Research Article

Association Between BMP15 and Gdf9 Gene Polymorphisms and Litter Size in Dorper and Local Sheep

Asrullah As[1], Chairdin Dwi Nugraha[2], Rafika Febriani Putri[1], Ari Ardiantoro[1], Tri Eko Susilorini[1], Wike Andre Septian[1], Kuswati Kuswati[1], Suyadi Suyadi[1]*

[1]Faculty of Animal Science, Brawijaya University, Malang 65145, Indonesia; [2]Research Center for Animal Husbandry, National Research and Innovation Agency Republic of Indonesia (BRIN), Bogor, 16911, Indonesia.

Abstract | Information on genetic diversity related to prolific traits in Dorper and Local sheep raised in Indonesian environments is still limited. This study aimed to identify polymorphisms in the *BMP15* and *GDF9* genes and evaluate their associations with litter size traits in full-blood Dorper and Local ewes at the CV. Kambing Burja farm, East Java, Indonesia. A total of 40 ewes (17 Dorper and 23 Local) were assessed for birth type and litter size. DNA was extracted from blood samples and analyzed using PCR, PCR-RFLP, and DNA sequencing. Six loci were examined: *BMP15* g.6393 C>G, *BMP15* g.6637 C>T, *BMP15* g.6790 C>T, *GDF9* g.792 G>A, *GDF9* g.3010 G>A, and *GDF9* g.913 T>C. Genotype and allele frequencies, Hardy–Weinberg equilibrium, heterozygosity, polymorphic information content (PIC), and associations with litter size were analyzed using SPSS version 26. The results showed that the *BMP15* g.6393, *BMP15* g.6637, *BMP15* g.6790, *GDF9* g.3010, and *GDF9* g.913T loci were monomorphic in both breeds and were not associated with litter size. However, a missense mutation at *GDF9*:g.792G>A in exon 1 was detected in Local sheep, producing two genotypes (GG and GA), while Dorper sheep remained monomorphic. The GA genotype frequency in Local sheep was 0.17, and GG was 0.83, with allele frequencies of 0.91 (G) and 0.09 (A). In Dorper sheep, only the GG genotype was found. While not statistically significant (P>0.05), the GA genotype at *GDF9*:g.792G>A showed a trend toward increased litter size, indicating potential for Marker-Assisted Selection (MAS) in Local sheep.

Keywords | Sheep, *BMP15* and *GDF9* genes, Litter size, Polymorphism, Association

Received | May 08, 2025; **Accepted** | August 04, 2025; **Published** | August 26, 2025
***Correspondence** | Suyadi Suyadi, Faculty of Animal Science, Brawijaya University, Malang 65145, Indonesia; **Email:** suyadi@ub.ac.id
Citation | As A, Nugraha CD, Putri RF, Ardiantoro A, Susilorini TE, Septian WA, Kuswati K, Suyadi S (2025). Association between BMP15 and Gdf9 gene polymorphisms and litter size in dorper and local sheep. Adv. Anim. Vet. Sci., 13(9):1970-1982.
DOI | https://dx.doi.org/10.17582/journal.aavs/2025/13.9.1970.1982
ISSN (Online) | 2307-8316

INTRODUCTION

Sheep are small ruminant livestock with significant development potential in Indonesia's smallholder farming systems due to their easy maintenance, adaptability to various environmental conditions, and role as a source of animal protein. However, according to BPS-Statistics Indonesia (2023), the national sheep population declined from 17.83 million in 2019 to 15.62 million in 2022, primarily due to inadequate genetic improvement programs, limited breeding technology adoption, and conventional management systems (Tienamurti *et al.*, 2020). To address these challenges, genetic improvement through crossbreeding has emerged as an important strategy to increase productivity. Crossbreeding between local sheep and exotic breeds like Dorper is done to combine Dorper's genetic superiority with Indonesian local sheep's adaptability. Local sheep are considered

superior due to their year-round breeding ability, prolific nature, and resistance to endoparasite attacks (Mangun *et al*., 2024).

Twin births significantly enhance maternal productivity indices over single births in sheep (Lahir *et al*., 2015). Litter size is an important reproductive trait for increasing livestock productivity and profitability (Maquivar *et al*., 2021). Despite its low heritability, this can be improved through selection and genetic quality enhancement (Putra *et al*., 2021). The ability to give birth to multiple offspring can rapidly increase population growthc even though pre-weaning weights may not be optimal (Sholikah *et al*., 2021). High litter size not only contributes to population growth but also accelerates the availability of quality breeding stock.

Traditional selection of prospective breeding ewes still relies on conventional methods through phenotypic characteristic assessment. Although long-used, its efficiency is limited due to the relatively long time required. Modern approaches such as Marker-Assisted Selection (MAS) offer potential to accelerate the selection process and improve accuracy in identifying individuals with desired genetic traits. MAS can reduce maintenance costs and enable earlier selection (Abd El-Hack *et al*., 2018).

Litter size and other reproductive traits results from interactions between various environmental and genetic factors. According to Scaramuzzi *et al*. (1993), ovulation rate, which is the number of eggs released in one estrus cycle, is a key factor affecting litter size. Ovulation rate is the result of complex processes of follicular development and growth in the ovary involving interactions between gonadotropin hormones, steroid hormones, and growth factors.

There are three main genes controlling prolific traits in sheep: *BMP15*, BMPR-1B, and *GDF9* (Davis, 2005). These genes have mutations related to high prolific traits that affect the bone morphogenetic signaling system in the ovary (Drouilhet *et al*., 2009). *BMP15* and *GDF9* act as ligands, while *BMPR1B* functions as a receptor (Di *et al*., 2021). BMP proteins play a role in regulating gonadotropin production in the anterior pituitary (Regan *et al*., 2018).

Various mutations in the *BMP15* gene have been reported, such as FecXI, FecXL, FecXH, FecXB, FecXBar, FecXR, FecXG, FecXO, and FecXGR (Davis *et al*., 2001; Hanrahan *et al*., 2004; Davis, 2005; Chu *et al*., 2007; Drouilhet *et al*., 2009; Lassoed *et al*., 2017). The *GDF9* gene also plays a crucial role in ovarian follicle development and ovulation rate (Elieser *et al*., 2018). Mutations in the *GDF9* gene such as FecGH, FecGT, FecGE, FecGF, and FecGV results in hyperprolificacy in heterozygous sheep and sterility in homozygous sheep (Melo *et al*., 2008; Mullen *et al*., 2013; Fiky *et al*., 2017).

Mutations in *BMP15* and *GDF9* genes show varying results regarding increased litter size. The G1 mutation in *GDF9* increases litter size in heterozygotes but causes infertility in homozygotes (Hanrahan *et al*., 2004; Abdelgadir *et al*., 2021). Kirikçi *et al*. (2021) found similar results in Turkey. In Chios sheep, Liandris *et al*. (2012) reported that G1 and G8 mutations significantly increased ovulation rates. However, the G8 mutation was not detected in Kazakh sheep (Amandykova *et al*., 2023), Kermani sheep (Khodabakhshzadeh *et al*., 2016), Iranian Shal sheep (Ghaffari *et al*., 2009), and Chinese Merino sheep (Guan *et al*., 2005).

Polymorphisms of genes controlling reproductive characteristics have been the focus of various studies on different sheep breeds in Indonesia (Maskur *et al*., 2016; Rahmawati *et al*., 2019; Abuzahra *et al*., 2024). However, information about genetic diversity of prolific traits in Local and Dorper sheep raised in Indonesia remains limited. Therefore, this research aims to identify polymorphism patterns in *BMP15* and *GDF9* genes in Local and Dorper sheep populations, and to understand the factors influencing genetic variation at these loci.

MATERIALS AND METHODS

Ethical approval

This study was approved by the ethical clearance committee of Brawijaya University, Indonesia (Ethical Clearance No.192-KEP-UB-2024). All procedures were conducted in accordance with institutional guidelines for the care and use of animals in research.

Management and feeding practices

All sheep were managed under standardized husbandry practices to ensure uniform management conditions throughout the study period. They were housed in elevated wooden-floored sheds to ensure proper ventilation and sanitation. The diet consisted of fresh Pakchong grass (*Pennisetum purpureum* cv. Thailand), provided at 10% of the ewe's body weight daily, supplemented with a formulated concentrate feed containing 14% crude protein. The ingredients used for making concentrate feed include cassava cobs, coffee husks, corn, kapok seeds, cassava peels, coconut meal, CGF (Corn Gluten Feed), SBM (Soybean Meal) or soybean meal, DDGS (Distillers Dried Grains), soy sauce residue, iodised salt, lime, sheep premix, molasses, FML (Fermented Mother Liquor), Soby (Sodium Bicarbonate), and pollard. The selection and usage of these ingredients are tailored based on the sheep's gender and age phase. Clean drinking water was provided ad libitum. Health checks were conducted biweekly to monitor the general condition of the flock.

