Volume 3, Issue 10, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

A STUDY OF FECUNDITY GENES IN KACHHI SHEEP OF SINDH, PAKISTAN

Shakeel Ahmed Tunio[1], Muhammad Naeem[*2], Atique Ahmed Behan[3], Asmatullah Kaka[4], Muhammad Bachal Bhutto[5], Javaid Ahmed Gaddahi[6], Zubair Ahmed Laghari[7], Nasir Rajput[8], Jahanzaib Khaliq[9]

[1,*2,3]*Department of Livestock and Management, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam*

[4]*Department of Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam*

[5,6,7]*Department of Veterinary Parasitology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam*

[8]*Department of Poultry, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam*

[9]*Department of Veterinary Physiology and Biochemistry, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam*

[1]satunio@sau.edu.pk, [*2]mnrajput@sau.edu.pk, [9]jahanzaibvet@gmail.com

DOI: https://doi.org/10.5281/zenodo.18058956

Keywords

Fecundity genes (BMP-15 and GDF-9), Reproductive performance, Genetic polymorphism

Article History

Received: 28 October 2025

Accepted: 12 December 2025

Published: 26 December 2025

Copyright @Author

Corresponding Author: *

Muhammad Naeem

Abstract

Background: *Kachhi sheep is an important medium sized, thin tailed breed of Sindh province of Pakistan. Despite having great importance and contribution in mutton, milk, wool production, and adaptation to harsh environments, Reproductive performance, especially fecundity, plays a key role in improving flock productivity and farmers' income. research on its genetic characterization is scant. It is therefore, seemed appropriate to plan a study on fecundity to observe the genetic polymorphism in Kachhi sheep based on BMP-15 and GDF-9 genes.* ***Methodology:*** *A total of 150 adult Kachhi sheep from different study areas (Hyderabad, Matiary, Mirpurkhas, Sanghar, Umerkot and Tharparkar) of Sindh province were selected for study purpose. Blood samples from selected animals were taken from the jugular vein aseptically. DNA was extracted from whole blood. DNA fragments with size of 141bp and 462bp for the BMP-15 and GDF-9 gene were amplified by using PCR with specific primers, respectively.* ***Results:*** *It was revealed that wild type homozygous genotype frequency of BMP15 gene was recorded higher in Tharparkar and Umerkot and little bit higher heterozygous genotype frequency was observed in Mirpurkhas. After the digestion of PCR products of GDF9 gene with Hha1 restriction enzyme, the wild type of homozygous genotype frequency of GDF9 gene of Kachhi sheep was recorded higher in Tharparkar, Umerkot and Sanghar and little bit higher heterozygous genotype frequency was observed in Hyderabad.* ***Conclusion:*** *Based on the results it was concluded that most of the studied sheep population possess wild type homozygous genotype, however a little bit heterozygous genotype was also recorded in BMP15 and GDF9 genes of Kachhi sheep.*

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

INTRODUCTION

Selection of breed based on phenotypic characteristics is not considered as an efficient method as compared to selection based on the genotype of animal which was found most effective to enhance the fertility traits in domestic animals (Nosrati *et al.,* 2019). The genetic variation in breeds or species could be described through gene isolation along with massive effect on ovarian function (Hanrahan *et al.,* 2004). Molecular polymorphisms and Biochemical polymorphisms considered as Molecular description tools (Meghen *et al.,* 1994).The molecular genetics tools have proposed new opportunities to improve breeding program in domestic animals to distinguish genomic regions or genes that controls traits of interest by using of DNA markers (Dekkers, 2012). In animal genetics to improve reproductive traits molecular markers considered as driving force and propose many opportunities and offer methods for determining polymorphism and genetic markers related to reproductive traits (Vignal *et al.,* 2002; Ghaffarri *et al.,* 2009)). In Pakistan (Babar *et al.,* 2017) described polymorphisms in different sheep and goat breeds, such as in Beetal, Teddy, and Pak Angora, which was linked with resistance to some major zoonotic and fatal diseases. Similarly, three significant genes have been studied by (Ghoreishi *et al.,* 2011)combined known as fecundity genes which improves the litter size of domesticated goats and plays vital role in management of ovulation rate.

The fecundity genes (BMP-15 and GDF-9) known as members of transforming growth factor β super family (TGFβ) that have been found to be critical progression of very earliest stages of folliculogenesis and then in late follicular growth (Galloway, 2000; Hanrahan *et al.,* 2004) and helpful in enhancing the chances of twin births in the sheep specie (Abdoli *et al.,* 2013). Genotyping consists of the degree of polymorphism, explanation of breeds in respect of comparative allelic frequencies, applying a point of neutrals reference markers and categorizing domestic animal breeds using genomic spaces between populations or strains (FAO, 2007). Various major genes influence Prolificacy in sheep that comprise three related oocyte-derived elements such as (FecB) BMPR-IB (Sauza *et al.,* 2001); FecG (GDF-9) and FecX (BMP-15) (Hanrahan *et al.,* 2004). Different mutations may occur in these genes resulting in increase or decrease the rate of ovulation and the impact of these mutations may even reach to the level of infertility in sheep (Hafezian, 2011). Bone morphogenetic protein (BMP-15) gene plays major role as controller of (GC) granulosa cell procedures in development of ovarian follicles and located on X chromosomes (Crawford *et al.,* 2011) and in reproductive performance which enhances ovulation rate and infertility and was first detected in sheep breeds (Hanrahan*et al.,* 2004). In mammals GDF-9 gene plays significant role in female reproductive tract during early folliculogenesis (Silva *et al.,* 2005). In some prolific breeds of sheep these mutations may yield greater ovulation rates, twin, and triplets in heterozygous, and entire initial ovarian failure in homozygous causing in total sterility (Galloway *et al.,* 2000). Five naturally occurring mutations were found in Belclare sheep breed in exon-II of BMP-15 gene and known as (FecXB) that shows superior ovulation rates (Davis, 2005). Furthermore, based on latest innovations in human, rodents, and sheep breeds, (BMP-15 and GDF-9) genes in domestic animals can be deemed to be new goals for fertility management (Mc Natty *et al.,* 2005).

