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Association of *INHA* Gene Polymorphisms with Litter Size Trait in Indonesian Thin-Tailed Sheep

M. Abuzahra[a], D. Wijayanti[b], M. H. Effendi[c,*], I. Mustofa[d], J. P. Munyaneza[e], L. A. Eid[f], & E. N. Ugbo[j]
[a]Doctoral Program in Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia
[b]Department of Animal Science, Perjuangan University of Tasikmalaya, Tasikmalaya, West Java, 46115, Indonesia
[c]Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia
[d]Department of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia
[e]Division of Animal and Dairy Science, Chungnam National University, Daejeon 34134, South Korea
[f]Joint Graduate School, Faculty of Veterinary Medicine, Yamaguchi University, Yoshida, Yamaguchi, Japan
[j]Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, Nigeria
*Corresponding author: mheffendi@yahoo.com
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ABSTRACT

The inhibin alpha (*INHA*) serves as a marker for the number of fully developed ovarian follicles and plays a crucial role in regulating the secretion of pituitary FSH (follicle-stimulating hormone) and the frequency of ovulation. This study aims to examine the effect of *INHA* gene polymorphisms on the litter size of thin-tailed sheep. Detection of single nucleotide polymorphisms (SNPs) in the *INHA* gene was performed using PCR and DNA sequencing techniques. A total of 45 ewes were included in the study. Three SNPs were identified: g.236311141G>C, g.236311367G>A, and g.236311368G>A. Further investigation of the g.236311367G > A variant revealed that individuals with the GA genotype had a significantly higher litter size than those with the AA or GG genotype ($p<0.05$). SNPs at positions g.236311141G/C and g.236311368G/A were non-synonymous mutations resulting in amino acid changes p.A225P and V301I, respectively. Our results suggest that g.236311367G>A loci may serve as a potential molecular marker for improving the litter size trait in thin-tailed sheep.

Keywords: INHA; litter size; molecular marker; sheep; single nucleotide polymorphism

INTRODUCTION

Indonesia's high population density and predominantly Muslim demographic drive a significant increase in demand for sheep meat during festive periods. However, local production constraints and suboptimal reproductive efficiency have escalated prices substantially. To prevent the extinction of native sheep populations, there is a critical need to focus on improved breeding practices and enhance reproductive efficiency (Yue *et al.*, 2023; Yuan *et al.*, 2019; Mazinani & Rude, 2020). The total sheep meat production in Indonesia was 50,702.06 tons in 2021, increased to 52,162.3 tons in 2022, and further increased to 52,998.8 tons in 2023, showing a relatively high annual increase (Badan Pusat Statistik, 2023). Thin-tailed sheep, also known as local lamb or village sheep, is an indigenous breed raised in Indonesia, and these sheep are predominantly raised for meat production (Putra *et al.*, 2021). The genetic diversity of indigenous sheep in Indonesia is reflected in their physical traits and ability to adapt to tropical conditions, which can vary based on their geographical origins and the local environments in which they are raised (Ibrahim *et al.*, 2020).

In sheep, litter size is considered a significant reproductive characteristic that offers substantial economic advantages (Tao *et al.*, 2021; Li *et al.*, 2022; Yuan *et al.*, 2019; Su *et al.*, 2022). Achieving rapid genetic enhancement of the reproductive traits through conventional breeding techniques is a formidable task because of limited heritability and the fact that most quantitative traits are influenced by multiple genes (Chen *et al.*, 2021; Vaishnav *et al.*, 2023; Haile *et al.*, 2020). Marker-assisted selection can be used to increase litter size and optimize production efficiency (Abd El-Hack *et al.*, 2018; Wijayanti *et al.*, 2022). *INHA* plays a pivotal role in regulating ovarian function and hormone secretion, impacting reproductive traits such as litter size. Inhibin is a heterodimer of alpha and beta subunits linked through two sulfur bonds. The beta subunits can be categorized into types A and B. Two variants of INH (*INHA* and INHB) have been identified. Inhibin A is composed of alpha subunits and beta A subunits; inhibin B is composed of alpha subunits and beta B subunits (Yu *et al.*, 2019). Inhibin belongs to the transforming growth factor-β (TGF-β) superfamily and primarily functions to inhibit the synthesis and release of follicle-stimulating hormone (FSH) (Dolatabady *et al.*, 2022).

Studies in pigs and cattle have suggested that *INHA* indicates the quantity of mature ovarian follicles and regulates the release of pituitary FSH and ovulation

