# Determining the Polymorphism of V397I SNP - published UHO Janet

*by* Gus Anto

**Submission date:** 02-Apr-2023 01:57PM (UTC+0700)
**Submission ID:** 2053280315
**File name:** ermining_the_Polymorphism_of_V397I_SNP_-_published_UHO_Janet.pdf (196.99K)
**Word count:** 2367
**Character count:** 12511

# Determining the Polymorphism of V397I SNP of Growth Differentiation Factor 9 (GDF9) gene in Indonesian Saanen Goats

Janet Mamutse [1*], Dattadewi Purwantini[1], Agus Susanto[1], Akhmad Sodiq[1]

[1] *Department of Animal Breeding, Faculty of Animal Science, Jenderal Soedirman University. Jl. Dr. Soeparno, Purwokerto Utara, Banyumas 53122, Central Java, Indonesia*
[*] *Corresponding author. Email: janetmamutse767@gmail.com*

**ABSTRACT**
Reproduction is an economically important trait. It is key to the profitability and sustainability of the goat industry. The Goat industry plays a major role in the Indonesian livestock industry, thus genetic improvement of reproductive traits of goats in Indonesia may be of great benefit to the industry. GDF9 gene is one of the most common and largely studied genes with regards to fecundity in goats. Many GDF9 gene SNPs have been reported and the V397I SNP has been studied extensively about to with fecundity in goats. Therefore, the current study aimed to determine the polymorphism of V397I SNP in Indonesian Saanen goats at Baturraden breeding centre. The study used T-ARMS-PCR for genotyping and the predicted alleles (186 bp for G allele and 275 bp for A allele) were reported. The population was monomorphic for genotype AG which is associated with relatively high litter size. Therefore, the study concluded that the T-ARMS-PCR method can be used successfully to genotype V397I SNP. Although the SNP was monomorphic, the findings of the study revealed that the SNP has the potential to be used as a marker for reproduction performance in indigenous breeds and also non-selected lines. The study also suggested that the polymorphism of the V397I SNP should be determined in larger sample size, various breeding centres or studs and (or) non-selected population of Indonesian goats.

***Keywords:*** *GDF9 gene, Reproduction, Saanen goats, T-ARMS-PCR, V397I SNP.*

## 1. INTRODUCTION

Reproduction is one of the most important traits that influence productivity and profitability of the goat industry [1]. It also helps to increase selection intensity and genetic progress of other production traits [2]. Due to climate change, adaptable and robust goat breeds are required to improve the goat industry, and reproduction is one of the traits that are linked to adaptability and robustness [3]. Therefore, genetic improvement of the trait will be of great benefit to the industry.

Reproductive traits are of high economic importance in goat farming. Due to the trait's low heritability and late expression during an animal's life, using traditional selection leads to slow genetic progress of reproduction performance. To address this problem in animal breeding, new selection methods such as marker-assisted selection will be a better alternative [4]. Previous studies indicate that litter size can be influenced by a set of genes referred to as fecundity genes [5].

The growth differentiation factor 9 (GDF9) gene is one of the most important fecundity genes that has been widely studied in goats which belongs to the transforming growth factor β (TGFβ) superfamily. The protein is one of the important fecundity genes which plays a critical role during early folliculogenesis as a growth and differentiation factor secreted by oocytes in mammals [6]). The GDF9 gene is located on caprine Chromosome 7 and has a coding region of 4720 base pairs and two exons. There are many single nucleotide polymorphisms (SNPs) that have been identified in the

gene and the SNPs have been reported to have influence on litter size in goats [2].

The most common SNP in the caprine GDF9 gene is V397I or c.1198G>A located on exon 2 [2]. Literature has shown that the V397I SNP was polymorphic in many goat populations (5,7,8), while non-polymorphic in other goat breeds [9]. There are also inconsistent thoughts as to whether caprine GDF9 gene with this SNP encodes for an important protein associated with litter size in goats (2). In some studies, there was no effect of this SNP on litter size, while in other studies there was an effect (2). In Indonesian goats, the V397I has not been studied, yet. The polymorphism of V397I SNP has definitely not been studied in Indonesian Saanen goats in Baturadden breeding center, yet.

