

# GDF9 gene polymorphism and its relation to litter size in East Java Pote goat germplasm

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**Abstract.** Pote goat is one of the local Indonesian goats from Bangkalan, Madura, which has not been clumped. Procurement of Pote goat breeds as germplasm in enriching genetic diversity can be done by genetic selection. This study aims to identify the GDF9 gene associated with litter size traits of Pote goats. The materials used in this study were 48 female goats that had given birth with a range of permanent incicivi (0,1,2,3,4), and with healthy conditions. This research method uses survey methods and observations in the laboratory. Sampling was done by purposive sampling. Pote goat blood samples were taken from a smallholder farm in Soket Laok Village, Bangkalan Regency, Madura, East Java. GDF9 gene has a length of 490 bp. DNA fragments were amplified and genotyped using PCR-RFLP method using Msp1 restriction enzyme. This study resulted in two genotype types (AG, and GG), and two alleles (A and G). Genotype frequencies of AG, and GG were 0.40, and 0.60. The frequencies of alleles A and G are 0.40 and 0.80. The a.2912 A>G mutation was significant with the litter size. The results showed an association between GDF9 gene diversity found with litter size traits in Pote goats ( $p < 0.05$ ). Polymorphisms in the GDF9 gene are associated with litter size of Pote goats, and can be used as genetic markers for selection on the litter size trait of Pote goats.

**Keywords:** Polymorphism, Association, GDF9 gene, and Pote Goat

## 1 Introduction

The origins of the Pote goat breed are uncertain, but it is flourishing in the Bangkalan area of Madura, East Java. The Pote goat is held as a dual-purpose breed for meat and milk production due to its ability to fulfill both roles. The Pote goat has a similar body structure to the Saanen goat, but it is suspected that the Pote goat is the result of crossing and white color selection between the Peranakan Ettawa goat and the Kacang goat, and may also have the genetic properties of Etawah and Senduro dairy goats [3]. The Pote goats in Bangkalan, originating from areas between 2-100 meters above sea level, are typically fed a combination of eight plant varieties, including *Moringa oleifera*, *Bambusa* sp., and *Artocarpus heterophyllus*, as well as natural grass, which is a commonly used plant with

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relatively high density [17]. The adaptability of Pote goats to these food sources provides an advantage in which they can sustain and boost their population, leading to greater economic benefits for the people.

Procuring breeding stock is crucial for germplasm preservation in order to maintain the genetic resources of the region. These resources are unique and have adapted to their environment over time. Pote goats are also included in goats that have not been clumped and have not been well identified. Pote goats are traditionally raised in rural areas, but due to inadequate education on proper husbandry management within the local community, their maintenance remains difficult. According to [5] there are two ways to enhance livestock production: environmental improvements and genetic improvements. Morphological appearance remains a prevalent factor in livestock selection and characterization today. Environmental factors like nutrition and climate can influence livestock morphology.

Twin births may be affected by hereditary factors transmitted from the parents to their offspring. Productivity of the parent is directly associated with litter size; thus, the larger the litter size, the greater the parent productivity [16]. [18] assert that three factors determine the litter size of goats, namely, the number of eggs produced during lambing and ovulation, fertilization, and conditions during pregnancy, as well as embryo death. Reproduction is a significant characteristic that impacts the productivity and profitability of the goat industry [2]. Traditional selection results in slow genetic gains in reproductive performance due to the trait's low heritability and its expression over the animal's lifespan. Marker-assisted selection methods provide a more effective alternative to livestock breeding problems [14]. Litter size in Pote goats can be manipulated by specific genes known as fecundity genes [19]. Measuring productivity is possible by observing phenotypic characteristics, specifically litter size, and genotypic characteristics, such as gene markers. Certain genes have been discovered that regulate litter size, and they could be utilized as DNA molecular markers for litter size selection. Among these genes is GDF9, which plays a role in regulating goat litter size.