frequency (Dolatabady *et al.*, 2022; Wasti *et al.*, 2020). In males, inhibin inhibits testicular spermatogenesis and stimulates testosterone secretion by Leydig cells. This impacts the reproductive capabilities of mammals (Bian *et al.*, 2023; Nikitkina *et al.*, 2021). *INHA* has been linked to reproductive success in the Suhuai pig and Dazu black goat (Wang *et al.*, 2021; Bian *et al.*, 2023). The highest levels of inhibin A were observed in poultry with the largest ovarian follicles. Inhibin B was primarily detected in F5 follicles. Inhibin upregulation in poultry is believed to support androgen production in theca cells (Wasti *et al.*, 2020). In chickens, *INHA* was suggested to regulate egg-laying (Cui *et al.*, 2021). Inhibin modulates reproductive processes through endocrine, paracrine, and autocrine mechanisms, exerting its effects primarily on the pituitary gland. *INHA* inhibits the release of FSH and exerts localized control on estrogen synthesis. Elevated levels of FSH and estrogen are associated with the increased ovulation in animals, hence enhancing the probability of multiple births. Consequently, *INHA* significantly impacts litter size (Bian *et al.*, 2023). These results indicate that an SNP in the 5′UTR regulatory region of the *INHA* gene is significantly associated with reproductive performance and could improve breeding in Suhuai pigs (Liu *et al.*, 2017a). Isa *et al.* (2017) found a significant effect of the CT genotype at the g.3234A>G locus on litter size in the West African Dwarf goat population. Additionally, Liu *et al.* (2017a) noted a significant association between the G759A mutation and litter size in the *INHA* gene of the Jining Grey goat does, suggesting that *INHA* could be a potential marker for high prolificacy in goats. Furthermore, Pillai & Venkatachalapathy (2020) reported that the genotypes of c.911T>C (PP and PQ) had a significant influence on litter size in the *INHA* gene of Malabari goats in India.

The *INHA* gene in sheep is composed of two exons and one intron. *INHA* gene is recognized as a promising candidate for studying litter size in livestock, but studies on *INHA* polymorphisms related to reproductive traits in Indonesian sheep are lacking. Enhancing reproductive performance is crucial in the sheep fattening and breeding industry. This improvement is particularly beneficial for small farmers, as it can enhance their economic livelihoods. In Indonesia, where livestock farming heavily depends on individual and small farms, improving the litter size trait in sheep could substantially increase farmer income.

The inhibin alpha (*INHA*) gene is currently recognized as a candidate gene related to litter size in sheep, goats, and pigs (Dolatabady *et al.*, 2022; Bian *et al.*, 2023; Liu *et al.*, 2017b). Previous studies have highlighted the specific selection of the *INHA* gene in high fecundity sheep. Exon 2 of the *INHA* gene has been particularly interesting, as different sheep breeds exhibit nucleotide variations in this exon. These variations are believed to contribute to the increased litter size and improved reproductive performance in sheep (Dolatabady *et al.*, 2022; Tian *et al.*, 2010). Identifying SNPs and analyzing their associations with litter size is crucial for understanding the genetic basis of reproductive traits. Hence, the primary objective of this investigation is to identify *INHA* polymorphisms in thin-tailed sheep through sequencing and analyzing their associations with litter size.

MATERIALS AND METHODS

Ethics Statement

The Faculty of Veterinary Medicine at Airlangga University approved the use of animals in experiments. The Animal Care and Use Committee (ACUC) granted ethical permission for this study (No: 1.KEH.117.09.2022). Animal experiments were conducted in strict adherence to local legislation and regulations governing animal care.

Animal and Sample Preparation

In total, 45 local thin-tailed sheep, aged 2-4 years with body condition score (BCS) of 2.5-4.0, were subjected to aseptic blood collection from the jugular vein, yielding approximately 5 mL of blood per ewe. EDTA was used as an anticoagulant. Genomic DNA was extracted from the whole blood according to Sambrook & Russel (2001) protocol. The sheep had been raised at Barakah Farm, Wonosari-Malang, Indonesia. The ewes were chosen through random selection. In the study, the animals were provided with leguminous and gramineous grasses equivalent to 10% of their body weight during the day. Additionally, they were given a daily ration of commercial concentrate feed, amounting to 5% of their body weights per head. The concentrate feed had a composition of 15% crude protein.

Primer Design, Sequencing, and Genotyping

The primers were designed using Primer 3 (https://primer3.ut.ee/) with the primer base sequence derived from the National Center for Biotechnology (NCBI) (https://www.ncbi.nlm.nih.gov/search/all/?term=NM_001308579.1) with access code NM_001308579.1. The target region was part of exon 2, spanning 511 bp (fragment 1 F: TATCCTCTCTGTTCCTGCTC and R: GATTCCCTTAGATGCAAGCA). PCR was conducted using the gradient PCR system T100 Thermal Cycler with a reaction volume of 30 µL, consisting of 2.5 µL genomic DNA, 0.5 µL each of forward and reverse primer, 12.5 µL Taq Green PCR Master Mix, and 14 µL ddH_2O. PCR amplification was performed using the following conditions: initial denaturation at 94 °C for 3 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, and elongation at 72 °C for 1 s; final extension at 72 °C for 5 min. PCR products were identified using 1.5% agarose gel electrophoresis with ethidium bromide. Then, a 25-µL PCR product with forward primer was submitted to the 1st BASE DNA for capillary electrophoresis. The DNA sequences were analyzed using the BioEdit program ver. 7.00 (Tom Hall, Ibis Therapeutics, California, USA) and SNPs were confirmed based on electropherogram results.

