SNPs" Identification of Bone Morphogenetic Protein 15(BMP15) gene in different Sheep and Goat breeds

Safia Bibi[*], Naila Kousar[*], Zoonaira Tanveer[*], Muhammad Sufyan[**], Arslan Haider[**], Sidra Ishfaq[*]

[*] The Government Sadiq College, Department of Zoology, Women University Bahawalpur, Pakistan.
[**] Department of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Pakistan.

Abstract- The sheep and goats form the backbone of financial safety systems and rural employment. The fertility rate increase and improvement in reproductive efficiency will eventually pave the way for farmers to benefit economically. Owing to the low heritability traits, it is difficult to accomplish reproductive enhancement through the selection process. In the present study, there are some SNPs in candidate gene BMP15 were genotyped for the prolificacy in a few breeds of sheep and goats to estimate association with the litter size. Differences in fecundity rate and litter size were observed within many types of breeds. At different loci in the BMP15 gene, many mutations were observed, contributing to the reproductive capability of examined breeds. Consequently, enormous efforts made in the search for genes that influence animal fecundity. The effect of many mutations in different breeds was highly significant in increasing the fertility rate in sheep and goat breeds.

Index Terms- BMP15gene, fertility, mutations, sheep, SNPs.

I. INTRODUCTION

The oocyte factor Bone Morphogenetic Protein 15 (BMP15) gene has established significant and vital importance in mammal female normal fertility. Biological functions of the recombinant BMP15 gene exhibit its capacity to granulosa cell promotion process included in the early growth of follicles, even that stimulating action to lock up follicle-stimulating hormone (FSH) provoked differentiation of granulosa cells. In-vitro biological activities of Bone Morphogenetic Protein 15 (BMP15) showed its liability in promoting premature follicle growth by inducement of mitosis in granulosa cells. Even that, at the same time, confines the FSH and encourages the development of follicles as mRNA expression by suppressing the FSH receptors [32].

[3] Studied the mutations in GDF9, BMPR-1B, and BMP15 genes' effects on the litter size and ovulation rate of sheep. Overall their assessment validated the important role of the BMP15 gene factor in the ovarian folliculogenesis process and ovulation control in sheep. [28] Reported two genes GDF9 and BMP15 were expressed differentially during the process of oocyte maturation and the BMP15 gene was completely expressed during the expansion of cumulus cells in porcine oocytes. Homozygous sheep for four types of mutations are sterile with the same phenotypes due to blockage of the folliculogenesis process. Significantly, rates of ovulation are higher in heterozygous animals because of the rapid and fast process of follicular development [22].

In the BMP15 rates of ovulation are more in heterozygous instead of the homozygous mutants which show the failure of primary ovarian as the result of entire sterility [19] [22] [3] [33]. Due to the influence of hormones oocytes are starting to prepare for ovulation [41]. Preovulatory surge is stimulated by estrogen, or by dramatically increasing LH that induces follicle maturation then it is ready to ovulate. In sheep which are mono-ovulatory species, some follicles become mature and grow to be dominant [49].

[53] Conducted a study to investigate and notice SNPs, as well as the effects of BMPR-IB and BMP15 genes on the litter size in sheep. Some types of sheep ranked as HU-Yang, Chinese Merino multiparous and Chinese Merino monotocous for the production of wool, and Chinese Merino multiparous for the production of mutton used in this study. Results show there is no polymorphism with the gene BMP15 among four lines. There was SNP A/G with the gene BMPR-IB at base 746 among four lines. Results have indicated that the BMPR-IB gene is major in affecting the sheep litter size, and might be used as a genetic marker for litter size selection.

In this field, a current challenge is the elucidation of fundamental molecular mechanisms. The crash is that there are no mutations in genes containing the progression of ovulation and folliculogenesis. Improved consideration of these types of mechanisms will give a marvelous insight into female fertility in the mammal local ovarian regulation on a physiological basis [29].

II. Methodology

From 2000 to 2020 data from different research papers and review articles were studied. This provided information on multiple functions of the BMP15 gene in many breeds of goats and sheep in different countries. Research Gate, PubMed, Google Scholar, and Wiley were used to collect the articles. Original and review articles were searched by using keywords/MeSH terms

"BMP15 gene", "single nucleotide polymorphisms", "goat", "sheep" "mutations" "characterizations" and "molecular markers. This systematic literature after initially searching the published data about the Bone Morphogenetic Protein 15(BMP15) gene, some repeated records were eliminated as the abstract was read. Further screening was done by considering their sample size together with the data regarding BMP15 gene mutations. Some articles having no proper reasoning were excluded. With their cress references remaining studies were retrieved by searching and suitable words related to the BMP15 gene were used to study "Fig 1". In some breeds heterozygosity is beneficial but in some cases causes infertility of many sheep and goats. The researcher's interest is increasing in the identification of fertility rate and also its effects on the reproductive system of different sheep and goat breeds.