The Kachhi sheep is a thin tailed, mutton and wool type medium sized sheep breed of Pakistan. It is mostly raised in the Ran of Katchh, District Tharparkar and neighboring desert areas of Sindh Province (Muhammad Ishaq & Zahoor-ul-Haq, 2007). The Kachhi sheep lives in a range of (arid) and (semi-arid) zones of Pakistan, where they are raised as valuable and effortless small ruminants (Wahid, 1982). The genetic characteristics of Kachhi breeds are not described, therefore, current study was proposed to identify the genotypic characterization and polymorphism of the (BMP-15) and (GDF-9) genes of Kachhi sheep, which will be beneficial to locate some genes that can be utilized in proliferation and as a result increasing the fecundity.

MATERIALS AND METHODS

Sample Collection

During current investigation a total of 150 apparently healthy adult Kachhi sheep, 25 from each study area (Hyderabad, Matiary, Mirpurkhas, Sanghar, Umerkot, and Tharparkar) of Sindh

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

province were selected according to the phenotypic standard of the breed. Blood samples (2 ml/ sheep) were collected aseptically from the jugular vein. Collected blood samples were placed into blood collection tubes containing (EDTA) as anticoagulant and transported immediately in an ice box to the Molecular laboratory department of veterinary parasitology, Sindh Agriculture University, Tandojam for further processing.

Genomic DNA Extraction

The DNA was extracted from the whole blood by using (Thermo, scientific Gene JET Genomic, DNA Purification kit, USA) according to manufacturer's procedures. Extracted DNA was kept at -20°C for further processing.

DNA Quantification

The quality and quantity of extracted DNA was checked by operating Spectrophotometer (Nano Drop ™ 1000, Thermo scientific, USA).

Primers used

The primers sequences and PCR reaction conditions are presented in (Table 1). Two specific primer pairs already published for amplification of BMP-15 and GDF-9 genes of Sangsari sheep of Iran (Kasiriyan *et al.*, 2011) were selected for genotyping in Kachhi sheep breed in different study areas of Sindh province.

Table 1: Primers used for Amplification of BMP-15 and GDF-9 genes of Kachhi sheep.

Gene	Primer	Gene sequences	Ann. Temp.	Size (bp)
BMP-15	B2-F	CACTGTCTTCTTGTTACTGTATTTCAATGAGAC	62 °C	141
	B2-R	GATGCAATACTGCCTGCTTG		
GDF-9	G9-F	GAAGACTGGTATGGGGAAATG	60 °C	462
	G9-R	CCAATCTGCTCCTACACACCT		

PCR Amplification

Primers were amplified by using (thermo cycler 2720 Applied Bio Sci. USA). The total volume of PCR reaction 20 µl contained 10 µl master mixes, 1ul primer (F), 1µl primer (R), 1µl of template DNA and 7µl of nuclease free water. The amplification for BMP-15primers (Table 1) was carried out by using 35 cycles at 94°C for 5 minutes, 94°C for 45 seconds, 62°C for 40seconds, 72 °C for 45 seconds followed by 72°C for 5minutes. Whileamplificationforprimer GDF-9 (Table 1) was conducted by using 35 cycles at 94°C for minutes, 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds followed by 72°C for 7 minutes.

RFLP Analysis

The resulted BMP-15 PCR products were digested at 37°C overnight with Hinf1 restriction enzyme and PCR products GDF-9 gene were digested at 37°C 4 h with Hhal1 restriction enzyme. The digestion reaction used for RFLP analysis included 10 µl of PCR product (0.5 µg 0f DNA), 2µl appropriate enzyme, 2ul buffer 10x in 18 µl nuclease-free water.

Gel Electrophoresis

The resulting fragments were identified by electrophoresis on 1% agarose gel for 30 minutes at constant voltage 120 volts. The ethidium bromide was used for staining gels and the predicted size of amplified PCR products was visualized as a single compact fluorescent band under (UV) light.

RESULTS

During the present study, polymorphism of (BMP-15 and GDF-9) genes in Kachhi breed was analyzed in different vicinities (Hyderabad, Matiari, Mirpurkhas,

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Sanghar, Umerkot and Tharparkar) of Sindh Province.