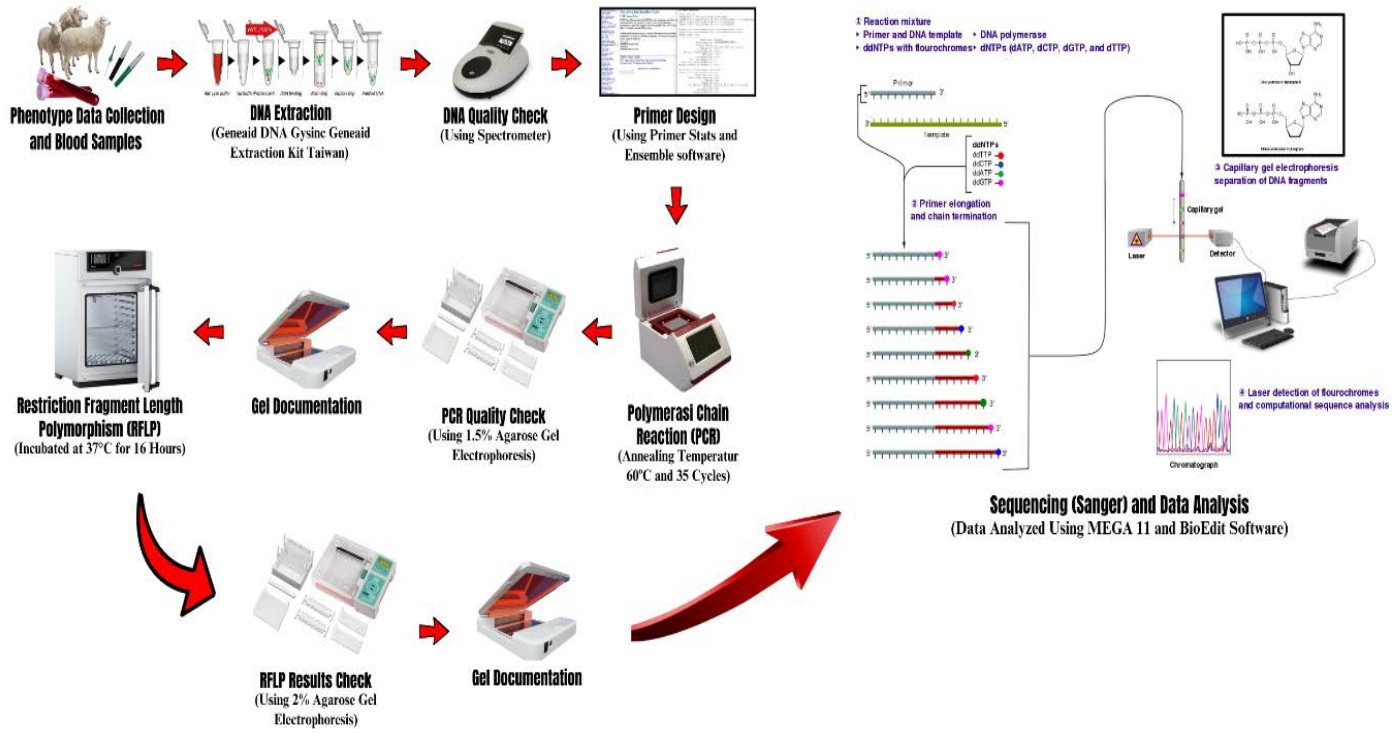

Figure 1: Workflow of genetic analysis.

Laboratory analysis

Genetic analysis was conducted through several sequential steps, including phenotypic data collection (litter size) and blood sample collection from ewes, DNA extraction and quality assessment, polymerase chain reaction (PCR) analysis, restriction fragment length polymorphism (RFLP) analysis, electrophoresis, gel documentation, sequencing analysis, and data analysis. The workflow of genetic analysis in this study is presented in Figure 1.

Sample collection

The study was conducted at CV Kambing Burja, located in Bedali Village, Lawang Subdistrict, Malang Regency, East Java, Indonesia (7°50'20" S, 112°37'50" E). The farm is situated at an altitude of approximately 550 meters above sea level, characterized by a tropical wet climate with an average temperature of 25–30°C and rainfall of 100–200 mm per month. The subjects of the research consisted of 40 ewes (17 Dorper full blood and 23 Local breeds, aged 1.5-3 years) that had given birth and whose litter sizes were recorded. The Dorper sheep used in this study were imported from Australia, characterized by standardized breeding systems and uniform genetic backgrounds, while the local sheep originated from smallholder farmers without structured breeding programs, resulting in greater genetic diversity and variable performance traits. The sheep's peripheral blood was used as biological material. Blood samples were collected in EDTA vacuum tubes via the jugular vein. Subsequently, the blood samples were delivered to containers with refrigerant and stored in a freezer (at -25°C) until they were used for deoxyribonucleic acid (DNA) analysis.

DNA extraction and quality assessment

DNA isolation was performed using the Geneaid DNA Gysinc Geneaid extraction kit (Taiwan) according to the manufacturer's protocol. The determination of quantitative indicators was carried out on the nanodrop spectrophotometer while the concentration of DNA. DNA qualitative characteristics were checked through agarose gel electrophoresis. Purified DNA was stored in a freezer at -25 °C before being used for PCR-RFLP (Polymerase Chain Reaction–Restriction Fragment Length Polymorphism) analysis.

PCR amplification and detection

The PCR reaction process was carried out with a total volume for each sample, consisting of 0.4 µl each of forward and reverse primers, 6.2 µl of Nuclease-Free Water (NFW), 7 µl of GoTaq Green, and 1 µl of DNA sample. This mixture was incubated in a PCR Thermocycler-Biorad machine with an initial DNA denaturation stage at 95°C for 5 minutes. The second stage consisted of 35 cycles, each comprising a denaturation process at 95°C for 10 seconds, primer annealing at 60°C for 20 seconds, and DNA extension at 72°C for 30 seconds. The final stage was primer extension (final extension) at 72°C for 5 minutes, ending with a cooling down process at 12°C for 2 minutes.

The DNA amplification results were visualized using 1.5% agarose gel through electrophoresis. Primers for the *BMP15* Gene with genbank accession number

(ENSOARG00000009372) and the *GDF9* Gene with accession number (ENSOARG00000013229) were designed by ourself using the Ensemble genbank website (https://asia.ensembl.org/). The primer designs were subsequently validated using Primer Stats software to assess primer length, melting temperature (Tm), GC content, hairpin structure formation, dimerization potential (self-dimer or cross-dimer), GC clamp positioning, and to avoid repetitive sequences or homopolymers. Following design validation, PCR amplification was performed and products were verified through electrophoresis and gel documentation. Results confirmed that all PCR products matched the expected target fragment lengths (Table 1 and Figure 2).

RFLP and sequencing analysis

RFLP analysis of the *BMP15* and *GDF9* genes was conducted by digesting PCR-amplified DNA fragments with specific restriction enzymes. The digestion reaction mixture consisted of 5 µL of PCR product, 0.9 µL of nuclease-free water, 0.7 µL of Tango buffer, and 0.4 µL of restriction enzyme (Table 1). The mixture was incubated at 37°C for 16 hours. Digested DNA fragments were separated on a 2% agarose gel, visualized using a Gel Documentation Blue Light System (Gite 965 GW), and fragment sizes were determined using a 100-bp DNA ladder. PCR products of both genes were subsequently subjected to Sanger sequencing. For sequencing, 20 µL of each product was aliquoted into individual tubes, sealed with aluminum foil and plastic wrap, and shipped to PT Genetika Science, Jakarta, for further analysis. PCR-RFLP analysis was performed once for each sample. However, if ambiguous or unclear restriction patterns were observed, the analysis was repeated to ensure accuracy. No discordant genotypes were detected between the PCR-RFLP and sequencing results. All genotypes identified by RFLP analysis were fully consistent with the corresponding DNA sequencing data, confirming the reliability of the genotyping results.

Data analysis

The data were analyzed using Microsoft Excel and SPSS version 26 to calculate genotype frequency, allele frequency, Hardy–Weinberg equilibrium, heterozygosity levels, polymorphic information content (PIC), and to perform gene–trait association analysis with litter size. Sequencing results were analyzed using BioEdit software to examine chromatogram peaks and identify the obtained DNA bases, and MEGA11 was used for DNA base alignment, sequencing, and detection of mutation variants.

RESULTS AND DISCUSSION

Birth types of dorper and local sheep

Reproductive traits such as birth type and litter size reflect the genetic potential and biological capacity of livestock to produce offspring. These traits are directly linked to reproductive efficiency and population development. Observations of reproductive performance in 17 Dorper sheep and 23 Local sheep are presented in Table 2.