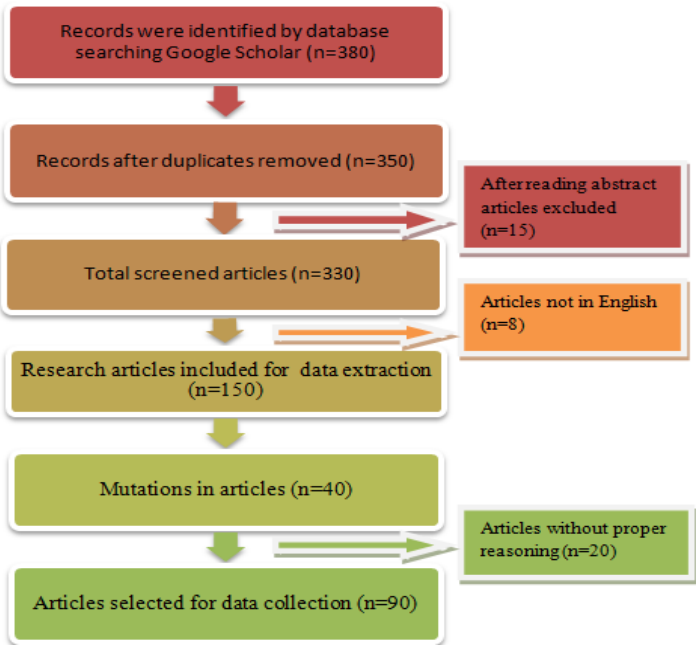

Figure 1: A flow chart for exclusion/inclusion criteria and for data selection strategy to identify mutations in the BMP15 gene of sheep and goats.

Table 3.1: SNPs in Sheep Breeds.

III. Results

Recently, a lot of studies on sheep on prolificacy genetics show the way to highlight the significance of chief gene instead of BMPR 1B, namely GDF9 and BMP15, which show the effects of ovulation rate and litter size through many mechanisms [15]. In sheep, maps of BMP15 gene X-chromosome of full-length sequencing codes 1179 nucleotides presented in two exons, are separated through the 5.4kb intron sequences, encodes 393 amino acid residues prepropeptide. A mature active peptide is 125 amino acids in length [22]. Many single nucleotide polymorphisms were observed in different breeds of sheep as studied by many scientists. A single study in Pakistan was found on sheep SNPs in the BMP15 gene. From 2000 to 2020 years in some countries including Pakistan number of mutations were observed as mentioned "Table 1".

Countries	Gene	Breeds	Nucleotide. Change	Amino acid change	References
Pakistan	BMP15	Balochi	g.171,172,173 Ins.CTT		[44]
		Balochi	g.3477 Del.T		
		Balochi	g.3478 Del. G		
		Balochi	g.3479 Del. A		
		Balochi	g.3296 Trans C>T		
		Balochi	g.4851 TranslT>G		

India	BMP15	Belclare/Cambridge	g.391C>T	Q239Ter	[22] [37]
		Romney	g.544C>T	Q23Stop	[19]
		Romney	g.579T>A	V31D	
		Lacaune	g.635G>A	C53Y	[3]
		Belclare	g.773G>T	S99 I	[22]
	BMP15	Chokla sheep	g.706G>A	Valine to Isoleucine	[8]
			g.783G>A	None	
			g.801G>A	None	
			g.931G>C	Alanine to Proline	
			g.893C>A	Alanine to Aspartic acid	
			g.891C>T	No	
			g.453G>T	No	
			g.606C>T	No	[47]
			g.612G>A	No	
			g.675G>A	No	[8]
		French Grivette		T317I	[14]
		Polish Olkuska		N337H	
Iran	BMP15	Inverdale	g.896T-A	V299D/V 31 D	[33]
		Hanna	g.871T-C	Q291Stop/Q23Stop	

		Belclare	g.1100T-G	S367I/S99I	
		Galway	g.718T-C	T239Stop/ NO	
		Lacanma	g.803G-A	C321Y/C53Y	
		Rasa aragonesa	g. 525-541 17nt Del	-	
		Grivette	g.950C-T	T317I	
		Olkuska	g.1009A-C	N337H	
California	BMP15	Inverdale	g.896T>A	Val 299Asp	[19]
		Hanna	g.871C>T	Gln 291 Term	
		Lacauna	g.962G>A	Cys 321 Tyr	[3]
		Rasa Aragonea	g. 525-541 del(17bp)	208 Term	[36] [33]
		Belclare/Cambridge	g.718C>T	Gln 239 Term	[22]
		Belclare/Cambridge	g.1100G>T	Ser 367 Ile	
China	BMP15	Inverdale		V31D	[22] [19]
		Hanna		Q23Ter	
		Cambridge		Q239Ter	
		Belclare		Q239Ter, S367I	
		Romney sheep	g.871C>T		[19]
			g.896T>A		
	BMP15 (exon 2)	Rasa aragonesa sheep	g.1172C>T		[14]

