



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating (2026): 4.61

VET 2025; SP-11(4): 253-257

© 2026 VET

www.veterinarypaper.com

Received: 23-01-2026

Accepted: 26-02-2026

Meghana K

Assistant Professor, Department of Animal Genetics and Breeding, NTR College of Veterinary Science (Sri Venkateswara Veterinary University), Gannavaram, Andhra Pradesh, India

Sudhakar K

Associate Professor and Head, Department of Animal Genetics and Breeding, NTR College of Veterinary Science, SVVU, Gannavaram, Andhra Pradesh, India

Vinoo R

Professor, Department of Animal Genetics and Breeding, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Narendranath D

Professor and Head, Department of Poultry Science, NTR College of Veterinary Science, SVVU, Gannavaram, Andhra Pradesh, India

Basavaiah M

Veterinary Assistant Surgeon, Veterinary Dispensary, Botlapalem, Prakasam, Andhra Pradesh, India

Evaluation of candidate gene for fecundity in *Gaddabolli* sheep of Andhra Pradesh

Meghana K, Sudhakar K, Vinoo R, Narendranath D and Basavaiah M

DOI: <https://www.doi.org/10.22271/veterinary.2026.v11.i4Sd.3231>

Abstract

A study was conducted on *Gaddabolli*, a lesser known indigenous sheep with its home tract in the dry land areas of Prakasam, Bapatla, Palnadu and Guntur districts of Andhra Pradesh with an objective to evaluate the presence of fecundity genes (*FecB* and *FecG^H*). The local farmers prefer this sheep to Nellore due to high twinning rate. During the study in the 27 flocks twinning rate was observed at 21 per cent of the animals and 210 blood samples were collected to screen for the *FecB* and *FecG^H* mutations by PCR-RFLP. Twenty six sheep were heterozygous (B+) and the rest were non-carriers (++) on *FecB/AvaII* analysis. No polymorphism at *GDF9/HhaI* locus existed and all individuals had the wild type except one sheep that was tested positive for presence of mutation.

Keywords: *Gaddabolli* sheep, *FecB* gene, *FecG^H* gene, Prolificacy

1. Introduction

Over generations of natural selection and domestication, India's diverse sheep breeds have evolved within specific agro-climatic zones each uniquely adapted to survive and thrive in its specific local environment. While India's sheep germplasm is vast, with 60 genetic groups as reported by the FAO (Scherf, 2000) [1], only 46 have been recognized to date (ICAR-NBAGR, 2025) [2], underscoring the need for the identification and characterization of lesser-known sheep. *Gaddabolli* sheep is one such lesser-known genetic group (Kondapu *et al.*, 2025) [3], that are distinct in their phenotype and are distinguishable from Nellore breed with its home tract in the dry land areas of Prakasam, Bapatla, Guntur and Palnadu districts of Andhra Pradesh. The local farmers prefer this sheep to Nellore due to high twinning rate which is about 20 to 25 per cent in the flocks that is uncommon in most of the Indian sheep breeds. The *FecB* (Booroola) gene, a point mutation in the *BMPT1B* (Bone Morphogenetic Protein Receptor Type 1B) gene, represents one of the most significant genetic discoveries in small ruminant science. Originally identified in the Booroola Merino but tracing its roots to the Indian Garole, this autosomal dominant mutation fundamentally alters the TGF- β signaling pathway in the ovaries. The high prolificacy in sheep is preferred as it benefits the farmer in terms of lamb production. *GDF9* gene (*FecG* locus) is a critical regulator of fertility in sheep that operates through a unique overdominance mechanism for high prolificacy. Hence, the study on *Gaddabolli* sheep to explore the genetic cause of prolificacy was undertaken by screening for the presence of mutation in Booroola gene (*FecB/BMPT1B*) and Growth differentiating factor gene (*FecG^H/GDF9*).

2. Materials and Methods

2.1. Experimental animals and sample collection

The present study does not warrant Institutional Animal Ethics Committee approval as no experimentation is conducted on the animals. Four milliliter of blood sample was collected from each of the 210 *Gaddabolli* sheep (Table 1) reared by farmers in their home tract with their consent in Guntur, Bapatla, Palnadu and Prakasam districts of Andhra Pradesh. The collected blood samples were transported to the laboratory in an ice packed container and stored at -20 °C.

