Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Exploration of the association between SMAD5 gene polymorphisms and litter size and growth traits in sheep (Ovis aries)

Dwi Wijayanti

wijayantidwi12@gmail.com

Universitas Perjuangan Tasikmalaya

Dela Ayu Lestari

Universitas Diponegoro, Central Java

Mutasem Abuzahra

National Research and Innovation Agency (BRIN)

Asep Setiaji

Universitas Diponegoro, Central Java

Andri Kusmayadi

Universitas Perjuangan Tasikmalaya

Nadiah S. Alzahrani

Albaha University

Weam I. Alnoqair

University of Hail

Research Article

Keywords: Garut sheep, Economic traits, Sequencing, SMAD5, SNP

Posted Date: November 3rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11574509/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background

SMAD Family Member 5 (SMAD5) is a downstream mediator of BMP signaling and plays a biological role in cell proliferation, differentiation, development, and reproduction. This study aimed to evaluate the effect of mutations in the *SMAD5* gene on litter size and growth traits in Garut sheep.

Methods and Results

A single nucleotide polymorphism (SNP) in the *SMAD5* gene of Garut sheep was detected using DNA pool sequencing and forced sequencing methods. Three genotypes AA, AG, and GG were identified, with AG being the most prevalent. The detected locus exhibited a moderate level of genetic diversity (PIC = 0.36). Statistical analysis revealed that the SNP and its genotypes were associated with body weight, hip width, and wither height, with the GG genotype displaying higher values than the other genotypes. We also observed an association between genotypes and litter size at first parity, where the AG genotype showed higher results compared to the others.

Conclusion

These findings provide evidence that polymorphism in the *SMAD5* gene was associated to growth traits and litter size at first parity, suggesting its potential utility for marker-assisted selection in Garut sheep breeding programs.

Introduction

In Indonesia, especially in West Java, Garut sheep represent a significant native genetic asset, offering substantial prospects for enhancing meat production and genetic development. In sheep production systems, traits related to reproduction and growth, such as litter size, body weight, body length, chest depth, chest girth, rump width, rump height, and withers height, serve as crucial indicators of productivity. Litter size directly determines the number of lambs produced per ewe, whereas growth traits reflect the productive performance and efficiency of lamb production [1–3]. Reproductive performance in Garut sheep is affected by genetic and non-genetic elements, with factors such as parity and birth type leading to differences in litter size and various reproductive characteristics [4]. Therefore, identification of genetic variants underlying these economically important traits is essential for developing more effective selection strategies.

The signaling pathway of transforming growth factor-beta (TGF-β) is crucial for reproductive development, including folliculogenesis and ovulation, as well as for cellular proliferation, differentiation, and tissue growth. The SMAD family members act as intracellular mediators in TGF-β/BMP signaling

pathways, making them potential candidate genes for traits related to reproduction and growth. Several studies have identified polymorphisms in TGF-β-related genes, including BMPR1B, BMP15, GDF9, and SMAD family genes, that may contribute to variation in ovulation rate and litter size in sheep [5–7].

The current state of the art indicates that molecular variation in genes involved in the TGF-β/BMP signaling pathway can contribute to differences in reproductive performance. Meanwhile, genome-wide and candidate-gene studies increasingly emphasize the importance of identifying breed-specific variants associated with economically important traits. In sheep, hundreds of SNPs have been reported to be associated with litter size, although most are located in non-coding regions, highlighting the importance of investigating additional candidate loci and breed-specific genetic variation [8]. In Garut sheep, the connection between GDF9 g.333G > A and litter size highlights the role of TGF-β superfamily genes in controlling genetic factors related to prolificacy [9]. However, information regarding SMAD5 polymorphisms and their potential associations with both reproductive and growth traits in Garut sheep remains limited.

Among these genes, SMAD5 is a downstream mediator of BMP signaling and has biological functions related to cell proliferation, differentiation, development, and reproductive processes. Previous research in Tibetan sheep identified genetic variation in SMAD5; however, the reported g.51537A > G variant was not significantly associated with litter size [10], indicating that the contribution of SMAD5 polymorphisms may be population or breed dependent. This observation highlights the importance of investigating additional SMAD5 variants in genetically distinct sheep populations.

In particular, the relationship between SMAD5 SNPs and the simultaneous variation of litter size and growth traits has not been adequately characterized in this indigenous Indonesian sheep population. Therefore, this study aimed to identify SNPs in the *SMAD5* gene and evaluate their associations with litter size and growth traits in Garut sheep. The results are expected to provide new information on the genetic basis of reproductive and growth variation in Garut sheep and to support the development of potential molecular markers for future marker-assisted selection and genetic improvement programs.

Materials and Methods

Animal preparation and sample collection

A total of 56 female Garut sheep with complete reproductive records (covering three lambing periods, aged 24–30 months, average body weight of approximately 44–48 kg) were selected from the Garut sheep flock at UPTD Margawati in Garut City, West Java, Indonesia, with the permission of the sheep breeding center. All animals were reared under uniform conditions regarding diet and management practices [4]. Additionally, data on litter size were obtained from UPTD Margawati. Growth traits specifically body weight, body length, chest depth, chest girth rump width, rump height, withers were measured [11]. Blood samples were collected via the jugular vein and stored in EDTA containing tubes for genomic DNA extraction.

Genomic DNA extraction

DNA was extracted from blood samples collected from the jugular veins of Garut sheep. The samples were placed in tubes containing ethylenediaminetetraacetic acid (EDTA) and stored at -20°C until extraction. Genomic DNA was isolated from whole blood using a DNA extraction kit (Geneaid-gSYNCTM) according to the manufacturer's instructions. To evaluate the quality of the obtained genomic DNA, electrophoresis was performed using a 1% agarose gel (w/v in 1x TAE buffer). Genomic DNA concentration and quality were assessed using a Nanodrop spectrophotometer, following the manufacturer's protocol (BioDrop LITE; Biodrop, UK) [12].

PCR Primers' Designing

Primer-BLAST at the National Center for Biotechnology Information (NCBI) was used to design primers for the *SMAD5* gene (GenBank NC_056058.1); the designed amplicon length was adjusted to cover the largest possible fragment while maintaining PCR accuracy. As a result, the sequences for the forward primer (5′- TACCTCCAGTGTTAGTGCCT – 3′) and the reverse primer (5′- GCTGAAATGGACTTCCTGGT – 3′) were obtained.