		Mounton Vendeen	C>T mutation noticed		[9]
Turkey		Tuj and Karakas sheep Ivesi sheep Akkaraman sheep Daglic sheep	G-A	Cys-Tyr	[19] [22] [37] [35]
			17bp Del	---	
			G-T	Ser-Ile	
			C-T	Glu-Stop	
			C-T	Glu-Stop	
			T-A	Val-Asp	
		Belclare and lacaune breed	g.239C-T	77Gln–Stop	[19] [55] [4] [22]
			g.367G-T	99Ser–Ile	
			g.299T-A	31Val–Asp	
			g.291C-T	23Glu–Stop	
			g.321G-A	53Cys–Tyr	
Europe	BMP15	Cambridge and Belclare	g.28-30CTT del	Leu (10)deletion	[22]
			g.718C-T	Gln (249)Stop. Sterility	
			g.747T-C	Unchanged Pro (249)	
			g.1100G-T	Ser 367Ile sterility	
		Hanna	g.871C>T		[22]

		Inverdale	g.896T>A		
		Booroola	g.718G>T		
		Olkuska sheep breed	g.231C>T		[14]
			g.230C>T		
			g.301G>C		
			g.404T>G		
			g.1009A>C		
	BMP15 (exon2)	Grivette	g.950C>T		
			g.747C>T		

As a precursor protein molecule, BMP15 is produced with a naturally active portion of protein residing in the C terminus. There are six mutations labeled as FecXI (Inverdale) and FecXH (Hanna) [19], FecXR (Rasa Aragonesa) [33], FecXL(Lacaune) [3], FecXB(Balclare) and FecXG(Galway) [22] has detected within BMP15 gene. All of these mutations in sheep prove the carrier is phenotypically homozygous having sterility because the hyperplasia in ovaries causes follicles inability at the primary stage of development. Heterozygous carriers illustrate an increased rate of ovulation at 0.8 and 2.4 over that of non-carrier flocks respectively [13, 33]. Like sheep, goats also different breeds were studied in many countries including Pakistan. In Pakistan only a single study for SNPs identified on Jining gray goat and Teddy goat breed. In many other countries, SNPs and also its effects on protein levels were also observed as mentioned "Table 2".

Table 3.2: SNPs in Goat Breeds.

Countries	Gene	Breed	Nucleotide. change	Amino acid change	References
Pakistan	BMP15	Teddy	g.6280T>G		[43]
		Teddy	g.6353G>A		
		Teddy	g.6443T>C		
		Teddy	g.6492A>G		
		Jining gray goat	g.982 T>C		
		Jining gray goat	g.5572 A>G		

China	BMP15	Jining gray goat	g.963A>G	S300G	[26] [18]
		Jining gray goat	g.1050C>G	L329V	
		Lehri goat	AGC (901bp)	Ser>Gly	[19]
			GGG (656bp)	Gly> Glutamic acid	
			TTA (200bp)	Leucine > Serine	
	BMP15 (Exon 1)	Markhoz goats	g.200G>T		[21]
	BMP15 (Exon 2)		g.573G>A		
			g.755T>G		
	BMP15	Boar goat and Funiu white goat	g.465T>G		[56]
			g.466C>G		
			g.510C>T		
			g.511T>C		
		Chaines goats	C>T		[19]
			T>A		
		Yunling black and Boer goat	g.825A-T		[10]
			g.1000A-C		
Iran	Exon 2	Baladi and alpine goats	g.757A>C		[50]
			g.760G>C		
			g.762G>A		
			g.769G>C		
		Iranian goats	G>T		[37]

