p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Higher Education, Research, and Technology, Republic of Indonesia, No. 225/E/KPT/2022

Tropical Animal Science Journal, June 2024, 47(2):141-148
DOI: https://doi.org/10.5398/tasj.2024.47.2.141
Available online at https://journal.ipb.ac.id/index.php/tasj

Detection of Polymorphisms in BMP15 and GDF9 Genes and Their Associations with Reproductive Traits in Black Bengal Goat of Bangladesh

M. A. Mou[a], M. F. A. Hridoy[a], N. H. Desha[b], S. Ahmed[b,#], & M. S. A. Bhuiyan[a,*]
[a]Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[b]Goat Production Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka 1341, Bangladesh
*Corresponding author: msabhuiyan.abg@bau.edu.bd
[#]Corresponding author: sadek_11@yahoo.com
(Received 05-11-2023; Revised 31-12-2023; Accepted 08-01-2024)

ABSTRACT

The Bone Morphogenetic Protein 15 (BMP15) and Growth Differentiation Factor 9 (GDF9) genes play important roles in follicular development, ovulation rate, and litter size in goats. This study aimed to investigate polymorphisms (SNP) in the coding sequences of BMP15 and GDF9 genes and to find possible associations between identified polymorphisms and reproductive traits in Bangladesh's Black Bengal goat (BBG). In total, 85 DNA samples of BBG were utilized for PCR amplification, gene fragments sequencing, and subsequent association studies using a generalized linear model implemented by R software. Sequence analysis revealed three SNPs (g.5875A>G, g.6051G>A, and g.6124C>G) in the exon 2 of the BMP15 gene, having one nonsynonymous mutation (g.6124C>G) that changed amino acid glutamic acid to glutamine (E270Q). Besides, the g.3764C>T polymorphism identified in the GDF9 gene was nonsynonymous in nature, changing the amino acid alanine to valine (A273V). The association analysis revealed that litter size and kidding interval differed significantly ($p<0.05$) for g.6051G>A and g.6124C>G SNP genotypes of the BMP15 gene in the BBG population. Besides, the combined genotypes derived from three BMP15 polymorphisms also significantly affected average litter size and at the 3rd parity. However, the g.3764C>T SNP genotypes of the GDF9 gene showed significant association with only average service per conception. Taken together, the identified SNPs of BMP15 and GDF9 genes showed potentials that could be used as molecular markers for improving the reproductive traits of BBG.

Keywords: Bangladesh; candidate gene; goat; polymorphism; reproduction

INTRODUCTION

Bangladesh is endowed with a promising goat breed, Black Bengal, potentially contributing to rural livelihoods by providing meat, milk, skin, and fertilizer. In Bangladesh, the goat population represented the country's second-largest livestock species. The total goat population of Bangladesh is about 26.47 million (DLS, 2021), of which 90% are Black Bengal and the remaining are exotic pure breeds (Jamunapari, Beetal, Sirohi, Boer etc.) or their crosses (Siddiky, 2017). The proverb "goat is the poor man's cow" still holds promise, especially for Bangladesh's landless, destitute, and marginal farmers who rely on subsistence farming. Chevon (goat meat) is the most expensive meat and has become a cultural icon in Bangladeshi cuisine, regardless of creed, caste, or religion. Litter size is a vital productivity index in any multiparous livestock species, including Black Bengal goat (BBG), and is associated with net economic returns to farmers. Due to their high prolificacy and sexual precocity, BBG has emerged as a promising genetic resource for validating the genetic mechanism of reproduction (Ahlawat *et al.*, 2015). Previous studies reported significant variations ($p<0.05$) in production and fitness traits among the individuals of BBG (Jalil *et al.*, 2016; Miah *et al.*, 2016; Talukder *et al.*, 2020; Faruque *et al.*, 2016). It is generally agreed that an animal producing twins or triplets contributes more than 1.5 times as much meat as a single offspring producing per kidding. As a result, improving reproductive traits has increased interest in goats.

Identifying molecular markers and their applications in animal breeding programs have been practiced worldwide for almost all farm animal species. Marker-assisted selection (MAS), combined with conventional selective breeding programs, is the most effective approach for improving reproductive traits (Ahlawat *et al.*, 2015). Genetic evidence showed the significant influence of Bone Morphogenetic Protein 15 (BMP15) and Growth Differentiation Factor 9 (GDF9) on the fecundity of goats (Zhao *et al.*, 2016; Arefnejad *et al.*, 2018; Bi *et al.*, 2020). Meanwhile, several polymorphisms in the candidate genes like BMP15 and GDF9 have been reported for their associations with prolificacy traits in different goat populations (Wang *et al.*, 2011; Jalbani *et al.*, 2017; Ghoreishi *et al.*, 2019). The

polymorphisms found in these two genes might have potential implications in the MAS program for further improvement of litter size in the Bangladeshi BBG population. Ahlawat *et al.* (2016) found a significant association between single nucleotide polymorphisms (SNPs) in the BMPR1B, BMP15, and GDF9 genes and litter size in Indian Black Bengal goats. Shaha *et al.* (2022) reported that a non-synonymous mutation at the G1330T locus of the GDF9 gene had significantly affected litter size in the Jamunapari goat of Bangladesh. Three SNPs (C735A, G754T, and C781A based on NCBI GenBank reference sequence: NM_001285588.1) of the BMP15 gene had a significant association with litter size in Bangladeshi BBG (Das *et al.*, 2021). Notably, the studies in BBG were based on previously reported SNP specific. To date, no neat information is available about polymorphisms on the entire coding sequences of BMP15 and GDF9 genes or litter size-specific association study considering data from wider samples of BBG of Bangladesh. Hence, this study aimed to detect polymorphisms in the coding sequences of two aforementioned genes and investigate possible associations between the identified polymorphisms and reproductive traits in BBG of Bangladesh.