Corresponding Author:

Sudhakar K

Associate Professor and Head, Department of Animal Genetics and Breeding, NTR College of Veterinary Science, SVVU, Gannavaram, Andhra Pradesh, India

Table 1: Details on the number of animals with twinnings sampled for DNA isolation

District	Mandal	Village	No of flocks	No of animals
Prakasam	Darsi	East Veerayapalem	2	20
		Ramchandrapuram	1	10
		Ganeshwarapuram	2	15
		Botlapalem	1	13
	Thalluru	Doskayalapadu	2	10
	Mundalmuru	Bhimavaram	1	12
		Purimetla	2	15
Bapatla	Ballikaruva	Guntupalle	2	15
	Addanki	Dharmavaram	3	16
	Paruchuru	Ramanayapalem	1	10
	Marturu	Jonnathalli	1	10
		Nagarajupalle	1	10
	Bapatla	Cheruvu	3	12
Palanadu	Chilakaluripeta	Pasumaaru	3	30
Guntur	Pedanandipadu	Varagani	2	12
Grand Total			27	210

2.2. DNA extraction

Genomic DNA was isolated from whole blood by high salt method (Miller *et al.*, 1988) [4], with modifications to suit the lab conditions. The genomic DNA was verified using 1% agarose gel electrophoresis and was quantified by using Nano spectrophotometer (Venchal Scientific, USA).

2.3. Selection of Fecundity genes for screening

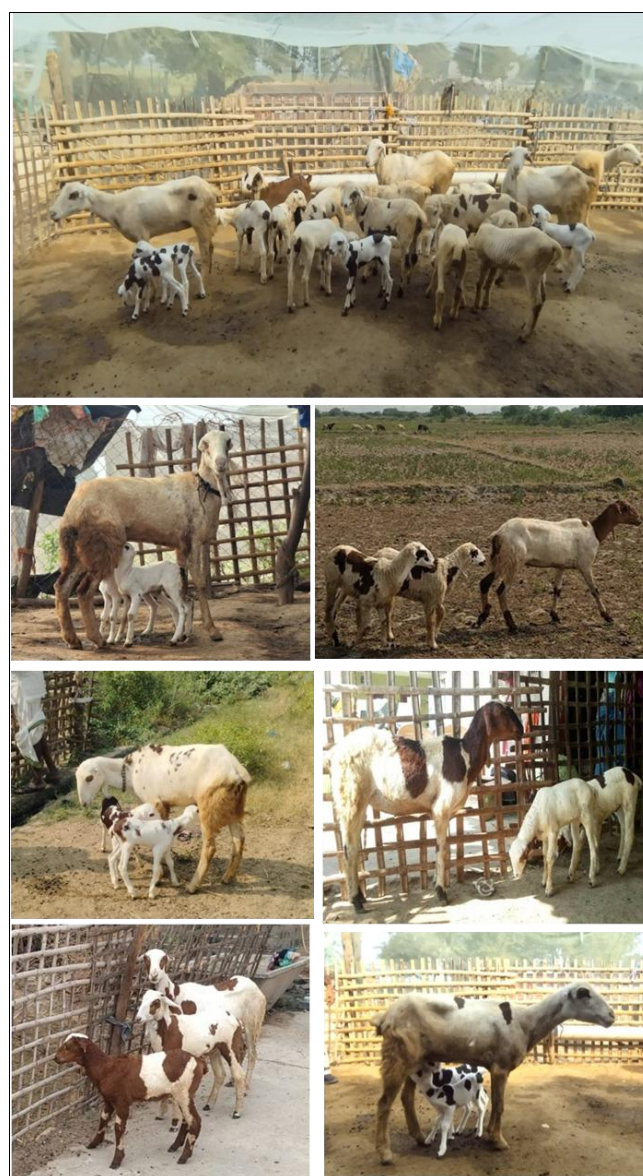
Owing to their significance for prolificacy in Sheep *FecB* and *FecG^H* genes were considered for the study. All the ewes and rams having twin lambs or a history of twinnings were screened for mutations in *FecB* using the primers F:5'-CCAGAGGACAATAGCAAAGCAAA-3', R:5'-CAAGATGTTTTTCATGCCTCATCAACAGGTC-3' (Abouheif *et al.*, 2011) [5], and for *FecG^H* using the primers F:5'-GAAGACTGGTATGGGGAAATG -3', R:5'-CCAATCTGCTCCTACACACCT -3' (Hanrahan *et al.*, 2004) [6]. The cycling conditions for amplification of *FecB* included initial denaturation for 3 min at 94 °C, followed by 35 cycles comprising of 45 s at 94 °C (denaturation), 45 s at 52 °C (annealing), and 45 s at 72 °C (extension) followed by final extension for 7 min at 72 °C. The corresponding cycling conditions for amplification of *FecG* were 94 °C / 3 min, 94 °C / 30 s, 58 °C / 30 s, 72 °C / 30 s and 72°C / 7 min. PCR products were subjected to 2% agarose gel. Subsequently, the PCR amplicons were subjected to RFLP with *AvaII* on *FecB* and *HhaI* for *FecG* respectively to observe the polymorphic patterns and thus genotype the animals for the respective locus. Seven milliliters of the digested samples was separated on 3.5 percent agarose gel for genotyping. Gels were visualized with Omega Fluor™ Plus Documentation Systems (BioExpress, USA). The agarose gels were scored based on the pattern observed as wild type, mutant and heterozygotes.

