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Original Research

# Molecular Characterization of Osmanabadi Breed of Goat at FecG Loci

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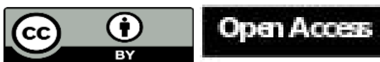

## Abstract

*The Osmanabadi breed of goat, renowned in Maharashtra for its quality chevon and high prolificacy, is the focus of a study on fecundity genes that enhance ovulation rates in sheep and goats. Notable genes include BMPR-1B (FecB), BMP-15 (FecX), and GDF-9 (FecG). Given the genetic similarities between sheep and goats, this research investigates polymorphic points in the GDF-9 gene in Osmanabadi goats. The study aims to genotype these goats at the FecG loci, examining eight known DNA variants in ovine GDF-9 (G1, G4, G6, G7, G8 [FecGH], FecGE, FecTT, and G1189A) that lead to amino acid changes. Blood samples from 165 Osmanabadi goats (162 females, 3 males) were collected and genotyped at the FecG loci following DNA isolation. PCR amplification of the GDF-9 gene's FecG loci was performed using tetra-primer sets specific to each locus. The T-ARMS PCR technique was used to screen for known ovine fecundity gene polymorphisms in caprine GDF-9. Despite various attempts, the T-ARMS-PCR of the G7 locus only amplified the control fragment at 343 bp. Consequently, the control PCR product of 20 individuals was sequenced using the G7 OF primer, allowing the analysis of other FecG mutations (FecGE and FecTT) and the G1189A mutation. The T-ARMS-PCR results on agarose gel electrophoresis for the G1 locus showed a wild-type fragment at 247 bp and a control fragment at 396 bp, with similar findings for the G4, G6, and G8 loci, indicating non-carrier animals. Sequencing revealed wild-type alleles at G7, FecGE, FecTT, and G1189A loci. Thus, Osmanabadi goats were found to be monomorphic, carrying wild-type alleles at all studied loci in the GDF-9 gene.*

**Keywords:** Osmanabadi Goat, Prolificacy, GDF-9 gene, Genotyping, T-ARMS PCR, Sequencing.

## Introduction

Prolificacy is measured as a female's ability to produce many young ones in their life span through a high ovulation rate. Differences among does/ ewes in litter size (single, twin, triplet) are largely due to non-genetic factors, such as management and nutrition. Nutrition and management vary yearly, but genetic change is permanent. It is challenging to obtain improvements in traits associated with reproduction through traditional selection since the kidding/ lambing percentage is lowly heritable. The strategy for increasing prolificacy at the genetic level can be selecting does/ ewes that are more likely to produce multiple births and selecting bucks/ rams that are more likely to sire prolific daughters (Davis *et al*., 2002).

The goat (*Capra hircus*) occupies a special niche in the Indian agricultural production system as it utilizes poor-quality grass and crop residues. The Osmanabadi breed of goat is one of the most prolific breeds of goat in Maharashtra. Osmanabadi goats derived their name from the district of Dharashiv (earlier known as Osmanabad). The breeding tract of these goats is Latur, Tuljapur, and Udgir towns of the Marathwada region of Maharashtra. The goats are large. The coat colour varies, but it is mostly black (73 percent), with the rest being white, brown, or spotted. Ninety percent of males are horned; females may be horned or polled. Average daily milk yield varies from 0.5 to 1.5 kg for a lactation length of about four months. The udder is small and round, having small teats placed laterally. The body weight in adult males ranges between 30.93 kg and 36.39 kg, whereas adult females weigh between 31.81 kg and 32.91 kg. Osmanabadi goats are considered for quality chevon and better prolificacy. In farmers' flocks, litter size is observed as 50 percent producing single, 40 percent producing twins, and 10 percent producing triplet kids in a kidding. In farm conditions, litter size is observed as 70.5 percent producing singles, 29.0 percent producing twins, and 0.5 percent producing triplets or more kids in a kidding (FAO, 1982; Banerjee, 1998).

The inheritance patterns and DNA testing of major genes for prolificacy in the 1980's have shown that a few major genes can significantly increase the reproductive performance of sheep flocks. Several mutations that increase ovulation rate in ewes have been discovered in the genes BMPR-1B (Bone Morphogenetic Protein Receptor 1B) known as FecB, BMP-15 (Bone Morphogenetic Protein 15), known as FecX, and GDF-9 (Growth Differentiation Factor 9) known as FecG (George, 2005). It is known that the FecB, FecX, and FecG mutations have significant effects on the litter size in sheep (Davis *et al*., 2002). A perusal of the literature reveals scanty information regarding the study of these loci in the goats. The genetic basis of caprine prolificacy remains to be explored. Identifying major genes influencing goats' prolificacy has become increasingly important to the goat industry. Improvement of reproductive traits through DNA tests in small ruminants, especially a small increase in litter size, can result in significant gains in profit.