Statistical Analyses

Genotyping data were calculated using Pop Gene version 1.32 (Yeh *et al*., 2022) to compute allele and genotype frequencies, assess polymorphism information content (PIC), evaluate heterozygosity (HE), determine the number of effective alleles, and use chi-square (χ^2) tests to derive the corresponding p-value (Nei & Kumar, 2000). Ewe populations with p-value < 0.05 from the χ^2 test were considered to adhere to the Hardy–Weinberg equilibrium. For statistical analysis, we used SPSS software, version 25. To investigate the correlation between genotype and litter size of Indonesian Thin-tailed sheep, the model was as follows (Bian *et al.,* 2023):

$$Y_{ij} = \mu + G_i + e_{ij}$$

where Y_{ij} is the litter size phenotype of the individual Indonesian Thin-tailed sheep, μ is the population mean, G_i is the effect of genotype, and e_{ij} is the random error effect.

STRING Database Predicts Protein–Protein Interactions

Proteins regulate several physiological processes and investigating the connections between proteins can help understand the regulation of traits such as litter size. To examine the pathways by which *INHA* influences litter size, we used the STRING database to predict protein–protein interaction networks of *INHA* (https://string-db.org).

RESULTS

PCR Amplicons of Sheep *INHA*

INHA was PCR amplified using primers shown in Figure 1. PCR products were separated using 1.5% agarose gels. The 511 bp size of amplified fragments matched the expected target fragments, signifying the robust specificity of the amplification process.

SNPs Identification by Sequencing

A total of 511 base pairs (bp) derived from exon 2 were sequenced. The insertion of genotype locations adhered to the latest version of the sheep genome assembly, Oar_Rambouillet_v1.0, linked to RefSeq (accession number NM_001308579.1). Sequence analysis of the entire population (n= 45) identified three polymorphisms (Figure 2). According to Table 1, the results obtained by sanger sequencing showed three-point mutations in exons 2 of the *INHA* gene in comparison to the reference sequence (NM_001308579.1). Table 1 provides information including the location and impact of amino acid substitution for single nucleotide polymorphisms (SNPs).

One synonymous polymorphism (SNP2) was found in exon 2 of *INHA*. Additionally, two non-synonymous SNPs, SNP1 (A225P), resulted in a change from arginine to proline, and SNP3 (V301I) led to a substitution from valine to isoleucine were detected. Based on Ensemble data, two novel SNPs (SNP1 and SNP3) identified in this study have not been documented in the other sheep breeds described in the manuscript. Furthermore, our study did not identify any heterozygous individuals harboring the predicted mutation (GC and TA for SNP1). Genetic analysis of Indonesian thin-tailed sheep showed that (g. 236311141G/C and g. 236311367G/A) SNPs loci were in Hardy-Weinberg equilibrium (p>0.05), but the g. 236311368G/A locus did not (p>0.05). The all-SNP sites were in a low polymorphic information content state (PIC < 0.25), as shown in Table 2.

Association of Polymorphism with Litter Size in Thin-Tailed Sheep

We analyzed all SNPs to assess their associations with litter size. Statistical analysis showed that the G/A genotype in SNP2 (g.236311367) or variant rs593506513, had a statistically significant association with litter size (Table 3). No significant associations were found between the other SNPs and the examined parameters related to litter size.

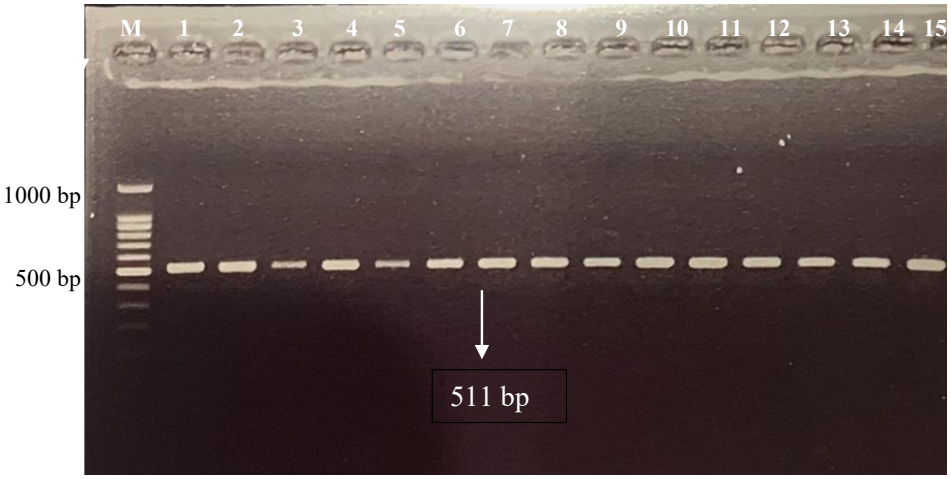

Figure 1. Polymerase chain reaction results of Indonesian Thin-Tailed Sheep for the *inhibin alpha* (INHA) gene. M= 100 bp DNA marker; Lines 1-15 show 511 bp PCR amplification results; bp= base pair.

