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Single Nucleotide Polymorphism of Partial GDF9 Gene in Three Local Goat of Indonesia Compare with Several Goat in Asia

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ABSTRACT

The GDF9 gene is a gene that affects the maturation of oocytes. GDF9 is expressed in oocytes and granulosa cell, it can stimulate granulose cell proliferation and regulate cumulus cell function from pre-ovulation to ovulation. The GDF9 gene is associated with an increase in the ovulation rate and litter size in animals. This study aims to determine the kinship relationship of local goats compared to goats in Asia on prolific traits and to determine the restriction mapping of the GDF9 gene in goats based on the different SNP locations. The local goat comes from the Bligon goat, Kacang goat and Kejobong goats which is compared to the GenBank data (EF446168, EU883989 and KY780296). GDF9 sequences were analyzed using BioEdit and sequencing results to identify Single Nucleotide Polymorphism (SNP) and using NEBCutter V2 to determine the restriction enzyme which recognized the sequence around SNP. The result shows that three variations of SNP were found in exon 2 (g.3615T>C, g.3760T>C and g.3855A>C). Identification of SNP position found 1 SNP position identified by restriction enzyme at g.3855A> C. The identified restriction enzyme is HpaII and MspI. The results of this study are expected to provide genetic information that will be used for further research on the relationship between GDF9 gene polymorphisms to animal prolific.

Keywords: GDF9, Goat, Phylogenetic, Restriction mapping, SNP

Introduction

Bligon is the one of goat breeds which has good fertility and productivity. In Yogyakarta, this goat population accounts for 60% from the whole goats population and takes up to 12.000 heads per year of their productivity. Kejobong goats are one of local breeds in Purbalingga, Central Java which has similar productivity with Bligon goats. Their population is 15.317 head in Kejobong distric, Purbalingga, Central Java. Kejobong and Bligon goats are known as local Indonesian goat breeds that have good productivity (Hanim *et al.,* 2020). The Bligon goat is a crossbreed between the Kacang goat and the Etawah cross-breed goat. The Bligon goat is the maximum several and great goat breed in Indonesia. Goat farming is one of the most important livelihood and food approaches for most Indonesian farmers (Kurniawati *et al.,* 2021). Kacang goat is an indigenous small goat breed of goat that is used as a meat source and is widely reared as a side business on farms in Indonesia due to the ability of these goats to reproduce and survive with simple rearing and feeding practices. These goats are commonly raised in small flocks that are confined in a simple colony house at night and tethered to graze, typically from noon through the late afternoon (Khalil *et al.,* 2019). Bligon goat has good reproductive performance. The average length of pregnancy for the Bligon goat was 5.5 months with a range of 5 to 6 months. The number of litter sizes produced by the Bligon goat is 1.74 with a range of 1 to 2. The level of litter size is influenced by genetic factors, age of the parent, body weight of the parent, and nutritional level (Murdjito *et al.,* 2011).

Prolific trait is a characteristic of animals that can give birth to one or more children per birth. Types of birth can be divided into single and twin. The birth of twins (more than one) is one thing that is highly expected because it can provide economic benefits (Tiesnamurti, 2002). To exploit the potential of increasing the prolific potency in goats, and these properties must be studied molecularly using genes relate to the nature of litter size or the nature of prolification. Several genes including GDF9. Growth Differentiation Factor (GDF9) is located on chromosome number 7 and consists of 2 exons and 1 intron with a length of 5644 bp. GDF9 could influences prolific traits of goats. It was a gene group of TGF beta superfamily which plays a role in the process of folliculogenesis and prolification.

GDF9 triggers the secretion of progesterone in luteal cells. The process of folliculogenesis was essential in follicular development. The GDF9 gene strongly influences increased ovulation rate. Polymorphism in the GDF9 gene was associated with litter size. Prolification trait was essential in determining the amount of litter size in goats (Mudawamah *et al.,* 2019). One point mutation of the GDF9 gene in Chinese goats that is associated with ovulation rate (Du *et al.,* 2008). Many significant associations have been reported between GDF9 polymorphism and ovulation rate, prolificacy, and fertility in sheep (Yuliana *et al.,* 2019). The study of polymorphism can be done by aligning the deoxyribonucleic acid (DNA) sequences. The program that usually uses DNA alignment and compares two or more sequences is the BioEdit program and Mega 10 (Anugratama and Hartatik, 2020). Research on the GDF9 polymorphism of local goats has not yet been carried out clearly. Therefore this study aims to identification the SNPs and restriction enzymes of partial GDF9 in local goat of indonesia. This information hopefully give beneficial for the further research on molecular genetic analysis as a marker for economical trait in livestock.

Materials and Methods

DNA Sample resources

The DNA materials come from sample extracts that have been isolated in previous studies sample, we used in study were 10 samples from extract DNA of Bligon goat, two samples of Kacang goat, and two samples Kejobong goat. Reference Genbank uses from NCBI with Genbank accession number EF446168, EU883989 and KY780296.