GDF-9 is an oocyte-secreted growth factor. GDF-9 protein has essential biological roles in follicle growth and development at various stages of folliculogenesis. Oocyte-secreted factors possess anti-apoptotic actions. GDF-9 also plays a role during the in-vitro maturation of oocytes and subsequent fetal viability. The addition of exogenous GDF-9 to mouse cumulus-oocyte complexes cultured with FSH before *in-vitro* fertilization and transfer to recipient females has shown an increase in viable fetuses. This suggests that GDF-9 supports embryo development and fetal viability (reviewed in Kaivo-oja, 2007).

Sheep have proved to be a valuable model to elucidate the importance of GDF-9 in female reproductive function. Normal ewes ovulate one or two oocytes per cycle. The Cambridge and Belclare sheep breeds were known to have highly prolific natures, showing extreme variation with some ewes ovulating up to 3–6 oocytes per cycle, some ovulating a normal number of oocytes and some ewes infertile. The ewes homozygous for the GDF-9S77F mutation were infertile, while heterozygous careers have an increased ovulation rate associated with a larger litter size than normal.

Considering the variation in the fecundity of Osmanabadi goats, it was expected that the variation in the fecundity/ prolific nature of Osmanabadi goats may also be because of the polymorphic nature of fecundity genes. Therefore, the present research work was carried out with the objective of genotyping Osmanabadi goats at FecG loci.

## Materials and Methods

Experimental material for the present study comprised 165 blood samples of Osmanabadi goats of either sex (162 females and three males). Blood Samples were collected from the Osmanabadi goats maintained at Sheep and Goat

farm, Mahud, Dist. Solapur (M.S.) by Punyashlok Aahilyadevi Maharashtra Sheep and Goat Development Corporation Ltd., Pune. The blood samples were collected aseptically in EDTA vacutainer and transported to the laboratory on ice. The samples were stored at 4°C till processing.

The genomic DNA was isolated from blood samples using the traditional Phenol: Chloroform: Isoamyl alcohol method of DNA isolation as described by Sambrook and Russel, 2001 with slight modifications. The quantity and quality of DNA were checked by spectrophotometer (NanoDrop ND-2000, Thermo, USA).

### *PCR Amplification for Genotyping FecG Loci of the GDF-9 Gene*

PCR amplification of FecG loci of the GDF-9 gene was carried out using the genomic DNA template. The PCR was performed using primer sets described by Polley *et al*., 2009 (Table 1). A simple tetra primer amplification refractory mutation system (T-ARMS) PCR was adopted for screening of known polymorphism of ovine fecundity gene GDF-9 in the caprine GDF-9, as described by Polley *et al*., 2009. The required oligonucleotides were synthesized from M/s. Bioserve Biotechnologies (India) Pvt. Ltd, Hyderabad, and used in the present investigation. T-ARMS-PCR technique adopts certain principles of the tetra-primer PCR method and the amplification refractory mutation system (ARMS, Newton *et al*., 1989). T-ARMS-PCR amplifies both wild-type and mutant alleles, together with a control fragment, in a single-tube PCR reaction. The region flanking the mutation was amplified by two common (outer) primers, producing a non-allele-specific control amplicon. Two allele-specific (inner) primers were designed in opposite orientations, and in combination with the common (outer) primers, they can simultaneously amplify both the wild type and the mutant amplicons. Both inner primers provided a deliberate mismatch at position −2 from the 3' terminus to increase the specificity of classical T-ARMS-PCR (Newton *et al*., 1989; Little, 2001). Allele-specific amplicons have different product lengths and can be easily separated by standard agarose gel electrophoresis because the mutation point is asymmetrically located concerning the common (outer) primers.

PCR amplification of Fec loci (G1, G4, G6, and G8) of the GDF9 gene was carried out in a final reaction volume of 25.5 µl. The PCR reaction was carried out using PCR SuperMix (Invitrogen) following the manufacturer's instructions. A master mix for at least 21 samples was prepared and aliquated 24.5 µl in each PCR tube. 1 µl genomic DNA (50-100 µg/µl) was added in each tube to make the final volume25.5 µl. A negative control, containing all the reaction components except the template DNA was also made to check for any contamination of the foreign DNA in the reaction components. Finally, the PCR tubes were kept in a pre-programmed thermal cycler. (Mastercycler nexus gradient, Eppendorf, Germany).