MATERIALS AND METHODS

Ethical Approval

The study was conducted following the protocol approved by the Animal Experimentation Ethics Committee of Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka (Approval no.: AEEC/BLRI00104/2023).

Phenotypic Data Collection, Blood Sampling, and DNA Extraction

A total of 85 Black Bengal goats having up to the 2nd or 3rd parity records were selected from four different BLRI-managed flocks (Savar, Dhaka; Nikhongchhari, Bandarban; Bhaluka, Mymensingh; and Godagari, Rajshahi). Four reproductive traits, such as days open (DO), service per conception (SC), litter size (LS), and kidding interval (KI), were included in this study, and relevant information was collected from the record sheet maintained by BLRI. Approximately 3.0 mL of blood was taken aseptically from the selected animals using venoject tubes coated with EDTA as an anticoagulant. Genomic DNA was extracted from goat whole blood samples using the AddPrep Genomic DNA Extraction Kit (ADD BIO INC., Daejeon, Republic of Korea) according to the manufacturer's instructions with some modifications in the protocol. The concentration and purities of isolated genomic DNA were assessed by a NanoDrop spectrophotometer (Model ND2000, Thermo Fisher Scientific, MA USA).

Primer Synthesis and PCR Amplification

Six pairs of primers (Supplementary Table 1) were selected from the published papers of Jalbani *et al.* (2017), Ghoreishi *et al.* (2019), and Zhao *et al.* (2016) to amplify entire coding sequences of the BMP15 and GDF9 genes. Primer synthesis was done by a commercial sequence service provider (Macrogen, Seoul, Republic of Korea). PCR amplification was carried out using TECHNE thermocycler (Bibby Scientific, Leicestershire, UK) in a 20 μL reaction comprising 1.5 μL of genomic DNA, 10 μL of 2× master mix (Prime Taq DNA polymerase 1 unit/10 μL, 20 mM Tris-HCl (pH-8.8), 100 mM KCl, 0.2% Triton® X-100, 4.0 mM $MgCl_2$, enzyme stabilizer, sediment, loading dye, and 0.5 mM each of dNTP), 2.0 μL of each primer (10 pmol/μL), and 4.0 μL deionized water (ADD BIO INC., Daejeon, Republic of Korea). The PCR thermal profile maintained the following cyclic condition: initial denaturation for 10 min at 95 °C; 32 cycles of denaturation at 95 °C for 30 s, annealing at 59~60 °C for 30 s, extension step at 72 °C for 1 min with a final extension at 72 °C for 10 min. The amplified PCR product was electrophoresed in 2% agarose gel stained with green gel dye and visualized by a digital gel documentation system (GDS-200, Sunil-Bio INC, Seoul, Republic of Korea).

Sequencing and Polymorphism Detection

A subset of pooled DNA samples was initially used to amplify each gene fragment, and the purified PCR products were sequenced bi-directionally from a commercial sequence service provider (Wuhan Tianyi Huayu Gene Technology Co. Ltd., Wuhan, China). The generated raw sequences were retrieved by the Chromas software (Version 2.6.6, Technelysium Pty. Ltd.) and were checked to detect polymorphisms in the coding sequences of BMP15 and GDF9 genes. Multiple sequence alignments along with reference sequences of *Capra hircus* (GenBank accession number: NW_017189516.1, complement: 15298841 to 15305343 and NC_030814.1, complement: 66023938 to 66028657 for BMP15 and GDF9 genes, respectively) were performed using CLUSTALW program. Multiple sequence alignment detected polymorphisms only in the exon 2 regions of the BMP15 and GDF9 genes. Finally, two pairs of primers were utilized for PCR amplification and Sanger sequencing of the fragments of both genes. A total of 81 and 72 sequence information were utilized for SNP genotyping purposes for the BMP15 and GDF9 genes, respectively.

Statistical Analysis

Genotypic and allelic frequencies were calculated from the sequence data according to Falconer & Mackay (1997). Single marker association analysis was carried out to evaluate the relationship between resultant genotypes of the BMP15 and GDF9 genes and reproductive traits in Black Bengal goats using the agricolae package in R (Mendiburu & Yaseen, 2021) software. Mean separation was tested using the pastecs package in R (Grosjean *et al.*, 2018). The genotype, management system, location, and parity were considered to have a fixed effect on reproductive traits, where the effects were calculated according to the following model:

$$Y_{ijklmno} = \mu + G_i + L_j + M_k + P_l + GL_m + GM_n + GP_o + e_{ijklmno}$$

Where, $Y_{ijklmno}$ was the dependent variable (reproductive traits), μ was the overall mean, G_i was the fixed effect of i^{th} genotype (1, 2, and 3 represent the respective genotypes), L_j was the fixed effect of j^{th} location (1, 2, 3, and 4 denote for four different locations), M_k was the fixed effect of k^{th} management system (1 and 2 for on-station and farmers' level), P_l was the effect of l^{th} parity (1, 2, and 3 for the 1st, 2nd, and 3rd parity), GL_m was the interaction between the fixed effects of genotype and location, GM_n was the interaction between the fixed effects of genotype and management system, GP_o was the interaction between the fixed effects of genotype and parity, and $e_{ijklmno}$ was the random error.

