacrylamide gel for electrophoresis in the 1×TBE buffer at 140 volts for 14-16 hours at 4°C. Silver staining was done to identify single strand conformation polymorphism (SSCP) or to score genotypes.

Cloning and Sequencing of BMP15 Segment

For cloning of BMP15 segment, one representative sample of three different SSCP pattern was cloned in pTZ57 / R vector. Ligation was confirmed by performing M13 PCR. Ligated product was transformed into competent E. coli cells and transformed cells were spread over on LA agar plates containing X-Gal and IPTG for blue white screening. White colonies proceeded for LB broth culture followed by plasmid extraction from overnight grown culture. The recombinant plasmid DNA was isolated and sequenced on ABI-PRISM – 310 DNA sequencer. All three SSCP variant patterns were sequenced at least two times.

Results

Total 60 samples (20 samples for each breed viz. Marwadi, Patanwadi and Dumba breeds) of Sheep having record for litter size were screened for BMP15 mutation. A DNA fragment with the expected size of 153 bp was amplified from exon 2 of BMP15 gene. The forced PCR-RFLP approach has been used previously to genotypic prolific sheep (Hanrahan et al., 2004) to decide whether they have the same mutation as $FecX^B$ in local breeds of Gujarat. The PCR product was digested with *Hinf1* restriction enzyme to produce two fragments of 121 bp and 32 bp for wild type. In present study, all animals of three different breeds were found wild-type (non carrier) with fragment size 121 bp and 32 bp.

PCR-SSCP and Sequencing of BMP15 Gene Variant

A specific fragment of BMP15 gene segment was subjected to single strand conformation polymorphism (SSCP). Three patterns were observed within 60 animals screened for BMP15 segment as shown in Fig. 2. Migration patterns of these single stranded DNA bands (conformers) of same molecular weight were different due to point mutations in their sequences resulting in the formation of different secondary structures. These DNA bands-variants were subjected to cloning and sequencing to confirm the nucleotide change within gene sequences. For cloning of BMP15 segment, one representative sample of three different SSCP pattern was cloned in pTZ57 / R vector. Ligated products showed expected band size i.e. 475 bp for BMP15 (321 bp BMP15 + 154 bp M13 fragment) on 2% agarose indicating successful ligation reaction (Fig. 2).

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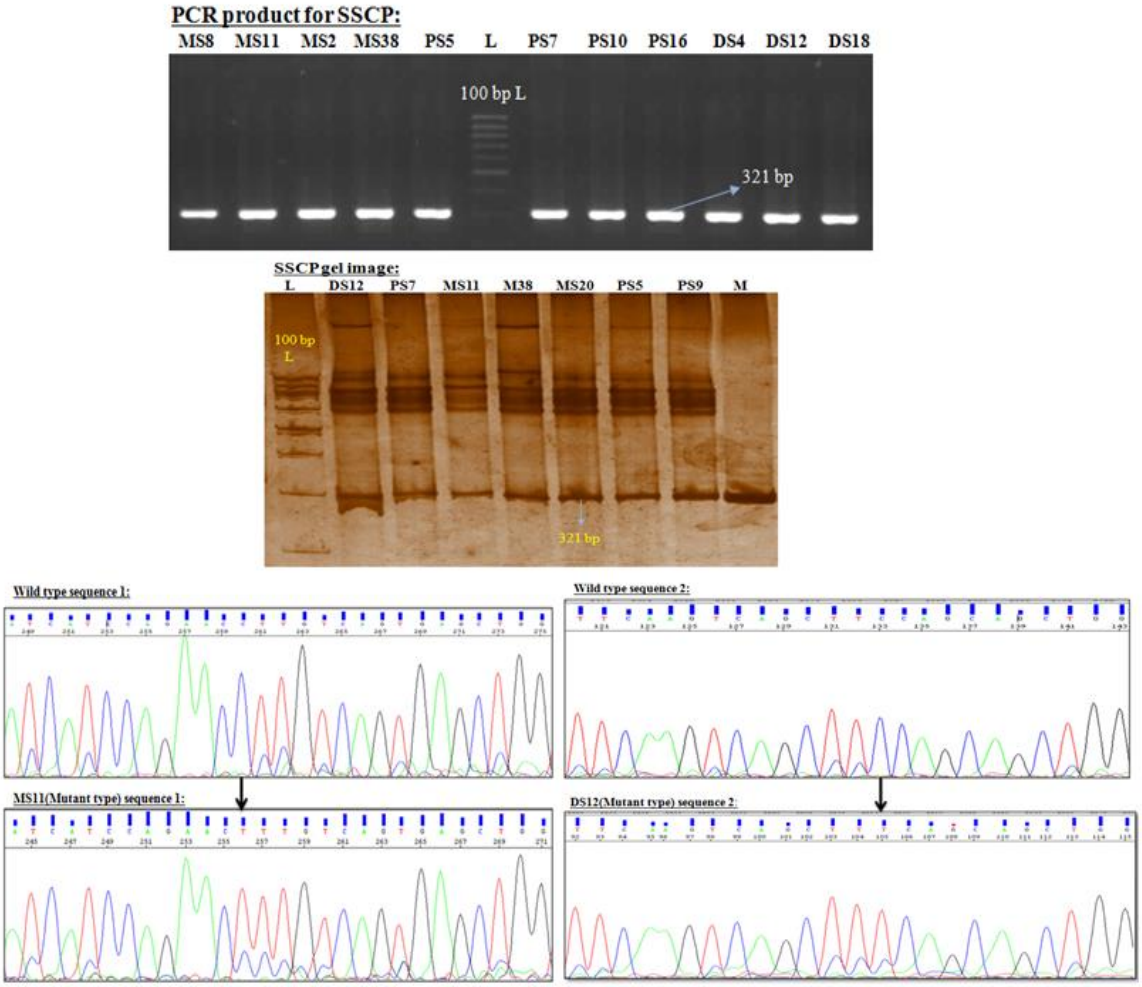