Table 1: Primer sequences and PCR-RFLP analysis conditions for *GDF9* and *BMP15* genotyping.

Gene	SNP	Primer sequences (5'-3')	Fragment length (bp)	Annealing temperatur	Enzyme
BMP15	g.6393 C>G	F:GCATAGCTTCTCTGAGCTTC R:GGTCTTCTGAACACTCTGAG	665 Exon 2)	60°C	HaeIII (GG/CC)
	g.6637 C>T	F:CGCTTTGCTCTTGTTCCC R:CTTTCAGGCCCATCATGC	573 (Exon 2)	60°C	HinfI (G/ANTC)
	g.6790 C>T	F:CTCAGAGTGTTCAGAAGACC R:CTCAAGTTGCTGTCTTCACC	735 (Exon 2)	60°C	SpeI (A/CTAGT)
GDF9	g.792 G>A	F:GGAGAAGCTCAGATTGTAGC R:GACAAGATGCTAACCTCCAG	568 (Exon 1)	60°C	HhaI (GCG/C)
	g.3010 G>A	F:CTGCCAAGTATAGCCCTTTG R:CCAGTGGTTGAACCTACATC	744 (Exon 2)	60°C	HpaI (GTT/AAC)
	g.913 T>C	F:GACTGGTATGGGGAAATGTG R:CAGTAAGATCAAGCACCAGG	591 (Exon 1)	60°C	MspI (C/CGG)

Table 2: Birth type and litter size dorper and local sheep.

Breed	Sample size	Litter size ($\bar{x}$ ± SD, Lambs)	Single birth (%)	Twins birth (%)	Triplet birth (%)	CV (%)[1]
Dorper	17	1.37 ± 0.58	67.50	27.50	5.00	42.59
Local	23	1.29 ± 0.61	61.70	31.92	6.38	42.77

[1]CV: Coefficient of variation, expressing the relative variability of litter size (%) in relation to its mean

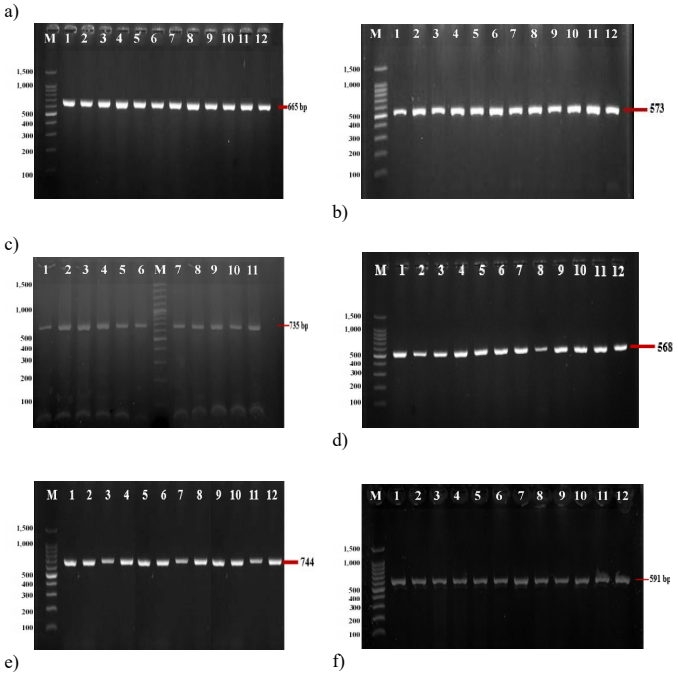

Figure 2: Electrophoretic analysis of PCR-amplified fragments of *BMP15* and *GDF9* genes. a) BMP15 g.6393 (Dorper and Local), b) BMP15 g.6637 (Dorper and Local), c) BMP15 g.6790 (Dorper and Local) , d) GDF9 g.792 (Local), e) GDF9 g.3010 (Dorper and Local), f) GDF9 g.913 (Dorper and Local).

The average litter size of Dorper sheep observed in this study shows variability compared to previous findings on Dorper crosses sheep in various countries. Some studies report both lower values (1.05–1.10) (Abebe *et al*., 2023; Tesema *et al*., 2020) and higher values (1.41–1.65) (Chen *et al*., 2015; Ji *et al*., 2023). The average litter size of Local sheep also varies. Purebred Texel sheep in Europe show values of 1.18–1.48 (Wolf *et al*., 2014; Schmidová *et al*., 2014), while in China and the Czech Republic, it reaches 1.56–1.89 (Tao *et al*., 2021; Štolc *et al*., 2011; Schmidová *et al*., 2014). Among Indonesian local sheep, values range from 1.35 to 2.1 (Sodiq *et al*., 2011; Lusi *et al*., 2022; Hudori *et al*., 2022; Hakim *et al*., 2019), whereas studies in Iran and New Zealand report values of 1.42–2.2 (Talebi *et al*., 2023; Najafabadi *et al*., 2020).

Regarding the litter size values we reported (1.29–1.37), these figures fall within the global average range for the respective breeds under semi-intensive to intensive management systems and align well with recent findings from similar tropical conditions. Litter sizes in local ewes crossed with Dorper rams ranged from 1.31–1.76, and with Awassi rams from 1.25–1.53, under tropical Indonesian conditions, with these values influenced by maternal age and parity (As *et al*., 2025). Similarly, purebred Indonesian local sheep have shown a litter size range of 1.35–2.1, with variation influenced by dam age, parity, nutrition, and management (Sodiq *et al*., 2011; Lusi *et al*., 2022).

The higher of CV values (42%) in Table 2 showed substantial variation in litter size and further indicates that environmental and management factors strongly affect reproductive performance, which may obscure the detection of genetic effects in a limited population. The frequency differences in litter size and birth type are influenced by genetic, physiological, and environmental factors, including gene mutations, genetic selection, management practices, and molecular mechanisms that regulate ovulation rates and follicular development. Montgomery (2024) highlights the complex interplay between genetic mutations and molecular mechanisms that influence ovulation rates and follicular growth in the ovaries, leading to variations in birth types. The *BMPR1B*, *BMP15*, and *GDF9* genes play a critical role in intra-ovarian signaling pathways, contributing signals from oocytes as the main driver of follicular regulation rather than circulating gonadotropin concentrations.

The diversity in age and parity of the sheep used in this study may also contribute to variations in litter size. Hudori *et al*. (2022) and Govur *et al*. (2015) note that factors such as maternal age, parity, body weight, genetics, nutrition, and management influence litter size. Breeding methods and genetic selection are additional factors affecting outcomes. Tao *et al*. (2021) state that inbreeding or outbreeding, selection of sires with superior genetic traits, and reproductive management interventions can lead to differences in reproductive performance. The complexity of these factors results in significant variation in litter size across populations and regions.

Although all sheep were reared under uniform management practices at the same commercial farm, including standardized housing, feed (Pakchong grass and 14% CP concentrate), and health monitoring, the high CV suggests substantial environmental or management influences on reproductive outcomes. Natural variation in age, parity, and physiological status likely contributed to the high coefficient of variation observed among individuals. Kandiwa *et al*. (2020) and Suyadi *et al*. (2019) highlight the importance of seasonal variation and nutritional conditions in determining litter size, especially in tropical environments that present challenges such as heat stress and feed availability. The intensive management system implemented in this research likely optimized reproductive performance across age groups through consistent health monitoring and preventive care.

Therefore, we conclude that the differences in polymorphism status and reproductive performance between our study and previous reports are most likely due to a combination of population structure, genetic background, and environmental factors. The high coefficient of variation observed further reinforces that environmental and

management factors can mask genetic effects, particularly in studies with limited population sizes. Further studies with broader population coverage and larger sample sizes are needed to comprehensively assess the impact of *GDF9* polymorphisms on reproductive performance.

PCR-RFLP ANALYSIS

The PCR-RFLP analysis results of the *BMP15* and *GDF9* genes shown in Figure 3 (*BMP15* g.6393 C>G (HaeIII), *BMP15* g.6790 C>T (SpeI), *GDF9* g.3010 G>A (HpaI), dan *GDF9* g.913 T>C (MspI)) indicate uniform DNA patterns (monomorphic) across all samples of Dorper and Local sheep. The restriction enzymes used did not detect any different restriction sites, and the sequencing results in Figure 3 validate these findings. However, at the *GDF9*|HhaI exon 1 locus, the Local sheep exhibited polymorphism (g.792G>A), while the Dorper sheep remained monomorphic. The HhaI enzyme recognizes the GCG/C cutting site, with a missense mutation involving a G>C base change at this locus.

