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Study of *BMP15* gene Polymorphisim in Lehri Goat Breed of Balochistan

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ABSTRACT

In this study only exonic regions of *BMP15* gene were studied which comprises 1185 nucleotide base pairs. Sequencing data analysis revealed that in Lehri goat there were two polymorphism at nucleotide positions number 200 and 901 observed as C>T and G>A respectively. A heterozygosity was also observed with triplets birth history at nucleotide positions 656 where sequences presenting both peaks of G and A at the same nucleotide position. DNA Sequencing analysis of BMP15 gene revealed two polymorphisms including changes of Serine (TCA) into Lucerine (TTA) codon 67 and Glysine (GGC) into Serine (AGC) at codon 301. Heterozygosity also observed changing Glutamatic acid (GAG) into Glysine (GGG) at codon 394. All changed amino acids are non-synonymous.
KEYWORDS*:* *BMP15* gene, Polymorphism, Heterozygosity, Lehri, Goat.

INTRODUCTION

Goats and sheep tender higher prospective for meat, milk and wool production but their efficiency of production can be improved and increased by introducing highly prolific goat and sheep. Pakistan is the third biggest goat producing country in the world (Khan et al., 2008). Out of three hundred goat breeds of the world, 30 goat breeds are in Pakistan (Khan et al., 2003). Pakistan has nearly 66.6 million goat population (Economical survey of Pakistan 2013-14). Small ruminants are generally preferred over large ruminants because of their small size and higher reproducibility rate. The importance of small ruminants in particular, has increased many folds in last decades due to the increase of human population. Although due to the gap between demand and supply, the price of mutton has been increased drastically in last few years. However, Sheep and goat are highly capable to ensure good quality meat supply and also provide animal origin protein. Lehri goat breed is a small size breed mainly found in Sibi and Bolan district of Balochistan. This local goat breed is characterized physically by black color, long hair, twins/ and or triplets births. Average adult body weight ranges from 30-35 kg. The prime objective of breeder to obtain maximum turnout and this could only be obtained by hereditary potential through appropriate selection techniques. It is generally accepted that the selection criteria with estimated goals will eventually increase the reproduction as well as production effectiveness in flock. The assortment for reproduction traits in particular is rather challenging to obtain because of low genetic traits, but this could be achieved through another selection technique like Marker Supported Selection is adopted with maximum accuracy in prediction of breeding value. Therefore, it is imperative to find out vital genes that can safe guard as marker influencing fertility in animals. In goats and sheep ovary 3 prominent genes were found namely *BMP-15* and *GDF-9* serves in oocytes (Juengel et al., 2002). *BMPR-1B* receptor found in granulose cells as well in oocytes in early stage and afterwards in antral follicles and minute quantity were found in thecal layer of sheep, goat, cattle and buffalo (Souza et al., 2002; Wilson et al., 2001 and Glister et al., 2004). *BMP-15* has got importance in terms of booroola phenotype and also termed as *GDF-9B* genetic code for protein synthesis in oocytes which enhance the formation of follicules and fecundity of sheep and goat (Galloway et al., 2000 and Bodensteiner et al., 1999). Role of *BMP-15* gene is not known in the course of time being how it works to manage granulose cell (McNatty et al., 2005). Recently, researchers argued that 5 mutations influence the prolificacy in *BMP-15* gene by expressing amino acid sequences vizFec^{XL}, Fec^{XB}, Fec^{XI}) or precipitate stop codons (Fec^{XG}, Fec^{XH}) on ovulation rate are considerably improved in heterozygosity (Galloway *et al*., 2000; Montgomery *et al*., 2001; Hanrahan et al., 2004; Davis, 2005; Bodin et al., 2007).

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BMP15 gene first recognized in Romney sheep and named as inverdale gene (Fec^X) is an X-linked gene in nature, plays important role to boost the ovulation rate in sheep (Davis et al., 1991, Davis et al., 1992). In this way, for achieving high performance of animals at early phase of life, detection of FecB (Booroola) is important in sheep and goat production. Markers that considerably donate various trait terminologies in ruminants have been inspiring forward an inside in genetics. If this sort of markers is acknowledged in goat and sheep breeds of Pakistan, recognition and deliberate breeding of elevated productive animals will result in fast perpendicular growth of sheep and goat.