3. Results and Discussion

3.1. Screening for *FecB* and *FecG^H* gene mutation

This genetic group of sheep were reported by the farmers to be having high prolificacy at the rate of 25-30 per cent in the flocks. During the survey in the sampled 27 flocks (Table 1) twinning rate was observed at 21 per cent (Figure 1). More twinnings were observed in flocks from Darsi Mandal. RFLP was employed to detect the polymorphic patterns and to determine genotypes of the animals for each of *FecB* and *FecG^H* gene in these populations in the sample under study. The *Gaddabolli* (n = 210) ewes, rams and lambs that were from families having two lambs in a lambing were selected for blood collection during the survey. The detail of samples

screened across different flocks is represented in Table 1.

**Fig 1:** Twinnings in Gaddabolli sheep

3.1.1. *FecB* genotyping

The 190 bp PCR amplicon (Figure 2a) of the *FecB* gene on digestion with *AvaII* restriction enzyme was supposed to produce a 160 bp band in homozygous carriers (BB), the non-

carrier 190 bp band (++), whereas heterozygotes produce both 160 and 190 bp bands (B+). The band pattern indicating different *FecB/AvaII* restriction patterns observed are presented in Figure 2b.

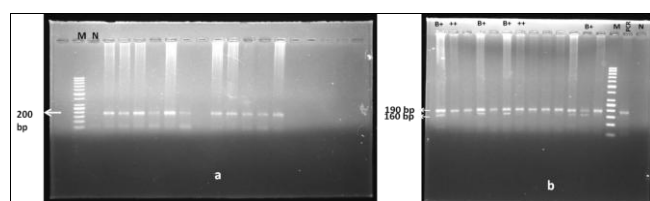


Fig 2: PCR amplification products of *FecB* gene and RFLP pattern of *FecB/AvaII* on agarose gel (M: 50 bp DNA marker, N: Negative control, PCR: PCR Control)

The study revealed the presence of only ++ and few B+ genotypes in the populations. In the present population that was screened 26 sheep (~12.4%) were heterozygous carriers (B+) and the rest were non-carriers for the *FecB* mutation (Table 2) and frequency of the allele was about 0.1.

Table 2: Distribution of genotypes among sheep screened for mutation at *FecB* and *FecG^H* locus

	<i>FecB</i>			<i>FecG^H</i>		
	B/B	B/+	+/+	-/-	+/-	+/+
Ewe	-	12	86	-	-	53
Ram	-	6	41	-	-	92
Lamb	-	8	57	-	1	64

In various studies on Indian sheep breeds that were non prolific viz. Deccani, Bannur (Nimbkar *et al.*, 2003) [7], Kenguri sheep (Asharani *et al.*, 2018) [8], Marwadi, Patanwadi and Dumba sheep (Mankad *et al.*, 2020) [9], Mecheri sheep (Sudhakar *et al.*, 2013) [10], Coimbatore, Mecheri and Tiruchy Black sheep (Saravanan *et al.*, 2020) [11], Deccani (Praveena *et al.*, 2017) [12], were reported to be non carriers of *FecB* mutation. However, all the three genotypes of *FecB* were detected in Bonpala sheep. In another study on forty Nellore sheep blood samples, there was only one ewe with (BB) homozygous, five ewes with (B/+) heterozygous and thirty four ewes with (+/+) homozygous genotype in *FecB* loci (Praveena *et al.*, 2017) [12].