However, different SNPs of GDF9 gene were identified in the Indonesian goats. Some SNPs were reported to be polymorphic in Garut goats (10), and novel SNPs were reported in Etawah breed. Non-polymorphic SNPs of the GDF9 gene were reported in 25 animals Kacang goats, 29 head PE goats, 35 Muara goats and 60 Samosir goats [11] and Kacang goats [12].

Therefore, the aim of this study was to determine the polymorphism of the V397I SNP of GDF9 gene of Indonesian Saanen goats at Baturraden breeding station. The study will give insight on the potential of the V397I SNP as a genetic marker for selection of litter size in the Saanen goat population at Baturadden breeding centre.

## 2. MATERIALS AND METHOD

### *2.1. Animal Sampling, Blood Collection and DNA Isolation*

The Indonesian Saanen goats at Baturraden breeding station were used in this study. A total of 60 does from parities 1 to 3 were randomly selected for blood collection. The blood was collected via a puncture on the jugular vein and put in polypropylene vacutainer tubes of containing EDTA (anticoagulant). The collected blood was stored under -20°C until the DNA is extracted. Genomic DNA was extracted from blood samples using the column-matrix method according to the manufacturer's protocol. The DNA was dissolved in elution buffer and the quality of the total genomic extraction was assessed by 1% agarose gel electrophoresis [9].

### *2.2. Genotyping*

The V397I SNP of caprine GDF9 gene (accession no. XM_013965446.2.) on mutation point 1850bp was genotyped using the T-ARMS-PCR method (13). The outer forward outer and reverse primers were designed using Primer-Blast NCBI. The sequence of the outer forward and reverse were 5' ATC GTC CCG TCA CCG CAG AGA CCC 3' and 5' CAG GTA CAC TTA GTG GCT ATC AT 3'; respectively. The inner forward and inner primers were designed manually with deliberate change of the allele complementary to the SNP of interest on the inner forward primers. The sequence of inner forward and inner primers were 5' ACA TCG GTA TGG CTC TCC GG 3' and 5' TGT TCT GCA CCA TGG TGT GAA T 3'; respectively [13]. Below is Figure 1 showing the primers used and the point of mutation.

The yellow- and green highlighted nucleotides are the positions of outer forward and inner forward; and inner reverse and outer reverse primers; respectively and the red-underlined nucleotide is the mutation point.

The thermal conditions were as follows; predenaturation (95°C/3min), denaturation (95°C/20sec, annealing (55.3°C/20sec), extension (72°C/15sec) and final extension (72°C/3min) for 34 cycles. The visualization of the DNA amplicons and a 50bp DNA ladder was done on 1.5 % agarose gels in 0.1X TBE buffer stained with ethidium bromide [14].

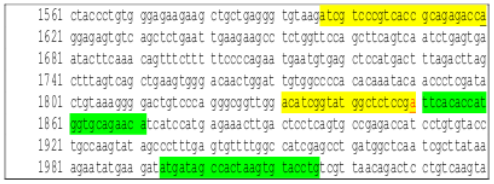


**Figure 1**. Primers and point of mutation of V397I SNP of caprine GDF9 gene

## 3. RESULTS AND DISCUSSION

The results of this study showed consistence with the expected alleles as determined by the output of the designed primers. The alleles were 186 bp (G allele) and 275 bp (A allele). However, no polymorphism was reported for the V397I SNP of caprine GDF9 gene in the Indonesian Saanen goats population at Baturraden breeding station. All the 60 animals showed the genotype GA. Below is figure 1 showing the results of the genotypes.