RESULTS

Detection of Polymorphisms

Multiple sequence analysis revealed three SNPs (g.5875A>G, g.6051G>A, and g.6124C>G) in the exon 2 of the BMP15 gene and only one SNP (g.3764C>T) in exon 2 of GDF9 gene (Figure 1). SNP position is based on the reference sequences on GenBank of *Capra hircus* GDF9 (NC_030814.1) and BMP15 (NW_017189516.1). The g.3764C>T polymorphism of the GDF9 gene is a nonsynonymous type that changes the amino acid alanine to valine (A273V). Among the three identified mutations of the BMP15 gene, only the g.6124C>G SNP is nonsynonymous in nature, resulting in the change of amino acid glutamic acid to glutamine (E270Q) (Table 1).

Genotypic and Allelic Frequencies

The genotypic and allelic frequencies for the four identified SNPs of GDF9 and BMP15 genes in the BBG population are summarized in Table 2. The major alleles for the identified SNPs (g.3764C>T, g.5875A>G, g.6051G>A, and g.6124C>G) were C, A, G, and C, respectively. The novel SNP of the BMP15 (g.5875A>G) resulted from genotypic frequencies were 0.84, 0.15, and 0.01, respectively, for AA, AG, and GG genotypes. The corresponding A and G allelic frequencies were 0.91 and 0.09, respectively. This study revealed the identified SNPs with all three possible genotypic combinations, including heterozygous genotypes, for all tested goats.

Association of SNPs with Litter Size

Table 3 recounted the effects of four SNP genotypes on the parity-wise litter size of BBG. This study found the 1st and 3rd parity litter size differed significantly ($p<0.05$) for g.6051G>A SNP genotypes and the g.6124C>G SNP genotypes also had a significant association with the 3rd parity litter size in the studied

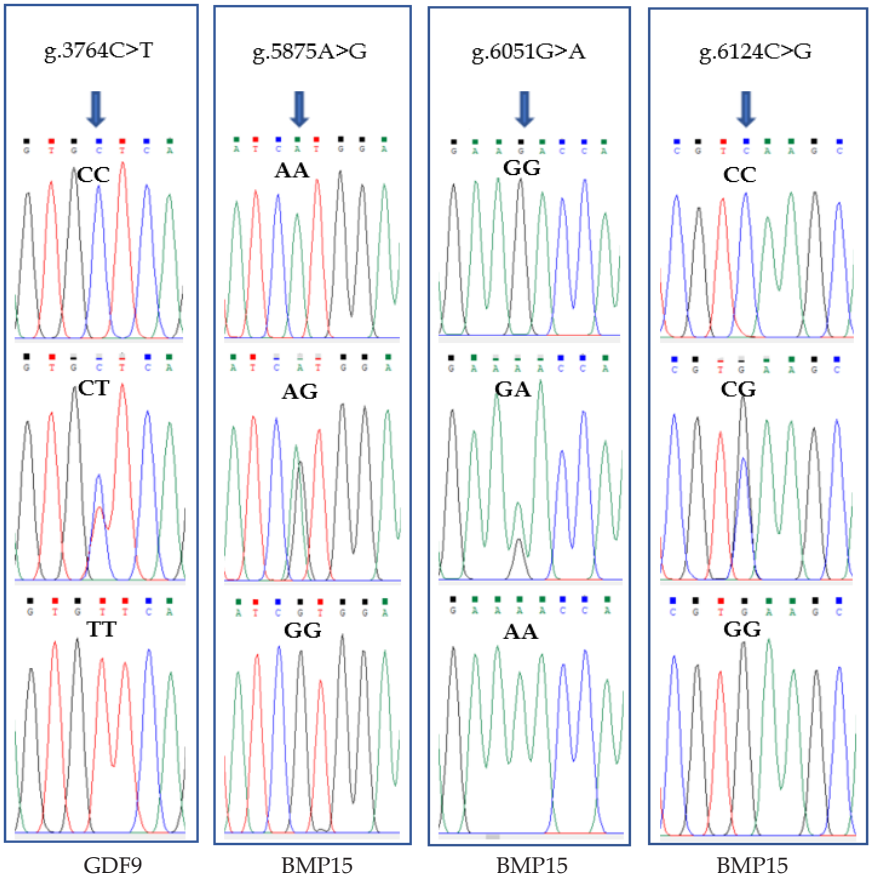

Figure 1. Partial sequence of all the genotypes (column wise) of Black Bengal goat and the identified single nucleotide polymorphisms in GDF9 (g.3764C>T) and BMP15 (g.5875A>G, g.6051G>A, and g.6124C>G) genes

Table 1. Identified polymorphisms in GDF9 and BMP15 genes of Black Bengal goat of Bangladesh

Gene	Location	SNP position[1]	Consequence	Amino acid substitution
GDF9	Exon 2	g.3764C>T	Non-synonymous	A273V
	Exon 2	g.5875A>G	Synonymous	I186I
BMP15	Exon 2	g.6051G>A	Synonymous	K245K
	Exon 2	g.6124C>G	Non-synonymous	E270Q

Note: [1]SNP position is based on the reference sequences of Capra hircus GDF9 (NC_030814.1, Chr. 7, Position: 66023938 to 66028657 bp) and BMP15 (NW_017189516.1, Chr. X, Position: 15298841 to 15305343 bp, reverse complement).

Table 2. Genotypic and allelic frequencies of four SNPs of GDF9 and BMP15 genes in Black Bengal goat

Gene	SNP	Genotype frequency[1]			Allele frequency	
GDF9	g.3764C>T	CC	CT	TT	C	T
		0.54 (39)	0.33 (24)	0.13 (09)	0.71	0.29
BMP15	g.5875A>G	AA	AG	GG	A	G
		0.84 (68)	0.15 (12)	0.01 (01)	0.91	0.09
	g.6051G>A	GG	GA	AA	G	A
		0.42 (34)	0.35 (28)	0.23 (19)	0.59	0.41
	g.6124C>G	CC	CG	GG	C	G
		0.46 (37)	0.37 (30)	0.17 (14)	0.64	0.36

Note: [1]Values in the parentheses indicate the number of observations in the respective genotypes. SNP position is based on the reference sequences of Capra hircus GDF9 (NC_030814.1, Chr. 7, Position: 66023938 to 66028657 bp) and BMP15 (NW_017189516.1, Chr. X, Position: 15298841 to 15305343 bp, reverse complement).