Primary objective of the current study was to identify SNPs (Single Nucleotide Polymorphisims) in *BMP15* gene and their influence on fecundity in Lehri goat breed of Balochistan. As this is first study of its kind at molecular level particularly on *BMP15* gene in Balochistan on a local goat breed with high prolificacy. Results of current study will help to ensure the mutton production and encourage the socio economic situation of small ruminant's farmers in the region.

MATERIAL AND METHODS

Animals were selected from different areas of district Bolan and Sibi of Balochistan province. Hundred (100) blood samples were collected from unrelated females of fifty (50) having single birth history and fifty (50) have multiple birth history (Twin, Triplets). The samples brought to Molecular Genetics Laboratory, Faculty of Veterinary and Animal Sciences, Lasbela University of Agriculture, Water and Marine Sciences Uthal, Balochistan and stored at -20ºC before further processing. Genomic DNA was extracted from blood by inorganic method using standard techniques already published (Sambrook and Russel, 2001).

Six pairs of primers (Table-1) six paires of primers designed for amplifying the Exon 1 and 2 of BMP15 gene sequence available in NCBI (Gene Bank Accession# EU743938) with help of Primer3 software (Rozen and Skaletsky, 2000). PCR amplifications were carried out in 25 µL reaction mixtures containing 50 ng of DNA template, 10 pmol of each primer, 2.5 mM $MgCl_2$, 100 µM of dNTPs mix and 1.5 U of Taq DNA polymerase (Fermentas, Thermo Fisher Scientific Inc. USA). PCR was carried out in BioRadthermocycler while using initial denaturation of 94 ºC for 4 min, 30 cycles of denaturation at 94 ºC for 30s, annealing at 60 °C for 45s and extension at 72 °C for 30s, followed by final extension at 72 °C for 5 min. The PCR products were purified and sequenced on an ABI 3130XL genetic analyzer (Applied Biosystems, Foster, CA, USA) using a Big Dye Terminator cycle sequencing kit (Applied Biosystems).

Table-1: Primer sequences of *BMP15* gene used in current study

Oligo Name	Sequence	Length	Annealing Temp.	Product Size(bp)
Primer Forward 1	CTGCCAGCCTTTCATTTTTC	20	53.4	420
Primer Reverse 1	TTTTCCCTAGGGTGTCCACTT	21	55.9	
Primer Forward 2	GATTCAGGAGCTGCTAGAAGAA	22	56.3	274
Primer Reverse 2	TGAAGCCTGACAGAAAACTGA	21	53.9	
Primer Forward 3	GCTTTGCTCTTGTTCCCTCT	20	55.4	420
Primer Reverse 3	TGCCACCAGAACTCAAGAAC	20	55.4	
Primer Forward 4	CCCAAAACTTGGACAGAGATG	21	55.9	420
Primer Reverse 4	ATGCAATACTGCCTGCTTGA	20	53.4	
Primer Forward 5	ACTCAGAGTGTTCAGAAGACCAAA	24	57.0	488
Primer Reverse 5	CTGGGCAATCATACCCTCAT	20	55.4	
Primer Forward 6	AGTGTTCCCTCCACCCTTT	19	54.9	348
Primer Reverse 6	GCCTCAATCAGAAGGATGCTA	21	55.9	

Sequences were analyzed manually by using Bioedit software (Hall et al., 1999) and blast against normal sequence by using multiple sequence alignment strategy using ClustalW software package (Thompson et al., 1997) for detecting the Single Nucleotide Polymorphism (SNPs) from the aligned sequences.

RESULTS

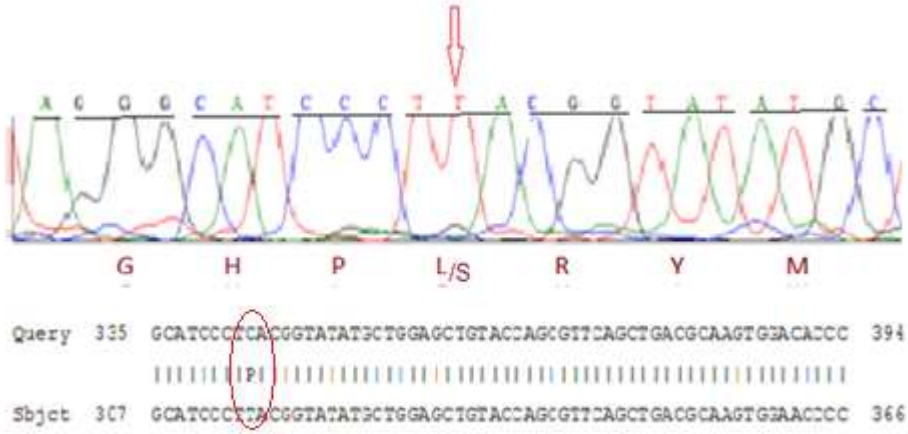

Fig-1: Showing c.200C>T (p.L67S) variant.