The GDF9 gene, which belongs to the TGF- $\beta$  superfamily of fecundity genes, has been extensively researched in goats. This gene plays a crucial role during the early stages of folliculogenesis by facilitating growth and differentiation and is secreted by oocytes in mammals [6]. GDF9 plays a crucial role in mammalian folliculogenesis development due to its necessity for typical oocyte maturation and embryo development. Without GDF9, embryos will cease development until they reach the blastocyst stage [8]. This study aims to analyze the relationship between the GDF9 gene's polymorphism and litter size in Pote goats found in Bangkalan, Madura. The genetic information discovered in this study will serve as a foundation for developing selection strategies to enhance the genetic quality of Pote goats.

## **2 Materials and Methods**

### **2.1 Materials**

The research material used was 48 female Pote goats with a range Permanent incicvi (0,1,2,3,4) from smallholder farms. The Pote goats were healthy, disease-free, and previously parturient. A survey method was used to collect data on the litter size of Pote goats and the PCR-RFLP method was used for laboratory observation to detect GDF9 gene polymorphism. The first stage of the research was carried out by collecting litter size data and taking blood samples of Pote goats taken in the jugular vein as much as 5 ml. Then, the

blood samples were analyzed at the Biotechnology Laboratory, Faculty of Animal Science, Universitas Brawijaya.

The second stage of research is the analysis conducted in the laboratory in the form of DNA Extraction, DNA Amplification (PCR), and Determination of GDF9 gene genotype by RFLP method. Research tools used during laboratory analysis were GD Column, 1.5 ml Tube, Beaker glass, Micropipet (Select Bio Products), Tip, Tube Rack, Centrifuge (HETTICH Micro 185), BioRad T100 Thermal Cycler, Mini Centrifuge Spindown, Microwave, Electrophoresis (BioRad Mupid-Exu), and Gel Documentation. Materials used during laboratory analysis were Genomic DNA Mini Kit (Promega), Agarose (1st Base Lot 1A1019FH9934), Ethanol absolute, Diamond Nucleic Acid Dye (Promega), Gel Loading Dye Blue, GoTaq Green Master Mix (Promega), Nuclease Free Water (Promega), TBE Buffer, and GDF9 gene primers (10 PM/μl).

DNA extraction in this study used the Genomic DNA Mini Kit (Promega), where DNA extraction is carried out as a process of separating DNA from other cell components such as proteins, carbohydrates, fats, and others (Hutami, et al., 2018). Then, followed by the process of DNA amplification or PCR, using primers. Primer sequences of the GDF9 gene in this study were designed based on the GenBank database with the access number: (ENSCHIG00000008167), consisting of forward primer (5'-GAATGAATGTGAGCTCCATGAC-3') and reverse primer (5'-CCCTTACATTGATAGATGCCAC-3'). The primers were 491 bp long and located in exon 2.

DNA amplification has 3 stages, namely denaturation, annealing, and extension in accordance with PCR conditions on each target gene fragment. The results of DNA amplification (PCR), followed by the determination of GDF9 gan genotype by RFLP method. PCR products were cut using a restriction enzyme, Msp1. The results of all processes can be seen after the electrophoresis process using MUPid Exu electrophoresis machine..

## 2.2 Data Analysis

### 2.2.1 Genotype and Allele Frequency

Allele frequency (1) is the ratio or percentage of a particular allele at a locus. Allele frequency is calculated based on the formula of Nei and Kumar [10]. Genotype frequency (2) is the ratio or percentage of certain genotypes in a population. Genotype frequency is calculated based on the formula of Nei and Kumar [10].

$$x_i = \frac{(\sum_{j=1}^n n_{ij})}{2N} \quad (1)$$

$$x_{ii} = \frac{n_{ii}}{N} \quad (2)$$

Description:

$\chi_i$  = i-th allele frequency

$n_{ii}$  = number of individuals with genotype ii

$n_{ij}$  = number of individuals with genotype ij

$N$  = total sample

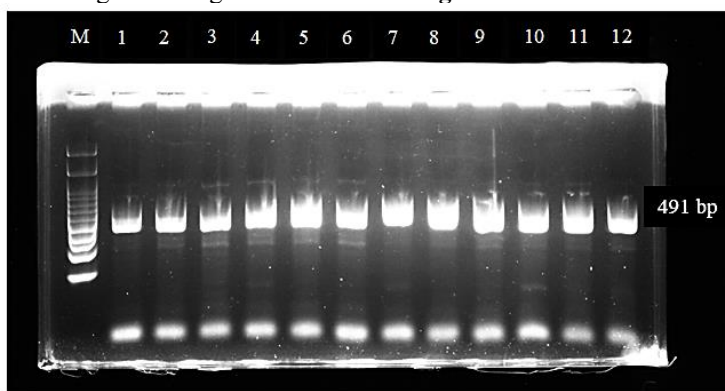
$\chi_{ii}$  = frequency of the i-th genotype

$N_i$  = number of individuals with genotype ii

### 3 Results and discussion

#### 3.1. Amplification of Growth Differentiation Factor 9 (GDF9) Gene by PCR Method in Pote Goat.

Identification of the GDF9 gene can be achieved through the PCR (Polymerase Chain Reaction) method, an in vitro DNA synthesis and amplification technique. According to [9] PCR techniques can be employed to identify mutations in gene fragments, and during the amplification process of gene fragments, components called primers are required. GDF9 gene amplification results in Pote goats were electrophoresed using a 1.5% agarose gel and observed with UV light on the gel documentation **Figure 1**.



**Fig 1.** PCR result of GDF9 gene of Pote goat. Description: M: marker DNA ladder Gene Aid 100 kb, 12 samples.

Based on the PCR results shown in Figure 2, it is evident that all GDF9 gene samples of Pote goats exhibit clearly visible DNA bands, indicative of proper DNA amplification or separation. According to [16] the purity of the DNA produced during the extraction, the selection of appropriate primers and the PCR conditions are important for good PCR results. The PCR results of the GDF9 gene in Pote goats exhibited targeted amplification of the desired fragment (491 bp) without any amplification of contaminant or unintended fragments.

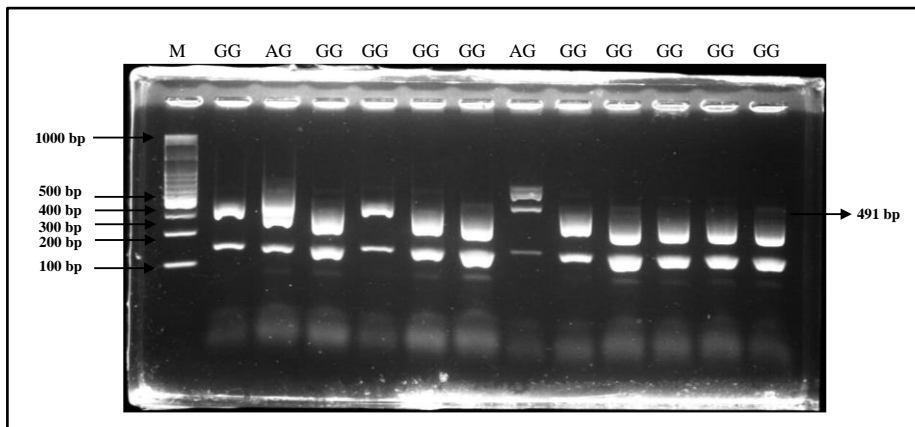
The annealing temperature choice significantly impacts PCR success, as [11] reported that temperature affects the process of attaching the polymer to the DNA template, because if the annealing temperature is too high, the primer will not be able to attach to the template, and if the temperature is too low, the primer will not be able to attach to the specific attachment site, causing thermapplication of unwanted fragments.