PCR Conditions

PCR amplifications targeting the specified 243 bp amplicons were conducted using a gradient thermocycler (Nexus, Eppendorf, Germany). The PCR process was executed in a total volume of 50 µL, which included 3 µL of genomic DNA, 1 µL each of forward and reverse primers, 25 µL of Taq Polymerase, and 20 µL of ddH_2O. The PCR protocol involved an initial denaturation at 95°C for 1 min, followed by 39 cycles of denaturation at 95°C for 15 s, primer annealing at 55.3°C for 15 s, and polymerase extension at 72°C for 10 s, concluding with a final extension at 72°C for 5 min. Before sequencing, the anticipated 243 bp of the PCR products were verified using 1% (w/v in 1x TAE buffer) agarose gel electrophoresis.

DNA Sequencing

Amplified 243 bp *SMAD5* gene fragments were purified using a commercial purification kit. The purified products were then sent for direct Sanger sequencing (Genetika Science, Jakarta, Indonesia) in both forward and reverse directions. The identification of nucleic acid variations was meticulously carried out using MEGA 11 by examining the original electropherogram. In accordance with the recommended technical parameters for visualized electropherograms only the distinct nucleotide peaks corresponding to the representative genotypes were included for alignment with the genomic sequences of the *SMAD5* gene (GenBank accession no. NC_056058.1), while less reliable readings were excluded from further analysis.

Protein Interaction Networks Predicted by STRING Database

Using the STRING database version 11.029 (https://string-db.org), we forecasted the networks of protein interactions that include SMAD5. This database serves as an effective resource for anticipating protein interactions [13].

Bioinformatic and in Silico Analyses

Bioinformatic analyses were conducted on the acquired SMAD5 sequences to explore sequence variation and evolutionary connections. MUSCLE, as implemented in MEGA 11, was utilized for multiple sequence alignment. The Neighbor-Joining method, accompanied by bootstrap analysis, was employed to deduce the phylogenetic relationships among the SMAD5 sequences. Additionally, the predicted SMAD5 protein sequences underwent further examination with suitable bioinformatic tools to evaluate sequence conservation, functional domains, and structural features.

Population Genetic Index Calculation

Genetic parameters including genotype frequency, allele frequency, heterozygosity (He), observed heterozygosity (Ho), effective allele numbers (Ne), and polymorphism information content (PIC) were evaluated utilizing Nei's method [14]. The genotypes of the SNPs were examined for adherence to Hardy–Weinberg equilibrium (HW) in accordance with the Hardy–Weinberg law.

Statistical Analysis

The connection between *SMAD5* gene polymorphisms and traits related to growth and reproduction, including litter size at first parity, average litter size over the initial three parities, average birth weight at first parity, and average birth weight across the first three parities, was analyzed using the general linear model (GLM) procedure in SPSS version 22. The statistical model was as follows:

$$Yij = \mu + Gi + eij$$

In this context, Yij represents the observed phenotypic value, μ denotes the mean value for the entire population, Gi signifies the fixed effect attributed to genotype, and eij stands for the random residual error. Since reproductive traits were examined separately as traits of the first parity and as average traits over the initial three parities, parity was inherently integrated into the trait definition. Consequently, it was not considered an additional effect in the model. The findings are presented as the average along with the standard deviation (SD), and statistical significance between mean values was analyzed by Tukey's post hoc test at $p < .05$. All animals used in the association analysis originated from the same

conservation farm and were maintained under uniform feeding and management practices, which reduced possible environmental differences among them.

Results

Polymorphism in Gene

A 243 bp segment of the *SMAD5* gene was successfully amplified in Garut sheep from 56 specimens using PCR and electrophoresis (Fig. 1). The sequencing peak maps for these genes illustrating the mutated sites are shown in Fig. 2.

Population Genetic Analysis of SMAD5 gene in Garut Sheep

Sequencing analysis revealed three distinct genotypes: AA, AG, and GG, with genotype frequencies of 0.16, 0.46, and 0.38, respectively (Table 1). The allele frequencies were determined to be 0.39 for the A allele and 0.61 for the G allele. These genotypes adhered to the Hardy-Weinberg equilibrium ($p < 0.05$). Furthermore, the observed heterozygosity (Ho) was 0.52, while the expected heterozygosity (He) was 0.48. The polymorphic information content (PIC = 0.36) suggested that the SNP exhibited moderate polymorphism, with values ranging from 0.25 to 0.50 (Tabel 2).

Table 1
Genotype and allele frequencies of *SMAD5* gene in the Garut sheep

Locus	Genotype	Genotype Frequency (no.)	Allele	Allele frequencies
rs162301116	AA	0.16(9)	A	0.39
	AG	0.46(26)	G	0.61
	GG	0.38(21)		

Table 2
Population genetic analysis of single nucleotide polymorphism of *SMAD5* in Garut sheep

SNP Locus	Homozygosity (Ho)	Heterozygosity (He)	Polymorphism information content (PIC)	Effective Allele Numbers (Ne)	Chi square test (p-value)
rs162301116					
	0.52	0.48	0.36	1.912	0.04

Association of SMAD5 genotypes with Growth traits

In a study involving 56 female Garut sheep, the influence of the loci in the *SMAD5* gene on seven growth traits was assessed (Table 3). The relationships between genotypes and these traits were analyzed using the General Linear Model (GLM). Within this model, three genotype categories were considered as

factors, and the age of the sheep was included as a fixed effect. The analysis showed that sheep with the GG genotype exhibited significantly higher body weight, rump width, and withers height compared to other genotypes ($p < 0.05$) among the growth traits. For the other traits, no significant differences were found between the genotype groups (Table 3).

Table 3
Association between locus within *SMAD5* gene and growth traits in Garut Sheep

Growth traits	**Genotype**			**p-Value**
	AA	**AG**	**GG**	
Body weight	**41.17 ± 7.36^{b}**	**49.80 ± 14.05^{ab}**	**50.23 ± 13.92^{a}**	**0.04**
Body length	79.89 ± 7.04	79.55 ± 4.93	80.28 ± 4.83	0.72
Chest depth	45.78 ± 2.53	46.07 ± 3.17	46.41 ± 3.06	0.59
Chest girth	84.27 ± 4.39	83.76 ± 5.54	85.32 ± 5.92	0.47
Rump width	**17.81 ± 0.68^{b}**	**20.08 ± 1.88^{b}**	**23.95 ± 2.24^{a}**	**0.02**
Rump height	68.33 ± 3.08	70.01 ± 4.70	69.16 ± 3.11	0.27
Withers height	**69.13 ± 4.28^{b}**	**71.05 ± 4.59^{b}**	**74.10 ± 3.30^{a}**	**0.02**
Note: cells with different letters (a, b) have a significant difference ($P < 0.05$)				

Association of SMAD5 genotypes with litter size traits of Garut Sheep

The analysis of the association between the *SMAD5* SNP and reproductive traits (Table 4) revealed a significant association with litter size at first parity ($p < 0.05$). Garut sheep with the AG genotype exhibited a significantly larger litter size (number of offspring per birth) at first parity compared to those with the AA and GG genotypes.