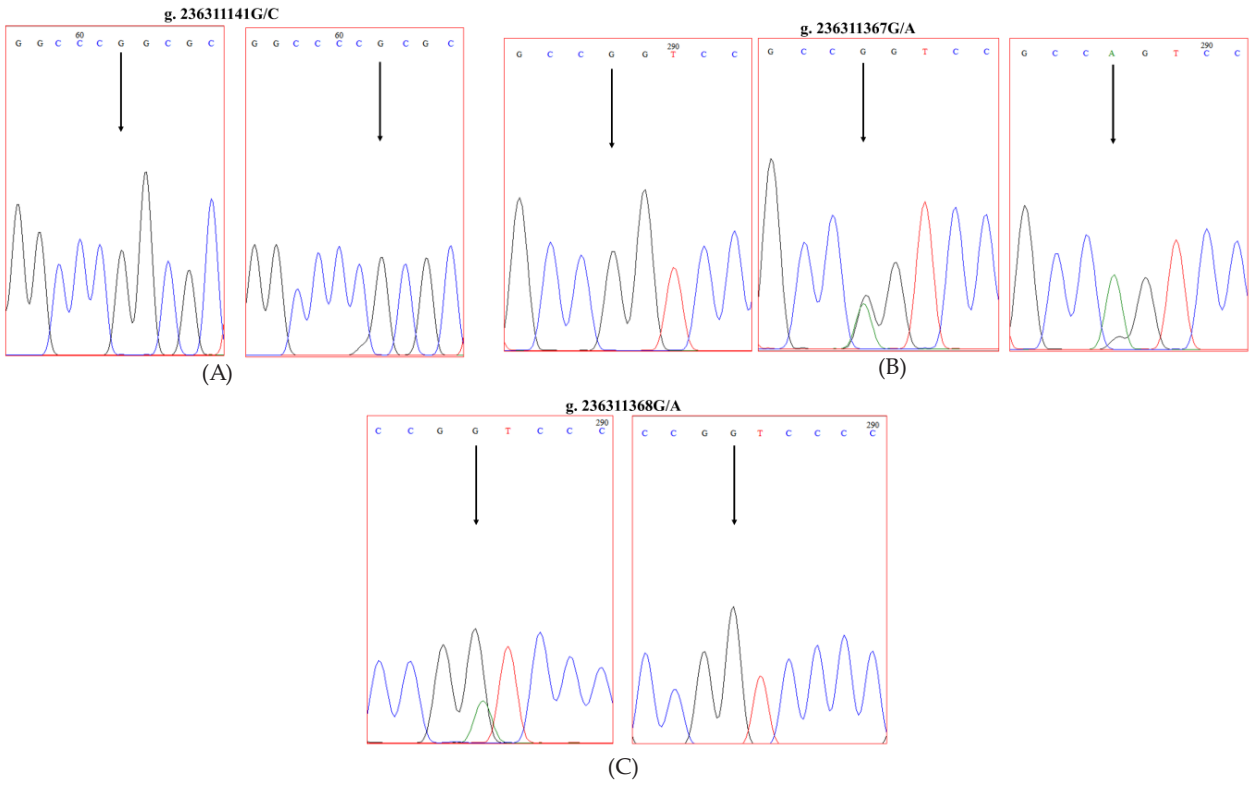

Figure 1. SNPs distribution of the *INHA* gene in Thin-tailed Indonesian sheep. (A-C) SNPs identified by sequencing.

Table 1. Details on SNPs locations and amino acid substitution effects in the *INHA* gene of Indonesian Thin-Tailed Sheep

Gene	Mutation type	SNP	Location	Mutation region	Amino acid change
INHA	G/C	SNP1	g. 236311141	Exon 2	A225P CCG/CCC
	G/A	SNP2	g. 236311367	Exon 2	P300P CCG/CCA
	G/A	SNP3	g. 236311368	Exon 2	V301I CCG/CCA

Table 2. Frequencies of alleles and genotypes of the *INHA* gene in thin tail sheep

Locus	Genotype			Allele frequency		Ho	He	PIC	*Ne*	Chi square test (p value)
g. 236311141G/C	GG	GC	CC	G	C	0.000	0.043	0.038	1.04	0.000
	(44)	(0)	(1)	0.98	0.02					
g. 236311367G/A	GG	GA	AA	G	A	0.044	0.085	0.073	1.09	0.001
	(42)	(2)	(1)	0.96	0.04					
g. 236311368G/A	GG	GA	AA	G	A	0.089	0.085	0.073	1.09	0.750
	(41)	(4)	(0)	0.96	0.04					

Note: PIC= polymorphism information content, Ho= observed heterozygosity, He= expected heterozygosity, Ne= effective allele numbers, HWE= Hardy–Weinberg equilibrium.

Table 3. Association of INHA gene polymorphism and litter size in Indonesian thin-tailed sheep

Locus	Genotype	N	LS (Mean ± SD)
g. 236311141G/C	GG	44	1.90±0.67
	CC	1	2.00
g. 236311367G/A	GG	42	1.85±0.60^{b}
	GA	2	3.00±1.41^{a}
	AA	1	2.00±0.00^{ab}
g. 236311368G/A	GG	41	1.92±0.68
	GA	4	1.75±0.50

Note: Means in the same column with different superscript differ significantly ($p<0.05$). LS=litter size.

Functional Protein Association Networks (STRING)

Interactions between mutant genes were examined using the STRING database. This study demonstrated the interaction between sheep *INHA* and its functional partners by examining the protein–protein interaction networks between *INHA* and growth differentiation factor 9 (GDF9), bone morphogenetic protein 15 (BMP15), FSH receptor (FSHR), and Anti-Mullerian hormone (AMH) (Figure 3).

