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Original Research

Study of FecX^{B} and FecX^{G} Mutations in a Group of Prolific Osmanabadi Goats

Mahendra Kumar[1], P. V. Nandedkar[2]*, M. S. Vaidya[3], S. A. Dhaware[4], S. S. Gaikwad[5] and P. R. Suryawanshi[6]

[1]M.V.Sc. Student, Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, INDIA
[2]Assistant Professors, Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, INDIA
[3]Professor and Head of Department, Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, INDIA
[4]Assistant Professors, Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, INDIA
[5]Assistant Professor, Animal Biotechnology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, INDIA
[6]Assistant Professor, Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, INDIA

*Corresponding Author: npanditv25@gmail.com

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Abstract

Study was planned on FecXB andFecXGgenes' (Alleles of BMP15 geneexonII region) polymorphism in unrelated prolific Osmanabadi goat breed (n=60) of Maharashtra with triplets (n=42), quadruplets (n=16) and above kidding (n=2) and amplified 153 bp ofFecXB and a 141 bp of FecXG genes were digested with DdeI and HinfI restriction enzymes, respectively. After DdeI and HinfI digestion of FecXB and FecXG found two fragments (122 and 31 bp) and (112 and 29 bp), respectively. All studied goats were found monomorphic for FecXB andFecXG genes. Previously reported sheep mutations hadn' t shown effect on prolificacy in studied goats. Species-specific differences in BMP15 may be associated with the differences in ovulation rate amongst species and hence, BMP15 gene may not be major gene associated with prolificacy/fecundity of Osmanabadi goats, therefore this study suggested to target other loci ofFecXB,FecXGgenes etc. in Osmanabadi goat and with larger samples.

Keywords: BMP-15, FecX^{B}, FecX^{G}, Fecundity, Goat, Prolificacy and Mutations

Introduction

India ranks second in the goat population of the World with 34 defined goats breeds, including Osmanabadi, Berari, Sangamneri and Konkan Kanyal from Maharashtra. Osmanabadi is famous meat goat breed in Marathwada region of Maharashtra state with desirable characteristics viz. hardy, early maturity, high prolificacy, high kidding rate, better growth rate, quality meat, disease resistance, heat tolerance, ability to survive under extreme climate. This breed is having a good prolificacy, Sahare *et al*. (2009) studied the kidding percentage and twinning ability in Osmanabadi goat and found 55.87 and 10.52 per cents, respectively and with 45-50 per cent dressing percentage. Prolificacy is reproductive character controlled by well-known *BMP15* gene (*Fec*X/*GDF9B*), a member of TGF-β super family with a crucial role in growth and differentiation (Juengel *et al.*, 2004). A number of mutations in fecundity genes had been found increased fecundity in different breeds of sheep, namely, *Fec*X^B in Belclare (Hanrahan *et al.,* 2004); *Fec*X^G in Galway (Hanrahan *et al.,* 2004); *Fec*X^Hin Hanna (Galloway *et al.*, 2000); *Fec*X^Iin Inverdale (Galloway *et al.*, 2000); *Fec*X^L in Lacaune (Bodin*et al*., 2003) and *Fec*X^R in Rasa Aragonesa sheep (Monteagudo *et al*., 2009). However, identification of useful genetic markers is the most and first crucial step in MAS (Wakchaure *et al.*, 2015). The present research was planned to study of *FecX*^{B} and *Fec*X^G mutations and their association with kidding pattern in a group of unrelated prolific Osmanabadi goats.