Table 4
Association analysis between *SMAD5* gene polymorphism and reproductive traits in Garut Sheeps

SNP	Genotype	n	Litter Size at First Parity	Average Litter Size Across First Three Parities	Average Birth Weight at First Parity (kg)	Average Birth Weight Across First Three Parities (kg)
rs162301116	AA	9	**1.00 ± 0.71^{c}**	1.11 ± 0.23	2.81 ± 0.28	2.11 ± 0.17
	AG	26	**1.69 ± 0.74^{a}**	1.96 ± 0.24	2.92 ± 0.17	2.72 ± 0.35
	GG	21	**1.52 ± 0.68^{ab}**	1.76 ± 0.17	2.63 ± 0.29	2.41 ± 0.35

Note: cells with different letters (a, b, c) have a significant difference ($P < 0.05$)

Predicted protein interaction networks involving SMAD5

The predicted protein–protein interaction network demonstrated that SMAD5 is functionally associated with several proteins integral to the BMP/TGF-β signaling pathway, including SMAD1, SMAD4, BMPR1A, BMPR1B, BMPR2, ACVR1, SMAD6, and SMAD7 (Fig. 3). This network suggests that SMAD5 may play a role in regulating BMP-mediated intracellular signaling through its interactions with receptor-regulated SMADs and the common mediator, SMAD4. Additionally, the inclusion of MAPK1 and MAPK3 implies potential cross-talk between the BMP/TGF-β and MAPK signaling pathways. The interaction network thus indicates that SMAD5 is functionally linked to proteins involved in BMP/TGF-β signaling, potentially connecting it to reproductive and growth-related traits.

Sequence Conservation and Phylogenetic Analysis of SMAD5 Protein

The alignment of SMAD5 protein sequences across ten mammalian species revealed significant conservation (Fig. 4). The SMAD5 sequence in Ovis aries exhibited a high degree of amino acid similarity with those in Capra hircus, Ovis canadensis, Bos taurus, Bos indicus, Bubalus bubalis, and the other species examined. Most positions in the alignment were conserved, with only a few amino acid changes observed. This substantial conservation suggests that the SMAD5 protein has been highly preserved among the studied mammalian species.

The phylogenetic tree of Neighbor-Joining (NJ) derived from SMAD5 protein sequences demonstrated a close association among Caprinae species (Fig. 5). *Capra hircus* and *Ovis canadensis* were grouped into a distinct cluster, supported by a bootstrap value of 64%, with *Ovis aries* (sheep) located next to this cluster. The node linking these sequences also had a bootstrap value of 64%. Other species, such as *Rattus norvegicus, Bos taurus, Bos indicus, Bubalus bubalis, Sus scrofa, Equus caballus*, and *Homo sapiens*, were found on separate branches. In summary, the topology suggested relatively close

connections among the SMAD5 protein sequences of Caprinae species, although the moderate bootstrap values (64–65%) indicate limited support for some internal branches.

Discussion

Evidence suggests that the discovery of a polymorphic site in the *SMAD5* gene of Garut sheep could play a role in the phenotypic differences observed in traits of economic significance. The level of polymorphism at this locus is moderate, indicating that the variant provides enough information for analyzing population genetics. It also holds promise as a candidate marker for genetic enhancement. Nevertheless, it is important to approach the link between an individual SNP and intricate quantitative traits with care, as growth and reproductive outcomes are influenced by numerous genes in addition to environmental and management elements.

The association between *SMAD5* polymorphisms and growth traits such as body weight, rump width, and withers height can be biologically explained by the known function of SMAD5 as an intracellular mediator in BMP signaling. Type I and type II receptors are activated by BMP ligands, leading to the phosphorylation of SMAD1/5/8 [15]. These proteins interact with SMAD4 and translocate into the nucleus to regulate the transcription of genes involved in cell proliferation, differentiation, and tissue development [16]. Consequently, variations in SMAD5 can influence the efficiency of BMP-mediated signaling and, ultimately, affect growth and tissue development [17, 18]. The BMP–SMAD1/5 pathway has also been shown to promote skeletal muscle growth, indicating that activation of this pathway may contribute to the maintenance of muscle mass and hypertrophy [19–21]. Although these mechanisms have primarily been demonstrated in mammalian models, they provide a biological basis for interpreting the relationship between *SMAD5* variations and body conformation traits in sheep.

The stronger associations observed between body weight and specific body conformation measurements in Garut sheep may reflect the role of BMP signaling in regulating musculoskeletal development. Body weight is a composite phenotype influenced by muscle, bone, and other tissues, whereas rump width and withers height represent structural dimensions closely linked to skeletal development and body size. BMP signaling is known to play a role in the differentiation and development of mesenchymal and skeletal tissues [22–24]. Therefore, the association between the favorable *SMAD5* genotype and these specific traits suggests that this locus contributes to variation in structural growth, rather than exerting a uniform effect across all morphological measurements. The absence of significant associations with other traits further implies that the effect of this SNP may be trait specific, or that the magnitude of the effect is insufficient to overcome environmental and genetic variation within the studied population.

The relationship between *SMAD5* polymorphism and litter size is highly significant, given that SMAD5 is a crucial component of the BMP signaling pathway involved in female reproductive biology. BMP signaling regulates ovarian development, folliculogenesis, oocyte related processes, and other reproductive events through SMAD dependent mechanisms [25, 26]. Specifically, BMP receptors can

activate SMAD1/5 signaling, which subsequently regulates transcriptional responses within reproductive tissues [27]. Experimental evidence indicates that disruptions to SMAD1/5 signaling can impair uterine receptivity and embryo implantation, thereby underscoring the pathway's importance for reproductive success [28, 29]. Thus, the association observed in Garut sheep supports the hypothesis that genetic variation in *SMAD5* may influence reproductive performance through the modulation of BMP-mediated ovarian or uterine functions.