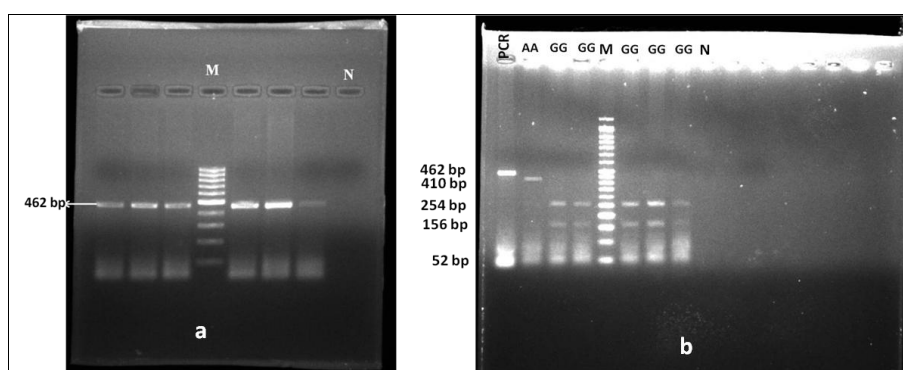


Fig 3: PCR amplification products of *FecG* gene and RFLP pattern of *GDF9/HhaI* on agarose gel

(a) M: 100 bp DNA marker, N: Negative control, b) M: 50 bp DNA marker, N: Negative control, PCR: PCR Control

At G1 locus of *GDF9* in Garole, two genotypes, mutant (A) and wild types (G) were detected with allele frequencies of 0.18 and 0.82, respectively (Polley *et al.*, 2010) [19]. At G1 locus of *GDF9* gene in Bonpala sheep, also an Indian sheep, three genotypes, namely, wild type (GG, 0.89), heterozygous

Though, all the local Kashmir sheep were prolific, lacked the mutation indicating that the prolificacy in those animals may be due to some other *Fec* gene (Ganai *et al.*, 2012) [13].

The observation on Gaddaboli sheep in the present study was in accordance to those in Nilagiri sheep, in which 12.99% of ewes were with multiple births (Rajendran, 2005) [14], and the frequency of the *FecB* allele was 0.14 (Sudhakar *et al.*, 2013) [10]. The varied frequencies of *FecB* allele was reported in Kendrapada sheep (Kumar *et al.*, 2008) [15]. In the current study, the *FecB* mutant allele occurred at a low frequency despite the prevalence of twin births. This suggests that other genetic factors may be at play, as previously noted by Davis *et al.* (2006) [16], who reported twin-producing sheep that lacked both the *FecB* and Inverdale mutations. This may be due to certain genes that are yet to be identified having a role in molecular regulatory mechanisms underlying sheep prolificacy.

3.1.2. *FecG^H* genotyping

A DNA fragment with the expected size of 462 bp was amplified from exon I of *GDF9* gene (Figure 3a). The G→A nucleotide change in *GDF9* exon I disrupts a *HhaI* restriction enzyme cleavage site (GCGC to GCAC). Restriction digestion of the PCR product from wild-type animals with *HhaI* should result in cleavage of the 462-bp product (at two internal *HhaI* sites) into fragments of 52, 156, and 254 bp (*FecG*⁺). But, DNA fragments containing the 'A' nucleotide was supposed to yield only two fragments of 52 and 410 bp (*FecG^H*). Animals heterozygous for the mutation have fragments of all four sizes (52, 156, 254, and 410 bp). Among the 210 sheep that were screened for *FecG^H* mutation no polymorphism at *GDF9/HhaI* locus existed and all individuals had the wild type except one sheep that was tested positive for presence of mutation showing 52 and 410 bp fragments (Figure 3b). The sheep that was harbouring *FecG^H* mutation was free from *FecB* mutation. The coexisting polymorphism of these two was reported in Kendrapada sheep (Dash *et al.*, 2017) [17]. Mutations of *FecG* (five single nucleotide polymorphisms (SNPs)) are associated with increased ovulation rates in heterozygous animals and sterility in homozygous animals (Hanrahan *et al.* 2004; Bodin *et al.* 2007) [6, 18].

(GA, 0.10), and mutant (AA, 0.01) were detected (Roy *et al.*, 2011) [20]. The changing concentrations of *GDF9* *in vivo* leads to incremental changes in ovulation rate in sheep (Hanrahan *et al.* 2004) [6].