GDF9 is secreted by mammalian oocytes and is a key regulator of follicular proliferation, ovulation and fertilization, and also improve the developmental competence of oocytes in females [15]. To date, the V397I SNP has been studied extensively in various goat breeds [2]. Therefore, it is much meaningful to study V397I SNP of GDF9 gene as a marker for improved litter size in goats.

Currently, among all known polymorphisms of goat GDF9 gene, Q320P and V397I had been extensively studied in various global goat breeds.

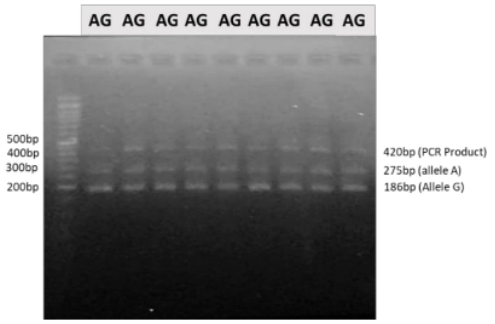


**Figure 2.** Results of T-ARMS-PCR genotyped V397I SNP of caprine GDF9 gene

Similar to the results reported in this study was another study that revealed that the V397I SNP was non-polymorphic in the Black Bengal Indian goats and the study genotyped 88 animals [9]. In contrast to the findings of this study, the V397I SNP was found to be polymorphic in several breeds. These studies used larger sample sizes; i.e., 641 goats of three breeds: Xinong Saanen, Guanzhong and Boer [5], 343 Indian goat breeds [8] and 164 does of Iran goat breeds [7]. According to these results, it can be assumed that the sample size in the current study was small. Therefore, further studies should be conducted on a larger sample size.

Reproduction performance has high variation in indigenous breeds than the selected lines of exotic sheep [16]. The goats that were used in this study are imported from Australia as a selected for high performance of the desirable traits such as productivity. Therefore, there are high chances that the V397I SNP was already selected for the genotype associated with the expression of high reproduction performance.

Further studies should consider DNA sequencing of the caprine GDF9 gene in the Indonesian Saanen goats to identify novel SNPs that may be polymorphic and may have the potential to be used in MAS of litter size in Indonesian Saanen goats at Baturradden breeding centre.

## 4. CONCLUSION

This current study determined the polymorphism of V397I SNP of caprine GDF9 gene in Indonesian Saanen goats using T-ARMS-PCR genotyping method. The results showed that the T-ARMS-PCR method can be used successfully to genotype V397I SNP. The findings of the study also showed no polymorphism of the SNP in the Saanen goats. Although the SNP was non-polymorphic, the research concluded that the V397I SNP of caprine GDF9 gene has the potential to be used as a marker in the population. Further studies should be conducted in a larger sample size.

## ACKNOWLEDGMENTS

I would like to express my gratitude to the Ministry of Research and Technology and Higher Education the Republic of Indonesia that funded this research. I am also grateful to the Animal Technology Laboratory, Jenderal Soedirman University that made it possible for the experiments of this study to be conducted.

## REFERENCES

[1] Fleming A, Baes CF, Martin AAA, Chud TCS, Malchiodi F, Brito LF, et al. Symposium review: The choice and collection of new relevant phenotypes for fertility selection. J Dairy Sci [Internet]. 2019;102(4):3722–34. Available from: http://dx.doi.org/10.3168/jds.2018-15470

[2] Mahmoudi P, Rashidi A, Rostamzadeh J, Razmkabir M. Association between c.1189G>A single nucleotide polymorphism of GDF9 gene and litter size in goats: A meta-analysis. Anim Reprod Sci [Internet]. 2019;209(April):106140. Available from: https://doi.org/10.1016/j.anireprosci.2019.106140

[3] Molotsi A, Dube B, Oosting S, Marandure T, Mapiye C, Cloete S, et al. Genetic traits of relevance to sustainability of smallholder sheep farming systems in South Africa. Sustain. 2017;9(8):1–18.