The current findings align with broader evidence indicating that genes within the BMP/TGF-β signaling network are key candidates for prolificacy traits in sheep. For instance, variants in the *BMPR1B* gene have been linked to differences in litter size across several sheep breeds, suggesting that genetic variation in components of the BMP–SMAD receptor pathway can influence reproductive performance [30–32]. However, the association identified with the *SMAD5* gene in Garut sheep cannot be equated to established major-effect prolificacy genes such as *BMPR1B, BMP15*, and *GDF9*. Instead, *SMAD5* likely represents an additional regulatory component contributing to reproductive variation through downstream signal transduction.

Interestingly, previous research on Tibetan sheep reported no significant association between *SMAD5* gene polymorphisms and litter size (number of offspring per birth), despite the gene's expression in reproductive tissues [10]. The discrepancy between those findings and the results of the present study likely reflects differences in the genetic background of specific sheep breeds, allele frequencies, linkage disequilibrium patterns, population structure, environmental conditions, or the functional characteristics of the variants under investigation. These differing results are significant as they indicate that the phenotypic effects of *SMAD5* polymorphisms may not be universal across sheep populations. Consequently, the association detected in Garut sheep may reflect a population-specific genetic effect, necessitating further validation in larger, independent populations.

Protein-protein interaction analysis further reinforces the biological interpretation of these association results. SMAD5 is predicted to interact with SMAD1, SMAD4, BMPR1A, BMPR1B, BMPR2, ACVR1, SMAD6, and SMAD7, placing it within a highly interconnected BMP/TGF-β signaling network. In the canonical pathway, BMPR1 and BMPR2 activate SMAD1/5, which subsequently form a complex with SMAD4 to regulate target gene expression [33]. Meanwhile, SMAD6 and SMAD7 function as inhibitory regulators that provide negative feedback within the pathway [34]. Therefore, genetic alterations affecting SMAD5 have the potential to influence signaling output, not only through its own activity but also through interactions with other pathway components.

The predicted interactions between SMAD5 and both MAPK1 and MAPK3 are also biologically relevant, given that BMP signaling can activate both the canonical SMAD dependent pathway and the non-canonical MAPK dependent pathway [35, 36]. This pathway cross talk provides a mechanism enabling extracellular signals to simultaneously regulate cell proliferation, differentiation, and tissue development [37]. Such signal integration may help explain why a single *SMAD5* variant can be associated with both growth and reproductive phenotypes. However, this protein interaction network merely depicts predicted

functional relationships and does not directly demonstrate that the identified SNPs alter protein-protein interactions. Therefore, functional validation such as gene expression analysis, allele-specific expression, protein activity assays, or reporter assays is required to determine the molecular consequences of these variants.

From a breeding perspective, these findings indicate that the *SMAD5* SNP is a potential candidate marker for simultaneously considering growth and reproductive performance in Garut sheep. This is highly relevant because improvements in growth and prolificacy are desirable for enhancing production efficiency; however, selection based solely on a single candidate SNP could lead to an overestimation of breeding value. Therefore, the observed association requires validation using larger populations, different livestock groups, and additional generations. Furthermore, incorporating other key genes within the BMP/TGF-β pathway alongside genomic information and conventional phenotypic selection will provide a more robust strategy for developing marker-assisted or genomic selection programs for Garut sheep.

Overall, these findings support *SMAD5* as a biologically plausible candidate gene linking growth and reproductive traits via the BMP/TGF-β signaling network. The combination of genotype-phenotype association analysis and protein interaction analysis provides a basis for proposing *SMAD5* as a potential molecular marker in Garut sheep. However, further functional studies are required to determine whether the identified SNP is the direct causative factor or exists in linkage disequilibrium with a functional variant affecting *SMAD5* expression or signaling activity.

Conclusion

In summary, association analysis revealed that the specific SNP is significantly associated with birth size at first parity, as well as with body weight, hip width, and withers height. Thus, *SMAD5* can serve as an effective DNA marker for selecting superior Garut sheep in marker-assisted selection (MAS) breeding programs.

Abbreviations

SMAD5, SMAD Family Member 5; PCR, polymerase chain reaction; GWAS, Genome-wide association studies; bp, base pair; InDels, insertion/deletions; HWE, Hardy-Weinberg equilibrium; SPSS, Statistical product, and service solutions; Ho, homozygosity; He, heterozygosity; Ne, effective allele numbers; PIC, polymorphism information content.

Declarations

Conflict of Interest No conflicts of interest, financial or otherwise, are declared by the authors.

AcknowledgementWe are grateful to UPTD Margawati in Garut City, West Java, Indonesia for providing the necessary samples collection and support during the execution of this research.

Author contributions DW: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing - original draft, Writing - review & editing. DAL and MA: Investigation, Methodology, Software, Validation, Writing – original draft, Writing - review & editing. AS and AK: Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Writing - review & editing. NSA: Formal analysis, Writing - review & editing. WIA: Data curation, Formal analysis. Writing - review & editing. All authors have read and agreed to the published version of the manuscript.

Funding This study was in part supported by the 2026 Regular Fundamental Research Scheme – Ministry of Higher Education, Science, and Technology (Kemdiktisaintek), Republic of Indonesia under Award Number 131/C3/DT.05.00/PL-MULTITAHUN LANJUTAN and 019/KP/LP2M-UP/05/2026.

Data availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate All procedures involving animals were conducted in accordance with the ethical review guidelines of the Faculty of Animal Science at Universitas Perjuangan Tasikmalaya and Universitas Diponegoro (Approval No. 61-09/A-21/KEP-FPP).

Clinical trial number Not applicable.

Generative AI statement The author(s) declare that no Generative AI was used in the creation of this manuscript.