Therefore, testing the temperature gradient is necessary because the selection of the correct annealing temperature depends on the primers utilized and the DNA target's characteristics. The optimal annealing temperature falls within the range of 50oC to 65oC. The optimal annealing temperature is in the range of 50oC to 65oC, in this study the temperature gradient used ranged from 58oC to 62oC, and at 62oC showed positive results and can then be used as a new annealing benchmark for samples that have not been PCR.

#### 3.2. Restriction Result of GDF9 Gene by RFLP Method.

The restriction enzyme used in this study is the MspI enzyme, which recognizes and cuts the specific DNA sequence C<sup>^</sup>CGG. Cutting with MspI restriction enzyme produced allele A and allele G, with allele A size of 491 bp and allele G size of 140 bp. Pote goats obtained

2 genotype variations AG with 3 fragments (491 bp, 351 bp, and 140 bp), and GG genotype with 2 fragments (351 bp and 140 bp) Figure 2.



**Fig. 2.** RFLP visualization results of GDF9 Gene with MspI restriction enzyme on 2% agarose gel.

This study resulted in the location of MspI enzyme restriction results, namely there are 3 cut points located at 491, 351, and 140, with one mutation point at 491 bp. Indicates that there is a SNP point a.2912 A>G, which is a nucleotide change from A to G (GAT> GGT), namely Asparagine> Glycine. The type of mutation that occurs is called a missense mutation, which is a sequence variant, which changes one or more bases, resulting in a different amino acid sequence but the length is maintained. According to [13] missense mutation is a change in a genetic code that causes the amino acids bound to the polypeptide chain to change. [4] Identified a missense mutation in Brazilian Santa Ines sheep that caused substitution of phenylalanine with cysteine (F345C) in GDF9 protein named FecGSI.

**Table 1.** Allele Frequency and Genotype Frequency of GDF9 gene in Pote Goat

| Genotype Frequency |         | Allele Frequency |      |
|--------------------|---------|------------------|------|
| AG (19)            | GG (29) | A                | G    |
| 0.40               | 0.60    | 0.40             | 0.80 |

Based on the results of the study (Table 1) shows that the frequency of genotype GG is higher (0.60) than the genotype AG (0.40) while the frequency of allele G (0.80) is higher than allele A (0.40). The differences in the results of genotype frequency and allele frequency in the Pote goat population in this study indicate the existence of polymorphism. [2] Found that populations with allele and genotype frequencies less than or equal to 0.99 exhibit genetic diversity. Polymorphism is an indicator of this diversity, which provides populations with the capability to adjust to environmental changes.

### 3.3. GDF9 Gene Genotype Association to Litter Size of Pote Goats.

This study shows that GDF9 gene polymorphism is associated with litter size of Pote goats. Based on the results of the study in Table 2, the GDF9 gene genotype polymorphism showed a significant effect on the average litter size of Pote goats ( $P<0.05$ ).

**Table 2.** Average litter size of Pote goats based on genotype

| <b>Genotype</b> | <b>n</b> | <b>Average Litter Size</b> |
|-----------------|----------|----------------------------|
| <b>AG</b>       | 17       | 1.6765 ± 0.43088           |
| <b>GG</b>       | 27       | 1.1852 ± 0,28244           |

Based on these results, it means that the GDF9 gene mutation is significantly associated with the litter size trait in Pote goats, and the genetic variant of the GDF9 gene is considered as a genetic marker for increased proliferation in Pote goats. In this study, the GDF9 gene heterozygous genotype AG had a higher average litter size than the homozygous genotypes GG. The results of research from [7] also show that homozygous segments are mostly associated with lower litter size groups, and there is an increase in the level of heterozygosity in higher litter size groups. This is in accordance with the results of [12] who discussed 5 different mutants from several sheep breeds that showed superiority in heterozygous genotypes of the BMP15 gene and GDF9 gene. These mutants increased ovulation rate and fecundity in the heterozygous genotype, but the homozygous genotype mutants had impaired oocyte development and maturation, resulting in underdeveloped ovaries and female infertility.