[4] Wakchaure R, Ganguly S. Marker Assisted Selection (MAS) in Animal Breeding: A Review. J Drug Metab Toxicol. 2015;06(05):6–9.

[5] An XP, Hou JX, Zhao HB, Li G, Bai L, Peng JY, et al. Polymorphism identification in goat GNRH1 and GDF9 genes and their association analysis with litter size. Anim Genet. 2013;44(2):234–8.

[6] Elvin JA, Clark AT, Wang P, Wolfman NM, Matzuk MM. Paracrine actions of growth differentiation factor-9 in the mammalian ovary. Mol Endocrinol. 1999;13(6):1035–48.

[7] Shokrollahi B, Morammazi S. Polymorphism of GDF9 and BMPR1B genes and their association with litter size in Markhoz goats. Reprod Domest Anim. 2018;53(4):971–8.

[8] Ahlawat S, Sharmaa R, Roy M, Mandakmale S, Prakash V, Tantia MS. Genotyping of novel SNPs in BMPR1B, BMP15, and GDF9 genes for association with prolificacy in seven Indian goat breeds. Anim Biotechnol. 2016;27(3):199–207.

[9] Polley S, De S, Batabyal S, Kaushik R, Yadav P, Arora JS, et al. Polymorphism of fecundity genes (BMPR1B, BMP15 and GDF9) in the Indian prolific Black Bengal goat. Small Rumin Res. 2009;85(2–3):122–9.

[10] Rahmawati RY, Sumadi, Hartatik T. Identification of single nucleotide polymorphisms in GDF9 gene associated with litter size in Garut sheep. Indones J Biotechnol. 2019;24(1):51–6.

[11] Batubara A, Noor RR, Frajallah A, Tiesnamurti B. Identifcation of Gdf9 Gene And its Relationship with The Prolific Traits on Indonesian Local Goats. 2005;1544–7.

[12] Rahayu S. Identifikasi Polimorfisme Gen GDF-9 dan BMP-15 pada Kambing Kacang. 2011;1(2):2011.

[13] Ahlawat S, Sharma R, Maitra A, Roy M, Tantia MS. Designing, optimization and validation of tetra-primer ARMS PCR protocol for genotyping mutations in caprine Fec genes. Meta Gene [Internet]. 2014;2:439–49. Available from: http://dx.doi.org/10.1016/j.mgene.2014.05.004

[14] Menck Soares MA, Rodrigues MT, Mognol GP, de Fátima Chasko Ribeiro L, da Conceição Silva JL, Costa Brancalhão RM. Polimorfismo do gene da alphas1-caseína em um rebanho leiteiro na região sudeste do Brasil. Rev Bras Zootec. 2009;38(6):1026–32.

[15] Wang X, Yang Q, Wang K, Yan H, Pan C, Chen H, et al. Two strongly linked single nucleotide polymorphisms (Q320P and V397I) in GDF9 gene are associated with litter size in cashmere goats. Theriogenology [Internet]. 2019;125:115–21. Available from: https://doi.org/10.1016/j.theriogenology.2018.10.013

[16] Molotsi AH, Taylor JF, Cloete SWP, Muchadeyi F, Decker JE, Whitacre LK, et al. Genetic diversity and population structure of South African smallholder farmer sheep breeds determined using the OvineSNP50 beadchip. Trop Anim Health Prod. 2017;49(8):1771–7.

# Determining the Polymorphism of V397I SNP - published UHO Janet

ORIGINALITY REPORT

| 17% | 15% | 12% | 4% |
| --- | --- | --- | --- |
| SIMILARITY INDEX | INTERNET SOURCES | PUBLICATIONS | STUDENT PAPERS |

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

3%

★ Submitted to SDM Universitas Gadjah Mada

Student Paper

| | | | |
| --- | --- | --- | --- |
| Exclude quotes | On | Exclude matches | Off |
| Exclude bibliography | On | | |