			C>T		
India	Exon 2	Indian goats	g.735G>A		[1]
			g.808C>G		
	BMP15 (promotor region)		g.242T>C		
			g.623G>A		
Indonesia		Kejobong	g.391A-G	Asp>Asn	[17]
			g.828T-C	Silent mutation	
			g.464C-G	premature stop codon	
			g.830C-G	silent mutation	
Iraq	BMP15	Iraqi goats	g.156T>G	Methionine> Arginine	[23]
			g.218G>T	Valine>leocine	
			g.102G>A	Arginine>Lysine	
			g.207G>A	Arginine>glutamine	
Bangladesh	BMP15	Black bengal	g.1061C>T	p.Ala354val	[2]
			g.781C>A	p.Pro261Thr	
			g.743C>A	p.Pro248His	
			g.735G>A	No change	
			g.754G>T	p.Gly252Cys	
			g.808C>G	p.Gln270Glu	
Egypt	BMP15	Damascus goats	g.751G>T and g.752G>C	Gly251Cys	[24]

			g.760G>C and g.762G>A	Glu254Gln	
			g.769G>A	Glu257Gln	
			g.805G>C	Glu269Gln	
		Baladi goats	g.751G>T and g.752G>C	Gly251Ala	
			g.757A>C	Lys252Gln	
			g.760G>C and g.762G>A	Glu254Gln	
			g.769G>A	Glu257Lys	
			g.769G>C	Glu257Gln	
			g.805G>C	Glu269Gln	
		Alpine goats	g.757A>C	Lys252Gln	
			g.762G>T	Glu254Asp	
			g.760G>C and g.762G>A	Glu254Gln	
			g.769G>A	Glu257Lys	
			g.769G>C	Glu257Gln	
			g.805G>C	Glu269Gln	
		Zaraibi goats	g.752G>C	Gly251Ala	
			g.757A>C	Lys252Gln	
			g.760G>C	Glu254Gln	
			g.769G>C	Glu257Gln	
			g.805G>C	Glu269Gln	
	BMP15	Kejobong breed	g.391A>G	Asp>Asn	[22]
			g.828T>C	Silent mutation	

			g.464C>G	Stop codon	
			g.830C>G	Silent mutation	

In Pakistan, only a single study was done on the BMP15 gene's SNPs, but in many other countries, various studies were done that also observed amino acid changes. The fecundity rate may increase or decrease through these nucleotide changes "Table 1". Similarly, in Pakistan, Iran, and Indian goat breeds studies showed only SNPs in nucleotide sequences, not their expressions on protein functions. In China, Indonesia, and some other countries goat breeds, BMP15 gene expression is also checked on protein level which is helpful in the breeding programs. All of the mutations are not causing infertility. Some mutations increase fertility, these types of mutations are more beneficial to breeders for specific purposes as mentioned "Table 2".

IV. Discussion

The prolificacy increased in si breeds of goats (Haimen, Boer, Nubi, Huanghuai, Jining Grey, and Matou) not correlated with known point mutations in BMP15 or BMPR1B gene [7,25]. [7] Reported either BMP 15gene is major which influences Jining Grey goat breed prolificacy or genetic molecular marker with such gene is closely linked.

Bone Morphogenetic Protein 15 gene significance was confirmed in the sheep fertility by identification of the two BMP15 additional point mutations BMP-15S99I and BMP-15Q-29 Term, in the Belclare and Cambridge sheep. These types of breeds show results in similar phenotypes as Hanna and Inverdale ewes [22].

It was shown by in vitro studies that BMP15 gene mutation C53Y damages the process of maturation of BMP15 protein, in the defective secretions as a result of proprotein and matures both [3]. Subsequently, two, a new mutant of BMP15 (BMP-1517bp-del and BMP-15C53Y) was identified in the sheep breeds Lacaune and Rasa Aragonesa respectively [3,36,33]. An interesting understanding is provided by in vitro studies, concerning the roles of endogenous GDF9 in BMP15 mutated gene sheep. In the Inverdale sheep, the mimic ovine BMP15 V31D mutation was identified as a human recombinant BMP15 mutation at amino acid position 31 from Ile to Asp (BMP15 I31D). [19] Expressed in the HEK293 cells, in BMP15 I31D processed proprotein and mature the dimeric BMP15 I31D normally secreted. When GDF9 and BMP15I31D were co-expressed, proprotein processing and mature protein secretion were concurrently disrupted [31]. Secretion and abnormal processing suggested by the formation of heterodimers BMP15I31D/GDF-9 are not vulnerable to usual proteolytic cleavage which is leading to the degradation in cells communicating these molecules [31].

At the early stage of the follicle growth for promotion, the BMP15 gene appears as important. Even restraining the number of preovulatory dominant follicles, for ovulation quota is a determination key in the cattle and sheep litter size [51].

An ovine gene BMP15 plays an important role in differentiation and growth at the early stage of ovarian follicles [42,51,27,16]. In Hanna and Inverdale sheep, mutation c. 718C>T of the BMP15 gene reported a rate of ovulation increased as heterozygous conditions, otherwise homozygotes are infertile [13]. The BMP15 (FecXG) gene was identified in a sheep breed Small Tailed Han [11].