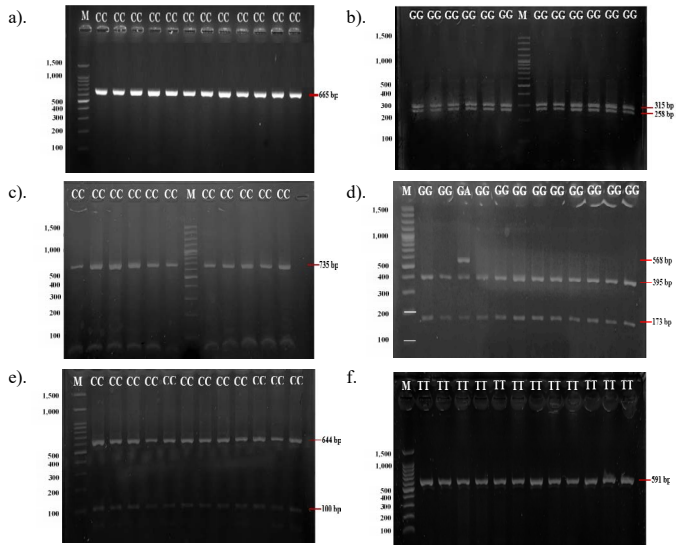

Figure 3: Results of PCR-RFLP analysis of *BMP15* and *GDF9* genes in dorper and local sheep.
a) BMP15 g.6393|HaeIII (Dorper and Local), b) BMP15 g.6637|HinfI (Dorper and Local), c) BMP15 g.6790|SpeI (Dorper and Local), d) GDF9 g.792|HhaI (Local), e) GDF9 g.3010|HpaI (Dorper and Local), f) GDF9 g.913|MspI (Dorper and Local).

The contrasting polymorphism patterns observed across different sheep populations demonstrate the breed-specific nature of genetic variation at fertility-related loci. While previous studies have documented polymorphic status at *GDF9* g.792 in Merino × Garut sheep (Putra *et al.*, 2022), Greek sheep (Liandris *et al.*, 2012), and Kazakh sheep (Amandykova *et al.*, 2023), while Fiky *et al.* (2017) identified polymorphisms at *GDF9* g.913 in Saidi and Ossimi breeds. In contrast, we found that Dorper sheep are monomorphic at *GDF9* g.792, suggesting that each breed has evolved its own distinct genetic landscape. This discrepancy underscores the critical influence of breed-specific genetic architecture on allelic diversity patterns.

The monomorphic status observed in Dorper sheep can be attributed to their distinct evolutionary and breeding history. As a composite breed developed through intensive selection for growth performance and environmental adaptability, Dorper sheep have likely experienced genetic bottlenecks and directional selection that reduced allelic diversity at certain loci. The absence of the A allele at the *GDF9* g.792 site may reflect either founder effects during breed establishment or selective sweeps that eliminated less favorable variants. Conversely, crossbred populations such as Merino × Garut maintain greater heterozygosity due to ongoing genetic admixture between parental breeds, preserving allelic variants that may have been lost in purebred lines.

This breed-dependent variation in polymorphism status has important implications for marker-assisted selection programs. The effectiveness of genetic markers varies significantly across populations depending on their allelic frequencies and linkage disequilibrium patterns. Our results suggest that genetic improvement strategies must be tailored to the specific genetic architecture of target populations, as universal application of polymorphism-based markers may not be appropriate across all sheep breeds.

It is important to note that the Local sheep included in this study were sourced from a single farm in East Java, and therefore represent a specific sub-population with potentially unique genetic and environmental backgrounds. Local sheep across Indonesia exhibit considerable diversity due to regional adaptations, different management systems, and minimal structured breeding programs. As such, the genetic patterns observed in this study particularly the allele frequencies and polymorphism status of the *GDF9* and *BMP15* loci may not be representative of all Indonesian local sheep populations. Future research should expand sampling to multiple geographic regions and sub-populations to better understand the broader genetic architecture and its association with prolificacy traits.

These findings highlight the dynamic nature of genetic diversity within livestock species, where population history, selection pressure, and breeding practices interact to shape contemporary allelic distributions. The heterogeneous polymorphism patterns across sheep breeds emphasize the necessity for population-specific genetic characterization before implementing molecular breeding programs, ensuring genetic improvement strategies align with each population's underlying genetic structure.

Sequencing analysis

The samples sequenced represent the parents of Dorper and Local sheep, with sample selection based on the visualization results of the RFLP analysis. The selected samples are from the polymorphic loci observed in this study and represent each birth type (single, twins, and triplets). The results of sequencing analysis of the *BMP15* g.6393 and *GDF9* g.792 genes are presented in Figures 4 and 5.

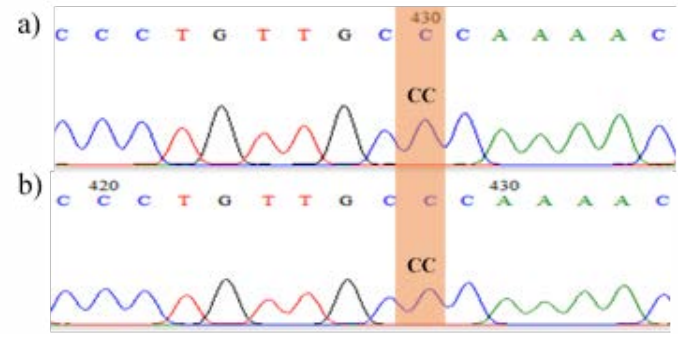

Figure 4: Sequencing results of BMP15 g.6393 gene (Line a: Local Sheep, Line b: Dorper Sheep) a) single birth type, and (b) twins birth type.

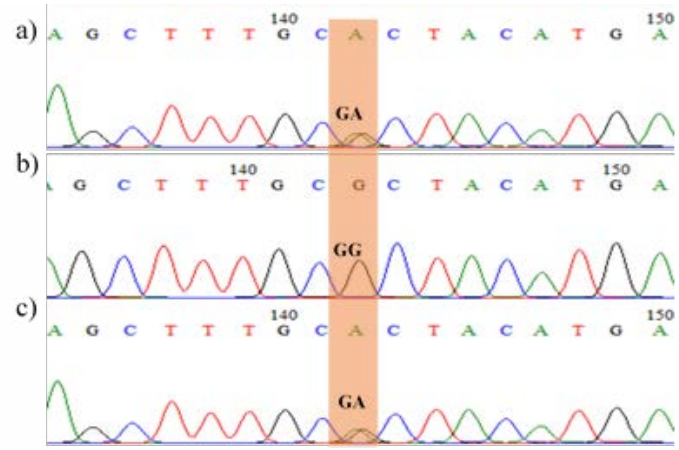

Figure 5: Results of GDF9 g.792 G>A gene sequencing (Line a, b, c: Local Sheep) (a) twin birth type, (b) single birth type, (c) triplet birth type.

The sequencing analysis of the *BMP15* g.6393 gene shows no base changes at the observed locus, indicating no amino acid changes. This result is consistent with the PCR-RFLP analysis, which showed a monomorphic pattern (Figure 3a). However, the Ensembl gene bank data for this loci *BMP15* g.6393 C>G shows a base change from Cytosine (C) to Guanine (G). This mutation is a missense mutation, which may potentially affect the function of the resulting protein.

The sequencing analysis of the *GDF9* g.792 G>A exon 1 gene revealed a DNA base substitution from Guanine (G) to Adenine (A), resulting in an amino acid change from Histidine (CAC) to Arginine (CGC). This mutation is classified as a *missense* mutation. These results are consistent with the PCR-RFLP analysis, which showed polymorphic results (Figure 3d).

Mutations in the *GDF9*|HhaI exon 1 locus are missense mutations. Irham *et al.* (2023) stated that a *missense* mutation occurs when a codon encoding one amino acid changes into a codon encoding a different amino acid, thereby altering the function of the resulting protein. The mutation in the *GDF9* g.792 G>A exon 1 gene has been extensively studied in various sheep breeds, such as MEGA sheep (Merino x Garut) by Putra *et al.* (2022), Kazakh sheep by Amandykova *et al.* (2023), Ujimqin sheep by Ji *et al.* (2023), Mehraban sheep by Ahmadi *et al.* (2016), and Garut sheep by Rahmawati *et al.* (2019). These studies indicate that livestock with the heterozygous GA genotype at the *GDF9* g.792 locus have a higher average litter size compared to those with the homozygous GG genotype.

Allele and genotype frequencies

The analysis results of allele and genotype frequencies are presented in Table 3. The *BMP15* g.6393 C>G (HaeIII), *BMP15* g.6790 C>T (SpeI), *GDF9* g.3010 G>A (HpaI), dan *GDF9* g.913 T>C (MspI) loci were monomorphic in both sheep breeds, while only the *GDF9*|HhaI locus in Local sheep was polymorphic.