References

1. Boareki MN, Brito LF, Cánovas Á, et al (2021) Estimation of genetic parameters and selection response for reproductive and growth traits in Rideau-Arcott sheep. Can J Anim Sci 101:134–142. https://doi.org/10.1139/cjas-2019-0152
2. Van Donkersgoed J (2025) Ewe litter size and lamb birth weight: Effects on lamb health, performance, and carcass traits. Can Vet J 66:911–914
3. Ziadi C, Bedhiaf-Romdhani S, Molina A (2024) Estimation of Genetic Parameters for Litter Size and Growth Traits in a Prolific Line of Tunisian Barbarine Sheep. Ruminants 4:256–266. https://doi.org/10.3390/ruminants4020018
4. Wijayanti D, Ardigurnita F, Frasiska N, et al (2025) Effect of parity and type of birth on reproduction performance of Garut ewes in Indonesia. Bulletin Peternakan 49:313–318. https://doi.org/10.21059/buletinpeternak.v49i1.100020
5. Çelikeloğlu K, Tekerli M, Erdoğan M, et al (2021) An investigation of the effects of BMPR1B, BMP15 and & GDF9 genes on litter size in Ramlıç and Dağlıç sheep. Arch Anim Breed 64:223–230. https://doi.org/10.5194/aab-64-223-2021

6. Han Y, Cao G, Chen W, et al (2025) The Role of TGF-β Signaling Pathway in Determining Small Ruminant Litter Size. Biology 14:786. https://doi.org/10.3390/biology14070786
7. Li M, He N, Sun R, et al (2022) Expression and Polymorphisms of SMAD1, SMAD2 and SMAD3 Genes and Their Association with Litter Size in Tibetan Sheep (Ovis aries). Genes 13:2307. https://doi.org/10.3390/genes13122307
8. Ghiasi H, Abdollahi-Arpanahi R (2021) The candidate genes and pathways affecting litter size in sheep. Small Ruminant Research 205:106546. https://doi.org/10.1016/j.smallrumres.2021.106546
9. Rahmawati RY, Sumadi S, Hartatik T (2019) Identification of single nucleotide polymorphisms in GDF9 gene associated with litter size in Garut sheep. Indones J Biotechnol 24:51. https://doi.org/10.22146/ijbiotech.42095
10. Sun R, Li M, He N, et al (2022) Molecular Characterization, Expression Profiles of SMAD4, SMAD5 and SMAD7 Genes and Lack of Association with Litter Size in Tibetan Sheep. Animals 12:2232. https://doi.org/10.3390/ani12172232
11. Wijayanti D, Kusmayadi A, Setiaji A (2025) Application of principal component factor analysis to identify key morphological traits in Garut ewes. Journal of Advanced Veterinary Research 15:843–846
12. Wijayanti D, Zhang S, Bai Y, et al (2022) Investigation on mRNA expression and genetic variation within goat SMAD2 gene and its association with litter size. Anim Biotechnol 18:1–9. https://doi.org/10.1080/10495398.2022.2077214
13. Wijayanti D, Bai Y, Zhu H, et al (2023) The 12-bp indel in the SMAD family member 2 gene is associated with goat growth traits. Animal Biotechnology 34:4271–4280. https://doi.org/10.1080/10495398.2022.2144342
14. Nei M (1973) Analysis of Gene Diversity in Subdivided Populations. Proc Natl Acad Sci USA 70:3321–3323. https://doi.org/10.1073/pnas.70.12.3321
15. Yang S, Ning G, Hou Y, et al (2022) Myoneurin regulates BMP signaling by competing with Ppm1a for Smad binding. iScience 25:104495. https://doi.org/10.1016/j.isci.2022.104495
16. Baas R, Sijm A, Van Teeffelen HAAM, et al (2016) Quantitative Proteomics of the SMAD (Suppressor of Mothers against Decapentaplegic) Transcription Factor Family Identifies Importin 5 as a Bone Morphogenic Protein Receptor SMAD-specific Importin. Journal of Biological Chemistry 291:24121–24132. https://doi.org/10.1074/jbc.M116.748582
17. Blázquez-Medela AM, Jumabay M, Boström KI (2019) Beyond the bone: Bone morphogenetic protein signaling in adipose tissue. Obesity Reviews 20:648–658. https://doi.org/10.1111/obr.12822
18. Qian S, Tang Y, Tang Q-Q (2021) Adipose tissue plasticity and the pleiotropic roles of BMP signaling. Journal of Biological Chemistry 296:100678. https://doi.org/10.1016/j.jbc.2021.100678
19. Mina E, Wyart E, Sartori R, et al (2023) FK506 bypasses the effect of erythroferrone in cancer cachexia skeletal muscle atrophy. Cell Reports Medicine 4:101306. https://doi.org/10.1016/j.xcrm.2023.101306

20. Wu M, Wu S, Chen W, Li Y-P (2024) The roles and regulatory mechanisms of TGF-β and BMP signaling in bone and cartilage development, homeostasis, and disease. Cell Res 34:101–123. https://doi.org/10.1038/s41422-023-00918-9
21. Yoshida T, Delafontaine P (2020) Mechanisms of IGF-1-Mediated Regulation of Skeletal Muscle Hypertrophy and Atrophy. Cells 9:1970. https://doi.org/10.3390/cells9091970
22. Chen G, Xu H, Yao Y, et al (2020) BMP Signaling in the Development and Regeneration of Cranium Bones and Maintenance of Calvarial Stem Cells. Front Cell Dev Biol 8:135. https://doi.org/10.3389/fcell.2020.00135
23. Grafe I, Alexander S, Peterson JR, et al (2018) TGF-β Family Signaling in Mesenchymal Differentiation. Cold Spring Harb Perspect Biol 10:a022202. https://doi.org/10.1101/cshperspect.a022202
24. Yang J, Ueharu H, Mishina Y (2020) Energy metabolism: A newly emerging target of BMP signaling in bone homeostasis. Bone 138:115467. https://doi.org/10.1016/j.bone.2020.115467
25. Cheng L, Zhang J, Li D, et al (2025) The impact of different ovarian stimulation protocols on the expression levels of GDF-9 and BMP-15 in cumulus cells of follicles. Front Endocrinol 16:1572388. https://doi.org/10.3389/fendo.2025.1572388
26. Fountas S, Petinaki E, Bolaris S, et al (2024) The Roles of GDF-9, BMP-15, BMP-4 and EMMPRIN in Folliculogenesis and In Vitro Fertilization. JCM 13:3775. https://doi.org/10.3390/jcm13133775
27. Park E-S, Woods DC, Tilly JL (2013) Bone morphogenetic protein 4 promotes mammalian oogonial stem cell differentiation via Smad1/5/8 signaling. Fertility and Sterility 100:1468-1475.e2. https://doi.org/10.1016/j.fertnstert.2013.07.1978
28. Monsivais D, Nagashima T, Prunskaite-Hyyryläinen R, et al (2021) Endometrial receptivity and implantation require uterine BMP signaling through an ACVR2A-SMAD1/SMAD5 axis. Nat Commun 12:3386. https://doi.org/10.1038/s41467-021-23571-5
29. Tang S, Cope DI, Vasquez YM, Monsivais D (2022) BMP/SMAD1/5 Signaling in the Endometrial Epithelium Is Essential for Receptivity and Early Pregnancy. Endocrinology 163:bqac043. https://doi.org/10.1210/endocr/bqac043
30. Han Y, Cao G, Chen W, et al (2025) The Role of TGF-β Signaling Pathway in Determining Small Ruminant Litter Size. Biology 14:786. https://doi.org/10.3390/biology14070786
31. Jia J, Chen Q, Gui L, et al (2019) Association of polymorphisms in bone morphogenetic protein receptor-1B gene exon-9 with litter size in Dorset, Mongolian, and Small Tail Han ewes. Asian-Australas J Anim Sci 32:949–955. https://doi.org/10.5713/ajas.18.0541
32. Zhang X, Zhang L, Sun W, et al (2020) Study on the correlation between BMPR1B protein in sheep blood and reproductive performance. Journal of Animal Science 98:skaa100. https://doi.org/10.1093/jas/skaa100
33. Yoo S-H, Kim JG, Kim B-S, et al (2016) BST2 Mediates Osteoblast Differentiation via the BMP2 Signaling Pathway in Human Alveolar-Derived Bone Marrow Stromal Cells. PLoS ONE 11:e0158481. https://doi.org/10.1371/journal.pone.0158481