At the primary stage of follicle development protein is expressed and it is onward and acts in the ovaries [45]. Through follistatin a binding protein action of the BMP15 gene is regulated it can bind within the BMP15 gene and inhibit the effect on oocytes. Expression of FSH receptor inhibited by BMP15 gene in ovaries, to maintain responsiveness of granulosa cells to FSH follistatin is significant [46]. In the heterozygous individuals rate of ovulation is increased due to BMP15 gene mutations. Multiple ovulations were shown in the heterozygous ewes, granulosa cells early maturation, and follicle size reduced. In comparison between antral and noncarriers, more follicles are shown by phenotype but some granulosa cells also expand receptors for the LH earlier. Conversely, most mutations in BMP15 gene development follicle block in homozygous individuals [41].

Genetics about the liter size of sheep has good reported with a number of very important sheep prolificacy genes. On prolificacy genetics, many studies in sheep highlight the significance of vital genes such as BMPR1B, GDF9, and BMP15. These genes are recognized to affect the rate of ovulation and litter size through a variety of mechanisms [15]. Bone Morphogenetic Protein 15 is measured as a vital gene having a possible role in sheep fecundity. BMP15 is also known as GDF9b, mapped to X-chromosome in sheep having full length 6648 nucleotide coding sequence and two exons are

separated through intron of 5309 nucleotides and also instruct 394 amino acids [19]. Therefore, the findings of major gene BMP15 mutations having various effects on fecundity, litter size, and rate of ovulation have made significant awareness in goat and sheep different breeds.

v. Conclusion

In this study identification of various genetic polymorphisms (SNPs) and their association with prolificacy may help enhance the reproductive efficiency among different goat and sheep breeds. Some SNPs bring change at the amino acid level. Since reproductive traits are polygenic and controlled by many genes. All of the genetic variations identified in the BMP15 gene help to carry out marker-assisted selection in a more accurate manner. The BMP15 genes with multiple SNPs may be suitable candidate genes for increasing the fecundity and litter size in sheep and goats. These SNPs may be further evaluated and selected as markers of fecundity in different breeds in the future.

References

[1] S. Ahlawat, R. Sharma, M. Roy, S. Mandakmale, V. Prakash, & M. Tantia, "Genotyping of novel SNPs in BMPR1B, BMP15, and GDF9 genes for association with prolificacy in seven Indian goat breeds," *Animal biotechnology*, *27*(3), 199-207. 2016.

[2] D1Ashutosh, S1 Mishuk , G. D. Mukta2 , D. Avijit2 3 and M. F. Omar1, "Polymorphism of fecundity genes (BMP15 and GDF9) and their association 2 with litter size in Bangladeshi prolific Black Bengal goat," bioRxiv preprint doi: https://doi.org/10.1101/2020.01.08.899443. January 9. 2020.

[3] L. Bodin, E. P. Di, E. S. Fabre, M. Bontoux, P. Monget, L. Persani, et al., "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. 2007.

[4] L. Bodin, F. Lecerf, C. Pisselet, M. SanCristobal, B. Bibe & P. Mulsant, "*How many mutations are associated with increased ovulation rate and litter size in progeny of Lacaune meat sheep,"* Paper presented at the Proceedings of the International Workshop on Major Genes and QTL in Sheep and Goats'.(Ed. L. Bodin.) pp. 2003.

[5] M. X. Chu, Z. H. Liu, C. L Jiao, Y.Q. He, L. Fang, S. C. YE, G. H. Chen, J. Y. Wang, "Mutations in BMPR-IB and BMP-15 genes are associated with litter size in Small Tailed Han sheep (Ovis aries)," J. Anim. Sci. 85, 598–603. 2007.

[6] Y. Chen, Q. Luo, D. Li, Y. Zhang, F. Yang, J. Yang, et al., "Relationship between BMPR1B polymorphism and litter size in six breeds or strains of sheep," *Journal of Xinjiang Agricultural University, 31*(2), 12-16. 2008.

[7] M. Chu, Z. Liu, C. Jiao., Y. He, L. Fang, S. Ye, et al., "Mutations in BMPR-IB and BMP-15 genes are associated with litter size in Small Tailed Han sheep (Ovis aries)," *Journal of Animal Science*, *85*(3), 598-603. 2007.

[8] V. Carcangiu, M. C. Mura, G. M. Vacca, M. Pazzola, M. L Dettori, S. Luridiana, et al., "Polymorphism of the melatonin receptor MT1 gene and its relationship with seasonal reproductive activity in the Sarda sheep breed," *Animal reproduction science*, *116*(1-2), 65-72. 2009.

[9] L. Chantepie, L Bodin, J. Sarry, F. Woloszyn, J. Ruesche, L. Drouilhet, et al., "Presence of causative mutations affecting prolificacy in the Noire du Velay and Mouton Vendéen sheep breeds," *Livestock science*, *216*, 44-50. 2018.