**Table 1:** Primer sequences used for amplification of FecG loci

| Gene | Locus | Primer | Primers sequence (5' to 3' ) | Product size (bp) |
|---|---|---|---|---|
| GDF-9 (AF078545) | G1 | IF(A) | 334 CTGCAGCCAGATGACAGAGCTTTTCA 359 | Mut 205<br>Wt 247<br>Co. 396 |
| | | IR (G) | 388 CGTATGCCTTATAGAGCCTCTTCATGTCGC 359 | |
| | | OF | 168 GCCTGGCTCTGTTTCCTATTAGCCTTG 188 | |
| | | OR | 537 TCTTCTTCCCTCCACCCATTAACCAATC 510 | |
| | G4 | IF (G) | 296 TTCACATGTCTGTAAATTTTACATGTGAGG 325 | Mut 212<br>Wt 261<br>Co. 417 |
| | | IR (A) | 350 GCTGAAGGATGCTGCAGCTGGTCGTT 325 | |
| | | OF | 90 CAACAACTCCATTTCTTTTCCCTTTCCTG 118 | |
| | | OR | 506 TAGGCAGATAGCCCTCTC TTCTGGTCAG 479 | |
| | G6 | IF (A) | 573 CAGCTCTGAATTGAAGAAGCCTCGGA 598 | Mut 193<br>Wt 223<br>Co. 362 |
| | | IR (G) | 625 ATTCACTCAGATTGACTGAAGCTGGCAC 598 | |
| | | OF | 403 TATCTGAACGACACAAGTGCTCAGGCTT 430 | |
| | | OR | 764 CTGGGACAGTCCCCTTTACAGTATCGAG 737 | |
| | G7 | IF (G) | 688 AGTCAGCTGAAGTGGGACAACTGGAGTG 715 | Mut 187<br>Wt 212<br>Co. 343 |
| | | IR (A) | 742 ATCGAGGGTTGTATTTGTGTGGGGCAAT 715 | |
| | | OF | 556 AGAGACCAGGAGAGTGCCAGCTCTGAAT 583 | |
| | | OR | 898 CGATGGCCAAAACACTCAAAGGGCTATA 871 | |
| | G8 | IF (T) | 763 AGGGCGGTCGGACATCGGTATGGATT 788 | Mut 146<br>Wt 108<br>Co. 198 |
| | | IR (C) | 817 TGATGTTCTGCACCATGGTGTGAACCGTAG 788 | |
| | | OF | 710 GGATTGTGGCCCCACACAAATACAACCC 737 | |
| | | OR | 907 CATCAGGCTCGATGGCCAAAACACTCAA 880 | |

*(Polley et al., 2009)*

*(OF – Outer Forward, OR – Outer Reverse, IF – Inner Forward, IR – Inner Reverse, Mut- Mutant, WT – Wild Type, Co – Control)*

The components for each PCR reaction included 1X PCR SuperMix (Invitrogen), 5 pmoles of each primer (IF, IR, OF, and OR), and 50-100ng of template DNA. The standardized PCR cycle included the annealing temperatures of 57°C for G1 & G8, 60°C for G4 & G61, 55°C for G7.

PCR amplification was confirmed by running 5 µl of PCR product mixed with 1µl of 6X gel loading dye from each tube on 1.7 per cent agarose gel at the constant voltage of 5V/cm for 50 minutes in 1X TAE buffer for G1 and G4 loci specific PCR products, the G6, G7and G8 loci specific PCR products were run on 2.5 percent agrose gel at a constant voltage of 5V/cm for 60 minutes. Ethidium bromide (1 percent) was incorporated in agarose gel @ 5µl/ 100 ml of gel. The amplified product was visualized as the compact fluorescent band/s of expected size under UV light and documented by a gel documentation system (UVP, UK).

### *Sequencing*

The control fragment of T-ARMS-PCR of the G7 locus was sequenced directly. The representative 20 PCR products were sequenced using G7 OF primer in an automated ABI 3730 XL sequencer using Sanger's di-deoxy chain termination method of sequencing by M/s. Bioserve Biotechnologies (India) Pvt. Ltd., Hyderabad, India. The nucleotide sequence data was edited and analyzed using DNAStar software (Version 6.0. DNASTAR. Madison, WI).

## Results and Discussion

The genomic DNA isolated from the blood samples of Osmanabadi goats had a concentration >100 ng/µl. The quality and purity of DNA were checked by UV spectrophotometry (Nanodrop spectrophotometer ND2000, Thermo Scientific). Most of the DNA samples had an O.D. ratio in the range of 1.8 to 2.0 ($OD_{260:280}$). The required amount of DNA was diluted approximately to 50-100 ng/µl as a final concentration with ultrapure water for further use. The remaining genomic DNA was stored at -20 °C.

The most crucial step in optimizing the PCR programme is the optimization of annealing temperature because it depends on the length and GC content of the primers used. A suitable annealing temperature was tested from a range of 50-60$^0$ C in the thermal cycler (Mastercycler nexus gradient, Eppendorf, Germany), and consistent results were obtained for G1, G4, G6, G7, and G8 loci at 57°C, 60°C, 60°C, 55°C and 57°C, respectively.