Table 3. Associations of four SNPs of GDF9 and BMP15 genes with litter size trait in Black Bengal goat (LSM±SE)

Locus	Genotype	LS1[1, 2]	LS2	LS3	ALS
g.3764C>T	CC	1.62±0.09 (39)	2.06±0.11 (33)	1.83±0.14 (23)	1.85±0.07 (39)
(GDF9)	CT	1.67±0.13 (24)	2.19±0.13 (21)	1.92±0.18 (13)	1.92±0.11 (24)
	TT	1.89±0.20 (9)	2.33±0.17 (9)	2.33±0.33 (6)	2.17±0.17 (9)
	Level of significance	NS	NS	NS	NS
g.5875A>G	AA	1.70±0.06 (67)	2.16±0.07 (58)	1.91±0.12 (33)	1.92±0.05 (67)
(BMP15)	AG	1.85±0.15 (13)	2.00±0.26 (10)	2.00±0.58 (3)	1.92±0.16 (13)
	Level of significance	NS	NS	NS	NS
g.6051G>A	GG	1.74±0.09^{ab} (34)	2.15±0.12 (27)	2.13±0.17^{a} (15)	1.95±0.08 (34)
(BMP15)	GA	1.86±0.10^{a} (28)	2.21±0.12 (24)	1.90±0.23^{ab} (10)	1.99±0.09 (28)
	AA	1.50±0.10^{b} (18)	2.00±0.15 (17)	1.64±0.20^{b} (11)	1.76±0.11 (18)
	Level of significance	*	NS	*	NS
g.6124C>G	CC	1.64±0.09 (36)	2.09±0.11 (32)	1.85±0.15^{ab} (20)	1.87±0.08 (36)
(BMP15)	CG	1.80±0.09 (30)	2.13±0.11 (24)	1.67±0.24^{b} (9)	1.91±0.08 (30)
	GG	1.79±0.15 (14)	2.25±0.18 (12)	2.43±0.20^{a} (7)	2.07±0.12 (14)
	Level of significance	NS	NS	*	NS

Note: [1]LS1, LS2, and LS3= litter size at the first, second, and third kidding, respectively; ALS= average litter size. [2]Values in the parentheses indicate the number of samples investigated. Means in the same row with different superscripts differ significantly ($p<0.05$).

population ($p<0.05$) of BMP15 gene. However, parity-wise litter size and average litter size did not differ significantly among the genotypes of g.3764C>T of GDF9 and g.5875A>G of BMP15 genes

Effects of Combined Genotypes on Litter Size

The effects of combined genotypes derived from BMP15 gene polymorphisms on the litter size of BBG are abridged in Table 4. There were eight combined genotypes where litter size at the first and second kidding had no significant effects. Contrary to expectations, a significant effect was observed on litter size at the third kidding and average litter size ($p<0.05$). Among the derived genotypes, AAGGCC had the maximum litter size (2.33±0.33), while the AGGGCG genotype possessed a minimum (1.00±0.00) at the third parity of kidding.

Association of SNPs with Reproductive Traits

Table 5 narrates the association between four SNPs of GDF9 and BMP15 genes and reproductive traits (DO, SC, and KI at the first three consecutive kidding, and KI). The association of g.3764C>T (GDF9) SNP genotypes resulted in a significant ($p<0.05$) effect only on ASC, whereas the other traits were found to be insignificant ($p>0.05$). The g.5875A>G SNP genotypes of the BMP15 gene had significant effects ($p<0.05$) on the kidding interval at the first and second parity and mean kidding interval, where the SNP (g.6051G>A) showed highly significant effects ($p<0.01$) on the traits

Table 4. Litter size trait in Black Bengal goat treated with combined genotypes derived from BMP15 gene polymorphisms

Genotype (n)	LS1 (n)[1]	LS2 (n)	LS3 (n)	ALS (n)
AAAACC (18)	1.50±0.12 (18)	2.00±0.15 (17)	1.64±0.20^{b} (11)	1.76±0.11^{b} (18)
AAGACG (16)	1.88±0.13 (16)	2.14±0.14 (14)	1.75±0.48^{b} (04)	1.97±0.11^{ab} (16)
AAGGGG (14)	1.79±0.15 (14)	2.25±0.18 (12)	2.43± 0.20^{a} (07)	2.07±0.12^{a} (14)
AAGGCG (10)	1.60±0.16 (10)	2.14±0.14 (07)	1.75±0.25^{b} (04)	1.80±0.14^{b} (10)
AGGACC (07)	1.86±0.26 (07)	2.17±0.31 (06)	2.50±0.50^{a} (02)	2.02±0.24^{a} (07)
AGGGCG (06)	1.83±0.17 (06)	1.75±0.48 (04)	1.00±0.00^{c} (01)	1.81±0.22^{b} (06)
AAGACC (05)	1.80±0.20 (05)	2.50±0.29 (04)	1.75±0.25^{b} (04)	2.00±0.10^{ab} (05)
AAGGCC (04)	1.75±0.25 (04)	2.25±0.25 (04)	2.33±0.33^{a} (03)	2.09±0.21^{a} (04)
Level of sig.	NS	NS	*	*

Note: [1]LS1, LS2, and LS3= litter size at the first, second, and third kidding, respectively; ALS= average litter size. n= Values in the parentheses indicate the number of samples investigated in the respective combined genotypes. Means in the same column with different superscripts differ significantly ($p<0.05$). * = $p<0.05$ and NS = $p>0.05$.