Genotypic polymorphism of BMP 15 gene

The analysis of amplification of DNA fragment from exon II of (BMP-15) gene with the size of 141 bp lengths was performed successfully as shown in Table 2 and Figure 1. The amplified PCR products were digested with Hinf I restriction enzyme at 37 °C for overnight for BMP-15 gene. The genotypes of everyone were identified by agarose gel electrophoresis. The PCR products digested with HinfI restriction enzyme for the BMP-15 gene showed higher homozygous wild type genotype (B+B+) as compared to heterozygous genotype. The wild type of genotype (B+B+) of BMP15 gene showed DNA fragments with size 30 and 111 bp length and heterozygous (B-B-) genotype showed 30,111 and 141 bp size DNA fragments Figure 2. Wild type homozygous genotype frequency of BMP15 gene was recorded higher in Tharparkar and Umerkot and little bit higher heterozygous genotype frequency was observed in Mirpurkhas study areas of Sindh province in Table 3.

Table 2: Number and size of bands resulted from Hinf1 enzyme digestion for BMP15 gene in Kachhi sheep breed.

BMP15		Genotype		
S. No.	Fragment size {bp}	{B+B+}	{B+B-}	{B-B-}
1.	141	-	-	141
2.	111	111	-	111
3.	30	30	-	30

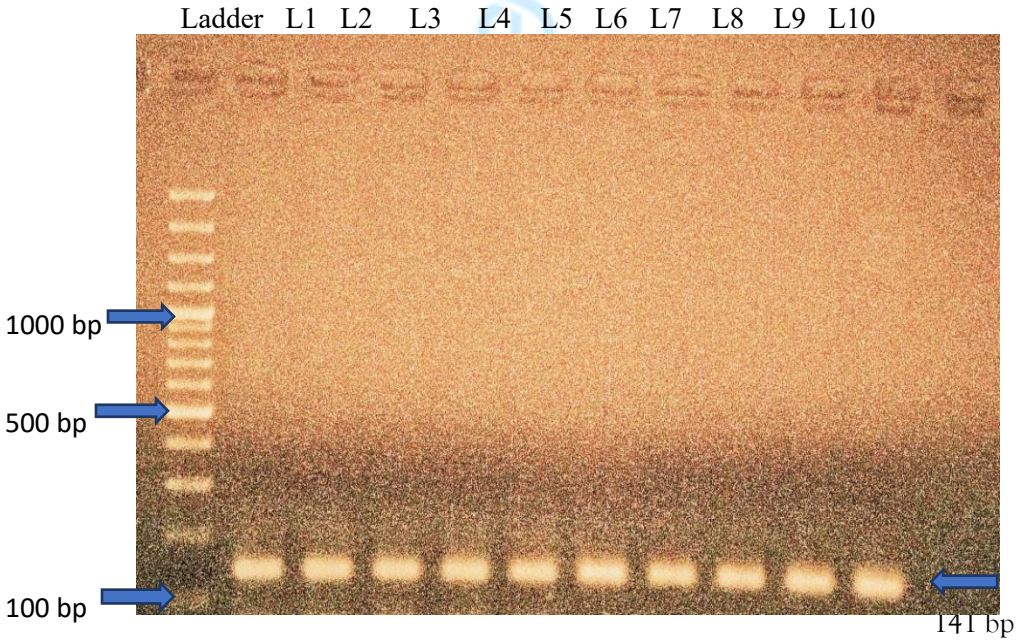

Figure 1: Amplification of PCR product (141 bp fragment) of exon 11 in BMP15 gene

Volume 3, Issue 10, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

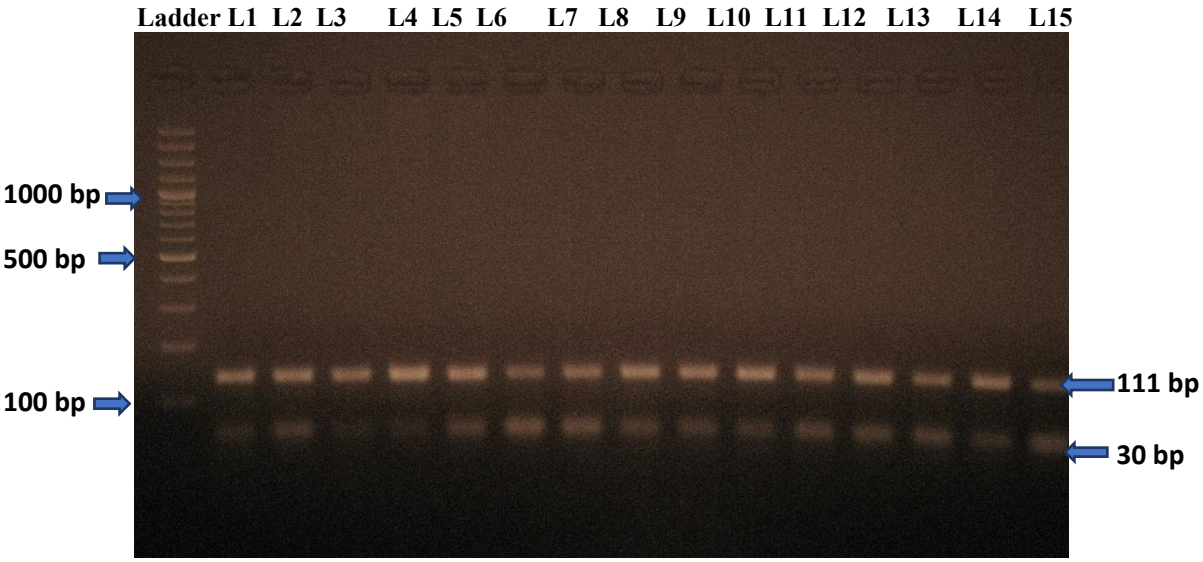

Figure 2: Digestion of PCR of exon 11 in BMP 15 gene by using Hinf 1 enzyme in Kachhi sheep