Fig. 2: PCR agarose gel, SSCP gel and Sanger sequencing of BMP15 gene variant identified by comparing electropherogram of wild-type and mutant type sequence

Sequences obtained by sequence analyser were curated and vector sequences were removed using Vecscreen programme. These filtered sequences were local aligned using BLASTn protocol available at NCBI. BLAST analysis of BMP15 gene segment sequence of *Ovis aries* bone morphogenic protein 15 (BMP15), mRNA in Genebank database using BLASTn protocol is shown in Fig. 3 (1-3).

Fig. 3: (1) Normal Sequence (wild type)

```
>☐ ref|NM_001114767.1|Ovisaries bone morphogenetic protein 15 (BMP15), mRNA
Length=1182

GENE ID: 100141303 BMP15|bone morphogenetic protein 15 [Ovis aries]
 (10 or fewer PubMed links)

 Score =  577 bits (312),  Expect = 1e-161, Identities = 312/312 (100%), Gaps =
0/312 (0%), Strand=Plus/Plus
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DOI 10.5455/ijlr.20190830034644

```
Query  2     CATGATGGGCCTGAAAGTAACCAGTGTTCCCTCCACCCTTTTCAAGTCAGCTTCCAGCAG  61
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  850   CATGATGGGCCTGAAAGTAACCAGTGTTCCCTCCACCCTTTTCAAGTCAGCTTCCAGCAG  909

Query  62    CTGGGCTGGGATCACTGGATCATTGCTCCCCATCTCTATACCCCAAACTACTGTAAGGGA  121
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  910   CTGGGCTGGGATCACTGGATCATTGCTCCCCATCTCTATACCCCAAACTACTGTAAGGGA  969

Query  122   GTATGTCCTCGGGTACTACACTATGGTCTCAATTCTCCCAATCATGCCATCATCCAGAAC  181
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  970   GTATGTCCTCGGGTACTACACTATGGTCTCAATTCTCCCAATCATGCCATCATCCAGAAC  1029

Query  182   CTTGTCAGTGAGCTGGTGGATCAGAATGTCCCTCAGCCTTCCTGTGTCCCTTATAAGTAT  241
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1030  CTTGTCAGTGAGCTGGTGGATCAGAATGTCCCTCAGCCTTCCTGTGTCCCTTATAAGTAT  1089

Query  242   GTTCCCATTAGCATCCTTCTGATTGAGGCAAATGGGAGTATCTTGTACAAGGAGTATGAG  301
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1090  GTTCCCATTAGCATCCTTCTGATTGAGGCAAATGGGAGTATCTTGTACAAGGAGTATGAG  1149