The *GDF9* g.792 locus showed that the homozygous GG genotype was dominant over heterozygous GA in Local sheep breed (0.83; 0.17), with G allele being predominant (0.91; 0.09). The *BMP15* g.6393 C>G (HaeIII), *BMP15* g.6790 C>T (SpeI), *GDF9* g.3010 G>A (HpaI), dan *GDF9* g.913 T>C (MspI) locus showed homozygous genotypes (CC, GG, CC, CC, and TT, respectively) with both genotype and allele frequencies of 1 across all sheep. These values were influenced by the limited sample size and samples originating from homogeneous environments and management systems. Moreover, detailed pedigree records were not available for all individuals in this study, limiting our ability to estimate inbreeding coefficients. However, based on management records, the local sheep originated from a limited number of dam lines, which may contribute to the observed genetic homogeneity and monomorphism at several loci. Damayanti *et al.* (2023) noted that variations in allele and genotype frequencies can be influenced by various factors such as selection, gene mutations, interpopulation mixing, inbreeding, and outbreeding. The presence of heterozygous individuals in the population is reflected in polymorphic loci.

Genetic polymorphism in a population can be assessed through allele and genotype frequencies. According to

Gunawan *et al.* (2017), a locus is considered polymorphic if its most common allele and genotype frequencies are less than 0.99. The polymorphism value correlates with the number of alleles found at each locus, with more alleles resulting in higher polymorphism values (Riyanto, 2015). Yuniarsih (2011) explains that variations in allele and genotype frequencies may vary due to selection, mutation, gene flow, or inbreeding. The presence of polymorphic loci in a population is indicated by the occurrence of heterozygous individuals. The allele and genotype frequency values of 1 (one) in this study resulted from the small sample size and homogeneous population, leading to no observed diversity.

Table 3: Allele and genotype frequencies of *BMP15* and *GDF9* genes.

Gene	Breed	Sample size	Allele frequency	Genotype frequency
BMP15 g.6393 C>G (HaeIII)	Dorper	17	C (1) G (0)	CC (1) GG (0) CG (0)
	Local	23	C (1) G (0)	CC (1) GG (0) CG (0)
BMP15 g.6637 C>T (HinfI)	Dorper	17	G (1) T (0)	GG (1) TT (0) GT (0)
	Local	23	G (1) T (0)	GG (1) TT (0) GT (0)
BMP15 g.6790 C>T (SpeI)	Dorper	17	C (1) T (0)	CC (1) TT (0) CT (0)
	Local	23	C (1) T (0)	CC (1) TT (0) CT (0)
GDF9 g.792 G>A (HhaI)	Dorper	17	G (1) A (0)	GG (1) AA (0) GA (0)
	Local	23	G (0.91) A (0.09)	GG (0.83) AA (0) GA (0.17)
GDF9 g.3010 G>A (HpaI)	Dorper	17	C (1) T (0)	CC (1) TT (0) TC (0)
	Local	23	C (1) T (0)	CC (1) TT (0) TC (0)
GDF9 g.913 T>C (MspI)	Dorper	17	T (1) C (0)	TT (1) CC (0) TC (0)
	Local	23	T (1) C (0)	TT (1) CC (0) TC (0)

Heterozygosity values, Hardy-Weinberg equilibrium, and degree of polymorphism

Data on heterozygosity values, Hardy-Weinberg equilibrium, and Polymorphic Information Content (PIC) can be seen in Table 4.

Table 4: Heterozigozity, hardy-weinberg equilibrium (H-W) and PIC value.

Gene	Breed	Sample size	H_e	H_o	(X^2)	PIC
BMP15 g.6393 C>G (HaeIII)	Dorper	17	0	0	0	0
	Local	23	0	0	0	0
BMP15 g.6637 C>T (HinfI)	Dorper	17	0	0	0	0
	Local	23	0	0	0	0
BMP15 g.6790 C>T (SpeI)	Dorper	17	0	0	0	0
	Local	23	0	0	0	0
GDF9 g.792 G>A (HhaI)	Dorper	17	0	0	0	0
	Local	23	0.172	0.191	0.247	0.157
GDF9 g.3010 G>A (HpaI)	Dorper	17	0	0	0	0
	Local	23	0	0	0	0
GDF9 g.913 T>C (MspI)	Dorper	17	0	0	0	0
	Local	23	0	0	0	0

Note: H_e= Heterozygosity expected, H_o= Heterozygosity observed, X^2 tabel 0.05=3.841, PIC= polymorphic information content.

The research results showed genetic equilibrium conditions at several analyzed loci. At the *BMP15* g.6393, *BMP15* g.6637, *BMP15* g.6790, *GDF9* g.3010, and *GDF9* g.913 locus, the Chi-square (X^2) values, observed heterozygosity (Ho), and expected heterozygosity (He) were 0, indicating low heterozygosity with equilibrium conditions where Ho=He. The *GDF9* g.792 locus was also in equilibrium with Ho > He, where Hardy-Weinberg equilibrium analysis using Chi-square (X^2) showed that both Dorper and Local sheep populations were in equilibrium (X^2calculated < X^2table). Although the Polymorphic Information Content (PIC) value was classified as low (<0.25) according to Carsono *et al.* (2014) classification, these results still indicate the presence of polymorphism or genetic diversity in the *GDF9* g.792 gene in both sheep populations, albeit at a low level.

The population in this study was not in Hardy-Weinberg equilibrium, which was likely caused by small population size and non-random mating that triggered genetic drift and inbreeding. The PIC values at all loci showed that only the GDF9 g.792 locus in Local sheep was polymorphic, although low (<0.25). According to Abramovs *et al.* (2020) and Noor (2010), Hardy-Weinberg equilibrium is achieved when genotype frequencies remain stable across generations without migration, selection, and mutation. Carsono *et al.* (2014) classified PIC values into three cat-

egories: highly informative (>0.5), moderately informative (0.25–0.5), and low (<0.25). Heterozygosity values also reflect the level of population diversity; low heterozygosity (<0.5) indicates low diversity, while high values (>0.5) indicate high diversity (Tambasco *et al.*, 2003). These results provide preliminary information regarding genetic diversity in Local sheep and Texel crosses, which can be used for further studies related to livestock productivity traits.

Association of *BMP15* gene with litter size

The analysis results showed that at all analyzed loci, only one genotype (monomorphic) was detected in each sheep breed: the CC genotype at *BMP15*|HAEIII and *BMP15*|SpeI loci, and the GG genotype at the *BMP15*|HinfI locus. Because no alternative genotypes such as CG, GT, or CT were found in the analyzed samples, there were no genotype variations that could be associated with increased litter size. The results of the association analysis between the *BMP15* gene and litter size are presented in Table 5. The difference in average litter size between the two sheep breeds indicates potential influence from other genetic factors or environmental interactions affecting these results. The mutation at the *BMP15* g.6637 C>T gene locus is also known as the FecXB variant. The research results showed no diversity in this study, similar to findings by Amandykova *et al.* (2023) in Kazakh sheep. In contrast, Liandris *et al.* (2012) found FecXB mutations in Chios and Karagouniki sheep. According to Galloway *et al.* (2000) and Hanrahan *et al.* (2004), the FecXB variant in heterozygous condition increases ovulation rate, while homozygotes cause ovarian atrophy and infertility. These heterogeneous observations demonstrate that despite examining identical genetic loci, significant intra-population genetic variation exists, with environmental variables such as nutrition, climatic conditions, and management practice influencing gene expression and epistatic interactions.

Each breed possesses genetic characteristics that affect ovulation rate and fertility capabilities. Scaramuzzi *et al.* (1993) explained that major genes like Fecβ and FecX control ovulation rate. Fecβ increases plasma FSH concentration, which accelerates follicular development and maturation without altering their initial number. FecX influences ovarian follicular development and increases fertility. The synergy of these two genes modifies sheep reproductive mechanisms, enabling an increase in the number of offspring per reproductive period.

Association of *GDF9* gene with litter size

The results of this study showed that *GDF9*|HpaI and *GDF9*|MspI loci were monomorphic, thus could not be associated with increased litter size in both sheep breeds. Meanwhile, the *GDF9* g.792 G>A loci in Local sheep had genotype variations that could be statistically analyzed for relationships. The results of the association analysis between the *GDF9* gene and litter size are presented in Table 6.

Table 5: Association of the *BMP15* gene with litter size.

Gene	Breed	Sample size	Genotype	Litter size (Mean± SD)
BMP15 g.6393 C>G (HaeIII)	Dorper	17	CC GG CG	1.29 ± 0.47 - -
	Local	23	CC GG CG	1.28 ± 0.46 - -
BMP15 g.6637 C>T (HinfI)	Dorper	17	GG TT GT	1.29 ± 0.47 - -
	Local	23	GG TT GT	1.28 ± 0.46 - -
BMP15 g.6790 C>T (SpeI)	Dorper	17	CC TT CT	1.29 ± 0.47 - -
	Local	23	CC TT CT	1.28 ± 0.46 - -

Table 6: Association of the *GDF9* gene with litter size.

