**SCREENING FOR POLYMORPHISMS IN LITTER SIZE TRAIT ASSOCIATED GENES IN GOATS OF BANGLADESH**



A thesis

by

**MISHUK SHAHA**

Roll No.: 118/02

Registration No.:486

Session: 2018-2019 (January-June)

*A thesis submitted to the*

*Department of Genetics and Animal Breeding,Faculty of Veterinary Medicine*

*ChattogramVeterinary and Animal Sciences University, Chattogram*

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**DEPARTMENT OF GENETICS AND ANIMAL BREEDING**

**FACULTY OF VETERINARY MEDICINE**

**CHATTOGRAM VETERINARY AND ANIMAL SCIENCES UNIVERSITY**

**CHATTOGRAM-4225**

**DECEMBER, 2019**

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***This is to certify that we have examined the above Master’s thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made***



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**DECEMBER, 2019**

**AUTHORIZATION**

I, MishukShaha assure that I have performed all works furnished here in this report. The information has been collected from books, national and international journals, websites and other references. All references have been acknowledged duly.

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**The Author**

**December, 2019**

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# List of Abbreviations

|  |  |
| --- | --- |
| A | Adenine |
| % | Percentage |
| ARMS-PCR | Amplification-Refractory Mutation System- Polymerase Chain Reaction |
| BER | Bangladesh Economic Review |
| BLAST | Basic Local Alignment Search Tool |
| *BMP15* | Bone morphogenetic protein-15 |
| *BMP4* | Bone morphogenetic protein-4 |
| *BMPR1B* | Bone morphogenetic protein receptor type-1B |
| bp | Base pair |
| BRAC | Bangladesh Rural Advancement Committee |
| C | Cytosine |
| *CART* | Cocaine and amphetamine-regulated transcript |
| *CDH26* | Cadherin 26 |
| CDS | Coding sequence |
| Chr | Chromosome |
| cm | Centimeter |
| CVASU | Chattogram Veterinary and Animal Sciences University |
| ddNTPs | Dideoxynucleotide triphosphates |
| DLS | Department of Livestock Services  |
| DNA | Deoxyribonucleic acid |
| dNTPs | Deoxynucleotide triphosphates |
| dsDNA | double stranded DNA |
| e.g. | Example |
| EDTA | Ethylene demine tetra acetic acid |
| FAO | Food and Agricultural Organization |
| FAOSTAT | Food and Agriculture Organization Corporate Statistical Database |
| *Fec* | Fecundity |
| *FSH* | Follicle Stimulating Hormone |
| *FSHR* | Follicle Stimulating Hormone Receptor |
| G | Guanosine |
| *GDF9* | Growth Differentiation Factor 9 |
| gDNA | genomic DNA |
| *GnRHR* | Gonadotropin-releasing hormone receptor |
| *IGF1* | Insulin like growth factor 1 |
| *INHβ* | Inhibin beta |
| *KDM6A* | Lysine Demethylase 6A |
| kg | Kilogram |
| *KISS-1* | Kisspeptin-1 |
| *KITLG* | KIT Ligand |
| *LHX4* | LIM Homeobox4 |
| *LHβ* | Luteinizing Hormone β-subunit |
| LSD | Least significant difference |
| MAS | Marker assisted selection |
| ml | Milliliter |
| NCBI | National Center for Biotechnology information |
| NGO | Non Governmental Organization |
| PCR | Polymerase Chain Reaction |
| *PITX2* | Paired Like Homeodomain 2 |
| *POU1F1* | POU Class 1 Homeobox 1 |
| *PRLR* | Prolactin Receptor |
| PRTC | Poultry Research and Training Centre |
| RNA | Ribonucleic acid |
| *SETDB2* | SET Domain Bifurcated Histone Lysine Methyl-transferase 2 |
| SNP | Single Nucleotide Polymorphism |
| T | Thymine |
| TAE | Tris-acetate-EDTA |
| TGFβ | Transforming growth factor β |
| UV | Ultraviolet |
| µl | Micro liter |

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**The Author**

December, 2019

**DEDICATION**

In the **memory** of all the Martyrs (30 millions) who sacrificed their lives in the Bangladesh Liberation War of 1971

**Dedicated** to the Father of the Nation Bangabandhu Sheikh MujiburRahman

 &

to the poor farmers who are keeping the indigenous goats for the rich!

**ABSTRACT**

A study was carried out to screen the goats of Bangladesh for the presence of polymorphisms in three prolificacy genes (*GDF9*, *BMP15* and *CDH26*) and to assess the association between polymorphisms and litter size. A total of 139 animals were screened comprising of 63 Black Bengal, 40 Jamnapari and 36 Crossbred goats.The selected animals had variable litter size and parity. Blood samples were collected from jugular vein using sterile vacutainer pack. Genomic DNA was extracted from collected blood samples using commercial DNA extraction kit. The fragments of exon 2 of *BMP15*, exon 2 of *GDF9* and exon 8 of *CDH26* gene were amplified using Polymerase Chain Reaction (PCR). PCR products were sequenced using DNASanger sequencing method. Association analysis for identified polymorphisms and litter size was done using a generalized linear model. Phylogenetic analysis was also performed to explore the genetic diversity of different goats.Ten single nucleotide polymorphisms (SNPs) were identified of which four (g.818C˃T, g.1073G˃A, g.1189G˃A and g.1330G˃T) were in *GDF9* gene, three (g.616G˃T, g.735G˃A and g.811G˃A) were in *BMP15* gene and three (g.975C˃T, g.1064G˃A and g.1035-1036CA˃TG) were in *CDH26* gene.Among them, seven SNPs were novel and three SNPs were known based on extensive literature search.The association analysis showed 818C>T and 1330G>T loci of *GDF9* gene recorded with a significantly (p<0.05) higher litter size. However, the effect of breed and parity was significant (p<0.05) on litter size in these three Bangladeshi goats. These results preliminarily showed that the *GDF9* gene might be a major gene that influences prolificacy of Bangladeshi goat.This study provides basic additional molecular data that would be useful for future researchon thelitter size trait in goats of Bangladesh. This attempt for goat fecundity research would also be paveda scientific basis for the conservation and utilization of goat genetic resources.

**Keywords:**Association analysis, goat, novel SNPs, prolificacy genes,sequencing.