## 4 Conclusion

Polymorphisms in the GDF9 gene are associated with litter size of Pote goats in Bangkalan, Madura, East Java, and can be used as genetic markers for selection on the litter size trait of Pote goats.

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## References

1. A. Fleming, C. F. Baes, A. A. A Martin, T. C. S. Chud, F. Malchiodi, L. F. Brito, & F. Miglior. *Journal of Dairy Science*, 102(4), 3722–3734. (2019)
2. A. Gunawan, C. Sumantri, & R. Juniarti. *Gen dan Keragaman Genetik Ternak*. IPB Press. Bogor. (2017)
3. A. Rohman, K. Kuswati, and T. E. Susilorini. *International Journal of Current Science Research and Review*, 06(10). (2023)
4. B.D.M. Silva, E.A. Castro, C.J.H. Souza, S.R. Paiva, R. Sartori, M.M. Franco, H.C. Azevedo, T.A.S.N. Silva, A.M.C. Vieira, J.P. Neves & E.O. Melo. *Animal Genetics*. 42(1): 89–92. (2011)
5. E. Wiyanto, & A.Y. Putra. *Jurnal Ilmiah Ilmu-Ilmu Peternakan*. 23(1), 55-60. (2020).
6. J. A. Elvin, A. T. Clark, P. Wang, N. M. Wolfman, & Matzuk, M. M. *Molecular Endocrinology*, 13(6), 1035–1048. (1999).
7. J. J. Wang, T. Zhang, Q.M. Chen, R.Q. Zhang, L. Li, S.F. Cheng, W. Shen and C.Z. Lei. *Frontiers in Genetics*, 11, 510062. (2020).
8. J. Sudiman, M. L. Sutton-McDowall, L. J. Ritter, M. A. White, D. G. Mottershead, J. G. Thompson, & R. B Gilchrist. *PLOS ONE*, 9(7). (2014).

9. L. B. Ketut, Maitriani, I. Nengah Wirajana, D. Sagung, & C. Yowani. In *Cakra Kimia (Indonesian E-Journal of Applied Chemistry)*. 3(2). (2015).
10. M. Nei, & S. Kumar. (2000). *Molecular evolution and phylogenetics*. Oxford University Press, USA.
11. N. P. D. Pertiwi, I. G. N. K. Mahardika, dan N. L. Watiniasih. *Journal of Biology*.19(2), 1-5. (2015).
12. P. W. Hedrick. Heterozygote Advantage. *Journal of Heredity*, 106(2), 141–154. (2015).
13. R. G. Almada, Y. Oktanella, & G. Ciptadi. *Journal of Tropical Animal Production*, 21(2), 102-110. (2020)
14. R. Wakchaure, S. Ganguly, P.K Praveen, A. Kumar, S. Sharma, & T. Mahajan. *J. Drug. Metab. Toxicol*, 6(5), e127. (2015).
15. S. Rahayu, S. B. Sumitro, T. Susilawati, dan S. Soemarno. 2006. *Biological Research Periodical*. 12(1), 7-11.
16. Sudrajad, P., S. D. Volkandari, M. Cahyadi, A. Prasetyo, K. Komalawati, S. Wibowo, & S. Subiharta. *Livestock and Animal Research*, 19(1), 1-12. (2021).
17. T. E. Susilorini, K. Kuswati, R. D. Wahyuni, P. Surjowardojo, & S. Suyadi. *AGRIVITA, Journal of Agricultural Science*, 44(2). (2022).
18. T. Kostaman, & I. K. Utama. *JITV*, 10(2), 106-112. (2005).
19. X. P. An, J. X. Hou, H. B. Zhao, G. Li, L. Bai, J. Y. Peng, Q. M. Yan, Y. X. Song, J. G. Wang, & B. Y. Cao. *Animal Genetics*, 44(2), 234–238. (2013).