Table 3: Genotype frequency analysis for BMP-15 gene in Kachhi sheep at different vicinities of Sindh.

S.No.	Study Area	Gene	Frequency	Genotype B+B + %		B+B %		B-B	%
2.	Hyderabad	BMP-15	25	23	92	-	-	2	8
3.	Matiari		25	23	92	-	-	2	8
4.	Mirpurkhas		25	22	88	-	-	3	12
5.	Sanghar		25	23	92	-	-	2	8
6.	Umerkot		25	24	96	-	-	1	4
	Tharparkar		25	24	96	-	-	1	4

B+B+ = Wild type homozygous genotype
B+B- =Mutant
B-B- = Heterozygous genotype

Genotypic polymorphism of GDF9 gene

The restriction analysis of amplification of DNA fragments from (exon 1) of GDF9 gene with the size of (462 bp) length was performed efficiently (Figure 3). The PCR products (amplified) were digested with restriction enzyme (Hha1) at 37 °C for 4 hrs and sheep genotype was identified by electrophoresis. The resulted digestion of PCR products with Hha1 restriction enzyme showed the wild type (G+G+) genotype of GDF-9 gene and three DNA fragments on two restriction sites with the size of 52, 156 and 254 bp and heterozygous genotypes (G-G-) of GDD-9 gene showed four DNA fragments with size of 52,156,254 and 410 (Table 4 and Figure 4). Wild type homozygous genotype frequency of GDF9 gene of Kachhi sheep was recorded higher in Tharpaparkar, Umerkot and Sanghar and little bit higher heterozygous genotype frequency was observed in Hyderabad as presented in Table 5.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Table 4: Number and size of bands resulted from Hha1 enzyme digestion for GDF9 gene in Kachhi sheep breed.

GDF9		Genotype		
S. No.	Fragment size (bp)	G+G+	G+G-	G-G-
1	410	-	-	410
2	254	254	-	254
3	156	156	-	156
4	52	52	-	52

