EFFECT OF *FECB* GENE POLYMORPHISM ON DIFFERENT REPRODUCTIVE TRAITS IN BLACK BENGAL GOAT

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ABSTRACT

The aim of this study was to investigate *FecB* gene polymorphism in Black Bengal goats and its effect on different reproductive traits like age at first kidding, service period, kidding interval and gestation period. A total 96 DNA samples (42 males and 54 females) were subjected to polymerase chain reaction to obtain amplified fragments of 190 bp. These fragments were allowed for polyacrylamide gel electrophoresis for the detection of Single Strand Conformational Polymorphism (SSCP). Polymorphism was revealed and three different SSCP variants were found which were designated as AA, AB and BB. The highest genotype frequency was observed for AB (0.38), which was followed by BB (0.33) and AA (0.29). Least-square analysis of variance showed non-significant effect of genotype on age at first kidding, service period, kidding interval and gestation Period. It suggested that genetic factor responsible for these traits is not related to this mutation.

KEY WORDS : FecB, Black Bengal Goat, PCR-SSCP, Reproductive traits

INTRODUCTION

Black Bengal Goat is one of the most prolific breed of India. A remarkable improvement in reproductive traits of this breed will result in enhanced economic gain. Earlier use of quantitative genetic methods has not caused considerable improvement in reproductive traits. For example, attempts to increase litter size by selection within a breed result in slow progress, because the heritability of litter size is low (Morris, 1990). Variation in reproductive traits is controlled by both genetic and environmental factors. If the genes associated with reproductive traits are identified, then marker assisted selection (MAS) can be employed in breed improvement. Also, due to the fact that these traits can be measured only in one sex, it can be considered for MAS.

The *FecB (Booroola* fecundity) gene is the first major gene to be described that affects ovulation rate and proliferation in sheep. The *FecB* gene is a single autosomal gene, which increases ovulation rate and litter size in sheep (Piper *et al.*, 1985; Montgomery *et al.*, 1992). Piper *et al.* (1985) and Piper and Bindon (1996) found that the effect of *FecB mutation* is additive for ovulation rate and each copy increases ovulation rate by about 1.6 and approximately one to two extra lambs in Booroola Merinos. Davis (2004) reported that one copy of the *FecB* gene increases ovulation rate in Booroola Merino by about 1.5 and two copies by 3.0. These extra ovulations in turn increase litter size by 1.0 and 1.5, respectively. High prolificacy in Booroola sheep is due to a non-conservative mutation (q249r) in a highly conserved intracellular kinase signaling domain of the bone morphogenetic protein receptor-1B (BMPR-1B) expressed in the ovary and granulosa cells (Wilson *et al*., 2001). Many aspects of the *FecB* gene, including reproductive endocrinology (Smith *et al.*, 1993), ovary development (Cognie *et al.*, 1998), litter size, organ development and body mass have been studied. This gene has an additive effect on litter size and ovulation rate, but has negative effects on fetal growth and development and body mass during gestation.

On this basis, there may be possibility that *FecB* gene might have some role in determining age at first kidding, service period, kidding interval and gestation period too. These traits are important

economic traits in goat breeding. Thus, considering the above mentioned facts, the present work has been designed to study the association analysis of *FecB* gene polymorphism with these reproductive traits in Black Bengal Goats.

MATERIALS AND METHODS

Collection of blood samples: A total of 96 Black Bengal goats were included in the present investigation. Blood samples (5 ml each) were collected from the jugular vein in vaccutainer tubes. An anticoagulant (EDTA) was mixed in blood. The blood samples were brought in ice as coolant to maintain low temperature.

Isolation of Genomic DNA : Genomic DNA was isolated and purified from white blood cells using proteinase-K digestion and standard phenol-chloroform extraction as per the standard protocol described by Sambrook *et al.* (1989).

Storage of DNA: 20 ml of stock solution of DNA was diluted serially to a concentration of 50-100ng/µl as working stock for further analysis. Rest of the stock solution of DNA was stored at -20°C.

Quality and quantity check : Quality and quantity check of isolated genomic DNA sample was done by agarose gel electrophoresis. On completion of electrophoresis, the gel was visualized under UV trans-illuminator (Bio-Rad) and quality and quantity was judged.

Polymerase Chain Reaction: A pair of synthetic oligonucleotide (primers) was required to prime DNA synthesis. The product size of *FecB* gene was 190 base pairs (bp) (Wilson *et al.*, 2001). Primers were synthesized by Xcelris Lab, Ahmedabad. Details of sequence are presented in Table 1.

Table 1: The Primer pair used for amplification of genomic DNA

FORWARD PRIMER	REVERSE PRIMER	PRODUCT SIZE (bp)
5'- CCAGAGGACAATAG CAAAGCAAA-3'	5'- CAAGATGTTTTCAT GCCTCATCAACAGG TC-3'	190

Optimization of PCR Reactions: Polymerase chain reaction was carried out in 10 µl reaction volume with about 1.5µl (50-100µg/µl) of genomic DNA. The reaction mixture consisted of 0.5µl(10mM) dNTPs, 0.5µl (25mM) MgCl2, 0.5µl (20ng / µl) each forward and reverse primer, 0.5µl (5 units/µl) Taqpolymerase and 1.5 µl 10X PCR buffer. The amplification reaction conditions were as follows: initial denaturation at 94°C for 3 min, followed by 33 cycles of 94°C for 30 sec, 58°C for 30 sec, 72°C for 30 sec and final extension at 72°C for 5 min.

Single Strand Conformational Polymorphism (SSCP) of FecB gene: Amplified PCR products were subjected for SSCP through polyacrylamide gel electrophoresis performed at 40 C for 4 hours at 200 V. After running was over, gel was kept for silver staining according to Bassam et al. (1991). The gel was visualized for banding pattern under light and documented. The data was analyzed with available computer software.