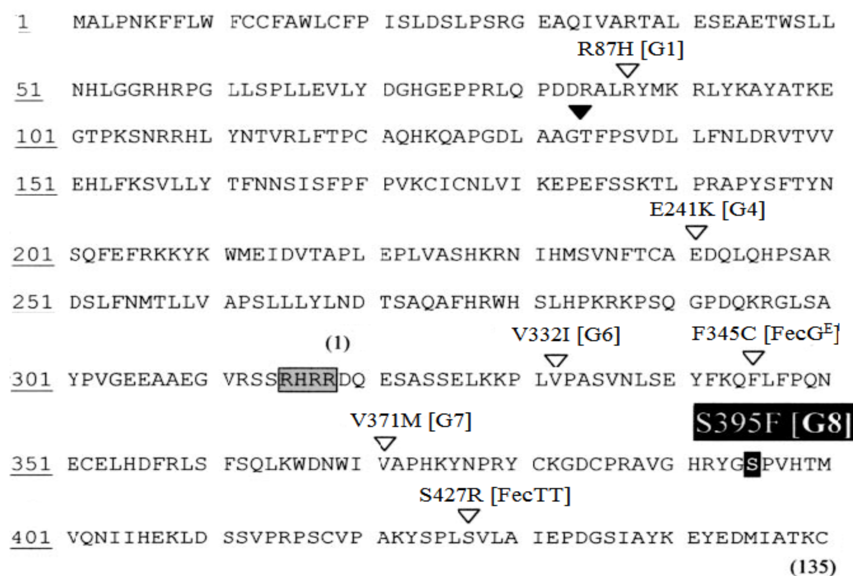


**Fig. 1:** Predicted amino acid sequence of sheep (Cambridge and F700-Belclare) GDF-9 protein.

*(Numbers at the start of each line indicate amino acid positions in the full-length unprocessed protein. Amino acid positions in the mature peptide are in parentheses. The RRHR furin protease cleavage site and predicted start of the mature processed peptide is shaded in gray. The filled triangle indicates the position of the single intron within the gene. The open triangles indicate positions of mutations that confer amino acid substitutions but are not associated with the sterility phenotype. The position of the G8 (FecG$^H$) mutation associated with sterility is shaded black, Hanrahan et al., 2004.)*

The predicted amino acid sequence of sheep GDF-9 protein is shown in Fig. 1. The primers (Table 1) used in the present study successfully amplified PCR products of 247 bp and 396 bp at G1 locus as shown in Fig. 2. Similarly, at G4 locus, 261 bp and 417 bp (Fig. 3), at G6 locus 223, bp and 362 bp (Fig. 4) and at G8 locus, 108 bp and 198 bp (Fig. 5) PCR products were successfully amplified. Whereas we could amplify only the control fragment of 343 bp at the G7 locus as shown in Fig 6.

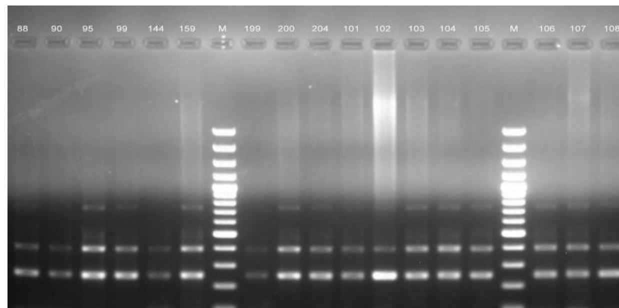


**Fig. 2:** T-ARMS PCR amplification of the GDF-9 G1 locus of the Osmanabadi goats.
(*LaneM: 100 bp DNA ladder, Lanes 88, 90, 95, 99, 144, 159, 199, 200, 204 and 101 to 108: PCR products atG1 locus (247bp and 396bp representing wild-type and control fragment, respectively)*

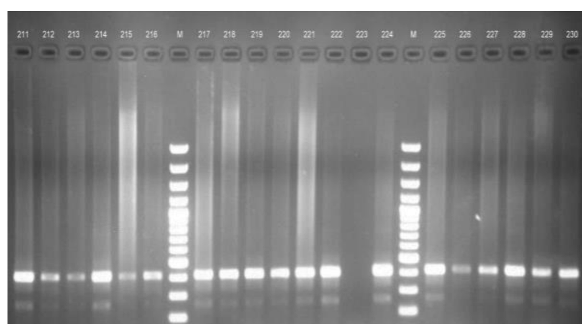


**Fig. 3:** T-ARMS PCR amplification of the GDF-9 G4 locus of the Osmanabadi goats.
*LaneM: 100 bp DNA ladder, Lanes 211 to 230: PCR products atG4 locus (261 bp and 417 bp representing wild-type and control fragment, respectively)*

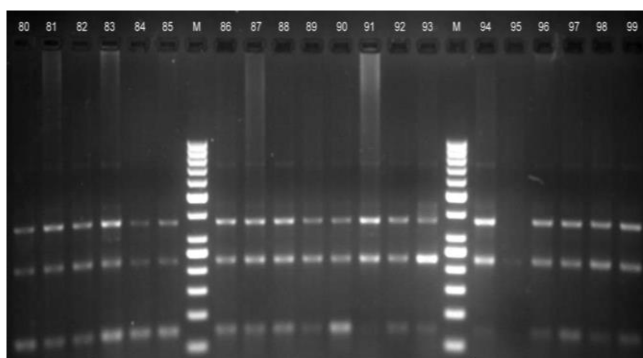


**Fig. 4:** T-ARMS PCR amplification of the GDF-9 G6 locus the Osmanabadi goats.
*LaneM: 50 bp DNA ladder, Lanes 80 to 99: PCR products at G6 locus (223 bp and 362 bp representing wild-type and control fragment, respectively)*

The observed bands of the G1 locus represented wild-type fragments at 247 bp and control fragments at 396 bp. Similar results were found for other loci G4 (261 bp and 417 bp), G6 (223 bp and 362 bp), and G8 (108 bp and 198 bp) corresponding to wild-type and control fragments representing normal non-carrier animals at respective loci. Our results were following the findings of Polley *et al*. (2009).