34. Zhang F, Sodroski C, Cha H, et al (2017) Infection of Hepatocytes With HCV Increases Cell Surface Levels of Heparan Sulfate Proteoglycans, Uptake of Cholesterol and Lipoprotein, and Virus Entry by Up-regulating SMAD6 and SMAD7. Gastroenterology 152:257-270.e7. https://doi.org/10.1053/j.gastro.2016.09.033
35. Loh H-Y, Norman BP, Lai K-S, et al (2023) Post-Transcriptional Regulatory Crosstalk between MicroRNAs and Canonical TGF-β/BMP Signalling Cascades on Osteoblast Lineage: A Comprehensive Review. IJMS 24:6423. https://doi.org/10.3390/ijms24076423
36. Wu M, Wu S, Chen W, Li Y-P (2024) The roles and regulatory mechanisms of TGF-β and BMP signaling in bone and cartilage development, homeostasis and disease. Cell Res 34:101–123. https://doi.org/10.1038/s41422-023-00918-9
37. Engel-Pizcueta C, Pujades C (2021) Interplay Between Notch and YAP/TAZ Pathways in the Regulation of Cell Fate During Embryo Development. Front Cell Dev Biol 9:711531. https://doi.org/10.3389/fcell.2021.711531

Figures

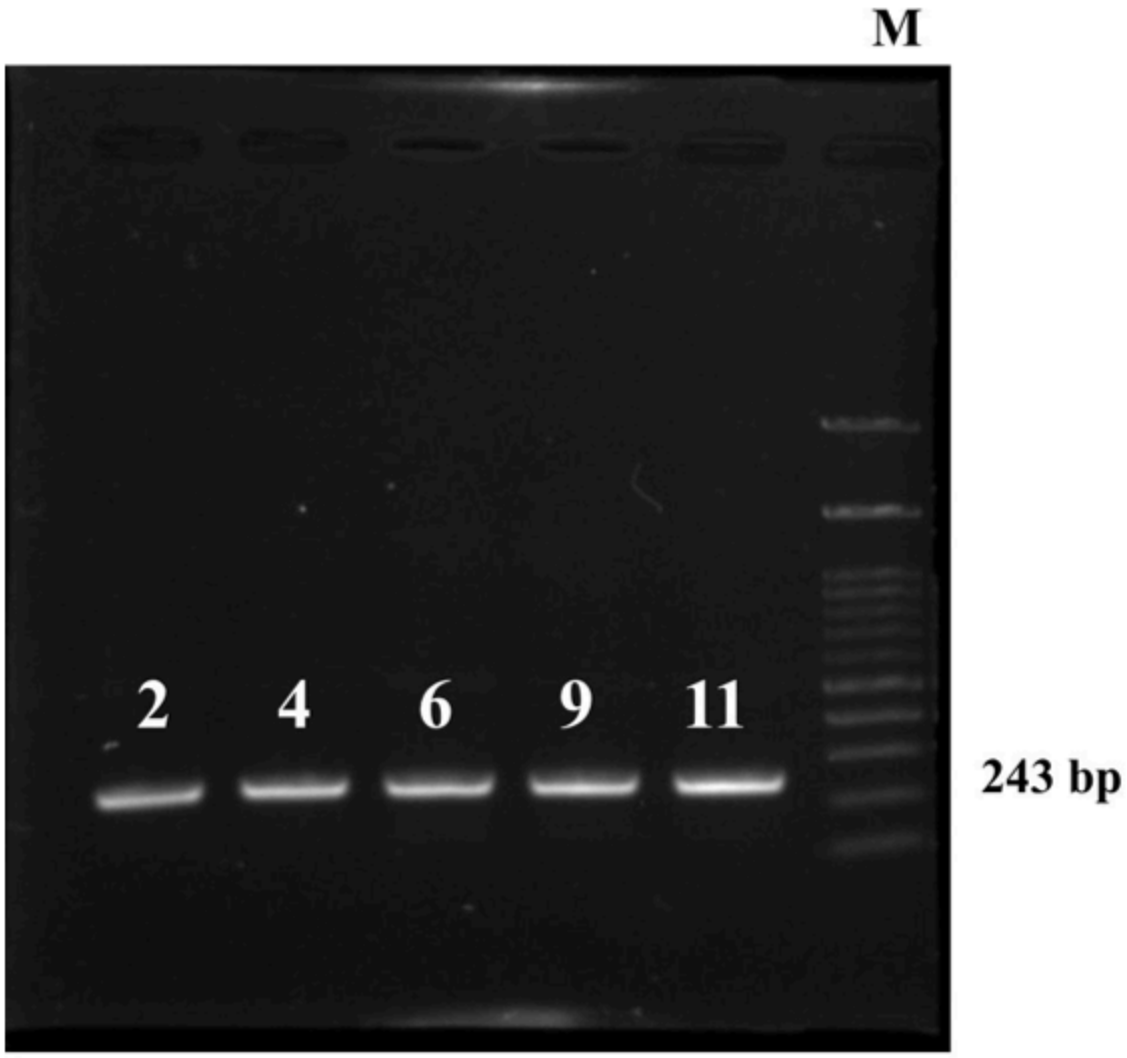


Figure 1

Electrophoresis results of polymerase chain reaction for the *SMAD5* gene in Garut sheep. M represents the 100 bp DNA marker. The numbered rows correspond to sample numbers and display the outcomes of the 243 bp PCR amplification. bp denotes base pairs.