RESULTS AND DISCUSSION

In the present investigation, PCR-SSCP studies were carried out on BMPR1B gene for detection of the point mutation of FecB gene in Black Bengal Goat. Gupta et al. (2005) reported that the PCR-SSCP method is one of the best method employed in detection of SNPs and nucleotide base change.

PCR-SSCP analysis of FecB Gene : With the primer having forward and reverse base sequence as 5'-CCAGAGGACAATAGCAAAGCAAA-3' and 5'-CAAGATGTTTTCATGCCTCATCAACAGGTC-3' (Supakorn et al., 2010) respectively, two allelic variants A and B (figure 1)were found and accordingly genotypes were designated as AA, AB and BB. Similar result was revealed by Polley et al. (2009) who worked upon BMPR1B gene (FecB) polymorphism in Black Bengal Goat and reported three types of genotype in FecB region. Also, Chu et al. (2010) found polymorphism of BMPR1B gene and obtained three types of genotypes in Jining Grey goats.

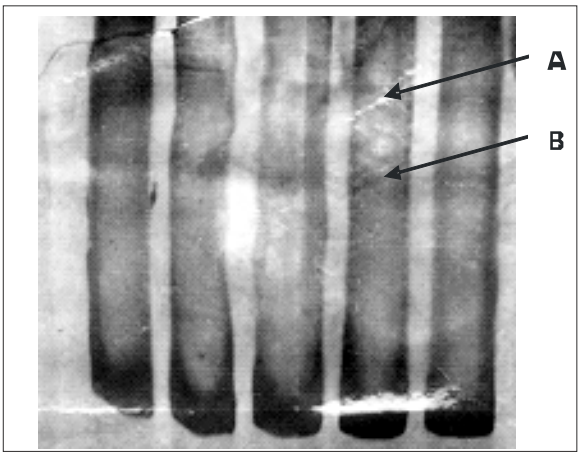

Figure 1: Two allelic variants of FecB gene

Genotype Frequency: In Black Bengal goat population, the highest genotype frequency was observed for AB (0.38), which was followed by BB (0.33) and AA (0.29) (Table 2).

Association Study: Least-square analysis of variance showed non-significant effect of genotype on age at first kidding, service period, kidding interval and gestation Period (Table 3 and 4). So, it can be suggested that genetic factor responsible for these traits is not related to this mutation.

Table 2: Allelic and genotype frequencies of Black Bengal goat for FecBgene

Gene	No. of Animal	Allele frequency		Genotype frequency		
		A	B	AA	AB	BB
FecB	96	0.48	0.52	0.29	0.38	0.33

Table 3: Least-squares analysis of variance showing effects of genotype on age at first kidding, service period, kidding interval and gestation period in Black Bengal goats

Source of variation	Age at first kidding			Service period			Kidding interval			Gestation period		
	d.f.	M.S.S.	F-value	d.f.	M.S.S.	F-value	d.f.	M.S.S.	F-value	d.f.	M.S.S.	F-value
Genotype	2	81.583	00.058	2	657.469	01.602	2	723.336	01.658	2	01.571	01.908
Residual	51	1409.701		51	410.289		51	436.300		51	00.823	

Table 4: Least-squares means for genotype affecting age at first kidding, service period, kidding interval and gestation period in Black Bengal goat

Genotype	Age at first kidding (days)	Service period (days)	Kidding interval (days)	Gestation period (days)
Population mean (μ)	453.35±05.16(54)	132.522±02.78(54)	278.47±02.87(54)	145.94±00.12(54)
AA	458.81±08.00(22)	128.81±04.31(22)	274.72±04.45(22)	145.90±00.19(22)
AB	454.62±09.38(16)	136.12±05.06(16)	282.25±05.22(16)	146.12±00.22(16)
BB	446.62±09.38(16)	132.62±05.06(16)	278.43±05.22(16)	145.81±00.22(16)

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REFERENCES :

Bassam *et al. (1991). Anal. Biochem.* **196**:80- 83.

Chu *et al. (*2010) .*Molecular Biology Reports* **37(8)**: 4033-4039

Cognie *et al.* (1998). *J. Reprod. Fertil.* 112:379-386

Davis G H. (2004). *Anim. Reprod. Sci.* **82–83**:247–253

Gupta *et al. (2005) .Indian J. Anim. Sci*. **31:**146-151.

Montgomery *et al. (1992). Endo. Rev.,13(2) : 309-327*

Morris C. A. (1990). Theoretical and realized responses to selection for reproductive rate. Proc. 4th World Congress on Genetics Applied to *Livestock Production Edinburgh,* **16**:309

Mulsant *(2001)* . Mutation in the bone morphogenetic protein receptor-IB is associated with increased ovulationrate in Booroola ewes.

Proc. Natl. Acad. Sci. **98**:5104–5109

Piper L R and Bindon B M. (1996) *Prolific Sheep* 152–160

Piper L R, Bindon B M and Davis G H. (1985). Genetics *of Reproduction in Sheep* 115–125

Polley *et al. (2009). Small Ruminant*

Research **85 : 122–129**

Sambrook *et al.* (1989) .Molecular cloning: A Laboratory Manual 2nd ed. *Cold spring Harbour, Cold Laboratory press, NY*

Smith *et al*. (1993) *Journal of Reproduction and Fertility* **98 :41–54.**

Supakorn C and Pralomkarn W. (2010) . Sheep *FecB* Gene Polymorphism Role In Thai Meat Goat Proliferation Rate. In : Proceedings *of the 9th* World Congress on Genetics Applied to Livestock *Production, Leipiz, Germany, 2010.*

Wilson *et al. (2001) Biology of Reproduction* **64:**1225-1235

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