Table 5. Associations of SNPs of GDF9 and BMP15 genes with reproductive traits in Black Bengal goat (LSM±SE)

Locus	Genotype	ADO (n)	ASC (n)	KI1 (n)	KI2 (n)	KI3 (n)	AKI (n)
g.3764C>T	CC	66.33±3.45 (16)	1.46±0.10^{b} (27)	185.08±6.85 (26)	194.75±6.61 (20)	211.09±11.51 (11)	186.54±6.61 (27)
(GDF9)	CT	66.38±5.27 (15)	1.55±0.13^{ab} (21)	190.53±6.82 (19)	201.94±9.34 (18)	216.45±10.81 (11)	195.51±7.51 (21)
	TT	65.33±3.98 (6)	1.89±0.28^{a} (9)	195.44±12.93 (9)	184.50±10.82 (8)	190.33±13.27 (6)	191.83±11.11 (9)
	Level of significance	NS	*	NS	NS	NS	NS
g.5875A>G	AA	68.43±4.27 (32)	1.60±0.09 (52)	188.60±5.37 (50)	197.00±5.95 (40)	209.68±9.58 (22)	189.80±5.24 (52)
(BMP15)	AG	60.47±1.22 (5)	1.67±0.22 (12)	172.83±9.08 (12)	182.00±10.41 (9)	212.50±2.50 (2)	173.26±9.12 (12)
	Level of significance	NS	NS	*	*	NS	*
g.6051G>A	GG	61.54±0.48 (16)	1.59±0.13 (29)	180.79±5.73^{b} (28)	192.75±6.02^{b} (20)	196.36±7.20 (11)	181.48±5.62^{b} (29)
(BMP15)	GA	63.92±3.31 (8)	1.75±0.16 (19)	176.74±8.46^{b} (19)	179.00±7.64^{b} (16)	197.60±12.72 (05)	175.60±7.57^{b} (19)
	AA	76.62±1.13 (13)	1.50±0.13 (16)	205.60±11.24^{a} (15)	215.31±13.26^{a} (13)	236.25±21.20 (8)	209.35±11.17^{a} (16)
	Level of significance	NS	NS	**	***	NS	***
g.6124C>G	CC	72.66±6.71 (20)	1.60±0.11 (31)	192.53±7.71 (30)	202.00±8.60^{a} (24)	221.54±14.53 (13)	194.19±7.57^{a} (31)
(BMP15)	CG	60.42±0.78 (10)	1.58±0.14 (20)	177.11±7.16 (19)	184.80±8.03^{b} (15)	182.00±13.74 (05)	178.76±6.97^{b} (20)
	GG	62.09±0.62 (7)	1.69±0.22 (13)	181.77±8.67 (13)	189.80±9.07^{ab} (10)	208.00±0.93 (6)	181.06±8.54^{ab} (13)
	Level of significance	NS	NS	NS	*	NS	*

Note: [1]ADO= average days open; ASC= average service per conception; KI1, KI2, and KI3= kidding interval at the first, second, and third kidding, respectively; and AKI= average kidding interval. n= Values in the parentheses indicate the number of observations in the respective reproductive traits. Means in the same column with different superscripts differ significantly at *** = $p<0.001$, ** = $p<0.01$ and * = $p<0.05$. NS= $p>0.05$.

mentioned above. The latter one (g.6124C>G) resulted in significant effects ($p<0.05$) only on the kidding interval at the 1st parity and mean kidding interval. All the heterozygous genotypes of the three SNPs of the BMP15 gene were associated with the minimum kidding interval with reference to the other genotypes.

DISCUSSION

BMP15 and GDF9 are the key genes belonging to the transforming growth factor-β superfamily, contributing to boosting the ovulation rate in animals and also play roles in oocyte maturation processes, ovulation, fertilization, and luteinization in mammals (Castro *et al.*, 2016). Few reports analyze the interaction between mutations in BMP15 and GDF9 genes with goat reproductive traits (Ahlawat *et al.*, 2015). Therefore, identifying novel SNP in the prolificacy genes and SNP-specific association analysis will assist in screening better-performed goats and improve population-level reproductive traits.

The analysis of the entire coding sequence depicted three nucleotide polymorphisms in exon 2 of the BMP15 gene, where g.5875A>G was a novel mutation. A plethora of mutations have been reported in the exon regions of BMP15 gene in prolific Jining Grey Goat (Chu *et al.*, 2007), Funiu white goat (Wang *et al.*, 2011), Teddy goat (Nawaz *et al.*, 2013), Lehri goat (Jalbani *et al.*, 2017), and Markhoz goat breed (Arefnejad *et al.*, 2018; Ghoreishi *et al.*, 2019). Polymorphisms of BMP15 (K245K and E270Q) in BBG found in this study have also been reported in Indian goat breeds (Maitra *et al.*, 2016), in Indian BBG (Ahlawat *et al.*, 2015), as well as in Bangladeshi BBG (Das *et al.*, 2021) which is similar to the current findings. In contrast, Polley *et al.* (2009) stated that the BMPR1B gene was polymorphic, whereas the BMP15 gene was monomorphic in Indian BBG, which contradicts with this finding.