The details of sheep that were carrying a copy of either *FecB* or *FecG* mutations were presented in Table 3. The sheep from Ganeshwarapuram village of Darsi mandal recorded more

twinings (9 out of 15; 60.0 per cent), followed by Pasumarru and East Veerayapalem villages each recorded 20.0 percent of animals with mutation (6 out of 30 and 4 out of 20 sheep respectively).

Table 3: Details of sheep that were carrying *FecB* and *FecG* mutations in the surveyed population

Gene	Animal no.	Village	Type of animal
<i>FecB</i>	F1- 13	East Veerayapalem	Lamb (Male)
	B4	East Veerayapalem	Ewe
	B5	East Veerayapalem	Ewe
	C4	Ramchandrapuram	Lamb (Male)
	D2	Ganeshwarapuram	Ewe
	D5	Ganeshwarapuram	Ewe
	E1	Ganeshwarapuram	Ewe
	F3	Ganeshwarapuram	Lamb (Male)
	F4	Ganeshwarapuram	Lamb (Female)
	F6	Ganeshwarapuram	Ram
	G4	Ganeshwarapuram	Ram
	H1	Ganeshwarapuram	Ewe
	J5	Ganeshwarapuram	Lamb (Male)
	K11	Botlapalem	Ewe
	L3	Botlapalem	Lamb (Male)
	M9	Doskayalapadu	Lamb (Female)
	M10	Doskayalapadu	Lamb (Male)
	N3	Doskayalapadu	Ewe
	O1	Bhimavaram	Ram
	Q1	Purimetla	Ewe
	S17	Pasumaaru	Ewe
<i>FecG^H</i>	YA13	Pasumaaru	Ram
	YA15	Pasumaaru	Ram
	YAF11	Pasumaaru	Ewe
	YVM3	Pasumaaru	Ram
	YVF2	Pasumaaru	Ewe
	B18	East Veerayapalem	Lamb (Male)

The *FecB/AvaII* analysis on the sampled sheep revealed only ++ and few B+ genotypes in them and ~12.4% individuals were found heterozygous carrier (B+) for the *FecB* mutation. According to Hardy Weinberg equation it is expected to find only two 'BB' sheep in the entire sample of 210 sheep. With an expected prevalence of only 1%, the absence of this genotype does not necessarily indicate a violation of genetic principles (Rodriguez *et al.*, 2009) [21], but rather reflects the low probability of capturing rare homozygotes in small sample known as sampling zero or random zero (Huang *et al.*, 2011) [22], due to the limits of the sample size.

Among the 210 sheep that were screened for *FecG^H* mutation no polymorphism at *GDF9/HhaI* locus existed and all individuals had the wild type except one sheep that was tested positive for presence of mutation.

4. Conclusion

The cause of frequent twinning in the flocks could be due the effect of other fecundity genes that segregate in other prolific sheep breeds, or may be a combination of gene products that stimulate/alter the ovulatory cycle. Further efforts are required to investigate the cause. However, other possible reasons may be attributed to good nutritional plan and body condition of sheep which was evident by their biometrical and growth traits that could have stimulated the ovulatory responses to some extent and in turn produce extra ova/lambs as also opined by Kandil (2002) [23].

5. Acknowledgements

The authors acknowledge Sri Venkateswara Veterinary

University for providing facilities to conduct this study.

Conflict of Interest

No potential conflict of interest was reported by the authors.