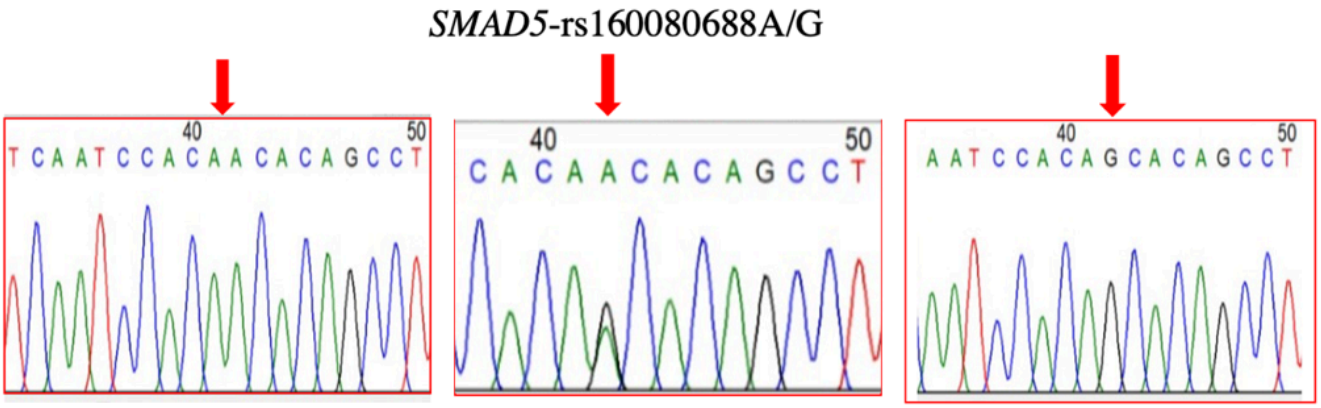


Figure 2

Distribution of *SMAD5* gene SNPs in Garut sheep. SNPs identified through sequencing.