On several attempts of using different annealing temperatures and/ or various reaction mixtures, T-ARMS-PCR of G7 locus could amplify only control fragment at 343 bp. The wild-type or mutant fragments were not amplified. Therefore, the control PCR product was sent for direct sequencing. For sequencing, out of total 165 animals, 20 representative animals including 17 does maintained in different sheds and all three males were selected. The T-

ARMS-PCR amplified product of these selected animals for G7 control fragment were sent for sequencing using G7 OF primer. The sequencing results on analysis using ClustalW method in the MegAlign Programme of DNAStar software (Lasergene v6.0) revealed G at nucleotide position 160 of the G7 control fragment of all the 20 PCR products. All the sequenced animals represented only wild type G7 allele in the GDF-9 gene of Osmanabadi breed of goat (Fig. 7).

The sequence analysis of the G7 T-ARMS-PCR control fragment enabled us to analyze the new FecG mutations viz. FecGE; T1034G (FecGSI) and FecTT; A1279C as described by Melo *et al*., 2008; Silva *et al*., 2010 and Nicol *et al*., 2009, respectively. The sequenced Osmanabadi goats revealed wild-type alleles at these loci representing non-carrier goats (Fig. 8 and 9). The G1189A mutation, as described by Wu *et al*., 2006 in the caprine GDF-9 gene, was also studied based on sequencing results of the G7 control fragment where we have observed wild-type G nucleotide at this locus. Major ovine Fec loci affecting prolificacy compared with the identified alleles in the Osmanabadi goats are presented in Table 2.

**Table 2:** Nucleotides observed in Osmanabadi goats at FecG loci

| Mutation | Amino acid change & position | Amino acid residue position in the mature peptide | Nucleotide observed in the Osmanabadi Goats |
|---|---|---|---|
| G1 (G260A) | R87H | − | G |
| G2 (C471T) | V157V | − | Not studied |
| G3 (G477A) | L159L | − | Not studied |
| G4 (G721A) | E241K | − | G |
| G5 (A978G) | E326E | 8 | Not studied |
| G6 (G994A) | V332I | 14 | G |
| G7 (G1111A) | V371M | 53 | G |
| G8 (C1184T) | S395F | 77 | C |
| $FecG^{E}$ ($FecG^{SI}$) (T1034G) | F345C | 27 | T |
| FecTT (A1279C) | S427R | 109 | A |
| A152G (A152G) | N51D | − | Not studied |
| T692C | L231T | − | Not studied |
| G423A | L141L | − | Not studied |
| G1189A | V397I | 56 | G |

Considering the wide range effect of fecundity genes on ovulation rate, artificial insemination, and embryo transfer programmes have been used to successfully introduce these genes *viz*. BMPR-1B (FecB, Booroola) and BMP-15 (FecXI, Inverdale) into other breeds in several countries. These artificial breeding technologies were very useful considering the availability of limited numbers of progeny-tested individuals (Davis, 2004). The goat breeding is just like the sheep, there is a need to find out the key mutations in the caprine candidate genes affecting the reproduction. The next challenge will be to increase the reproductive capability, including reproductive seasonality and litter size. This can lead to a rapid and economical method to improve the breeding speed in the goat industry. GDF-9, BMP-15, and BMPR-1B genes are known to enhance the primary and preantral follicular growth *in vitro* and *in vivo*, which are expressed in all the stages of normal ovarian follicle development (Hayashi *et al*, 1999; Vitt *et al*, 2000; Hanrahan *et al*, 2004). These genes are found to be increasing ovulation in sheep breeds, especially FecGH (G8) also known as high fertility, mutation of GDF-9 gene in Belcare and Cambridge breeds, FecXG, FecXB, FecXI, FecXH, and FecXL mutations of BMP15 gene in several sheep breeds, and FecB mutation in BMPR-1B gene (Davis, 2005) in Garol breed of sheep.

The FecB mutation popularly known as Booroola in the BMPR-1B gene was reported to be present in the Booroola Merino (Australia), Souza *et al*., 2001, Garole (India), Davis *et al*., 2002, Javanese (Indonesia), Davis *et al*., 2002, Small Tailed Han (China), Davis, 2005 and Hu (China), Davis, 2005 breeds of sheep. There were no reports of FecB polymorphism in any Indian goat breed until now except Black Bengal goats (Polley *et al*., 2009). None of the known polymorphism in the GDF-9 gene (G1, G4, G6, G7, and G8 or FecGH) and in the BMP15 gene (FecXG, FecXH, FecXI, FecXL and FecXB) observed in sheep were found in the tested Black Bengal goats (Polley *et al*., 2009). The Belclare/ Cambridge sheep (GDF-9 and BMP-15; Hanrahan *et al*., 2004), the Lacaune sheep (FecXL in

BMP15; Bodin *et al*., 2002) and Small Tailed Han sheep (BMPR1B and BMP15; Chu *et al*., 2007) were the sheep breeds where mutations in two different genes have been detected. Chu *et al*. (2007) reported that the BMP15 gene is either a major gene that influences the prolificacy of the Jining Grey goat or a molecular genetic marker in close linkage with such a gene.