Three nonsynonymous polymorphisms, i.e., A273V, Q320P, and V397I, respectively, were reported in the coding sequence of GDF9 in Indian goat breeds, whereas the g.3764C>T SNP (A273V) is similar to the

present investigation (Ahlawat *et al.,* 2012; Maitra *et al.,* 2016). In the follow-up research, Ahlawat *et al.* (2015) genotyped these three non-synonymous polymorphisms in Indian BBG and confirmed the C818T (A273V) polymorphism, consistent with this study. However, the identified g.3764C>T SNP of GDF9 in the studied BBG population has also been reported in prolific Beetal goats (Hadizadeh *et al.,* 2014), Cameroon native goats (Wouobeng *et al.,* 2020), and Jamunapari and crossbred goats (Shaha *et al.,* 2022) that supports the present finding. In the history of recorded polymorphisms, the identified SNP loci in the exon region of the GDF9 gene of different goat breeds were similar different from our identified SNP (Feng *et al.,* 2011; An *et al.,* 2012; Arefnejad *et al.,* 2018; Ghoreishi *et al.,* 2019; Bi *et al.,* 2020; Das *et al.,* 2021).

This study figured out a novel polymorphism, g.5875A>G, in exon 2 of BMP15 with 0.84, 0.15, and 0.01 genotypic frequencies for the AA, AG, and GG genotypes, and the resultant allelic frequencies of A (0.91) and G (0.09), respectively. However, Ahlawat *et al.* (2015) estimated allelic frequency for 735G>A SNP (K245K) as G: 0.635 and A: 0.365, which are close to the present study, and for the C808G (E270Q) mutation as C: 0.741 and G: 0.259, which are slightly apart from this study. The above stated two polymorphisms are similar to the g.6051G>A (K245K) and g.6124C>G(E270Q) mutations of this study.

Maitra *et al.* (2016) reported G as the major allele for G735A and C for C808G polymorphisms in the BMP15 gene of Indian BBG with allelic frequencies of 0.635 and 0.741, respectively and are close to the current estimation. However, the estimated genotypic and allelic frequencies of the BMP15 (K245K and E270Q) gene in BBG (Das *et al.,* 2021), in Jamunapari, and Crossbred (for K245K) (Shaha *et al.,* 2022) differed slightly from the present estimation. The genotypic and allelic frequencies of the GDF9 gene (A273V) estimated in this study differed slightly from those observed in Indian goat breeds (Ahlawat *et al.,* 2012) and Beetal goats (Hadizadeh *et al.,* 2014) respectively. However, the reported allelic frequencies of GDF9 for the C818T polymorphism of BBG (Ahlawat *et al.,* 2015; Maitra *et al.,* 2016) coincide with the present result. Notably, the genotype and allele frequency are breed or population-specific and differ largely from population to population due to SNP distribution, breeding strategy, sample size, and the genetic make-up of the animal.

No significant difference was observed for the g.5875A>G polymorphism on the litter size of BBG. Similar to the current findings, Ahlawat *et al.* (2016) reported non-significant effects of genotype on litter size for the G735A and C808G mutations of BMP15 and C818T, A959C, and G1189A polymorphisms of GDF9 gene. On the other hand, the g.6051G>A locus showed significant association with litter size at the first and third kidding and the g.6124C>G SNP was significantly associated with litter size at the third kidding. For G735A (K245K) polymorphism, Das *et al.* (2021) reported parity-wise mean litter size as 1.92, 2.64, and 2.88, respectively in BBG. Likewise, Shaha *et al.* (2022) also found the mean litter size in Jamunapari and Crossbred goats as 2.00 and 1.50, respectively, for the same SNP (G735A) of BMP15 gene and is homogenous to the present findings. In contrast, Wang *et al.* (2011) observed AA, AB, and BB genotypes (for 456T→G, 466C→G, 510C→T, and 511T→C SNPs) in the exon 2 of BMP15, where the BB genotype always appeared to have the maximum litter size at the three consecutive parities and the average litter size was found as 1.62±0.32, 1.60±0.42, and 2.28±0.93, respectively ($p<0.05$). Chu *et al.* (2007) revealed G963A and G1050C mutations of exon 2 of the BMP15 gene in Jining Grey goats where the mean litter size of the heterozygous AB (2.58) larger than the homozygous AA (1.45) genotype with significant associations ($p<0.01$). Conversely, Ghoreishi *et al.* (2019) found a g.755T>G polymorphism in exon 2 of the BMP15 gene, where Markhoz does had a BB genotype with 1.171 and a Bb genotype with 1.402 mean litter size. Altogether, sample size and variability in the performance data are essential parameters to have a significant association for any SNP genotyped study.

The combined genotypes derived from BMP15 gene polymorphisms had significant ($p<0.05$) effects on litter size at the third kidding and average litter size. Along with the highest average litter size (2.09) found for AAGGCC combined genotypes, 1.76 for the AAAACC genotype resulted in a lower average litter size ($p<0.05$), which is comparatively higher than the result of Ahlawat *et al.* (2015). A significant effect of combined genotypes has been reported ($p<0.05$) on litter size in Xinong Saanen, Guanzhong, and Boer goats; Markhoz goats, and small-tail Han sheep (An *et al.,* 2012; Ghoreishi *et al.,* 2019; Wen *et al.,* 2021) and the results are agreed partially to the present findings.

This study shows an insignificant ($p>0.05$) association among the genotypes of the g.3764C>T polymorphism on litter size at different parities. The identified non-synonymous mutation (g.3764C>T) has been reported by Ahlawat *et al.* (2015 and 2016) in Indian BBG with a non-significant effect of genotypes on litter size and is in agreement with the present study. Earlier studies reported polymorphisms in the GDF9 gene had a significant association ($p<0.01$) with litter size in Jining Grey goats (Feng *et al.,* 2011), in Cashmere goats (Zhao *et al.,* 2016), and in Markhoz goats (Arefnejad *et al.,* 2018) that contradicts to the present findings. Conversely, Polley *et al.* (2009) found GDF9 as monomorphic in Indian BBG, while Das *et al.* (2021) found GDF9 as polymorphic with dissociation of genotypes on litter size in Bangladeshi BBG. Moreover, Shaha *et al.* (2022) reported C818T with amino acid replacement A273V in the exon 2 of the GDF9 gene in Jamunapari and Crossbred goats in Bangladesh, where CC and CT genotypes had mean litter sizes of 1.50 and 1.83 in both of the breeds, which is close to this findings.