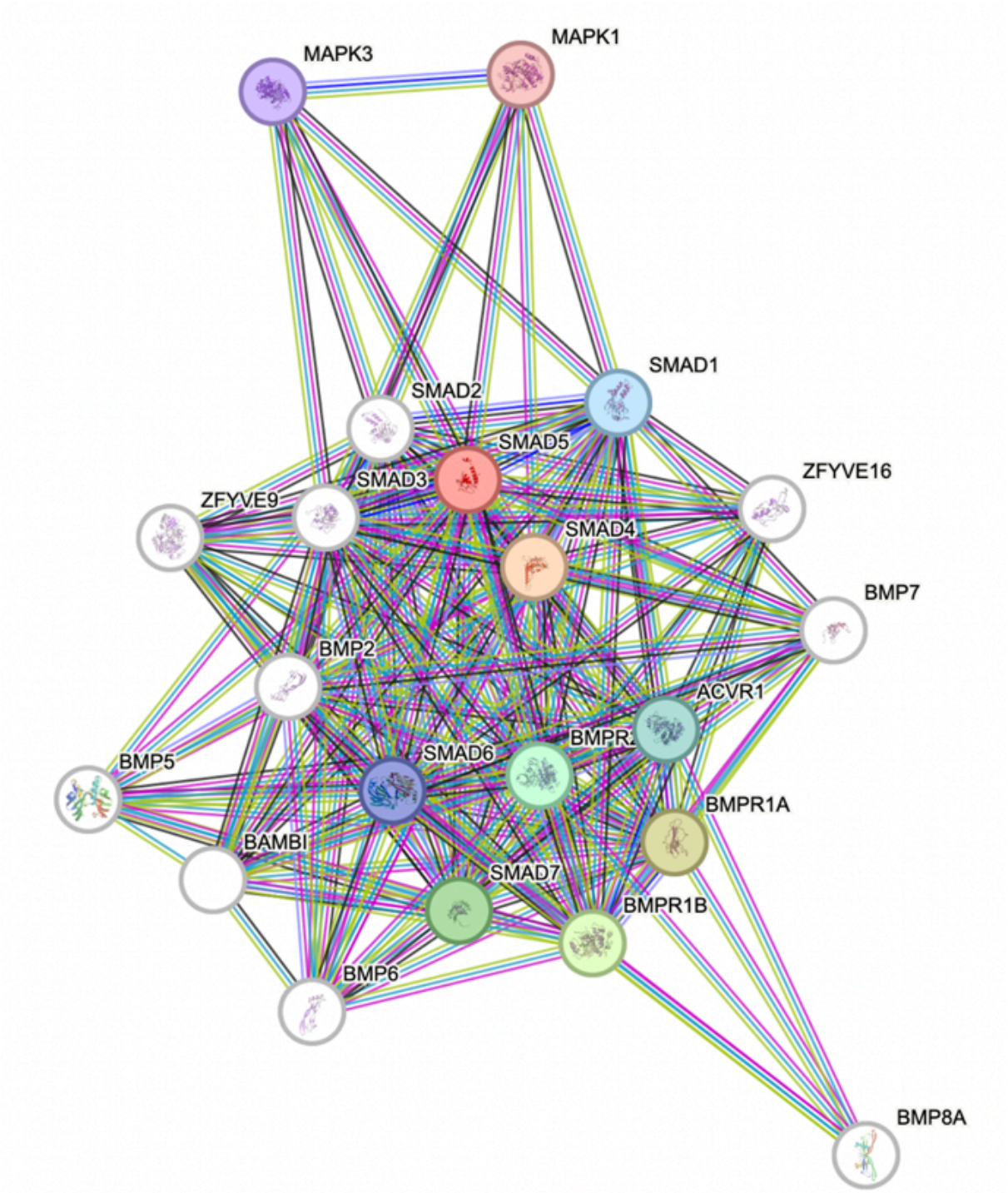


Figure 3

Network summary view shows *SMAD5* gene interaction

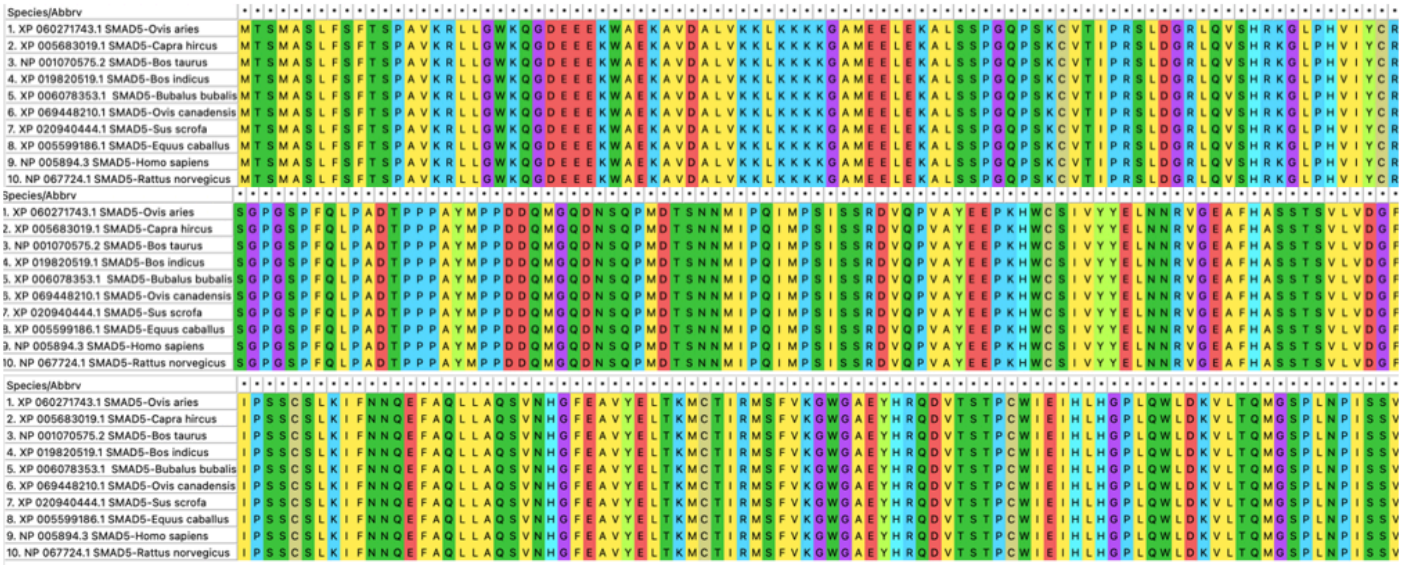


Figure 4

Multiple sequence alignment of SMAD5 protein sequences from 10 mammalian species. Identical amino acid residues are indicated by asterisks (*), whereas conserved and semi-conserved substitutions are indicated by colons (:) and dots (.), respectively.

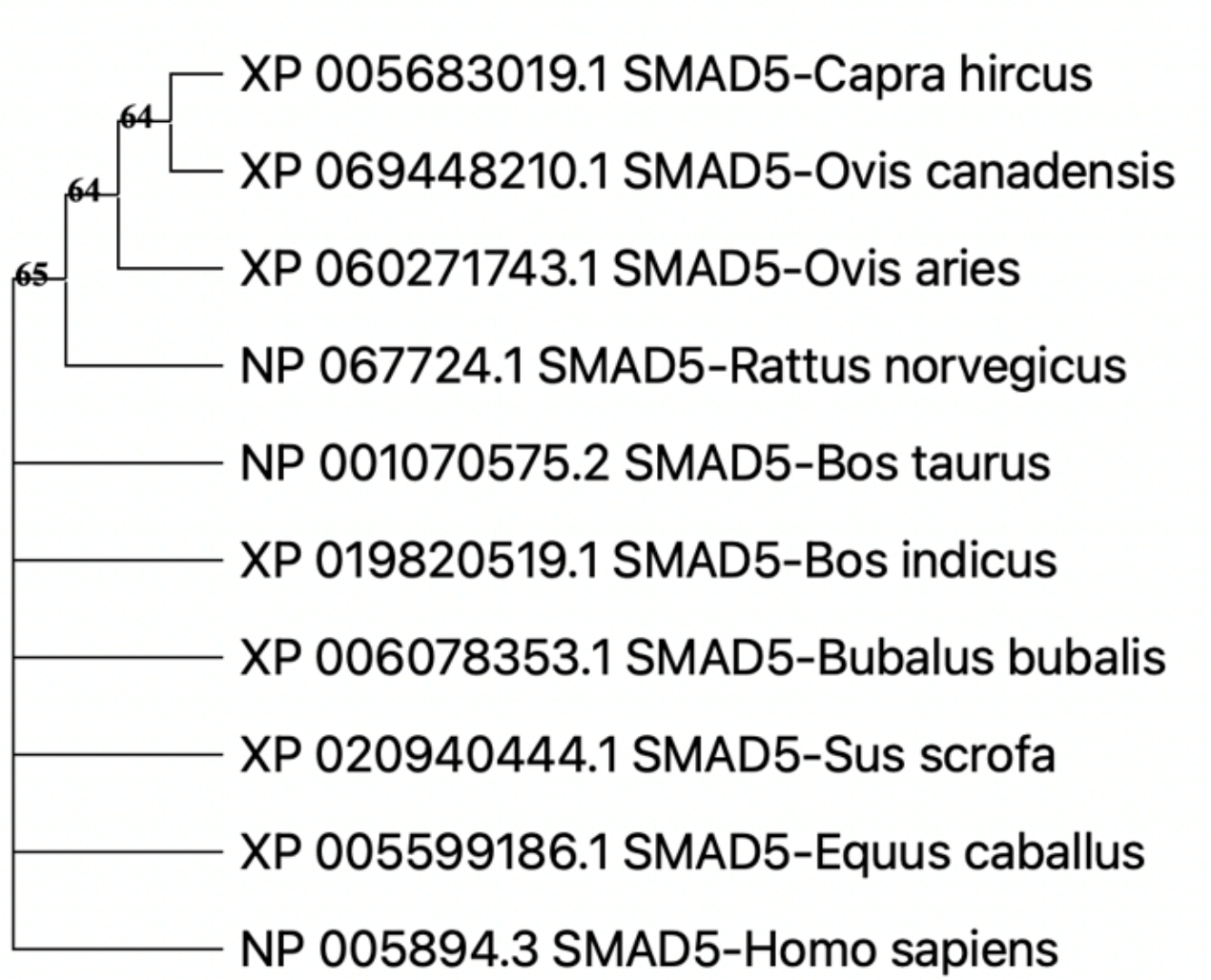


Figure 5

Neighbor-Joining phylogenetic tree based on SMAD5 protein sequences from 10 mammalian species. Bootstrap values (%) based on 1,000 replicates are shown at the nodes.