[10] H. X. Cui, S. M. Zhao, M. L. Cheng, L. Guo, R. Q Ye, W. Q. Liu, et al., "Cloning and Expression Levels of Genes Relating to the Ovulation Rate of the Yunling Black Goat1," *Biology of reproduction*, *80*(2), 219-226. 2009.

[11] M. X. Chu, C.L. Jiao, Y.Q. He, J. Y. Wang, Z.H. Liu, G. H, Chen, "Association between PCR-SSCP of bone morphogenetic protein 15 gene and prolificacy in Jining Grey goats" Anim. Biotechnol. 18, 263–274. 2007.

[12] G. H. Davis, "Major genes affecting ovulation rate in the sheep," Genet. Sel. Evol, 37: S11-S23. 2004.

[13] G. H. Davis, L. Balakrishnan, I.K Ross, T., Wilson, S. M. Galloway, B. M Lumsden, J. P. Hanrahan, M. Mullen, X. Z. Mao, G. L. Wang, Z. S. Zhao, Y.Q. Zeng, J .J. Robinson, A. P. Mavrogenis, C. Papachristoforou, C. Peter, R. Baumung, P Cardyn, I. Boujenane, N. E. Cockett, E. Eythorsdottir, J .J. Arranz, and D .R Notter, "Investigation of the Booroola (FecB) and Inverdale (FecXI) mutationa in 21 prolific breeds and strains of sheep sampled in 13 countries," Animal Reproduction Science, 92: 87-96. 2006.

[14] J. Demars, S. Fabre, J. Sarry, R. Rossetti, H. Gilbert, L Persani, et al., "Genome-wide association studies identify two novel BMP15 mutations responsible for an atypical hyperprolificacy phenotype in sheep," *PLoS genetics*, *9*(4). 2013.

[15] G. H. DAVIS, "Major genes affecting ovulation rate in sheep," Genet. Sel. Evol., 37: 11-23. 2005.

[16] S. Fabre, A. Pierre, P. Mulsant, L. Bodin, E. P. di, L. Persani, P. Monget, D . Monniaux, "Regulation of ovulation rate in mammals: Contribution of sheep genetic models," Reprod. Biol. Endocr. 2006, 4, doi:10.1186/1477-7827-4-20.

[17] A. Febriana, S. Sutopo, & E. Kurnianto, "Identification of BMP15 Exon 2 for fecundity traits by PCR-RFLP and nucleotide sequencies in Kejobong goat," *Journal of Indonesian Tropical Animal Agriculture, 42*(4), 220-226. 2017.

[18] T. Feng, Y.-z. ZHAO, M.-x. CHU, R. DI, Y.-j. ZHANG, & L. FANG, "Bone morphogenetic protein 15 as a candidate gene for prolificacy of Jining Grey Goat," *畜牧兽医学报, 1*. 2009.

[19] S. M. Galloway, K. P. McNatty, L. M. Cambridge, M. P. Laitinen, J. L. Juengel, T. S. Jokiranta, et al., "Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner," *Nature genetics, 25*(3), 279-283. 2000.

[20] F. Guan, S.-R. Liu, G.-Q. Shi, & L.-G. Yang, "Polymorphism of FecB gene in nine sheep breeds or strains and its effects on litter size, lamb growth and development," *Animal reproduction science, 99*(1-2), 44-52. 2007.

[21] H. Ghoreishi, J. Shayegh, A. Barzegari, N. Maheri-Sis, A. Gorbani, A. B. Bedoustani, et al., "Sequence assigning of the bone morphogenetic protein 15 (BMP15) genes in Markhoz goats," *Advances in Environmental Biology*, 1850-1854. 2011.

[22] J. P. Hanrahan, S. M. Gregan, P. Mulsant, M. Mullen, G. H. Davis, R. Powell, et al., "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. 2004.

[23] A. H. A. Hayder, "Molecular and Physiological Role of BMP15 Gene Associated with Ovulation Rate and Fertility of Iraq Goat," Journal of Animal and Veterinary Advances 17(5): 104-110, 2018. ISSN: 1680-5593. 2018.

[24] H. Heikal & W. E. Naby, "Genetic improvement of litter size in four goat breeds in egypt using polymorphism in bone morphogenetic protein 15 gene," *Adv. Anim. Vet. Sci, 5*(10), 410-415. 2017.

[25] G. H. Hua, S. L. Chen, J. T. Ai, L.G. Yang, "None of the polymorphism of ovine fecundity major genes FecB and FecX was tested in goat," Anim. Reprod. Sci. 108, 279–286. 2008.