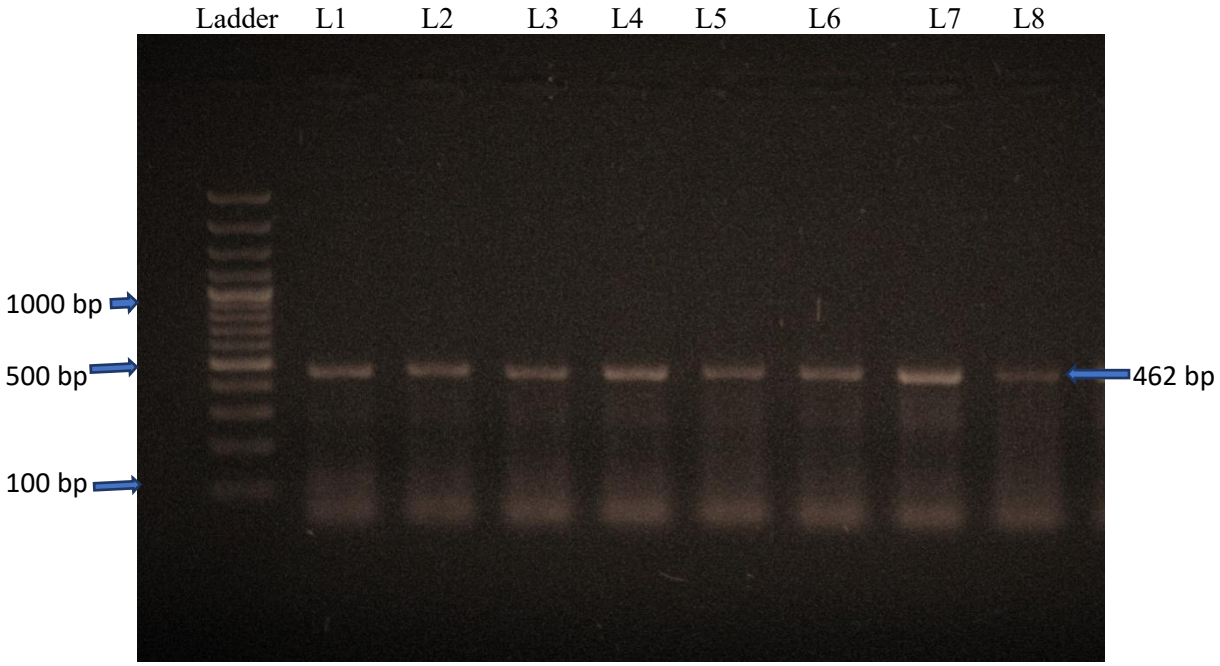

Figure 3: Amplification of PCR product (462 bp fragment) of exon 1 in GDF9 gene

Frontier in Medical & Health Research
ISSN: (e) 3007-1607 (p) 3007-1593

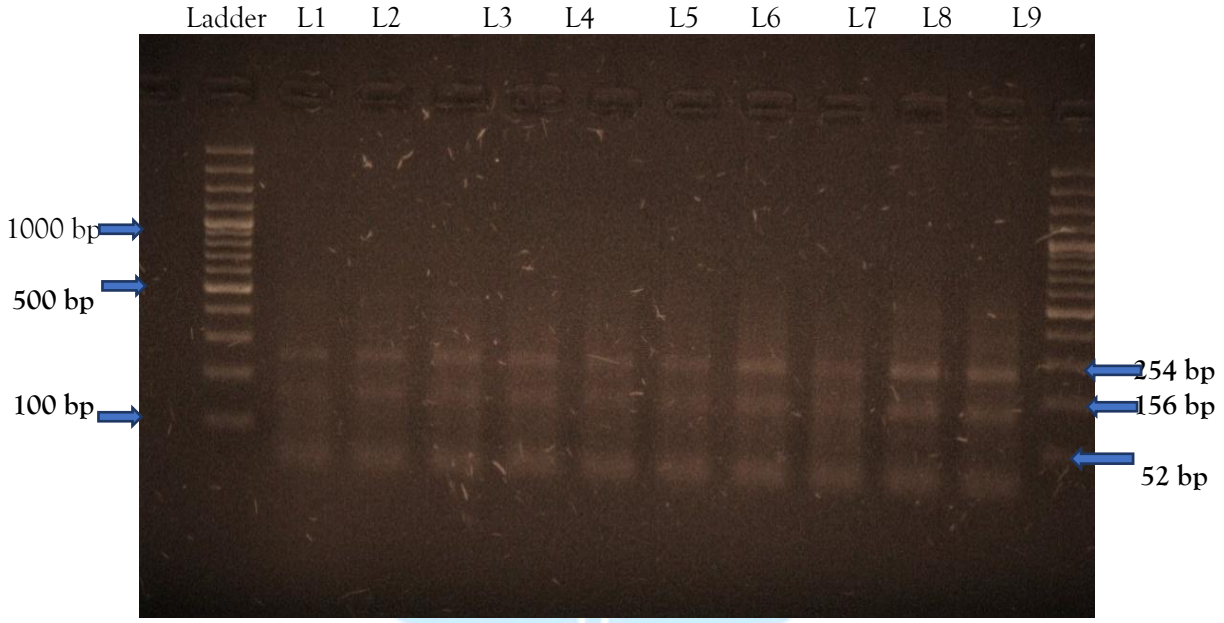

Figure 4: Digestion of PCR of exon 1 in GDF9 by using Hhal 1 enzyme in Kachhi sheep

Table 5: Genotype frequency analysis for GDF-9 gene in Kachhi sheep at different study areas of Sindh province

S.No.	Study Area	Gene	Frequency	Genotype G+G+	%	G+G-	%	G-G-	%
2.	Hyderabad	GDF-9	25	22	88	-	-	3	12
3.	Matiari		25	23	92	-	-	2	8
4.	Mirpurkhas		25	23	92	-	-	2	8
5.	Sanghar		25	24	96	-	-	1	4
6.	Umerkot		25	24	96	-	-	1	4
	Tharparkar		25	24	96	-	-	1	4

G+G+ = Wild type homozygous genotype
G+G- =Mutant
G-G- = Heterozygous genotype

DISCUSSION

Present research work was aimed to analyze polymorphic variations of the fecundity genes BMP-15 and GDF-9 in Kachhi sheep in different areas of Sindh province. In present research work polymorphic variations of the genes coding for BMP-15 and GDF-9 were analyzed. Majority of PCR products digested with restriction enzyme (HinfI) for the BMP-15 gene showed homozygous wild type genotype (B+B+) as compared to heterozygous genotype. The wild type of genotype (B+B+) of BMP15 gene showed DNA fragments with size 30 and 111 bp length and heterozygous (B-B-) genotype showed 30,111 and 141 bp size DNA fragments. Moreover, wild type homozygous genotype frequency of BMP15 gene was recorded higher in Tharparkar and Umerkot and little bit higher heterozygous genotype frequency was observed in Mirpurkhas, current results are in agreement with (Hanrahan et al., 2004), reported that the digestion of PCR products by Hinf I for the wild homozygous

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

individuals (+/+) will lead to cutting 141bp segment and creating 111 and 30bp segments while for the heterozygous genotype the 141 and 11bp segments are obtained, and for the homozygous mutant genotype (B/B) only the 141bp segment is acquired. In present study, majority of the PCR products digested by the Hinf I only showed 111 and 30bp segments implying that the exon 2 in BMP15 is a monomorphic locus in Kachhi sheep. Same results were obtained by (Shokrollahi, 2015) reported that PCR products for both exons of BMP15 gene were

500 bp in length. Three restriction enzymes (SpeI, Xbaland and HinfI) were used but enzymes could not cut the PCR product and majority of the individuals declared as homozygous with wild type genotype. The same type of study was conducted by (Farag et al., 2018) who studied different sheep and goat breeds, reported that all the animals possessed the homozygous wild type genotypes (AA) and no polymorphism was observed.