The present study also analyzed the associations of identified SNPs with reproductive traits (ADO, ASC, AKI, and KI at the first, second, and third kidding, respectively). This study demonstrated a significant association ($p<0.05$) with ASC for g.3764C>T genotypes of the GDF9 gene, where BMP15 genotypes showed different scenarios. The former two SNPs of BMP15 (g.5875A>G and g.6051G>A) significantly affected the

first, second, and average kidding intervals. In contrast, the latter polymorphism, g.6124C>G, was significantly associated with ($p<0.05$) only the second KI and AKI. However, Ahlawat *et al.* (2015) were ambivalent to our findings, where none of the genotypes showed significant effects ($p>0.05$) on various reproductive traits like litter size, age at sexual maturity, age at first service, and age at first kidding.

CONCLUSION

Sequence analysis detected four polymorphisms in the coding sequences of the BMP15 and GDF9 genes in the BBG population of Bangladesh. Both individual SNP and the resultant combined genotypes of the BMP15 gene showed a significant association with the litter size and average kidding interval traits in the BBG population. The present findings revealed the influence of BMP15 and GDF9 fecundity genes for reproductive traits, specifically litter size, in the BBG population of Bangladesh that can be used in a marker-assisted selection program.

CONFLICT OF INTERESTS

The authors declare no conflict of interest related to this manuscript.

ACKNOWLEDGEMENT

This study was funded by a research project entitled "Analysis of Candidate Genes for Growth, Prolificacy and Milk Production Traits in Black Bengal Goat of Bangladesh", Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh. The authors are also grateful to the goat farmers for providing reproductive data and blood samples.

REFERENCES

Ahlawat, S., R. Sharma, & A. Maitra. 2012. Analysis of coding DNA sequence of *GDF9* gene in Indian goats for prolificacy associated markers. Indian J. Anim. Sci. 82:721-725.

Ahlawat, S., R. Sharma, M. Roy, M. S. Tantia, & V. Prakash. 2015. Association analysis of novel SNPs in *BMPR1B*, *BMP15* and *GDF9* genes with reproductive traits in Black Bengal goats. Small Rumin. Res. 132:92-98. https://doi.org/10.1016/j.smallrumres.2015.10.010

Ahlawat, S., R. Sharma, M. Roy, S. Mandakmale, V. Prakash, & M. S. Tantia. 2016. Genotyping of novel SNPs in *BMPR1B*, *BMP15*, and *GDF9* genes for association with prolificacy in seven Indian goat breeds. Anim. Biotechnol. 27:199-207. https://doi.org/10.1080/10495398.2016.1167706

An, X. P., J. X. Hou, H. B. Zhao, G. Li, B. Bai, Y. J. Peng, Q. M. Yan, Y. X. Song, J. G. Wang, & B. Y. Cao. 2012. Polymorphism identification in goat *GNRH1* and *GDF9* genes and their association analysis with litter size. Anim. Genet. 44:234-238. https://doi.org/10.1111/j.1365-2052.2012.02394.x

Arefnejad, B., Y. Mehdizadeh, A. Javanmard, M. J. Zamiri, & A. Niazi. 2018. Single nucleotide polymorphisms (SNPs) in two oogenesis specific genes (*BMP15*, *GDF9*) and their association with litter size in Markhoz goat (Iranian Angora). Iran. J. Appl. Anim. Sci. 8:91-99.

Bi, Y., J. Li, X. Wang, L. He, K. Lan, L. Qu, X. Lan, X. Song, & C. Pan. 2020. Two novel rare strongly linked missense SNPs (P27R and A85G) within the GDF9 gene were significantly associated with litter size in Shaanbei White Cashmere (SBWC) goats. Front. Vet. Sci. 7:406. https://doi.org/10.3389/fvets.2020.00406

Castro, F. C. D., M. H. Cruz, & C. L. Leal. 2016. Role of growth differentiation factor 9 and bone morphogenetic protein 15 in ovarian function and their importance in mammalian female fertility-A review. Asian-Australas. J. Anim. Sci. 29:1065-1074. https://doi.org/10.5713/ajas.15.0797

Chu, M. X., C. L. Jiao, Y. Q. He, J. Y. Wang, Z. H. Liu, & G. H. Chen. 2007. Association between PCR-SSCP of bone morphogenetic protein 15 gene and prolificacy in Jining Grey goats. Anim. Biotechnol. 18:263-274. https://doi.org/10.1080/10495390701331114

Das, A., M. Shaha, M. D. Gupta, A. Dutta, & O. F. Miazi. 2021. Polymorphism of fecundity genes (*BMP15* and *GDF9*) and their association with litter size in Bangladeshi prolific Black Bengal goat. Trop. Anim. Health Prod. 53:1-8. https://doi.org/10.1007/s11250-021-02679-2

DLS. 2021. Annual Report on Livestock, Division of Livestock Statistics, Ministry of Fisheries and Livestock, Farmgate, Dhaka, Bangladesh.

Falconer, D. S. & T. F. C. MacKay. 1997. Introduction to Quantitative Genetics. 4th ed. Longman Pub Group, England.