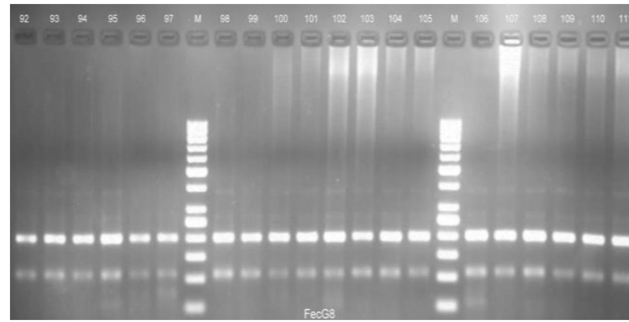


**Fig. 5:** T-ARMS PCR amplification of the GDF-9 G8 locus of the Osmanabadi goats.
*LaneM: 50 bp DNA ladder, Lanes 92 to 111: PCR products at G8 locus (108 bp and 198 bp representing wild-type and control fragment, respectively)*

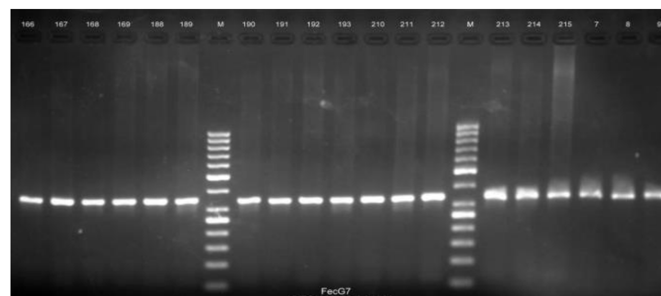


**Fig. 6:** T-ARMS PCR amplification of the GDF-9 G7 locus of the Osmanabadi goats.
*LaneM: 50 bp DNA ladder, Lanes 165 to 215 and 7 to 9: PCR product G7 locus (343 bp representing control fragment).*

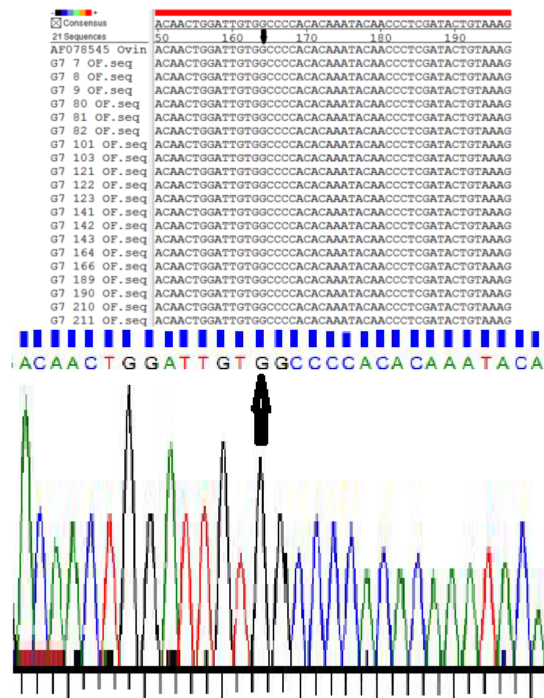


**Fig. 7:** Nucleotide sequence alignment of locus G7 of ovine GDF-9 gene (Acc. No. AF078545), with that of Osmanabadi goats. The bold vertical arrow represents a known polymorphic point at the G7 locus.

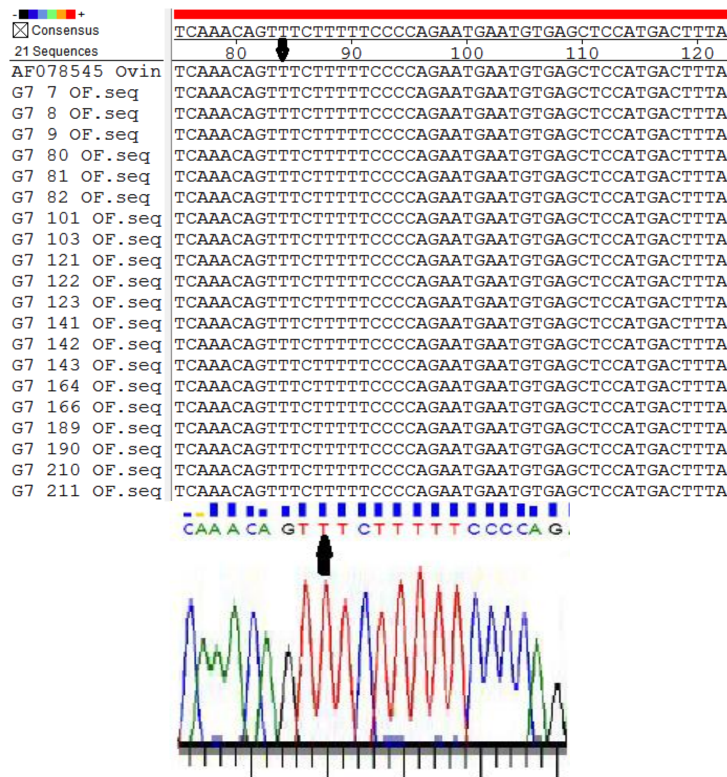


**Fig. 8:** Nucleotide sequence alignment of locus $FecG^E$ of ovine GDF-9 gene (Acc. No. AF078545), with that of Osmanabadi goats. The bold vertical arrow represents known polymorphic point at $FecG^E$ locus.