Query  302   GGTATGATTGCC  313
             ||||||||||||
Sbjct  1150  GGTATGATTGCC  1161
```

Fig. 3: (2) For MS11(mutant)

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>☐ ref|NM_001114767.1|Ovisaries bone morphogenetic protein 15 (BMP15), mRNA
Length=1182

GENE ID: 100141303 BMP15 | bone morphogenetic protein 15 [Ovis aries]
(10 or fewer PubMed links)

 Score =  571 bits (309),  Expect = 7e-160, Identities = 311/312 (99%), Gaps =
0/312 (0%), Strand=Plus/Plus

Query  2     CATGATGGGCCTGAAAGTAACCAGTGTTCCCTCCACCCTTTTCAAGTCAGCTTTCAGCAG  61
             ||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct  850   CATGATGGGCCTGAAAGTAACCAGTGTTCCCTCCACCCTTTTCAAGTCAGCTTCCAGCAG  909

Query  62    CTGGGCTGGGATCACTGGATCATTGCTCCCCATCTCTATACCCCAAACTACTGTAAGGGA  121
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  910   CTGGGCTGGGATCACTGGATCATTGCTCCCCATCTCTATACCCCAAACTACTGTAAGGGA  969

Query  122   GTATGTCCTCGGGTACTACACTATGGTCTCAATTCTCCCAATCATGCCATCATCCAGAAC  181
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  970   GTATGTCCTCGGGTACTACACTATGGTCTCAATTCTCCCAATCATGCCATCATCCAGAAC  1029

Query  182   CTTGTCAGTGAGCTGGTGGATCAGAATGTCCCTCAGCCTTCCTGTGTCCCTTATAAGTAT  241
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1030  CTTGTCAGTGAGCTGGTGGATCAGAATGTCCCTCAGCCTTCCTGTGTCCCTTATAAGTAT  1089

Query  242   GTTCCCATTAGCATCCTTCTGATTGAGGCAAATGGGAGTATCTTGTACAAGGAGTATGAG  301
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1090  GTTCCCATTAGCATCCTTCTGATTGAGGCAAATGGGAGTATCTTGTACAAGGAGTATGAG  1149

Query  302   GGTATGATTGCC  313
             ||||||||||||
Sbjct  1150  GGTATGATTGCC  1161
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Fig. 3: (3) For DS12(mutant)

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>☐ ref|NM_001114767.1|Ovisaries bone morphogenetic protein 15 (BMP15), mRNA
Length=1182

GENE ID: 100141303 BMP15 | bone morphogenetic protein 15 [Ovis aries]
(10 or fewer PubMed links)

 Score =  571 bits (309),  Expect = 7e-160 Identities = 311/312 (99%), Gaps =
0/312 (0%) Strand=Plus/Plus

Query  2     CATGATGGGCCTGAAAGTAACCAGTGTTCCCTCCACCCTTTTCAAGTCAGCTTCCAGCAG  61
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  850   CATGATGGGCCTGAAAGTAACCAGTGTTCCCTCCACCCTTTTCAAGTCAGCTTCCAGCAG  909

Query  62    CTGGGCTGGGATCACTGGATCATTGCTCCCCATCTCTATACCCCAAACTACTGTAAGGGA  121
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  910   CTGGGCTGGGATCACTGGATCATTGCTCCCCATCTCTATACCCCAAACTACTGTAAGGGA  969

Query  122   GTATGTCCTCGGGTACTACACTATGGTCTCAATTCTCCCAATCATGCCATCATCCAGAAC  181
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  970   GTATGTCCTCGGGTACTACACTATGGTCTCAATTCTCCCAATCATGCCATCATCCAGAAC  1029

Query  182   TTTGTCAGTGAGCTGGTGGATCAGAATGTCCCTCAGCCTTCCTGTGTCCCTTATAAGTAT  241
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1030  CTTGTCAGTGAGCTGGTGGATCAGAATGTCCCTCAGCCTTCCTGTGTCCCTTATAAGTAT  1089

Query  242   GTTCCCATTAGCATCCTTCTGATTGAGGCAAATGGGAGTATCTTGTACAAGGAGTATGAG  301
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1090  GTTCCCATTAGCATCCTTCTGATTGAGGCAAATGGGAGTATCTTGTACAAGGAGTATGAG  1149

Query  302   GGTATGATTGCC  313
             ||||||||||||
Sbjct  1150  GGTATGATTGCC  1160
```