Prolificacy is determined basically by the number of ova released in each estrous cycle i.e., ovulation rate (Gougeon, 1996). Ovulation rate is under the control of single genes as Booroola gene (Piper *et al*., 1985). Prolificacy is measured as capability of a dam to produce more number of young ones in her life span through high rate of embryo survival and ovulation. High litter size (twinning and above percentage) is economically important trait that improve goat efficiency in terms of producing more numbers of offspring, milk and meat (Akingbade *et al*., 2004). Since lambing/kidding percentage is lowly heritable, it is difficult to find selection improvements in traits related with reproduction. These genes for prolificacy are known as fecundity genes. Three types of fecundity genes have been identified in the sheep, viz. *BMPR1B* or ALK6 known as *FecB* located on chromosome 6 (Souza *et al.* 2001), GDF9 known as *FecG* located on chromosome 5 (Hanrahan *et al.,* 2004), and *BMP15* (*GDF9B*) known as *FecX* located on X-chromosome (Galloway *et al.,* 2000; Hanrahan *et al.,* 2004; Alinaghizadeh *et al.,* 2010). *BMP* genes are members of the TGF-β super-family, which are multifunctional cytokines and are scattered in a variety of cells. The *BMP15* gene regulates granulose cell proliferation and differentiation by promoting granulosa cell mitosis, stimulating kit ligand expression and suppressing FSH-R expression; hence, plays an important role in female mammals' fertility (Moore *et al*., 2003; Juengel *et al*., 2002). There are *FecG*^{H} (high fertility), *FecX*^{H} (Hanna), *FecX*^{B} (Belclare), *FecX*^{I} (Inverdale), *FecX*^{G} (Galway) or *FecX*^{L} (Lacaune) alleles of BMP-15 gene reported till date (Bodin *et al.*, 2007; Hanrahan *et al.*, 2004; Galloway *et al.*, 2000; McNatty *et al*., 2005). *FecX*^{B} and *FecX*^{G} genes are the two important genes regulating the ovulation rate and litter size of sheep. Association between polymorphisms in *FecX*^{B} and *FecX*^{G} genes with economically important traits in sheep, have been established by many researchers. Considering importance of these genes in prolificacy, present study was carried out to detect PCR-RFLP polymorphism in *FecX*^{B} and *FecX*^{G} genes in prolific Osmanabadi goats using with *DdeI* and *HinfI* restriction enzymes, respectively.

Materials and Methods

Present study was carried out to detect polymorphism in *FecX*^{B} and *FecX*^{G} genes through blood samples collected from unrelated prolific Osmanabadi goats (n=60) having known history of triplets, quadruplets and above kidding, from Parbhani and adjoining districts of Marathwada region after the approval of Institutional Animal Ethics Committee (IAEC) of College of Veterinary and Animal Sciences, Parbhani (Resolution No. IAEC 55/19 Dt.02.03.2019). Genomic DNA was isolated from blood. Its quality and quantity judged by agarose gel electrophoresis and Nano Drop™ Lite Spectrophotometer, respectively. A 153 bp fragment of *FecX*^{B} and a 141 bp fragment of *FecX*^{G} from exon II region of BMP-15 gene were successfully amplified by using following reported primers.

PCR conditions for *FecX*^{B}(exon II) gene amplification were (1) Initial Denaturation at 94°C - 5 min; (2) 30 cycles of Denaturation at 94°C - 30 sec, Annealing at 57.5°C - 30 sec and Extension at 72°C - 30 sec; and (3) Final Extension at 72°C - 10 min; and for *FecX*^{G}(exon II) gene were (1) Initial denaturation at 94°C - 5 min; (2) 30 cycles of Denaturation at 94°C - 30 sec, Annealing at 63°C - 30 sec and Extension at 72°C - 30 sec; and (3) Final extension at 72°C - 10 min. PCR-RFLP of *FecX*^{B} and *FecX*^{G}genes was done using with *DdeI* and *HinfI* restriction enzymes, respectively.

Table 1: Gene, primer sequence, PCR product (bp) and restriction enzyme

Gene	Forward (F) and Reverse (R) Primer sequence	PCR Product	Restriction enzyme
FecX^B	F-5' GCCTTCCTGTGTCCCTTATAAGTATGTTCCCCTTA 3'	153 bp	*DdeI*
	R-5' TTCTTGGGAAACCTGAGCTAGC 3'		(5'-C↓TNAG-3')
FecX^G	F-5' CACTGTCTTCTTGTTACTGTATTTCAATGAGAC 3'	141 bp	*HinfI*
	R-5 'GATGCAATACTGCCTGCTTG 3'		(5'-G↓ANTC-3')

Statistical Analysis: The data generated during the experiment was planned to subject statistical analysis using the method (Chi-Square Test) described by Jones, D. H. (1994).

Results and Discussion

a. Optimization of PCR-RFLP of *FecX*^{B} and *FecX*^{G} (exon II) gene

PCR of genomic DNA requires optimization of various parameters. Primer concentration, template DNA concentration, annealing temperature and Mg++ concentration were found to affect the PCR product specificity and its yield. The very important step in PCR programme was the optimization of annealing temperature (AT) of primers because it depends on GC content and the length of the primers used. A suitable annealing temperature was tested from a range of 55-64°C in the gradient thermal cycler and consistent results were obtained for *FecX*^{B} and *FecX*^{G} gene at 57.5 and 63 °C.