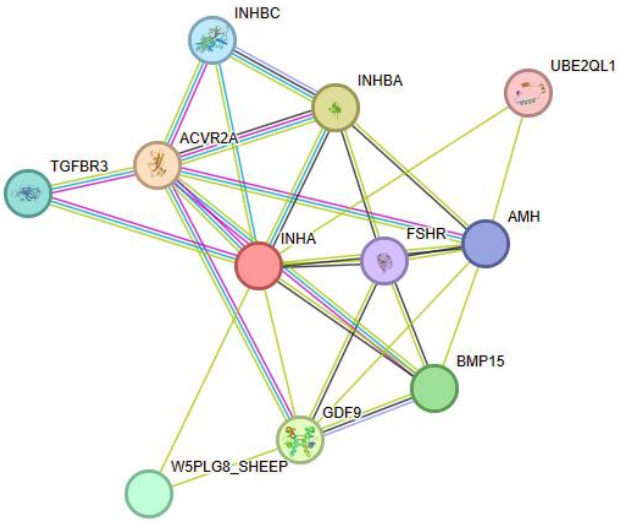

Figure 3. The network summary view shows the inhibin alpha (*INHA*) gene interaction. *INHA* gene concurrence of sheep with line color indicates the type of interaction evidence. Note: Inhibin subunit beta C (INHBC), Inhibin beta A chain (INHB), Uncharacterized protein (UBE2QL1), AMH_N domain-containing protein (AMH), Bone morphogenetic protein 15 (BMP15), Growth differentiation factor 9 (GDF9), Uncharacterized protein (W5PLG8), Follicle-stimulating hormone receptor (FSHR), Transforming growth factor beta receptor 3 (TGFBR3), Activin receptor type-2A (ACVR2A).

DISCUSSION

INHA plays a pivotal role in the suppression of FSH release by the pituitary gland and interacts with GDF9, BMP15, and FSHR (Yu *et al.*, 2019). Immunization against inhibin improves ovarian follicular development, ovulation rate, and transferable embryos by increasing FSH secretion (Dolatabady *et al.*, 2022). *INHA*—synthesized by oocytes—is a member of the TGF-β superfamily, which regulates follicular development (Grieco *et al.*, 2011; Wang *et al.*, 2021; Wasti *et al.*, 2020; Li *et al.*, 2016). *INHA* performs crucial functions in regulating the ovulation rate in many species (Pillai & Venkatachalapathy, 2020). Given the significant roles played by *INHA* in female fertility, it is a potential candidate for enhancing reproductive traits in sheep (Dolatabady *et al.*, 2022). In sheep, *INHA* has been scarcely studied, and the influence of *INHA* polymorphisms on litter size in thin-tailed sheep is unclear. Previous research by Dolatabady *et al.* (2022) identified novel mutations in four Iranian sheep breeds exon 2 of the *INHA* gene, including two SNPs (317C>A and 683C>T) resulting in amino acid changes, with frequencies varying among sheep breeds. In our study, three SNPs were detected within the amplified portion of *INHA* in thin-tailed sheep. Two SNPs, c.674G/C and c.901G/A, resulted in alterations in amino acids at positions 225 and 301 in the *INHA* sequence. A common SNP, rs593506513 (SNP2), was found in both studies, indicating its presence across different sheep breeds. This shared SNP underscores its potential significance in sheep genetics and reproduction, warranting further research into its functional effects in different breeds.

Only one locus had three INHA genotypes at the gene location investigated, whereas the remaining four loci had two *INHA* genotypes in the population (Table 3). The three SNPs variant exhibited low polymorphism (0.25 > PIC). Our findings revealed the absence of individuals with the heterozygous TA genotype at this genetic locus. Our study has limitations because the sample size of sheep included was relatively small. Consequently, expanding the sample size would allow for the inclusion of three genotypes, thereby resulting in the increased PIC value. In addition, some sheep deviated from HWE ($p \leq 0.05$) at the examined loci (Table 3), suggesting a potential influence of selection.

Non-synonymous mutations are responsible for amino acid substitutions and can significantly impact complex traits. For instance, point mutations in *BMP15*, *MTNR1A*, *BMP7*, and *BMP2* (Calvo *et al.*, 2020; Zhang *et al.*, 2019; He *et al.*, 2019) have been shown to affect litter size significantly. There has been a growing focus on the significance of synonymous mutations in influencing reproductive characteristics. A synonymous mutation in *MTNR1A* had a strong correlation with reproductive seasonality in the Rasa Aragonesa sheep (Martínez-Royo *et al.*, 2012). Consistently, a synonymous mutation in the TGF-β-induced factor homeobox 1 gene was strongly associated with litter size in the small-tailed Han sheep (Wang *et al.*, 2020). Significant genetic variation was identified within the luteinizing hormone beta polypeptide gene, demonstrating a strong association with litter size in the small-tailed Han sheep (Wang *et al.*, 2020). Consistently, a synonymous mutation in *FSHR* was highly correlated with litter size in both small-tailed Han sheep and Hu sheep (Pan *et al.*, 2014). These findings underscore the significance of genetic alterations as valuable markers for enhancing sheep fertility.