Gene	Breed	Sample Size	Genotype	Litter size (Mean ± SD)
GDF9 g.792 G>A (HhaI)	Dorper	17	GG AA GA	1.29 ± 0.47 - -
	Local	23	GG AA GA	1.24 ± 0.44 - 1.50 ± 0.58
GDF9 g.3010 G>A (HpaI)	Dorper	17	CC TT TC	1.29 ± 0.47 - -
	Local	23	CC TT TC	1.28 ± 0.46 - -
GDF9 g.913 T>C (MspI)	Dorper	17	TT CC TC	1.29 ± 0.47 - -
	Local	23	TT CC TC	1.28 ± 0.46 - -

The *GDF9*|HhaI exon 1 (g.792G>A) gene polymorphism showed no significant association with litter size (P > 0.05) in Local sheep. However, the GA genotype tended to produce higher litter sizes compared to the GG genotype, indicating potential applications in marker-assisted selection for improving reproductive performance. In contrast, the *GDF9*|HpaI and *GDF9*|MspI loci were

monomorphic and showed no variation related to litter size.

The lack of significant association between the *GDF9* g.792G>A mutation and litter size in this study differs from several previous reports that showed a positive correlation between this mutation and prolificacy. The *GDF9*|HhaI exon 1 g.792 G>A mutation in this study, in heterozygous condition (GA), has been associated with increased ovulation rate and litter size in various sheep breeds. This aligns with findings by Paz *et al.* (2015) in Chilota sheep, while Gorlov *et al.* (2018) reported that Volgograd sheep with GA genotype had higher litter size (1.88) compared to GG (1.22), as did Salks (GA: 1.80; GG: 1.13) and Ujimqin sheep (GA: 1.19; GG: 1.16) as found by Ji *et al.* (2023). Additionally, Getmantseva *et al.* (2019) reported that heterozygous sheep had higher birth weights compared to homozygotes.

However, several factors may explain the discrepancy between these results and previous studies. First, the low frequency of the A allele (0.09) in the local sheep population is likely the primary reason for the absence of statistical significance. With such a low frequency, the number of heterozygous individuals (GA) was limited (17%), and no homozygous AA individuals were observed in the sample. Second, the small sample size (n=23 for local sheep) may have reduced the statistical power of the analysis. In genetic association studies, particularly for minor alleles with low frequencies, a large sample size is essential to detect their effects reliably. Third, the absence of homozygous AA individuals may be an artifact of sampling or could genuinely reflect selective pressure against the A allele. Previous studies by Abdoli *et al.* (2013) and Hanrahan *et al.* (2004) reported that AA homozygotes cause infertility due to arrested follicular development. This suggests the possibility of negative selection against the A allele in its homozygous form, ultimately reducing its frequency in the population. Although the association was not statistically significant, the presence of the GA genotype may be related to a trend toward increased litter size. However, this result is preliminary and should not yet be used as a basis for Marker-Assisted Selection (MAS) without further validation involving larger sample sizes and more comprehensive genetic studies.

The absence of AA homozygotes for the *GDF9* g.792G>A mutation in our dataset, combined with its low allele frequency (0.09), suggests several potential biological and methodological explanations. First, as reported in other studies, the AA genotype may be lethal or lead to infertility, thereby preventing its presence in the breeding population and limiting the allele's propagation to heterozygous individuals only. Second, the persistence of the A allele at low frequency may reflect a form of balancing selection, wherein heterozygotes (GA) potentially benefit from a reproductive advantage such as a subtle increase in litter size, while homozygotes are selected against. Third, the observed allele frequency may be partially shaped by sampling bias, as the animals were sourced from a single farm representing a limited subpopulation, possibly influenced by founder effects or restricted gene flow. Together, these factors may explain the continued presence of the A allele despite the absence of AA individuals. Broader sampling across diverse populations is needed to clarify the underlying genetic dynamics and validate these hypotheses.

The *GDF9* g.913 T>C locus showed no genotype diversity or association with litter size in our study, contrasting with findings by Fiky *et al.* (2017) who reported polymorphism and significant associations in Saidi and Ossimi sheep breeds. Several factors likely contribute to this lack of diversity in our population. The small sample size may have limited our ability to detect rare variants, while the relatively homogeneous environment and strong natural selection pressure for locally adaptive alleles could have reduced genetic variation over time. Additionally, genetic drift in small, isolated populations often leads to the random loss of allele variants (Bradford *et al.*, 1986; Roberts, 2000). The lack of genetic diversity in the observed population can be attributed to inbreeding management practices, where individuals with close genetic relationships are bred to produce offspring, resulting in a risk of genetic homogeneity and ongoing decline in genetic diversity. Furthermore, genetic drift occurring in small and isolated populations can lead to the loss of allele variants within the population.

CONCLUSSION

Polymorphism was identified at the *GDF9*|HhaI exon 1 locus, characterized by a point mutation (g.792G>A), resulting in two genotypes (GG and GA) in Local sheep. Although the association was not statistically significant, the presence of the GA genotype may be related to a trend toward increased litter size. However, this result is preliminary and should not yet be used as a basis for Marker-Assisted Selection (MAS) without further validation involving larger sample sizes and more comprehensive genetic studies. The lack of genetic variability at these loci suggests limited diversity within the sampled population. Additionally, the high phenotypic variability and relatively small sample size may have masked potential genetic associations. Future studies should employ larger and stratified populations, and incorporate genomic or pedigree-based relationship data to more accurately disentangle genetic from environmental

effects on prolificacy traits in sheep.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the members of the Animal Biotechnology Research Group Laboratory Faculty of Animal Science Brawijaya University, and Directorate General of Higher Education, The Ministry of Education, Culture, Research and Technology, Indonesia through the PMDSU scholarship. We would like to thank the Brawijaya University for providing funding through the UB Stars scheme 2025, and CV. Kambing Burja for facilitating this research.

NOVELTY STATEMENT

This study provides preliminary insights into the polymorphism of BMP15 and GDF9 genes in full-blood Dorper and Indonesian local sheep raised under tropical conditions, and evaluates their associations with litter size traits. The results revealed that the GDF9 g.792G>A locus in local sheep is polymorphic and may contribute to enhanced reproductive performance, while the BMP15 gene and other GDF9 loci were found to be monomorphic. These findings enrich the currently limited local genetic data and highlight the potential application of Marker-Assisted Selection (MAS) in sheep breeding programs targeting prolificacy traits in Indonesia.

AUTHOR'S CONTRIBUTION

A. As, S. Suyadi, T. E. Susilorini, and K. Kuswati conceptualized the study, developed the methodology, provided resources, and supervised the study. W. A. Septian and A. Ardiantoro conducted the investigation, laboratory analysis, and data visualization. A. As and S. Suyadi wrote the original manuscript. R. F. Putri and C. D. Nugraha revised the manuscript. All authors agreed to the final version of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abd El-Hack ME, Abdelnour SA, Swelum AA, Arif M (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

Abdelgadir AZ, Musa LM, Jawasreh KI, Saleem AO, El-Hag F, Ahmed MKA (2021). G1 point mutation in growth differentiation factor 9 gene affects litter size in Sudanese desert sheep. Vet. World, 14(1): 104–112. https://doi.org/10.14202/vetworld.2021.104-112

Abdoli R, Zamani P, Deljou A, Rezvan H (2013). Association of BMPR-1B and GDF9 genes polymorphisms and secondary protein structure changes with reproduction traits in Mehraban ewes. Gene, 524(2): 296–303. https://doi.org/10.1016/j.gene.2013.03.133

Abebe A, Berhane G, Getachew T, Gizaw S, Haile A (2023). Reproductive performance and productivity of local and Dorper × local crossbred ewes under community-based management system, Ethiopia. Heliyon, 9(9): e19906. https://doi.org/10.1016/j.heliyon.2023.e19906

Abramovs N, Brass A, Tassabehji M (2020). Hardy-Weinberg equilibrium in the large scale genomic sequencing era. Front. Genet., 11: 210. https://doi.org/10.3389/fgene.2020.00210

Abuzahra M, Wijayanti D, Effendi MH, Mustofa I, Lamid M (2024). Association of melatonin receptor 1A with litter size in sheep: A review. F1000 Res., 12: 900. https://doi.org/10.12688/f1000research.134890.2

Ahmadi A, Afraz F, Talebi R, Farahavar A, Vahidi SMF (2016). Investigation of GDF9 and BMP15 polymorphisms in Mehraban sheep to find the missenses as impact on protein. Iran. J. Appl. Anim. Sci., 6(4): 863–872.