In the present research, primer concentration tested was 5-10 pmole and at 6 pmole primer concentration, the amplified PCR products were found to be better. Hence the conditions of PCR were optimized with respect to above parameters. All the reactions were carried out in 25 µl volume containing 80-100ng of template DNA. After optimization of denaturation, annealing and extension temperature in Thermal cycler, the following PCR conditions for *FecX*^{B} and *FecX*^{G} (exon II) gene amplification were used: (1) Initial Denaturation at 94°C - 5 min; (2) 30 cycles of Denaturation at 94°C - 30 sec, Annealing at 57.5 and 63 °C - 30 sec and Extension at 72°C - 30 sec; and (3) Final Extension at 72°C - 10 min.

b. Agarose gel electrophoresis of PCR product of *FecX*^{B} gene (exon II)

The reported polymorphic site (Hanrahan *et al.*, 2004) had been considered for the amplification 153 bp *FecX*^{B} gene and 141 bp *FecX*^{G} gene (exon II) in all the sixty prolific Osmanabadi goat DNA samples using aforesaid PCR conditions. Each PCR product (5 µl) mixed with 1 µl of gel loading dye (6X) was run in 1.4% w/v agarose gel added with 1% ethidium bromide at a constant 70V for 60 min in 0.5X TBE buffer, however PCR products were separated on 6% PAGE by Deldar-Tajangookeh *et al*. (2009). All amplified PCR products were visualized as a single band of expected size (153 bp and 141 bp) under UV transilluminator. PCR product size was judged by running with 100 bp DNA ladder in the agarose gel, and image analysis was done under Gel Documentation System (*Biovis*).

c. *Dde*I and *HinfI*-PCR-RFLP of *FecX*^{B} and *FecX*^{G} gene (exon II)

All the goats (n=60) were genotyped for detection of polymorphism in exon II region of *FecX*^{B} and *FecX*^{G} gene using PCR–RFLP technique. 153 bp and 141 bp amplified *FecX*^{B} and *FecX*^{G} gene (exon II) was subjected to digestion with *Dde*I and *HinfI* restriction enzyme (5'-C↓TNAG-3') & (5'-G↓ANTC-3') for determination of *FecX*^{B} and *FecX*^{G}-RFLP pattern. After *DdeI* and *HinfI* digestion, the PCR products were electrophoresed on 2.5 % agarose gel by submarine gel electrophoresis apparatus at constant voltage of 70 V for 80-90 min. Migration of each fragment was compared with the DNA marker lane and fragments size was estimated.

As per the literature, restriction digestion analysis of the 153 bp and 141 bp PCR product of exon II region of the *FecX*^{B} and *FecX*^{G} gene with *DdeI* and *HinfI* enzyme revealed two genotypic patterns in the sheep. The first pattern, with an undigested single fragment of 153 bp and 141 bp, was designated the AA genotype (lacking restriction site) and the second pattern with two fragments (122 and 31 bp) & (112 and 29 bp), was designated to as BB, genotype (Godara *et al*., 2011). In the present study, after *DdeI* and *HinfI* restriction enzyme digestion of 153 bp and 141 bp *FecX*^{B} and *FecX*^{G} gene (exon II) in all the prolific Osmanabadi goats, we have noticed two fragments (122 and 31

bp) & (112 and 29 bp). All the prolific Osmanabadi goats under study were monomorphic for the *FecX*^{B} and *FecX*^{G} gene (exon II) region (Plate 1& 2).

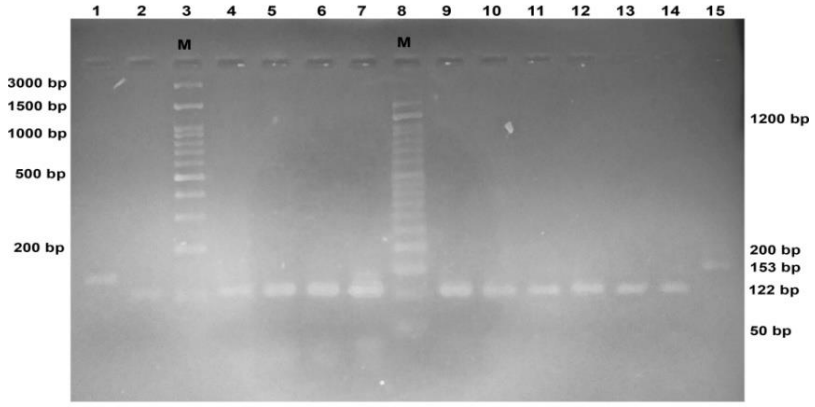

Plate 1: *DdeI*-PCR RFLP products of *FecX*^{B} gene (153bp)