Furthermore, in concurrence with present study (Iman et al., 2019) investigated that Hinf 1 enzyme did not detect any polymorphism in Barki and Rahmani sheep and absent of FecG mutation. Results are also in agreement with the findings of (Mustafa et al., 2018; Wang et al., 2018) detected that only wild type genotype in lohi sheep breed and absence of mutation. In contrast of present findings, the FecB mutation was noted in different sheep breeds (Yan et al., 2005; Davis et al., 2006; Nanekarani et al., 2016). The current results also agree with (Zhi-gang et al., 2021) reported that no polymorphism was observed in Cele black sheep which indicated that this sheep breed is not linked with the sum of high producing lambs. Furthermore, in another study same findings were observed by (Abdoli et al., 2018) revealed that polymorphism of exon I of (BMP-15) gene in Iranian fat-tailed ewe was observed. The Polymorphism of exon II of bone morph genic protein 15 gene in Sangsari sheep has been recorded by (Hafezian, 2011) in which $FecX^G$ mutations were observed in all the animals and all was found homozygous for this mutation (BB). Moreover, evaluation of polymorphic strains for $FecX^G$ and FecB loci in Baluchi sheep directed those genetic influences responsible for (twinning or multiple lambing) ratio is not linked to mutated alleles at $FecX^G$ and FecB major genes (Morad et al., 2001). Additionally in another investigation (Zhi-gang et al., 2021) observed smallest litter size in homozygous (BB) genotype instead of heterozygous (B+) allele and wild-type genotype (++) with highest litter size. In current investigation the length of amplified PCR product was recorded 462 bp from (exon 1) of GDF-9 gene.

Amplification of DNA fragments with the size of 462 bp from GDF9 was performed successfully. After the digestion of PCR products of GDF9 gene with Hha1 restriction enzyme, the wild type (G+G+) genotype of GDF9 gene showed three DNA fragments with the size of 52, 156 and 254 bp and heterozygous genotypes (G-G-) of GDF9 gene showed four DNA fragments with size of 52,156,254 and 410. Furthermore wild type homozygous genotype frequency of GDF9 gene of Kachhi sheep was recorded higher in Tharparkar, Umerkot and Sanghar and little bit higher heterozygous genotype frequency was observed in Hyderabad same results were recorded by (Hanrahan et al., 2004), stated if there is a change of G to A, the wild genotype (++) will produce three fragments with sizes of 254, 156 and 52bp. If such a change occurs in the mutated allele (G), two fragments with sizes of 52 and 410bp will be produced. Heterozygous genotype (G/+) in both types of alleles will make four fragments with sizes of 254, 156, 410, and 52bp. In our study, similar band patterns to those of Hanrahan et al. (2004). The results of current research are agreed with the findings of (Escobar-chaparro et al., 2017) revealed that in Katahdin and Dorper sheep breeds the monomorphic wild type of genotype (AA) was observed in majority of animals for GDF9 gene. In line with the outcomes of (Eghbalsaied et al., 2017) worked on litter size in Iranian sheep, reported that in Iranian sheep breeds one genotype (AA) was observed. The polymorphic genotypes (AA, AB, and BB) and G1 were recorded with wild type genotype (AA) in Baluchi and Sangsari sheep breeds (Moradband et al., 2011; Hafezian, 2011). Moreover, in another investigation conducted by (Farag et al., 2018) revealed that in GDF-9 gene occurrence of same mutation indicated the wild type of genotype (AA) genotype in all the individual of Assan hill goat breed. In contrast to present study in two sheep breeds the polymorphic (AA, AB, and BB) genotypes

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

were observed (Cambridge and Belclare). Also conflicting to these observations by (Moradband et al., 2011) revealed that during the state of heterozygosis GDF-9 gene could improve ovulation and lambing rate. Mutations in growth differentiation factor 9 (GDF9) showed various impacts on ovulation rate and produce infertility in some cases (Abdoli et al., 2016). Ovulation rate of sheep increased with the different mutations in the genes GDF9 and BMP15 (Rout et al., 2018). Furthermore, it was also observed that the sheep can produce more lambs if keeping at least one mutant allele in the GDF9 gene as compared to wild type allele as reported by (Hanrahan et al., 2004) stated that Sheep are considered as fertile and possess improved ovulation rate with single copy of muted $FecG^{H}$ (GDF9) gene. In agreement with present findings (Jawasreh et al., 2017) reported that homozygous individuals of GDF9 genes produce 0.792 more lambs born per lambing than that of heterozygous individuals. In conflict (Miller et al., 1988) stated that sheep were observed infertile with primary ovarian failure homozygous for the mutation $FecG^{H}$ (GDF9) gene.

CONCLUSION

The current study's findings indicate that the The majority of the sheep population under study had a wild type of homozygous genotype, according to the data; nevertheless, Kachhi sheep also had a slightly heterozygous genotype in the BMP15 and GDF9 genes.