In this study, a g.236311367G>A synonymous mutation exhibited a significant association with litter size in Indonesian thin-tailed sheep. Specifically, individuals with the GA genotype displayed larger litter sizes than those with the AA or GG genotype ($p<0.05$). Synonymous mutations, once overlooked, are now recognized for their significant roles in influencing reproductive traits. For instance, a synonymous mutation in the melatonin receptor 1A gene was identified in Rasa Aragonesa sheep, showing a strong association with reproductive seasonality (Martínez-Royo *et al.*, 2012). In Small Tail Han sheep, a synonymous mutation in the luteinizing hormone beta polypeptide gene was found to be highly correlated with litter size (Wang *et al.*, 2020). Additionally, a synonymous mutation in the follicle-stimulating hormone receptor gene was associated with litter size in various sheep breeds, including Small Tail Han and Hu (Pan *et al.*, 2014). These discoveries highlight the potential of synonymous mutations as valuable markers for enhancing sheep fecundity. Many studies have reported that *INHA* is involved in reproductive function (Wang *et al.*, 2021; Wasti *et al.*, 2020; Bian *et al.*, 2023; Cui *et al.*, 2021; Yu *et al.*, 2019). The SNP at g.236311367G > A was reported in four Iranian Indigenous sheep (Dolatabady *et al.*, 2022).

The connection between the BMP15 prodomain and *INHA* is firmly established, indicating that the BMP15 prodomain might oversee the collaboration between BMP15 and GDF9. Despite no direct proof of a physical interaction between the prodomains of GDF9 and *INHA*, considering the functions of prodomains in related TGFB family members, it's plausible that the GDF9 prodomain could also interact with *INHA* to modulate their post-secretion functions (Heath *et al.,* 2017). AMH signaling is crucial for determining sex and regulating gonadal function by controlling the number of follicles and selecting the dominant one. In the ovarian function regulation within the TGF-β family, AMH and inhibin play complementary roles. The heightened expression of *INHA* in the ovaries of FecBBB/B+ sheep, responsible for encoding the inhibin-α subunit, suggests a potential association with inhibin levels (Ma *et al.,* 2023). Protein interaction networks play a critical role in understanding complicated features such as reproduction, as many physiological processes depend on the connections between many proteins. The *INHA* protein interacts with multiple proteins involved in follicular growth, including *GDF9, AMH, FSHR*, and *BMP15* (Chu *et al.,* 2007). In the g.236311367G>A gene of *INHA*, thin-tailed sheep with mutant-type alleles (GA, AA) have bigger litters than ewes with wild-type alleles (GG). These results show that *INHA* may play a part in the development of ovarian follicles, especially when there is a synonymous mutation. It may work with *GDF9, AMH, FSHR*, and *BMP15*. This could explain the variations in fertility reported in thin-tailed Indonesian sheep. Our findings indicate that a synonymous mutation in *INHA* may contribute to improving litter size traits in sheep.

CONCLUSION

This study identified a total of three SNPs sites (g. 236311141, g. 236311367, and g. 236311368) in the *INHA* gene of Indonesian Thin-tailed sheep. Among these loci, the g.236311367G>A SNP was found to have a significant association with litter size. The g.236311367G>A locus has the potential to serve as a genetic marker for reproduction traits, including litter size, for future breeding purposes.

CONFLICT OF INTEREST

The authors declare no conflict of interest with any organization or third party regarding the material discussed in this research.

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REFERENCES

Abd El-Hack, M. E., S. A. Abdelnour, A. A. Swelum, & M. Arif. 2018. The application of gene marker-assisted selection and proteomics for the best meat quality criteria and body measurements in Qinchuan cattle breed. Mol. Biol. Rep. 45:1445-1456. https://doi.org/10.1007/s11033-018-4211-y

Bian, Z., K. Li, S. Chen, C. Man, F. Wang, & L. Li. 2023. Association between *INHA* gene polymorphisms and litter size in hainan black goats. PeerJ 11:e15381. https://doi.org/10.7717/peerj.15381

Badan Pusat Statistik. 2023. Produksi Daging Domba menurut Provinsi, 2021-2023. https://www.bps.go.id/id/statistics-table/2/NDgzIzI=/produksi-daging-domba-menurut-provinsi.html

Calvo, J. H., L. Chantepie, M. Serrano, M. P. Sarto, L. P. Iguacel, M. Á. Jiménez, J. L. Alabart, J. Folch, S. Fabre, & B. Lahoz. 2020. A new allele in the BMP15 Gene (FecXRA) that affects prolificacy co-segregates with FecXR and FecXGR in rasa aragonesa sheep. Theriogenology 144:107–111. https://doi.org/10.1016/j.theriogenology.2020.01.010

Chen, S., L. Tao, X. He, R. Di, X. Wang, & M. Chu. 2021. Single-nucleotide polymorphisms in FLT3, NLRP5, and TGIF1 are associated with litter size in small-tailed han sheep. Arch. Anim. Breed. 64:475–486. https://doi.org/10.5194/aab-64-475-2021