Amandykova M, Orazymbetova Z, Kapassuly T, Kozhakhmet A, Khamzina S, Iskakov K, Dossybayev K (2023). Detection of genetic variations in the GDF9 and BMP15 genes in Kazakh meat–wool sheep. Arch. Anim. Breed., 66(4): 401–409. https://doi.org/10.5194/aab-66-401-2023

Badan Pusat Statistik (BPS) (2023). Populasi domba menurut provinsi (ekor) 2020–2022. https://www.archive.bps.go.id/indicator/24/473/1/populasi-domba-menurut-provinsi.html

As A, Purwoagung AS, Susilorini TE, Septian WA, Nugraha CD, Putri RF, Ardiantoro A, Suyadi S (2025). The effect of ewe age on birth weight and litter size of texel cross sheep mated with dorper and awassi rams. Adv. Anim. Vet. Sci., 13(8): 1672-1680. https://doi.org/10.17582/journal.aavs/2025/13.8.1672.1680

Bradford GE, Quirke JF, Sitorus P, Inonu I, Tiesnamurti B, Bell FL, Fletcher IC, Torrel DT (1986). Reproduction in Javanese sheep: Evidence for a gene with large effect on ovulation rate and litter size. J. Anim. Sci., 63: 418–431. https://doi.org/10.2527/jas1986.632418x

Carsono N, Lukman PN, Damayanti F, Susanto U, Sari S (2014). Identifikasi polimorfis marka-marka molekuler yang diduga berkaitan dengan karakter daya hasil tinggi pada 30 genotip padi. Chim. Nat. Acta, 2(1). https://doi.org/10.24198/cna.v2.n1.9141

Chen X, Sun H, Tian S, Xiang H, Zhou L, Dun W, Zhao X (2015). Increasing litter size in a sheep breed by marker-assisted selection of BMPR1B A746G mutation. J. Genet., 94: 139–142. https://doi.org/10.1007/s12041-015-0470-8

Chu MX, Liu ZH, Jiao CL, He YQ, Fang L, Ye SC, Chen GH, Wang JY (2007). Mutations in BMPR-IB and BMP-15 genes are associated with litter size in Small Tailed Han sheep (*Ovis aries*). Anim. Reprod. Sci., 61: 598–603. https://doi.org/10.2527/jas.2006-324

Damayanti H, Susilorini TE, Efendi AZ, Ardiantoro A, Prasetyo G, Hidayah N, Septian WA, Suyadi (2023). Identification of genetic diversity of bone morphogenetic protein (bmp15) genes in Senduro goats and Boerawa goats using PCR-sequencing. Adv. Anim. Vet. Sci., 13(6): 1160–1168. https://doi.org/10.17582/journal.aavs/2025/13.6.1160.1168

Davis GH (2005). Major genes affecting ovulation rate in sheep. Genet. Sel. Evol., 37: 11–24. https://doi.org/10.1051/

gse:2004026
Davis GH, Dodds KG, Wheeler R, Jay NP (2001). Evidence that an imprinted gene on the X chromosome increases ovulation rate in sheep. Proc. Natl. Acad. Sci., 221: 216–221. https://doi.org/10.1095/biolreprod64.1.216.
Di R, Wang F, Yu P, Wang X, He X, Mwacharo JM, Pan L, Chu M (2021). Detection of novel variations related to litter size in BMP15 gene of Luzhong mutton sheep (Ovis aries). Animals, 11(12): 3528. https://doi.org/10.3390/ani11123528
Drouilhet L, Lecerf F, Bodin L, Fabre S, Mulsant P (2009). Fine mapping of the FecL locus influencing prolificacy in Lacaune sheep. Anim. Genet., 40(6): 804–812. https://doi.org/10.1111/j.1365-2052.2009.01919.x
Elieser AS, Maharani D, Budisatria GS, Batubara A, Hariyono DNH, Sari APZNL (2018). Study of growth differentiation factor 9 (GDF9) gene polymorphism in Indonesian goats. Proc. Int. Conf. Food Agric., 9: 6–10. https://publikasi.polije.ac.id/index.php/ProceedingICOFA/article/view/1265/857
Fiky ZAE, Hassan GM, Nassar MI (2017). Genetic polymorphism of growth differentiation factor 9 (GDF9) gene related to fecundity in two Egyptian sheep breeds. J. Assist. Reprod. Genet., 34: 1683–1690. https://doi.org/10.1007/s10815-017-1007-2
Galloway SM, McNatty KP, Cambridge LM, Laitinen MPE, Juengel JL, Jokiranta S, McLaren RJ, Luiro K, Dodds KG, Montgomery GW, Beattie AE, Davis GH, Ritvos O (2000). Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. Nat. Genet., 25(3): 279–283. https://doi.org/10.1038/77033
Getmantseva L, Bakoev N, Shirokova N, Kolosova M, Bakoev S, Kolosov A, Usatov A, Shevtsova V, Kolosov Y (2019). Effect of the GDF9 gene on the weight of lambs at birth. Bulg. J. Agric. Sci., 25(1): 153–157. https://journal.agrojournal.org/page/en/details.php?article_id=1573
Ghaffari M, Nejati-Javaremi N, Rahimi-Mianji G (2009). Lack of polymorphism in the oocyte derived growth factor (GDF9) gene in the Shal breed of sheep. S. Afr. J. Anim. Sci., 39(4). https://doi.org/10.4314/sajas.v39i4.51127
Gorlov IF, Kolosov YA, Shirokova NV, Getmantseva LV, Slozhenkina MI, Mosolova NI, Bakoev NF, Leonova MA, Kolosov AY, Zlobina EY (2018). GDF9 gene polymorphism and its association with litter size in two Russian sheep breeds. Rendiconti Lincei. Scienze Fisiche e Naturali, 29: 61–66. https://doi.org/10.1007/s12210-017-0659-2
Govur WA, Rasad SD, Solihati N (2015). Pengaruh umur terhadap bobot dan diameter ovarium serta kualitas oosit pada domba lokal. Students e-J., 4(4): 1–13.
Guan F, Ai JT, Pang XS, Liu SR, Shi GQ, Yang LG (2005). Detection of the polymorphisms of *GDF9* and *BMP15* genes in sheep. Life Sci. Res., 9(2): 184–188.
Gunawan A, Sumantri C, Juniarti R (2017). Gen dan keragaman genetik ternak. IPB Press, Bogor.
Hakim FR, Arifin M, Rianto E (2019). Growth pattern and productivity of female Wonosobo sheep in Wonosobo District, Central Java Province, Indonesia. IOP Conf. Ser. Earth Environ. Sci., 247(1): 012044. https://doi.org/10.1088/1755-1315/247/1/012044
Hanrahan JP, Gregan SM, Mulsant P, Mullen M, Davis GH, Powell R, Galloway SM (2004). 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*). Biol. Reprod., 70: 900–909. https://doi.org/10.1095/biolreprod.103.023093
Hudori HA, Pratama FEA, Andini P, Cnawp RP, Chairina RL (2022). Produktivitas peternakan domba menggunakan sistem kawin alam di CV gumukmas multi farm kabupaten jember. Conf. Appl. Anim. Sci. Proc. Ser., 3: 42–46. https://doi.org/10.25047/animpro.2022.335
Irham LM, Puspitaningrum AN, Adikusuma W, Mugiyanto E, Brahmadhi A, Djalilah GN, Satria RD, Septama AW (2023). Identifikasi variasi gen yang bersifat missense/nonsense pada dermatomyositis dengan memanfaatkan database genomik dan bioinformatik. Maj. Farm. Farmakol., 27(1): 5–9.
Ji X, Cao Z, Hao Q, He M, Cang M, Yu H, Ma Q, Li X, Bao S, Wang J, Tong B (2023). Effects of new mutations in *BMPRIB, GDF9, BMP15, LEPR,* and *B4GALNT2* genes on litter size in sheep. Vet. Sci., 10(4): 258. https://doi.org/10.3390/vetsci10040258
Kandiwa E, Nguarambuka U, Chitate F, Samkange A, Madzingira O, Mbiri P, Bishi AS, Mushonga B (2020). Production performance of sheep and goat breeds at a farm in a semi-arid region of Namibia. Trop. Anim. Health Prod., 52: 2621–2629. https://doi.org/10.1007/s11250-020-02283-w
Khodabakhshzadeh R, Mohammadabadi MR, Esmailizadeh AK, Shahrebabak HM, Bordbar F, Namin SA (2016). Identification of point mutations in exon 2 of *GDF9* gene in Kermani sheep. Pol. J. Vet. Sci., 19(2): 281–289. https://doi.org/10.1515/pjvs-2016-0035
Kirikci K, Cam MA, Mercan L (2021). Investigation of G1 (c.260G>A) polymorphism in exon 1 of GDF9 gene in Turkish sheep breed Karayaka. Turk. J. Vet. Anim. Sci., 45(1): 191–197. https://doi.org/10.3906/vet-2009-40
Lahir MS, Karyono T, Suyadi S (2015). Pengaruh tipe kelahiran terhadap produktivitas induk domba ekor gemuk di Kabupaten Situbondo. J. Ilmu-Ilmu Peternakan, 25(1): 24-30.
Lassoued N, Benkhlil Z, Woloszyn F, Rejeb A, Aouina M, Rekik M, Fabre S, Bedhiaf-Romdhani S (2017). FecX Bar: A novel BMP15 mutation responsible for prolificacy and female sterility in Tunisian Barbarine sheep. BMC Genet., 18: 1–10. https://doi.org/10.1186/s12863-017-0510-x
Liandris E, Kominakis A, Andreadou M, Kapeoldassi K, Chadio S, Tsiligianni T, Ikonomopoulos I (2012). Associations between single nucleotide polymorphisms of GDF9 and BMP15 genes and litter size in two dairy sheep breeds of Greece. Small Rumin. Res., 107(1): 16–21. https://doi.org/10.1016/j.smallrumres.2012.04.004
Lusi D, Yurmiati H, Ramdani D (2022). Pengaruh sistem pemeliharaan terhadap produktivitas induk domba Garut. J. Produksi Ternak Terapan, 3: 73. https://doi.org/10.24198/jptt.v3i2.42288
Mangun M, Riyanti DF, Duma Y, Mumu MI (2024). Dinamika populasi dan tipe kelahiran domba Palu di Kecamatan Mantikulore Kota Palu. J. Ilm. AgriSains, 25(1): 34–42. https://doi.org/10.22487/jiagrisains.v25i1.2024.34-42
Maquivar MG, Smith SM, Busboom JR (2021). Reproductive management of rams and ram lambs during the pre-breeding season in US sheep farms. Animals, 11(9): 2503. https://doi.org/10.3390/ani11092503
Maskur M, Tapaul R, Kasip L (2016). Genetic polymorphism of bone morphogenetic protein receptor 1B (BMPR-1B) gene and its association with litter size in Indonesian fat-tailed sheep. Afr. J. Biotechnol., 15(25): 1315–1319. https://doi.