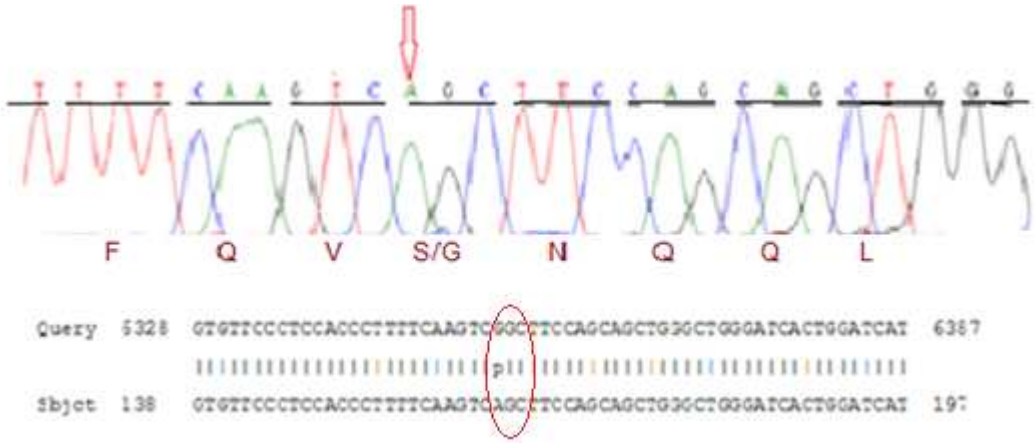

Fig-2: Showing c.901G>A (p.S301G) variant.

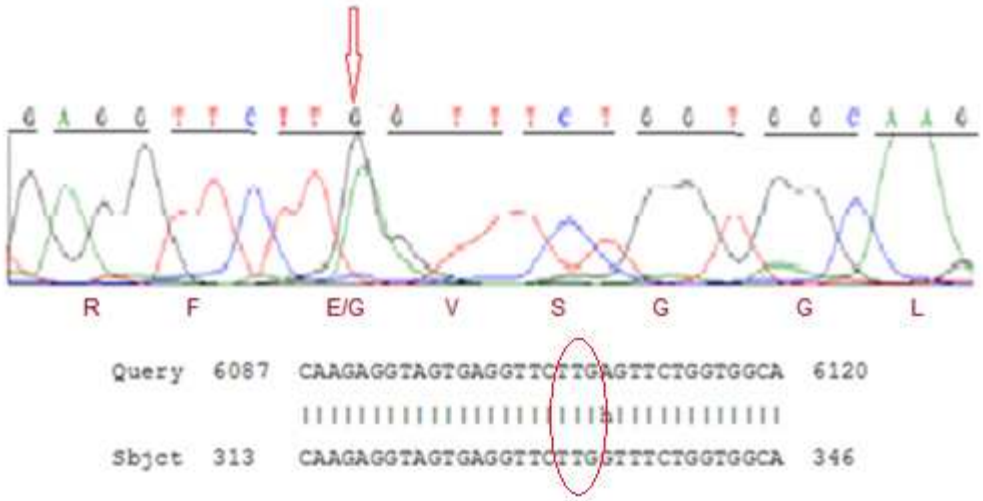

Fig-3: Showing c.656A>G (p.E221G) variant.

The polymorphic sites identified in Lehri goat are shown in (Table-02). Sequencing data analysis revealed that in Lehri goat breed there were two polymorphism at nucleotide position 200 (Exon 1) and 901(Exon 2) were observed as C>T and G>A respectively. A hetrozygosity was also observed in Exon 2 with triplets birth history, in nucleotide positions 656 where sequences revealed both peaks of G and A at the same nucleotide position.