Analysis of SSCP- Variant Sequence using Clustal W

All the three different patterns obtained by SSCP were sequenced and the obtained sequences were analyzed by clustalW, the result was showed in Fig. 4. The two different change in the bases were observed, one in the MS11 sample at position 53, C-T base change and the second in the DS12 sample at position 182, C-T base change compared to the reference sequences as shown below-

```
PS5R            TCATGATGGGCCTGAAAGTAACCAGTGTTCCCTCCACCCTTTTCAAGTCAGCTTCCAGCA 60
MS38R           TCATGATGGGCCTGAAAGTAACCAGTGTTCCCTCCACCCTTTTCAAGTCAGCTTCCAGCA 60
PS7R            TCATGATGGGCCTGAAAGTAACCAGTGTTCCCTCCACCCTTTTCAAGTCAGCTTCCAGCA 60
DS12R           TCATGATGGGCCTGAAAGTAACCAGTGTTCCCTCCACCCTTTTCAAGTCAGCTTCCAGCA 60
MS11R           TCATGATGGGCCTGAAAGTAACCAGTGTTCCCTCCACCCTTTTCAAGTCAGCTTTCAGCA 60
                ****************************************************** *****

PS5R            GCTGGGCTGGGATCACTGGATCATTGCTCCCCATCTCTATACCCCAAACTACTGTAAGGG 120
MS38R           GCTGGGCTGGGATCACTGGATCATTGCTCCCCATCTCTATACCCCAAACTACTGTAAGGG 120
PS7R            GCTGGGCTGGGATCACTGGATCATTGCTCCCCATCTCTATACCCCAAACTACTGTAAGGG 120
DS12R           GCTGGGCTGGGATCACTGGATCATTGCTCCCCATCTCTATACCCCAAACTACTGTAAGGG 120
MS11R           GCTGGGCTGGGATCACTGGATCATTGCTCCCCATCTCTATACCCCAAACTACTGTAAGGG 120
                ************************************************************

PS5R            AGTATGTCCTCGGGTACTACACTATGGTCTCAATTCTCCCAATCATGCCATCATCCAGAA 180
```

```
MS38R           AGTATGTCCTCGGGTACTACACTATGGTCTCAATTCTCCCAATCATGCCATCATCCAGAA 180
PS7R            AGTATGTCCTCGGGTACTACACTATGGTCTCAATTCTCCCAATCATGCCATCATCCAGAA 180
DS12R           AGTATGTCCTCGGGTACTACACTATGGTCTCAATTCTCCCAATCATGCCATCATCCAGAA 180
MS11R           AGTATGTCCTCGGGTACTACACTATGGTCTCAATTCTCCCAATCATGCCATCATCCAGAA 180
                ************************************************************

PS5R            CCTTGTCAGTGAGCTGGTGGATCAGAATGTCCCTCAGCCTTCCTGTGTCCCTTATAAGTA 240
MS38R           CCTTGTCAGTGAGCTGGTGGATCAGAATGTCCCTCAGCCTTCCTGTGTCCCTTATAAGTA 240
PS7R            CCTTGTCAGTGAGCTGGTGGATCAGAATGTCCCTCAGCCTTCCTGTGTCCCTTATAAGTA 240
DS12R           CTTTGTCAGTGAGCTGGTGGATCAGAATGTCCCTCAGCCTTCCTGTGTCCCTTATAAGTA 240
MS11R           CCTTGTCAGTGAGCTGGTGGATCAGAATGTCCCTCAGCCTTCCTGTGTCCCTTATAAGTA 240
                * **********************************************************

PS5R            TGTTCCCATTAGCATCCTTCTGATTGAGGCAAATGGGAGTATCTTGTACAAGGAGTATGA 300
MS38R           TGTTCCCATTAGCATCCTTCTGATTGAGGCAAATGGGAGTATCTTGTACAAGGAGTATGA 300
PS7R            TGTTCCCATTAGCATCCTTCTGATTGAGGCAAATGGGAGTATCTTGTACAAGGAGTATGA 300
DS12R           TGTTCCCATTAGCATCCTTCTGATTGAGGCAAATGGGAGTATCTTGTACAAGGAGTATGA 300
MS11R           TGTTCCCATTAGCATCCTTCTGATTGAGGCAAATGGGAGTATCTTGTACAAGGAGTATGA 300
                ************************************************************

PS5R            GGGTATGATTGCCA 314
MS38R           GGGTATGATTGCCA 314
PS7R            GGGTATGATTGCCA 314
DS12R           GGGTATGATTGCCA 314
MS11R           GGGTATGATTGCCA 314
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Fig. 4: CLUSTAL 2.0.12 multiple sequence alignment of Marwadi, Patanwadi and Dumba representative sheep samples sequence

Discussion