Conflict of Interest

No conflicts of Interest were disclosed.

Contribution Statement

Dr. Muhammad Naeem, Dr. Atique Ahmed Behan, Dr. Asmatullah and Shakeel Ahmed Tunio designed and conducted the study. Dr. M. Bachal Bhutto, Dr. Javaid Gadahi, Dr. Zubair A. Laghari and Shakeel Ahmed Tunio carried out lab experiments. Dr. M. Naeem, Dr. Nasir Rajput analyzed the data and helped during blood sample collection from different study areas of study. Dr. Atique Ahmed Behan, Dr. Asmatullah, and Dr. Jahanzaib Khaliq revised the manuscript for important contents.

REFERENCES

Abdoli, R., Mirhoseini, S. Z., Ghavi Hossein-Zadeh, N., Zamani, P., & Gondro, C. (2018). Genome-wide association study to identify genomic regions affecting prolificacy in Lori-Bakhtiari sheep. *Animal Genetics*, *49*(5), 488-491. https://doi.org/10.1111/age.12700

Abdoli, R., Zamani, P., Deljou, A., & Rezvan, H. (2013). Association of BMPR-1B and GDF9 genes polymorphisms and secondary protein structure changes with reproduction traits in Mehraban ewes. *Gene*, *524*(2), 296-303. https://doi.org/10.1016/j.gene.2013.03.133

Babar, M. E., Ali, A., & Hussain, T. (2017). Goat Genome Studies in Pakistan. *Sustainable Goat Farming for Livelihood Improvement in South Asia*, 161.

Bodin, L., Di Pasquale, E., Fabre, S., Bontoux, M., Monget, P., Persani, L., & Mulsant, P. (2007). A novel mutation in the bone morphogenetic protein 15 gene causing defective protein secretion is associated with both increased ovulation rate and sterility in Lacaune sheep. *Endocrinology*, *148*(1), 393-400. https://doi.org/10.1210/en.2006-0764

Crawford, J. L., Heath, D. A., Reader, K. L., Quirke, L. D., Hudson, N. L., Juengel, J. L., & McNatty, K. P. (2011). Oocytes in sheep homozygous for a mutation in bone morphogenetic protein receptor 1B express lower mRNA levels of bone morphogenetic protein 15 but not growth differentiation factor 9. *Reproduction*, *142*(1), 53-61. https://doi.org/10.1530/REP-10-0485

Davis, G. H. (2005). Major genes affecting ovulation rate in sheep. *Genetics Selection Evolution*, *37*(Suppl. 1), S11-S23. https://doi.org/10.1051/gse:2004026

CM Dekkers, J. (2012). Application of genomics tools to animal breeding. *Current genomics*, *13*(3), 207-212. https://doi.org/10.2174/138920212800543057

Eghbalsaied, S., Khorasgani, F. R., Amini, H. R., Farahi, M., Davari, M., Pirali, A., ... & Atashi, H. (2017). Variant GDF9 mRNA is likely not the main cause of larger litter size in Iranian Lori-Bakhtyari, Shal, Ghezel, and Afshari sheep

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

breeds. *Archives Animal Breeding*, *60*(2), 119-129. https://doi.org/10.5194/aab-60-119-2017

Escobar-Chaparro, R. A., Guillén, G., Espejo-Galicia, L. U., Meza-Villalvazo, V. M., Pena-Castro, J. M., & Abad-Zavaleta, J. (2017). qPCR and HRM-based diagnosis of SNPs on growth differentiation factor 9 (GDF9), a gene associated with sheep (Ovis aries) prolificacy. *3 Biotech*, *7*(3), 204. https://doi.org/10.1007/s13205-017-0837-z

FAO. (2007). Production Yearbook, Food and Agriculture Organization of United Nations, Rome, Italy.

Farag, I., Aboelhassan, D., Ghaly, I., Darwish, H., Darwish, A., & Eshak, M. (2018). Effect of genetic polymorphism of BMP15 gene on improving twin production in Egyptian small ruminants. *Res J AnimVet Sci*, *10*(1), 6-14. DOI: 10.22587/rjavs.2018.10.1.2

Galloway, S. M., McNatty, K. P., Cambridge, L. M., Laitinen, M. P. E., Juengel, J. L., Jokiranta, T. S., ... & Dodds, K. G. (2000). G. Montgomery W, Beattie AE, Davis GH, and Ritvos O. Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nat. Genet*, *25*, 279-283.

Ghaffari, M., Nejati-Javaremi, A., & Rahimi, G. (2009). Detection of polymorphism in BMPR-IB gene associated with twining in Shal sheep using PCR-RFLP method. *Int. J. Agric. Biol*, *11*(1), 97-99.

Ghoreishi, H., Shayegh, J., Barzegari, A., Maheri-Sis, N., Gorbani, A., Bedoustani, A. B., & Yosefabad, S. F. (2011). Sequence assigning of the bone morphogenetic protein 15 (BMP15) genes in Markhoz goats. *Advances in Environmental Biology*, 1850-1854.