[26] Cl. Jiao, M.-x. CHU, J.-y. WANG, L. FANG, & S.-c. YE, "Cloning and sequence analysis of exon 2 of BMP15 gene in Jining Grey Goats," *China Anim Husbandry Veter Med, 12*, 53-56. 2007.

[27] J. L. Juengel, K. P. McNatty, "The role of proteins of the transforming growth factor-β superfamily in the intraovarian regulation of follicular development" Hum. Reprod. Update 2005, 11, 144–161.

[28] H.K. Li, T. Y. Kuo, H. S. Yang, L.R. Chen, S. S. L. Li, & H. W. Huang, "Differential gene expression of bone morphogenetic protein 15 and growth differentiation factor 9 during in vitro maturation of porcine oocytes and early embryos," *Animal reproduction science, 103*(3-4), 312-322. 2008.

[29] W. X. Liao, R. K. Moore, & S. Shimasaki, "Functional and molecular characterization of naturally occurring mutations in the oocyte-secreted factors bone morphogenetic protein-15 and growth and differentiation factor-9," *Journal of Biological Chemistry, 279*(17), 17391-17396. 2004.

[30] S.-F. Liu, Y.-L. Jiang, & L. Du, "Studies of BMPR-IB and BMP15 as candidate genes for fecundity in Little Tailed Han sheep," *Yi chuan xue bao= Acta genetica Sinica, 30*(8), 755-760. 2003.

[31] W. X. Liao, R. K. Moore, F. Otsuka, S. Shimasaki, "Effect of intracellular interactions on the processing and secretion of bone morphogenetic protein-15 (BMP-15) and growth and differentiation factor-9," Implication of the aberrant ovarian phenotype of BMP-15 mutant sheep. J Biol Chem 278:3713–3719. 2003.

[32] R. K. Moore, & S. Shimasaki, "Molecular biology and physiological role of the oocyte factor, BMP-15," *Molecular and cellular endocrinology, 234*(1-2), 67-73. 2005.

[33] L. V. Monteagudo, R. Ponz, M. T. Tejedor, A. Laviña, & I. Sierra, "A 17 bp deletion in the Bone Morphogenetic Protein 15 (BMP15) gene is associated to increased prolificacy in the Rasa Aragonesa sheep breed," *Animal reproduction science, 110*(1-2), 139-146. 2009.

[34] G. W. Montgomery, S. M. Galloway, G. H. Davis, & K. P. McNatty, "Genes controlling ovulation rate in sheep," *REPRODUCTION-CAMBRIDGE-, 121*(6), 843-852. (2001).

[35] L. V. Monteagudo, R. Ponz, M. T. Tejedor, A. Laviña, & I. Sierra, "A 17 bp deletion in the Bone Morphogenetic Protein 15 (BMP15) gene is associated to increased prolificacy in the Rasa Aragonesa sheep breed," *Animal reproduction science, 110*(1-2), 139-146. 2009.

[36] A. Martinez-Royo, J. Jurado, J. Smulders, J. Marti, J. Alabart, A. Roche, et al., "A deletion in the bone

morphogenetic protein 15 gene causes sterility and increased prolificacy in Rasa Aragonesa sheep," *Animal genetics*, *39*(3), 294-297. 2008.

[37] K. P., McNatty, P., Smith, L., Moore, K., Reader, S., Lun, J., Hanrahan, et al., "Oocyte-expressed genes affecting ovulation rate," *Molecular and cellular endocrinology*, *234*(1-2), 57-66. 2005.

[38] S. Margulis, R. Abir, C. Felz, S. Nitke, H. Krissi, B Fisch, "Bone morphogenetic protein 15 expression in human ovaries from fetuses, girls and women," Fertil. Steril. 92, 1666–1673. 2009.

[39] A. Martinez-Royo, J.J. Jurado, J.P. Smulders, J. I. Marti, J.L. Alabart, A. Roche, E. Fantova, L. Bodin, P. Mulsant, M. Serrano, J. Folch, J. H. Calvo, "A deletion in the bone morphogenetic protein 15 gene causes sterility and increased prolificacy in Rasa Aragonesa sheep," Anim Genet 39:294–297. 2008.

[40] L.V. Monteagudo, R. Ponz, M. T. Tejedor, A. Lavina, I. Sierra, "A 17 bp deletion in the Bone Morphogenetic Protein 15 (BMP15) gene is associated to increased prolificacy in the Rasa Aragonesa sheep breed," Anim Reprod Sci 110:139–146. 2009.

[41] G. W. Montgomery, S. M. Galloway, G. H. Davis, K. P. McNatty, "Genes controlling ovulation rate in sheep," Reproduction, vol. 121, ss. 843-852. 2001.