Table-2: Codon modifications of BMP15 gene in Lehri goat

Serial #	Variation Position #	Codon #	Reference Sequence	Changed sequence	Reference Amino Acid	Changed Amino Acid
1	200bp	67	TCA	TTA	Serine	Leucine (Nonsynonymous)
2	901bp	301	GGC	AGC	Glycine	Serine (Nonsynonymous)
3	656bp	221	GAG	GGG	Glutamic Acid	Glycine (Nonsynonymous)

Codon variation of BMP15 gene in Lehrigoat breed presented in Table-02. DNA Sequencing analysis of BMP15 gene revealed that two polymorphisms were detected including changes of Serine (TCA) into Lucerine (TTA) at codon 67 and Glysine (GGC) into Serine (AGC) at codon 301. The heterozygosity was also observed with changing of Glutamatic acid (GAG) into Glysine (GGG) at codon 394. All the changed amino acids are non-synonymous shown in (Table-02).

DISCUSSION

In the previous era, growing kids and ewes were born mainly scarce due to the employ of the proliferation plan. Assortment plays crucial role in animal breeding but noticeably slower and create approximately twenty percent enhancement in prolificacy rate. Recently molecular markers studies have brought innovative preference that allocate the local sheep and goat farmers to generate a significant intensity of prolificacy (number of kids), growth performance, approvable fleece and skin production. A number of research works have been carried out worldwide, which put light on significance of prolificacy gene viz., *BMP15*, *BMPR1B* and *GDF9* (Davis, 2005). *BMP15* gene is a prominent gene having the credible position in the fecundity of goat (Galloway, et al.,2000).Studies suggest that heterozygosity in *BMP15* gene enhance ovulation rate and litter size whereas animals having homozygosity have been reported unproductive (Hanrahan et al., 2004). In current study we identified a hetrozygosity c.656A>G (p.E394G) in exon 2 (Fig 3) with triplet birth history similarly study conducted by (Juengel et al., 2004) analyzing the *GDF9, BMPR1B* and *BMP15*) also called as fecundity genes report that polymorphic variations observed in these genes have been found genetically synchronized the litter size and ovulation rate in domesticated sheep and goats. Studies conducted on Chinese goat breeds suggested that in goats with higher prolificacy is different with that of sheep (Chu et al., 2007, Hua et al., 2008). Where is Wang et al., (2011) in their study suggest that BMP15 gene in Chinese goat breeds of Henan province is polymorphic and considered to affect the prolificacy in Funiu white goats especially. The other two variations c.200C>T (p.S67L) and c.901G>A (p.G301S) observed in the goats with single birth history. Previous studies reported mutations i.e. Fec^{XH} in Hanna sheep, Fec^{XI} in Inverdale sheep (Galloway *et al.,* 2000), Fec^{XG} in Galway sheep (Hanrahan et al., 2004), Fec^{XL} in Lacaune sheep (Bodin et al.,2003) and Fec^{XB} in Belclare (Hanrahan et al., 2004) suggest that all these variations have a positive effect on ovulation rate. It has been found that the ovulation rate would be increase up among heterozygous ewes for any of these mutations whereas the homozygous ewes are infertile due to failure or malfunction of normal ovarian follicular development (Bodin et al., 2003 and Hanrahan et al., 2004; Ghoreishi et al., 2011). Study conducted by Montgomery et al (2001) suggest that the variation associated with BMP15 gene increase prolificacy in many breeds of sheep which has endorsed by Galloway et al (2000), Hanrahan et al., 2004 and McNatty et al., (2005) suggesting that variations in genes like BMP15 improve the ruminant reproduction and increase the sheep breeding system. Whereas study conducted by Gholibeikifard (2013) on Balochi sheep breed of Iran found no polymorphisms in BMP15 gene.

Three polymorphisms identified by Ghoreishi et al (2011) at exon 1, C200T and two at exon 2, G573A and T755G respectively in Markhoz goats with high prolificacy. Study was conducted by Nawaz et al., (2013) on *BMP15* gene in two goat breeds Teddy and Beetal of Pakistan. Six novel polymorphic sites identified in Teddy goat breed and concluded that these finding furnished significant explanations that *BMP15* gene is a major gene which increase the fecundity in Teddy goat. Studies suggest that BMP15 gene play a crucial role in female fertility including human (Di Pasquale et al., 2004), early developmental stages, and in cellular functions both postnatal and adult animals (Chena et al., 2004)

Conclusion

In this study we have concluded that in Lehri goat breed, the BMP15 gene is polymorphic and may be a most important gene which affects the prolificacy in this breed. The identification of the polymorphism in BMP15 gene increases the prolificacy and ovulation rate. The identified Hetrozygosity in Lehri goat breed which play an important role in prolificacy and increases fertility.

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