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

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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 (TGFB) 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 encodes 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 Baturraden 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].

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1561 ctaccctgtg ggagaagaag ctgctgaggg tgtaaatog tcocgtcaac gcagagacca
1621 ggagagtgtc agotctgaat tgaagaagcc tctggtcca gcttoagtca atctgagtga
1681 atacttcaaa cagtttcttt tccccagaa tgaatgtgag ctccatgact ttagacttag
1741 ctttagtcag ctgaagtggg acaactggat tctggcccca caaataca acoctogata
1801 ctgtaaaagg gaetgtccca gggcgtgtgg acaatgggat ggcctctccg tcacacacac
1861 tcacacacac tcatccatg agaaacttga ctctcaatgt ccgagacat cctgtgtacc
1921 tgccaagtat agcccttga gtgtttggc catcgagcct gatggctcaa tccgtataaa
1981 agaatatgaa gat tcacacacac tcaactaaagtc tcacacac cgt taacagactc ctgtcaagta
    
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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.

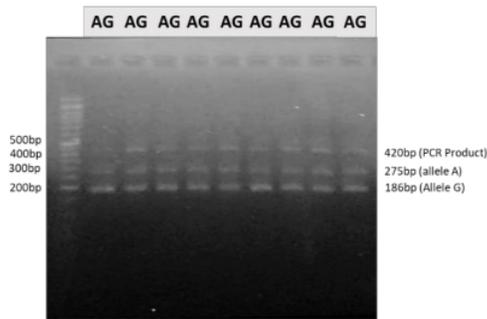


Figure 2. Results of T-ARMS-PCR genotyped V397I SNP of caprine GDF9 gene

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 Baturraden 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.

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