[42] K. P. McNatty, L.G. Moore, N. L. Hudson, L. D. Quirke, S.B. Lawrence, K. Reader, J. P. Hanrahan, P. Smith, N. P. Groome, M. Laitinen, et al., "The oocyte and its role in regulating ovulation rate: A new paradigm in reproductive biology," Reproduction 2004, 128, 379–386.

[43] A. Nawaz, M. E. Babar, T. Hussain, A. Nadeem, F. Bilal, K. Javed, et al., "Identification of molecular markers in BMP15 gene of Pakistani goat breeds," *Int. J. Adv. Res, 1*(8), 62-68. 2013.

[44] A. Nawaz, K. Masroor, E. Babar, A. Nadeem, T. Hussain, F. Bilal, et al., "Identification of molecular markers in bone morphogenetic protien15 (BMP15) gene of Balochi sheep," *Pakistan Journal of Zoology*, *45*(5). 2013.

[45] L. Nicol, S. C. Bishop, R. Pong-Wong, C. Bendixen, L-E. Holm, S. M. Rhind, S. A. McNeilly, "Homozygosity for a single base-pair mutation in the oocytespecific GDF9 gene results in sterility in Thoka sheep," Reproduction, vol. 138, ss. 921–933. 2009.

[46] R. K. Pramod S. K. Sharma, R. Kumar, & A. Rajan, "Genetics of ovulation rate in farm animals," *Veterinary World*, *6*(11), 833. 2013.

[47] J. Pelletier, L. Bodin, E. Hanocq, B. Malpaux, J. Teyssier, J. Thimonier, et al., "Association between expression of reproductive seasonality and alleles of the gene for Mel1a receptor in the ewe," *Biology of reproduction*, *62*(4), 1096-1101. 2000.

[48] K. R. Pramod, S. K. Sharma R. Kumar, A. Rajan, "Genetics of ovulation rate in farm animals," Veterinary World, vol. 6(11), ss. 833-838. 2013.

[49] O. V. Sjaastad, K. Hove, & O. Sand, *Physiology of domestic animals*: Scan. Vet. Press. 2010.

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

[51] S. Shimasaki, R.K. Moore, F. Otsuka G. F. Erickson, "The bone morphogenetic protein system in mammalian reproduction," Endocr. Rev. 25, 72–101. 2004.

[52] L. Tian, H.-B. Han, & X.-H. Chen, "Preponderance genotype of BMPR-IB improves the pregnant rate of embryo-transfer in sheep," *PIBB*, *11*, 1074-1079. 2006.

[53] Q. Wang, F. Zhong, H. Li, X. Wamg, S. Liu, X. Chen, et al., "Detection of major gene on litter size in sheep," *Yi chuan= Hereditas*, *27*(1), 80-84. 2005.

[54] Q. Wang, F. Zhong, H. Li, X. Wang, S. Liu, & X. Chen, "The polymorphism of BMPR-IB gene associated with litter size in sheep." *Grass-feeding Livestock, 2*, 20-23. 2003.

[55] T. Wilson, X.-Y. Wu, J. L. Juengel, I. K. Ross, J. M. Lumsden, E. A. Lord, et al., "Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells," *Biology of reproduction*, *64*(4), 1225-1235. 2001.

[56] Y. Wang, L. Yuanxiao Z. Nana, & B. Junyan, "Polymorphism of exon 2 of BMP15 gene and its relationship with litter size of two Chinese goats," *Asian-Australasian Journal of Animal Sciences*, *24*(7), 905-911. 2011.

[57] F. Zhong, X. Wang, S. Liu, H. Li, X. Chen, J. Yin, et al., "Study on the polymorphism of BMPR-IB gene associated with litter size in small-tailed Han sheep and Xinjiang Duolang sheep," *China Herbivore*, *25*(6), 15-16. 2005.

[58] Y. Zuo-qing, Y. Bo-hui, & G. Jian, "Polymorphism of GDF9 and FecB Gene in Two Types of Crossbred Sheep and Their Effects on the Litter Size and Body Weight [J]," *China Herbivores*, *2*. 2008.

AUTHORS

First Author – Safia Bibi, M.Phil Zoology, The Government Sadiq College, Department of Zoology, Women University Bahawalpur, Pakistan.

Second Author – Naila Kousar, Mphil Zoology, The Government Sadiq College, Department of Zoology, Women University Bahawalpur Pakistan.

Third Author – Zoonaira Tanveer, M.Phil Zoology, The Government Sadiq College, Department of Zoology, Women University Bahawalpur, Pakistan.

Correspondence Author – Safia Bibi, M.Phil Zoology, The Government Sadiq College, Department of Zoology, Women University Bahawalpur, Pakistan.