Chu, M. X., Z. H. Liu, C. L. Jiao, Y. Q. He, L. Fang, S. C. Ye, G. H. Chen, & J. Y. Wang. 2007. Mutations in BMPR-IB and BMP-15 genes are associated with litter size in small tailed han sheep (*Ovis aries*), J. Anim. Sci. 85:598–603. https://doi.org/10.2527/jas.2006-324

Cui, Z., X. Shen, X. Zhang, F. Li, F. K. Amevor, Q. Zhu, Y. Wang, D. Li, G. Shu, Y. Tian, & X. Zhao. 2021. A functional polymorphism of inhibin alpha subunit at MiR-181b-1-3p-binding site regulates proliferation and apoptosis of chicken ovarian granular cells. Cell Tissue Res. 384:545–560. https://doi.org/10.1007/s00441-020-03356-w

Dolatabady, M. M., S. Safari, P. Aslanlo, H. Soleimani, & J. H. Zad. 2022. Polymorphism of the ovine BMP15, INHBA and INHA candidate genes for litter size in four Iranian indigenous sheep using PCR-sequencing. JLST 10:1–8.

Grieco, V., B. Banco, A. Ferrari, A. Rota, M. Faustini, & M. C. Veronesi. 2011. Inhibin-α immunohistochemical expression in mature and immature canine sertoli and leydig cells. Reprod. Domest. Anim. 46:920–923. https://doi.org/10.1111/j.1439-0531.2011.01784.x

Haile, A., T. Getachew, T. Mirkena, G. Duguma, S. Gizaw, M. Wurzinger, J. Soelkner, O. Mwai, T. Dessie, A. Abebe, Z. Abate, T. Jembere, M. Rekik, R. N. B. Lobo, J. M. Mwacharo, Z. G. Terfa, G. T. Kassie, J. P. Mueller, & B. Rischkowsky. 2020. Community-based sheep breeding programs generated substantial genetic gains and socioeconomic benefits. Animal 14:1362–1370. https://doi.org/10.1017/S1751731120000269

He, X., Z. Zhang, Q. Liu, & M. Chu. 2019. Polymorphisms of the melatonin receptor 1A gene that affects the reproductive seasonality and litter size in small tail han sheep. Reprod. Domest. Anim. 54:1400–1410. https://doi.org/10.1111/rda.13538

Heath, D. A., J. L. Pitman, & K. P. McNatty. 2017. Molecular forms of ruminant BMP15 and GDF9 and putative interactions with receptors. Reproduction 154:521-534. https://doi.org/10.1530/REP-17-0188

Ibrahim, A., I. G. S. Budisatria, R. Widayanti, & W. T. Artama. 2020. The genetic profiles and maternal origin of local sheep breeds on Java Island (Indonesia) based on complete mitochondrial DNA D-loop sequences. Vet. World 13:2625-2634. https://doi.org/10.14202/vetworld.2020.2625-2634

Isa, A. M., M. N. Bemji, M. Wheto, T. J. Williams, & E. M. Ibeagha-Awemu. 2017. Mutations in inhibin alpha gene and their association with litter size in Kalahari Red and Nigerian goats. Livest. Sci. 203:106-109. https://doi.org/10.1016/j.livsci.2017.07.012

Li, M., N. He, R. Sun, Y. Deng, X. Wen, & J. Zhang. 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

Li, W., S. Chen, H. Li, Z. Liu, Y. Zhao, L. Chen, X. Zhou, & C. Li. 2016. A New insertion/deletion fragment polymorphism of inhibin-α gene associated with follicular cysts in large white sows. J. Vet. Med. Sci. 78:473–476. https://doi.org/10.1292/jvms.14-0489

Liu, C., Q. Wei, P. Li, Y. Wu, W. Wu, Q. Li, R. Huang, & F. Shi. 2017a. Association of polymorphism of 5′ UTR region at the INHA gene and the litter size in the Suhuai pig. Journal Nanjing Agricultural University 40:1119-1124.

Liu, Q. Y., Y. Q. He, Y. Ge, M. X. Chu, M. Jin, Y. J. Zhang, J. Y. Wang, X. K. Ma, R. Di, D. W. Huang, & N. Li. 2017b. Polymorphism of inhibin a gene and its relationship with litter size in Chinese indigenous goat. JAPS 27:1488-1495. https://www.thejaps.org.pk/docs/v-27-05/12.pdf

Martínez-Royo, A., B. Lahoz, J. L. Alabart, J. Folch, & J. H. Calvo. 2012. Characterisation of the Melatonin Receptor 1A (MTNR1A) Gene in the Rasa Aragonesa Sheep Breed: Association with Reproductive Seasonality. Anim. Reprod. Sci. 133:169–175. https://doi.org/10.1016/j.anireprosci.2012.06.018

Mazinani, M. & B. Rude. 2020. Population, world production and quality of sheep and goat products. Am. J. Anim. Vet. Sci. 4:291–299. https://doi.org/10.3844/ajavsp.2020.291.299

Ma, X., S. Liu, & S. Tian. 2023. A metaanalysis of mRNA expression profiling studies in sheep with different FecB genotypes. Anim. Genet. 54:225-238. https://doi.org/10.1111/age.13304