org/10.5897/AJB2015.15093

Melo E, Silva B, Castro E, Silva T, Paiva S, Sartori R, Franco M, Souza CJ, Neves J (2008). A novel mutation in the growth and differentiation factor 9 (GDF9) gene is associated, in homozygosis, with increased ovulation rate in Santa Ines sheep. Biol. Reprod., 78(s1): 141b. https://doi.org/10.1093/biolreprod/78.s1.141b

Montgomery GW (2024). Genetic regulation of ovulation rate and multiple births. Reprod. Fertil. Dev., 36(14). https://doi.org/10.1071/RD24083

Mullen MP, Hanrahan JP, Howard DJ, Powell R (2013). Investigation of prolific sheep from UK and Ireland for evidence on origin of the mutations in BMP15 (FecXG, FecXB) and GDF9 (FecGH) in Belclare and Cambridge sheep. PLoS One, 8(1): e53172. https://doi.org/10.1371/journal.pone.0053172

Najafabadi AH, Khansefid M, Mahmoud GG, Zhou H, Hickford JG (2020). Identification of polymorphisms in the oocyte-derived growth differentiation factor 9 (GDF9) gene associated with litter size in New Zealand sheep (*Ovis aries*) breeds. Reprod. Domest. Anim., 55(11): 1585–1591. https://doi.org/10.1111/rda.13813

Noor RR (2010). Genetika Ternak (6th Ed.). Penebar Swadaya, Jakarta.

Paz E, Quiñones J, Bravo S, Montaldo HH, Sepúlveda N (2015). Genotyping of *BMPR1B, BMP15* and *GDF9* genes in Chilean sheep breeds and association with prolificacy. Anim. Genet., 46(1): 98–99. https://doi.org/10.1111/age.12254

Putra WPBP, Margawati ET, Pardede S, Soetrisno E, Raadsma HW (2022). Detection of G1 point mutation (c.2039G>A) in *GDF9* gene of MEGA (Merino × Garut) sheep. Veterinaria, 71(3): 357–364.

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(1): 51-56. https://doi.org/10.22146/ijbiotech.42095

Regan SLP, Knight PG, Yovich JL, Leung Y, Arfuso F, Dharmarajan A (2018). Involvement of bone morphogenic proteins (BMP) in the regulation of ovarian function. Vitam. Horm., 107: 227–261. https://doi.org/10.1016/bs.vh.2018.01.015

Riyanto R (2015). Identifikasi variasi genetik kerbau lokal Jawa Timur (*Bubalus bubalis*) dari wilayah yang berbeda berbasis mikrosatelit. Bioscientist, 3(2): 66–72.

Roberts JA (2000). Frequency of the prolificacy gene in flocks of Indonesian thin tail sheep: A review. Small Rumin. Res., 36(3): 215–226. https://doi.org/10.1016/S0921-4488(99)00137-6

Scaramuzzi RJ, Adams NR, Baird DT, Campbell BK, Downing JA, Findlay JK, Henderson KM, Martin GB, McNatty KP, McNeilly AS, Tsonis CG (1993). A model for follicle selection and the determination of ovulation rate in the ewe. Reprod. Fertil. Dev., 5(5): 459–478. https://doi.org/10.1071/RD9930459

Sholikah D, Hilmia N, Hidayat R (2021). Identifikasi sifat kuantitatif domba Dorper jantan generasi pertama yang diberi pakan komplit di PT. Agro Investama. J. Nutr. Trop. Trop. Anim. Feed Sci., 3(2): 61–70. https://doi.org/10.24198/jnttip.v3i2.36315

Schmidová J, Milerski M, Svitaková A, Vostrý L, Novotná A (2014). Estimation of genetic parameters for litter size in Charollais, Romney, Merinolandschaf, Romanov, Suffolk, Šumava and Texel breeds of sheep. Small Rumin. Res., 119(1–3): 33–38. https://doi.org/10.1016/j.smallrumres.2014.02.004

Sodiq A, Yuwono P, Santosa SA (2011). Litter size and lamb survivability of Batur sheep in upland areas of Banjarnegara Regency, Indonesia. Anim. Prod., 13(3).

Štolc L, Ptáček M, Stádník L, Lux M (2011). Effect of selected factors on basic reproduction, growth and carcass traits and meat production in Texel sheep. Acta Univ. Agric. Silvic. Mendel. Brun., 59(5): 247–252. https://doi.org/10.11118/actaun201159050247

Suyadi S, Septian WA, Furqon A, Susilorini TE, Nasich M (2019). Reproduction index of Kacang goat dam reared under closed population in Buduran sub-district, Sidoarjo Regency, East Java, Indonesia. IOP Conf. Ser. Earth Environ. Sci., 391(1): 012007. https://doi.org/10.1088/1755-1315/391/1/012007

Talebi R, Ghaffari MR, Fabre S, Mardi M, Kazemi Alamouti M (2023). Comparison of the growth performance between pure Moghani sheep and crosses with Texel or Booroola sheep carrying major genes contributing to muscularity and prolificacy. Anim. Biotechnol., 34(8): 3495–3506. https://doi.org/10.1080/10495398.2023.2165933

Tambasco DD, Paz CCP, Tambasco-Studart M, Pereira AP, Alencar MM, Freitas AR, Coutinho LL, Packer IU, Regitano LDA (2003). Candidate genes for growth traits in beef cattle crosses Bos taurus × Bos indicus. J. Anim. Breed. Genet., 120(1): 51–56. https://doi.org/10.1046/j.1439-0388.2003.00371.x

Tao L, He X, Wang X, Di R, Chu M (2021). Litter size of sheep (*Ovis aries*): Inbreeding depression and homozygous regions. Genes, 12(1): 109. https://doi.org/10.3390/genes12010109

Tesema Z, Deribe B, Kefale A, Lakew M, Tilahun M, Shibesh M, Belayneh N, Zegeye A, Worku G, Yizengaw L (2020). Survival analysis and reproductive performance of Dorper x Tumele sheep. Heliyon, 6(4): e03840. https://doi.org/10.1016/j.heliyon.2020.e03840

Tiesnamurti B, Sinulingga SE, Gatenby RM (2020). Small ruminant community breeding program in Indonesia. Wartazoa, 30: 163-175. https://doi.org/10.14334/wartazoa.v30i3.1935

Wolf BT, McLean MBL, Davies OD, Griffiths JB (2014). Performance of purebred Welsh Mountain and crossbred ewes in a hill environment. Livest. Sci., 165(1): 181–188. https://doi.org/10.1016/j.livsci.2014.04.007

Yuniarsih P (2011). Eksplorasi gen growth hormone exon 3 pada kambing Peranakan Etawah (PE), Saanen dan Pesa melalui teknik PCR-SSCP.