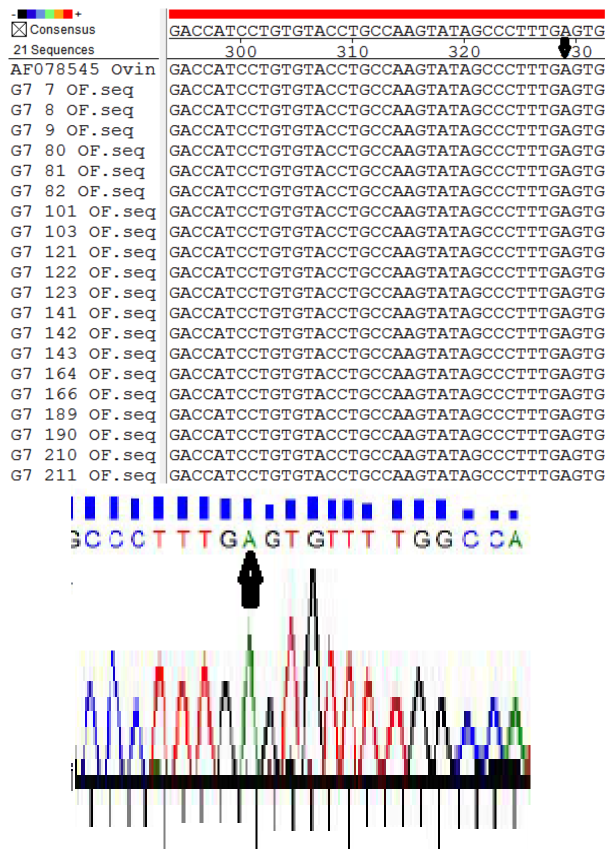


**Fig. 9:** Nucleotide sequence alignment of locus FecTT of ovine GDF-9 gene (Acc. No. AF078545), with that of Osmanabadi goats. The bold vertical arrow represents known polymorphic point at FecTT locus.

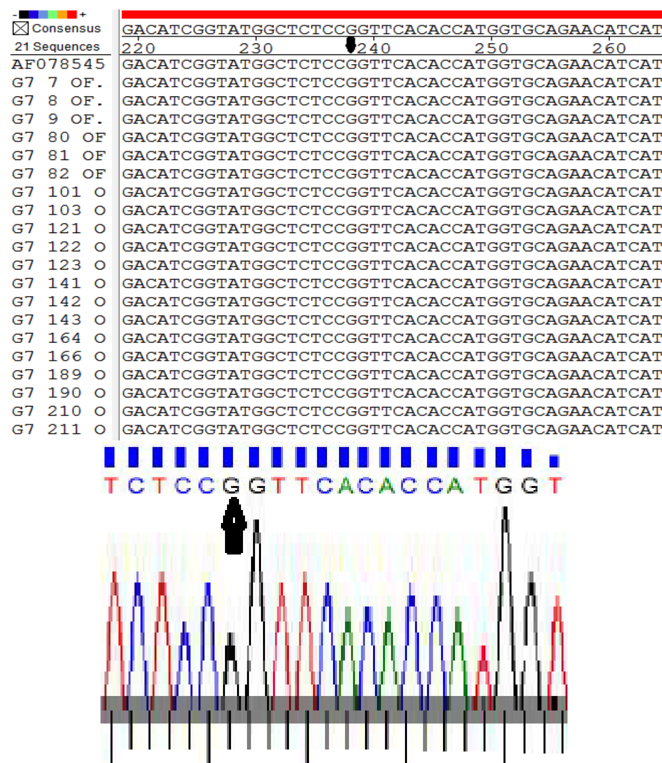


**Fig. 10:** Nucleotide sequence alignment of the locus (G1189A) of ovine GDF-9 gene (Acc. No. AF078545), with that of Osmanabadi goats. The bold vertical arrow represents a known polymorphic point at the (G1189A) locus.