Kasiriyan, M. M., Hafezian, S. H., & Hassani, N. (2011). Genetic polymorphism BMP15 and GDF9 genes in Sangsari sheep of Iran. *International Journal of Genetics and Molecular Biology*, *3*(1), 31-34.

Hanrahan, J. P., Gregan, S. M., Mulsant, P., Mullen, M., Davis, G. H., Powell, R., & Galloway, S. M. (2004). Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (Ovis aries). *Biology of reproduction*, *70*(4), 900-909. https://doi.org/10.1095/biolreprod.103.023093

Kasiriyan, M. M., Hafezian, S. H., & Hassani, N. (2011). Genetic polymorphism BMP15 and GDF9 genes in Sangsari sheep of Iran. *International Journal of Genetics and Molecular Biology*, *3*(1), 31-34.

McNatty, K. P., Smith, P., Moore, L. G., Reader, K., Lun, S., Hanrahan, J. P., ... & Juengel, J. L. (2005). Oocyte-expressed genes affecting ovulation rate. *Molecular and cellular endocrinology*, *234*(1-2), 57-66. https://doi.org/10.1016/j.mce.2004.08.013

Meghen, C., MacHugh, D. E., & Bradley, D. G. (1994). Genetic characterization and West African cattle.

Miller, S. A., Dykes, D. D., & Polesky, H. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic acids research*, *16*(3), 1215. https://doi.org/10.1093/nar/16.3.1215

Moradband, F., Rahimi, G., & Gholizadeh, M. (2011). Association of polymorphisms in fecundity genes of GDF9, BMP15 and BMP15-1B with litter size in Iranian Baluchi sheep. *Asian-Australasian journal of animal sciences*, *24*(9), 1179-1183. https://doi.org/10.5713/ajas.2011.10453

Mottershead, D. G., Sugimura, S., Al-Musawi, S. L., Li, J. J., Richani, D., White, M. A., ... & Gilchrist, R. B. (2015). Cumulin, an oocyte-secreted heterodimer of the transforming growth factor-β family, is a potent activator of granulosa cells and improves oocyte quality. *Journal of Biological Chemistry*, *290*(39), 24007-24020. https://doi.org/10.1074/jbc.M115.671487

Mourad, M., Gbanamou, G., & Balde, I. B. (2001). Carcass characteristics of West African dwarf goats under extensive system. *Small Ruminant Research*, *42*(1), 81-85. https://doi.org/10.1016/S0921-4488(01)00211-5

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Ishaq, M., & ul Haq, Z. (2007). Small ruminants farming in Pakistan. *Annals of Arid Zone*, *46*(3 & 4).

Nosrati, M., Asadollahpour Nanaei, H., Amiri Ghanatsaman, Z., & Esmailizadeh, A. (2019). Whole genome sequence analysis to detect signatures of positive selection for high fecundity in sheep. *Reproduction in Domestic Animals*, *54*(2), 358-364. https://doi.org/10.1111/rda.13368

Rout, M., Panigrahi, S., Pradhan, S., Routray, A., & Swain, B. (2018). Genetic basis of fecundity in sheep. *J Pharm Innov*, *7*(7), 314-6.

Shokrollahi, B. (2015). Investigation of BMP15 gene polymorphisms associated with twining in Markhoz goat. *Biharean Biologist*, *9*(1), 1-4.

Silva, J. R. V., Van Den Hurk, R., Van Tol, H. T. A., Roelen, B. A. J., & Figueiredo, J. R. (2005). Expression of growth differentiation factor 9 (GDF9), bone morphogenetic protein 15 (BMP15), and BMP receptors in the ovaries of goats. *Molecular Reproduction and Development: Incorporating Gamete Research*, *70*(1), 11-19. https://doi.org/10.1002/mrd.20127

Sunder, J., Kundu, A., Kundu, M. S., Sujatha, T., & De, A. K. (2019). Farming practices and morphometric characterization of Andaman Local Goat. *Indian J. Anim. Res*, *53*(8), 1097-1103. https://doi.org/10.18805/ijar.B-3615

Souza, C. J. H., MacDougall, C., Campbell, B. K., McNeilly, A. S., & Baird, D. T. (2001). The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (BMPR1B) gene. *Journal of Endocrinology*, *169*(2), R1. https://doi.org/10.1677/joe.0.169R001

Vignal, A., Milan, D., SanCristobal, M., & Eggen, A. (2002). A review on SNP and other types of molecular markers and their use in animal genetics. *Genetics selection evolution*, *34*(3), 275-305. https://doi.org/10.1051/gse:2002009

Wahid, A. (1982). General pattern of production, breeds, management, diseases, nutrition, performance and problems [of sheep along with wool characteristics and methods of its improvement].

Sunder, J., Kundu, A., Kundu, M. S., Sujatha, T., & De, A. K. (2019). Farming practices and morphometric characterization of Andaman Local Goat. *Indian J. Anim. Res*, *53*(8), 1097-1103. DOI: 10.18805/ijar.B-3615

Niu, Z. G., Qin, J., Jiang, Y., Ding, X. D., Ding, Y. G., Tang, S., & Shi, H. C. (2021). The identification of mutation in BMP15 gene associated with litter size in Xinjiang Cele black sheep. *Animals*, *11*(3), 668. https://doi.org/10.3390/ani11030668