Financial Support

Not available

References

- Scherf BD. World watch list for domestic animal diversity. 3rd ed. Food and Agriculture Organization; 2000.
- ICAR-NBAGR. Sheep breeds of India. 2025.
- Kondapu M, Krovvidi S, Regula V, Dhulipalla N, Maachepalli B. Phenotypic characterization of Gaddabolli sheep. *Journal of Livestock Science*. 2025;16:580-589.
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*. 1988;16:1215.
- Abouheif MA, Al-Owaimer AN, Shafey TM, AlShaikh MA, Aljumaah RS. Polymorphism of Booroola *FecB* gene in prolific individuals from Najdi and Naeimi sheep breeds of Saudi Arabia. *Journal of Animal and Veterinary Advances*. 2011;10:1262-1264.
- Hanrahan JP, Gregan SM, Mulsant P, Mullen M, Davis GH, Powell R, *et al.* Mutations in the genes for oocyte-derived growth factors *GDF9* and *BMP15* are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). *Biology of Reproduction*. 2004;70:900-909.
- Nimbkar C, Ghalsasi PM, Maddox JF, Pardeshi VC, Sainani MN. Expression of *FecB* gene in Garole and crossbred ewes in Maharashtra, India. In: Proceedings of the 15th Conference of AAABG; Melbourne, Australia. 2003;111-114.
- Asharani A, Appannavar M, Yatish MH, Hussain M, Kasaralikal V, Suranagi M, *et al.* Molecular variants of *FecB* and *BMP15* fecundity genes in sheep. *International Journal of Livestock Research*. 2018;8:185-195.
- Mankad P, Patel N, Rank D. Screening of Marwadi, Patanwadi and Dumba sheep breeds for presence of fecundity gene polymorphism by PCR-RFLP and sequencing. *International Journal of Livestock Research*. 2020;10:63-74.
- Sudhakar A, Rajendran R, Rahumathulla PS. Detection of Booroola (*FecB*) mutation in Indian sheep—Nilagiri. *Small Ruminant Research*. 2013;1:55-57.
- Saravanan R, Kumarasamy P, Anil Kumar R, Thiruvankadan AK, Murali N. Molecular screening of *FecB* gene mutation through PCR-RFLP method in different sheep breeds of Tamil Nadu. *International Journal of Genetics*. 2020;12:725-727.
- Praveena K, Ramana DBV, Pankaj PK. Booroola gene (*FecB*) polymorphism and its liaison with litter size in indigenous sheep breeds of Telangana, India. *Journal of Animal Research*. 2017;7:227-231.
- Ganai TAS, Misra SS, Shabir M. Polymorphism analysis of *BMP1B* gene by forced RFLP and PCR-SSCP techniques and expression of the mutation in introgressed sheep. *Tropical Animal Health and Production*. 2012;44:277-283.
- Rajendran R. Genetic analysis of reproduction traits and survivability of Nilagiri sheep and its synthetic crosses

- [PhD thesis]. Chennai: Tamil Nadu Veterinary and Animal Sciences University; 2005.
15. Kumar S, Mishra AK, Kolte AP, Dash SK, Karim SA. Screening for Booroola (FecB) and Galway (FecXG) mutations in Indian sheep. *Small Ruminant Research*. 2008;80:57-61.
 16. Davis GH, Balakrishnan L, Ross IK, Wilson T, Galloway SM, Lumsden BM, *et al*. Investigation of the Booroola (FecB) and Inverdale (FecXI) mutations in 21 prolific breeds and strains of sheep sampled in 13 countries. *Animal Reproduction Science*. 2006;92:87-96.
 17. Dash S, Maity A, Bisoi PC, Palai TK, Polley S, Mukherjee A, *et al*. Coexistence of polymorphism in fecundity genes BMPR1B and GDF9 of Indian Kendrapada sheep. *Exploratory Animal and Medical Research*. 2017;7:33-38.
 18. Bodin L, Di Pasquale E, Fabre S, Bontoux M, Monget P, Persani L, *et al*. A novel mutation in the bone morphogenetic protein 15 gene causing defective protein secretion is associated with both increased ovulation rate and sterility in Lacaune sheep. *Endocrinology*. 2007;148:393-400.
 19. Polley S, De S, Brahma B, Mukherjee A, Vinesh PV, Batabyal S, *et al*. Polymorphism of BMPR1B, BMP15 and GDF9 fecundity genes in prolific Garole sheep. *Tropical Animal Health and Production*. 2010;42:985-993.
 20. Roy J, Polley S, De S, Mukherjee A, Batabyal S, Pan S, *et al*. Polymorphism of fecundity genes (FecB, FecX, and FecG) in the Indian Bonpala sheep. *Animal Biotechnology*. 2011;22:151-162.
 21. Rodriguez S, Gaunt TR, Day INM. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *American Journal of Epidemiology*. 2009;169(4):505-514.
 22. Huang Z, Yi K, Liu Y. Optimal sampling algorithms for frequency estimation in distributed data. In: *Proceedings IEEE INFOCOM*. 2011:1997-2005.
 23. Kandil O. Twin production in sheep. In: *Proceedings of the 9th International Congress on Biotechnology in Animal Reproduction*; Madras Veterinary College, Chennai, India. 2002:200.

How to Cite This Article

Meghana K, Sudhakar K, Vinoo R, Narendranath D, Basavaiah M. Evaluation of candidate gene for fecundity in *Gaddaballi* sheep of Andhra Pradesh. *International Journal of Veterinary Sciences and Animal Husbandry*. 2026;SP-11(4):253-257.

Creative Commons (CC) License

This is an open-access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 International (CC BY-NC-SA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.