Monomorphism in *FecX*^{B} gene (153bp) of Osmanabadi goats when digested with *DdeI* Lane No.3: 100bp DNA ladder; Lane No.8: 50bp DNA ladder; Lane No. 1 and 15: 153bp (*FecX*^{B}); Lane No. 2, 4-7, 9-14: *DdeI* digested PCR product of *FecX*^{B} gene (153bp) with 122 bp and 31 bp (degraded in gel so couldn't recognized in agarose gel) fragments.

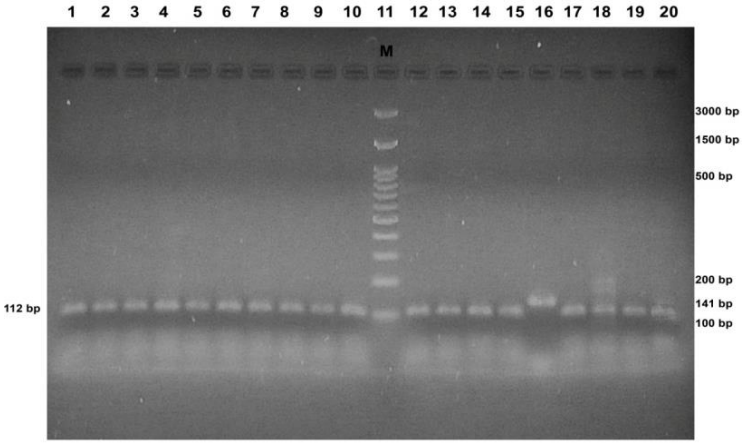

Plate 2: *HinfI* –PCR-RFLP products of *FecX*^{G} gene (141 bp)

Monomorphism in *FecX*^{G} (exon 2) gene of Osmanabadi goats when digested with *HinfI* Lane No.11: 100bp DNA ladder, Lane No. 16: 141bp *FecX*^{G} PCR product, Lane No. 1-10 and 12-20, 112bp *FecX*^{G} digested product.

In the present study, after *DdeI* and *Hinf-I* restriction enzyme digestion of 153 bp and 141 bp *FecX*^{B} and *FecX*^{G} genes (exon II), we have noticed two fragments (122 and 31 bp) and (112 and 29 bp), indicating monomorphic pattern for the *FecX*^{B} and *FecX*^{G} gene (exon II) region in all the unrelated prolific Osmanabadi goats with triplets, quadruplets and above kidding, from Parbhani and adjoining districts of Marathwada region. As we have noticed monomorphism in the *FecX*^{B} and *FecX*^{G} (exon II) gene after digestion with *DdeI* (5'-C↓TNAG-3') and *HinfI* (5'-G↓ANTC-3') restriction enzymes, respectively, there is no possibility of application of any statistical tool. Reported mutations in sheep hadn't shown effect on the difference of prolificacy in Osmanabadi goats under study. Hashimoto *et al*. (2005) suggested that species-specific differences in *BMP15* processing may be associated with the differences in ovulation rate amongst species and hence, *BMP15* gene may not be regarded as the major gene associated with the prolificacy or fecundity of Osmanabadi goats.

Conclusion

We have observed only one BB genotype (122 bp& 31 bp) and (112 bp& 29 bp) after *DdeI* and *HinfI* restriction enzyme digestion of *FecX*^{B} and *FecX*^{G} (exon 2) gene PCR product (153 bp) and (141 bp), concluding that all

unrelated prolific Osmanabadi goats are monomorphic for $FecX^B$ and $FecX^G$ (exon 2) gene studied. As all the goats have monomorphic genotypes for $FecX^B$ and $Fec\ X^G$ genes studied. This study suggests that detailed investigation needs to be carried out to find variation at other loci of $FecX^B$ and $FecX^G$ genes or other genes in Osmanabadi goat and also to study larger sample size (unrelated Osmanabadi goats) with twin, triplets, quadruplets and above kids.

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Conflict of Interests

There is no conflict of interest.

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