Nikitkina, E., A. Krutikova, A. Musidray, & K. Plemyashov. 2021. Search for Associations of FSHR, INHA, INHAB, PRL, TNP2 and SPEF2 Genes polymorphisms with semen quality in Russian holstein bulls (pilot study). Animals 11:2882. https://doi.org/10.3390/ani11102882

Pan, X., S. Liu, F. Li, W. Wang, C. Li, Y. Ma, & T. Li. 2014. Molecular characterization, expression profiles of the ovine FSHR gene and its association with litter size. Mol. Biol. Rep. 41:7749–7754. https://doi.org/10.1007/s11033-014-3666-8

Pillai, H. B. & R. T. Venkatachalapathy. 2020. Association of inhibin alpha gene polymorphism with litter size and growth in Malabari goats of India. Small Rumin. Res. 192:106188. https://doi.org/10.1016/j.smallrumres.2020.106188

Putra, W. P. B., Y. Alwi, & H. Suryani. 2021. Morphometrics characterization of Thin-Tail sheep in lowland and highland areas. Trop. Anim. Sci. J. 44:386-398. https://doi.org/10.5398/tasj.2021.44.4.386

Sambrook, J. & D. Russell. 2001. Molecular Cloning: a Laboratory Manual. 3rd ed. Cold Spring Harbor Laboratory Press, New York.

Su, P., Y. Luo, Y. Huang, Z. Akhatayeva, D. Xin, Z. Guo, C. Pan, Q. Zhang, H. Xu, & X. Lan. 2022. Short variation of the sheep PDGFD gene is correlated with litter size. Gene 844:146797. https://doi.org/10.1016/j.gene.2022.146797

Tao, L., X. He, X. Wang, R. Di, & M. Chu. 2021. Litter size of sheep (*Ovis aries*): inbreeding depression and homozygous regions. Gene 12:109. https://doi.org/10.3390/genes12010109

Tian, X., H. Sun, & Y. Wang. 2010. Genetic polymorphism of INHA gene and its effect on litter size in three sheep breeds. Journal Northwest A F University Natural 38:23-29.

Vaishnav, S., A. Chauhan, A. Ajay, B. L. Saini, S. Kumar, A. Kumar, B. Bhushan, & G. K. Gaur. 2023. Allelic to Genome Wide Perspectives of Swine Genetic Variation to litter size and its component traits. Mol. Biol. Rep. 50:3705-3721. https://doi.org/10.1007/s11033-022-08168-5

Wang, S. Z., E. Guang-Xin, Y. Zeng, Y. G. Han, Y. F. Huang, & R. S. Na. 2021. Three SNPs within exons of INHA and ACVR2B genes are significantly associated with litter size in dazu black goats. Reprod. Domest. Anim. 6:936–941. https://doi.org/10.1111/rda.13927

Wang, W., Y. La, F. Li, S. Liu, X. Pan, C. Li, & X. Zhang. 2020. Molecular characterization and expression profiles of the ovine Lhβ gene and its association with litter size in Chinese indigenous small-tailed han sheep. Animals 10:460. https://doi.org/10.3390/ani10030460

Wasti, S., N. Sah, D. L. Kuehu, Y. S. Kim, R. Jha, & B. Mishra. 2020. Expression of follistatin is associated with egg formation in the oviduct of laying hens. Anim. Sci. J. 91:e13396. https://doi.org/10.1111/asj.13396

Wijayanti, D., S. Zhang, Y. Yang, Y. Bai, Z. Akhatayeva, C. Pan, H. Zhu, L. Qu, & X. Lan. 2022. Goat SMAD family member 1 (SMAD1): MRNA expression, genetic variants, and their associations with litter size. Theriogenology 193:11–19. https://doi.org/10.1016/j.theriogenology.2022.09.001

Yu, D. Y., R. Z. Wu, Y. Zhao, Z. H. Nie, L. Wei, T. Y. Wang, & Z. P. Liu. 2019. Polymorphisms of four candidate genes and their correlations with growth traits in blue fox (*Alopex lagopus*). Genes 717:143987. https://doi.org/10.1016/j.gene.2019.143987

Yuan, Z., Li, W., Li, & Yue. 2019. Association of polymorphisms in candidate genes with the litter size in two sheep breeds. Animals 9:958. https://doi.org/10.3390/ani9110958

Yue, L., W. Li, Y. Pan, X. Lan, Q. Zhang, & C. Pan. 2023. Polymorphism of GTF2A1 gene is associated with litter size in sheep. Theriogenology 208:194–200. https://doi.org/10.1016/j.theriogenology.2023.06.004

Zhang, Z., X. He, Q. Liu, J. Tang, R. Di, & M. Chu. 2020. TGIF1 and SF1 polymorphisms are associated with litter size in small tail han sheep. Reprod. Domest. Anim. 55:1145–53. https://doi.org/10.1111/rda.13753

Zhang, Z., Q. Liu, R. Di, W. Hu, X. Wang, X. He, L. Ma, & M. Chu. 2019. Single nucleotide polymorphisms in BMP2 and BMP7 and the association with litter size in small tail han sheep. Anim. Reprod. Sci. 204:183–92. https://doi.org/10.1016/j.anireprosci.2019.04.001