Faruque, M. O., M. P. Choudhury, C. H. Ritchil, F. Tabassum, M. A. Hashem, & A. K. F. H. Bhuiyan. 2016. Assessment of performance and livelihood generated through community-based goat production in Bangladesh. SAARC J. Agric. 14:12-19. https://doi.org/10.3329/sja.v14i2.31241

Feng, T., C. X. Geng, X. Z. Lang, M. X. Chu, G. L. Cao, R. Di, L. Fang, H. Q. Chen, X. L. Liu, & N. Li. 2011. Polymorphisms of caprine *GDF9* gene and their association with litter size in Jining Grey goats. Mol. Biol. Rep. 38:5189-5197. https://doi.org/10.1007/s11033-010-0669-y

Ghoreishi, H., S. F. Yosefabad, J. Shayegh, & A. Barzegari. 2019. Identification of mutations in *BMP15* and *GDF9* genes associated with prolificacy of Markhoz goats. Arch. Anim. Breed. 62:565-570. https://doi.org/10.5194/aab-62-565-2019

Grosjean, P., F. Ibanez, & M. Etienne. 2018. Pastecs: Package for Analysis of Space-time Ecological Series. R Package version 1.3.21.

Hadizadeh, M., M. R. Mohammadbadi, A. Niazi, A. Esmailizadeh, & Y. M. Gazooei. 2014. Search for polymorphism in growth and differentiation factor 9 (*GDF9*) gene in prolific Beetal and Tali goats (*Capra hircus*). J. Biodivers. Environ. Sci. 4:186-191.

Jalbani, M. A., H. A. Kaleri, A. H. Baloch, N. Bangulzai, A. G. Bugti, F. Ashraf, R. R. Kaleri, M. Jan, G. A. Bugti, & A. N. Khosa. 2017. Study of *BMP15* gene polymorphism in Lehri goat breed of Balochistan. Journal Applied Environmental Biological Sciences 7:84-89.

Jalil, M. A., M. M. Kabir, M. P. Choudhury, & M. A. Habib. 2016. Productive and reproductive performance of Black Bengal goat under farming condition in Bangladesh. Asian-Australasian J. Biosci. Biotechnol.1:235-245. https://doi.org/10.3329/aajbb.v1i2.61570

Maitra, A., R. Sharma, S. Ahlawat, K. Borana, & M. S. Tantia. 2016. Fecundity gene SNPs as informative markers for assessment of Indian goat genetic architecture. Indian J. Anim. Res. 50:349-356. https://doi.org/10.18805/ijar.6708

Mendiburu, F. & M. Yaseen. 2021. Agricolae: Statistical Procedures for Agricultural Research. R. Package Version 1.4.0.2020.

Miah, G., A. Das, T. Bilkis, M. M. Momin, M. A. Uddin, M. A. Alim, M. S. Mahmud, & O. F. Miazi. 2016. Comparative study on productive and reproductive traits of Black Bengal and Jamnapari goats under semi-intensive condition. Scientific Research Journal IV:1-7.

Nawaz, A., M. E. Babar, T. Hussain, A. Nadeem, F. Bilal, K. Javed, & K. Muhammad. 2013. Identification of molecular markers in *BMP15* gene of Pakistani goat breeds. Int. J. Adv. Res. 1:62-68.

Polley, S., S. De, S. Batabyal, R. Kaushik, P. Yadav, J. S. Arora, S. Chattopadhyay, S. Pan, B. Brahma, T. K. Datta, & S. L. Goswami. 2009. Polymorphism of fecundity genes (*BMPR1B, BMP15* and *GDF9*) in the Indian prolific Black Bengal goat. Small Rumin. Res. 85:122-129. https://doi.org/10.1016/j.smallrumres.2009.08.004

Shaha, M., G. Miah, A. Lima, O. F. Miazi, M. D. Gupta, & A. Das. 2022. Identification of polymorphisms in *GDF9* and *BMP15* genes in Jamunapari and crossbred goats in Bangladesh. Trop. Anim. Health Prod. 54:350. https://doi.org/10.1007/s11250-022-03347-9

Siddiky, M. 2017. Dairying in South Asian region: Opportunities, challenges and way forward. SAARC J. Agric.15:173-187. https://doi.org/10.3329/sja.v15i1.33164

Talukder, M. A. I., M. M. Rahman, M. A. Alam, M. A. Hossain, & M. A. Hemayet. 2020. Effects of genetic and environmental factors on productive performances of Hilly Brown Bengal goat at Naikhongchari. J. Biosci. Agric. Res. 26:2192-2203. https://doi.org/10.18801/jbar.260220.268

Wang, Y., L. Yuanxiao, Z. Nana, W. Zhanbin, & B. Junyan. 2011. Polymorphism of exon 2 of *BMP15* gene and its relationship with litter size of two Chinese goats. Asian-Australas. J. Anim. Sci. 24:905-911. https://doi.org/10.5713/ajas.2011.10432

Wen, Y. L., X. F. Guo, L. Ma, X. S. Zhang, J. L. Zhang, S. G. Zhao, & M. X. Chu. 2021. The expression and mutation of *BMPR1B* and its association with litter size in small-tail Han sheep (*Ovis aries).* Arch. Anim. Breed. 64:211–221. https://doi.org/10.5194/aab-64-211-2021

Wouobeng, P., J. S. Kouam, F. Meutchieye, M. Yacouba, G. Achieng, J. C. Tutah, C. Mutai, & M. Agaba. 2020. Polymorphism of prolificacy genes (*BMP15, BMPR1B* and *GDF9*) in the native goat (*Capra hircus*) of Cameroon. Genet. Biodivers. J. 4:28-43. https://doi.org/10.46325/gabj.v4i1.69

Zhao, J., S. Liu, X. Zhou, R. Zhang, Y. Li, X. Wang, & Y. Chen. 2016. A non-synonymous mutation in *GDF9* is highly associated with litter size in Cashmere goats. Anim. Genet. 47:630-631. https://doi.org/10.1111/age.12447