Hanrahan *et al*. (2004) reported eight DNA variants in GDF9 of Cambridge and Belclare sheep including G1, G4, G6, G7 and G8. Out of these eight polymorphisms, three nucleotide changes did not alter amino acids (G2, G3 and G5). Four mutations of the eight SNPs resulted in amino acid changes viz., G1 (G260A; R87H), G4 (G721A; E241K), G6 (G994A; V332I) and G7 (G1111A; V371M). Li *et al*. (2003) identified one single nucleotide mutation (A152G; N51D) in the GDF-9 gene in Hu, Dorset, and Suffolk sheep by PCR-single strand conformation polymorphism (SSCP), which resulted in an amino acid change from Asparagine to Aspartic acid. Roy *et al*., 2011 also observed G1 and G4 of GDF-9 gene to be polymorphic in the Bonpala ewes, at G1 locus, three genotypes, namely, wild type (GG,0.89), heterozygous (GA, 0.10), and mutant (AA, 0.01) were detected, at G4 locus they have observed wild type (AA, 0.01), heterozygous (AG,0.14) and mutant (GG, 0.85) genotypes. Moradband *et al*. (2011) observed all three genotypes in the Baluchi sheep population at G1 locus, with allele FecG+ having the highest frequency (0.82), whereas allele FecG1 had the lowest frequency (0.18). Our results differ from these observations as we have observed only wild-type genotypes at these loci in the Osmanabadi goats.

Our results at FecG loci are in agreement with the findings of Dutta *et al*., 2013, Where they observed none of the ten SNPs identified in sheep GDF-9 gene, including four mutations (FecGH, FecGE, FecTT, and G1) associated with fecundity in Assam Hill goats. Hua *et al*., 2008 and He *et al*., 2010 observed that none of the mutations in GDF-9 and BMP-15 genes, including FecGH was detected in goat breeds viz., Haimen, Boer, and Huanghui, and concluded that these mutations had no noticeable effect on the difference of prolificacy in goats. They cannot be regarded as the major gene associated with the fecundity of goats. The mutations G1, G4, G6, G7, and A152G were observed before the furin processing site in the unprocessed protein and, therefore, are unlikely to affect the mature active coding region.

The mutation G8 (C1184T; S395F), also known as FecGH, results in an amino acid change from serine to phenylalanine at residue 395, which replaces an uncharged polar amino acid with a non-polar one at residue 77 of the mature coding region. In the present investigation, the T-ARMS-PCR results revealed a wild-type allele at the site of G8 (FecGH); none of the Osmanabadi goats were found polymorphic at this locus.

Melo *et al*. (2008) and Silva *et al*. (2010) detected FecGE (FecGSI) mutation (T1034G; F345C) in GDF-9 gene of Brazilian Santa Ines sheep. Another new mutation (A1279C), named as FecTT was found in GDF-9 gene of Icelandic Thoka sheep, resulting in a non-conservative amino acid change from serine to arginine, S109R in the C-terminus of the mature GDF-9 protein (Nicol *et al*., 2009). In our study, we have sequenced the control fragment of

G7 T-ARMS-PCR product from twenty representative goats, including three males, where we observed the sites FecGE and FecTT were carrying wild-type allele; none of the sequenced Osmanabadi goats showing mutant allele at these sites.

Few point mutations (G423A and G1189A; V397I) in exon 2 of GDF9 gene had been detected extensively in several goat breeds, in which mutation G423A was detected in Jining Grey, Liaoning Cashmere and Boer goats (Wu *et al*., 2006; Feng *et al*., 2010), Wendeng Dairy and Beijing native goats (Wu *et al*., 2006) and Guizhou White goats (Feng *et al*., 2010), Indonesian goats (Elieser *et al*., 2018), tropical goat breeds of Kerala (Sasi *et al*., 2020), Egyptian small ruminants (Aboelhassan *et al*., 2021), Jamunapari and crossbred goats in Bangladesh (Shaha *et al*., 2022), Iraqi goats (Ali and Al-Azzawi, 2022), Tibetan Cashmere Goat (Song *et al*., 2023), East Java Pote goat (Imaniah *et al*., 2023) and Shami Goats (Hussein *et al*., 2023). The mutation G423A was observed in the unprocessed protein and, therefore, is unlikely to affect the mature active coding region of the GDF-9 protein. Therefore, the G423A site was not included in the present investigation. Mutation G1189A was identified in Jining Grey, Liaoning Cashmere and Boer goats: Wendeng dairy and Beijing native goats (Wu *et al*., 2006; Feng *et al*., 2010), Guizhou White goats (Du *et al*., 2008; Feng *et al*., 2010). In the present study, the analysis of G7 control fragment sequences from 20 representative goats revealed the presence of wild-type allele G at the mutation site G1189A; none of the sequenced Osmanabadi goats revealed the presence of mutant alleles at this locus.

## Conclusions

In the present investigation, Osmanabadi goats were found to be monomorphic, carrying wild-type alleles at the G1, G4, G6, G7, G8 (FecGH), FecGE, FecTT and G1189A loci in the GDF-9 gene, therefore it can be observed that fecundity of Osmanabadi goats is independent of FecG mutations. For genotyping the G7 locus a different method, viz., PCR-RFLP, PCR-SSCP or direct sequencing, can be adopted/ developed since the T-ARMS-PCR of G7 yielded only the control fragment. The T-ARMS-PCR method can be a rapid and economical tool for discriminating G1, G4, G6 and G8 genotypes in the goats.

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## Contribution by Authors

Equal contribution. All authors declared that 'written informed' consent was obtained from the approved parties for the publication of this article and accompanying images.

## Conflict of Interests

There is no conflict of interest.

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