BMP15 mutations are associated with sterility and increased ovulation rate in sheep. *BMP15*gene is code for distinct proteins that are members of the transforming growth factor beta (TGFb) superfamily. The present study was carried out to find the mutation in the exon 2 of the BMP15 gene. Hanrahan and co-workers (Hanrahan *et al.*, 2004) reported the FecXB mutation at 1100bp of the coding nucleotide. The nucleotide change is from G to T which causes the amino acid change from Sre(S) to Ile(I). In the present study the forced PCR-RFLP was used to amplify a 153bp of PCR product and digested with Hinf I which gave two bands of 121bp and 32bp respectively. The FecB mutation is present in Booroola Merino (Mulsant *et al.*, 2001; Souza *et al.*, 2001; Wilson *et al.*, 2001), Garole (Davis *et al.*, 2002), Javanese (Davis *et al.*, 2002), Small Tailed and Han (Liu *et al.*, 2003). The result was not in accordance to that obtained by Hanrahan (Hanrahan *et al.*, 2004). Beiyao and his co-worker (Beiyao *et al.*, 2013) found the mutation in BMPR-B/FecB was found in Bayanbulak sheep. Independence test results of the two flocks demonstrate that the FecB locus has a significant effect on the lambing number of Bayanbulak sheep, while BMP15 have corresponds to a G to A transition, leading to a substitution of the Cys residue (TGT) at position 53 of the mature BMP15 some activity on their own, both human and ovine GDF9 and BMP15 together have been shown to work synergistically (McNatty *et al.*, 2005; Peng *et al.*, 2013). In the present study, no mutation was found in all sheep breeds (Marwadi, Patawadi, and

Dumba) of Gujarat, indicates the low litter size in these sheep breeds. The present study was also carried out to detect the mutation in another fragment of exon 2 of BMP15 gene as reported by (Bodin *et al.*, 2007), they reported the mutation protein with a Tyr (TAT).

In the present study, the PCR product was also subjected to SSCP, which gave a different pattern. The sequencing of a representative of these three different patterns was carried out. Out of three, one was matching to the reference sequence of Ovis aries bone morphogenic protein 15 (BMP15), mRNA. In the other two patterns two mutations were found at position 910bp and 1030bp and the change is from C to T nucleotide in both samples respectively. The mutation reported by the (Bodin *et al.*, 2007) was not found in the three local breed of Gujarat which may be due to the low litter size in these breeds. The difference could be due to a lower prolificacy potential from the background genotype, to environmental factor such as the relatively low nutritional value of the tropical forages available to these ewes, or to a combination of these factors.

On the other hand, due to small size of the sample studied in this research, this is a probability that the mutant allele was to available in the sample. Therefore, there is a need to undertake a further research on a relatively larger size sample for the population. A number of other mutant genes affecting lambing rates have been also detected for which the local sheep breed (Marwadi, Patanwadi and Dumba) may be studied.

In present study, due to small samples studied it was found no any mutation in BMP15 gene but further studies are needed with large sample size along with other fecundity genes, especially ewes with twins and triplets in any of these breeds.

Conclusion

BMP15 gene segment study for this study showed no mutation in each of 20 populations of Marwari, Patanwari and Dumba sheep breeds. The study was also carried out to detect the mutation in the fragment of exon 2 of BMP15 gene. The PCR product was subjected to SSCP, which gave three patterns. Among these, two patterns two mutations were found at position 910bp and 1030bp and the change is from C to T nucleotide in both samples respectively. These two were matching to the reference sequence of Ovis aries bone morphogenic protein 15 (BMP15), mRNA (NM_001114767.1). The mutation studied for this study which increases the ovulation rate was not found, these indicates the low litter size in the local sheep breeds.

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