DNA amplification and sequencing

Ten genomic DNA samples from Bligon goat, two samples from Kacang goat, and two samples of Kejobong goat were amplified used PCR method to get targeted sequence. The primer design uses bioinfo.ut.ee/primer3-0.4.0. At positions 3549 to 4004 along 456 bp, the primers used were forward primer CTCCTCTTGAGCCTCTGGTG and reverse primer TCCAGTTGTCCCACTTCAGC.. Polymorphism Chain Reaction performed in a total reaction of 23 µl, containing 12,5 µL PCR Kit (KAPA BIOSYSTEMS, USA), 9,5 µL DDW, 2 µL of DNA, 0,5 µL of both forward and reverse primers. The reactions were performed using a thermal cycle (PEQLAB Primus 25 advanced, Germany) with a pre-denaturation temperature at 94°C for 1 minute, followed by 35 cycles of reaction; denaturation at 94°C for 1 minute, annealing at a temperature of 57°C for 1 minute and extension at 72°C for 1 minute, then the last step was a final extension at 72°C for 5 minutes. The quality of the PCR product was determined using gel electrophoresis (1%), the thick appearance of DNA bands were the preferred result (Albakri and Hartatik, 2021). The sequencing was done by LPPT UGM.

Sequence comparison analysis

Single nucleotide polymorphism identification, comparison sequences, and amino acid change were performed using BioEdit software. A total of 14 sequences of GDF9 gene (ten sample Bligon goat, two sample Kacang goat, two sample Kejobong goat) were aligned using ClustalW on BioEdit ver. 7.2.5 to reveal the SNPs and to perform the amino acid change. Identification of SNP position based on GenBank accession number EF446168.2 as sequence reference. Identification of restriction enzymes was recognized by using Nebcutter V2 which available online in http://nc2.neb. com/NEBcutter2/. Individual target sequences of genes are entered in NEBCutter V2 with all other parameters in their default settings. The program will calculate the positions of all restriction enzymes and then display all restriction enzymes that recognize the target sequence. Specific restriction enzymes that can recognize targets are determined by the appearance of a red line under a sequence (Albakri and Hartatik, 2021).

Results and Discussion

Study reference

The target DNA in this study was GDF9 gene fragment based on genbank accession number EF446168.2. Based on the alignment results, 3 SNP positions were found in positions of GDF9 from 3549 to 4004 along 456 bp found three SNPs g.3615T> C, g.3760T> C and g.3855A>C. Single Nucleotide Polymorphism can cause change in amino acids. These changes can be either silent or missense. The silent mutation occurs if the SNP only changes the DNA but does not change the amino acid. Missense mutation will occur if the SNP not only change the DNA but also an amino acid. Previous research by Xue-qin *et al.* (2009) stated that it was found in the polymorphism analysis of the GDF-9 gene in exon 2 of white goats Guizhou Province. The goat has a heterozygous genotype that mutates at 791 bp (G / A) which results in a substitution of valine to isoleucine in the residue of 79 mature peptide GDF-9 gene. Due to the mutation of valine into isoleucine, it will increase the methyl group in the amino acid chain.

In SNP g.3615T>C there is 1 genotype, namely CC is indicated by a single peak on base C. SNP g.3760T>C has 1 genotype, namely TT is indicated by single peak on base T. SNP g.3855A>C has 2 genotypes namely AA and AC, with AA genotype marked single peak on base A, and AC genotype marked double peak on base A and C.

Based on the results of the analysis of changes in the amino acid SNP g.3615T>C, there change the amino acid from valine to alanine. The position of SNP g.3760T>C did not changes the

amino acid, namely proline. The position of SNP g.3855A>C did not change the amino acid, namely proline, the X sign indicates heterozygous. Research by Ghoreishi et al. (2019) detected the GDF9 SNP in Markhoz goats with mutations at position g.183C>A which did not change the amino acid Leucine. Previous research, Du et al. (2008) detected a heterozygous genotype (g.1189G>A mutation) of the GDF9 gene in Guizhou white goats, as many as 8 samples from 33 high-productivity broods, of which 8 mothers were found to produce 3 offspring per birth rather than 112 low-productive goats with homozygous genotype. Aboelhassan *et al.* (2021) stated that the genetic polymorphism of the GDF9 gene was revealed to affect the nature of fecundity in animals, where heterozygous genotypes were found to cause an increase in ovulation rates and consequently lead to an increase in litter size in livestock brooders, compared to homozygous broods in wild animals. Changes in amino acids can be seen in Table 1.

The restriction enzymes found in GDF9 are HpaII and MspI with SNP g.3855A> C. There was one fragment AA with a size of 456 bp and three fragments AC with sizes 456 bp, 306 bp and 149 bp. Restriction mapping can be seen in Table 2.

3541 tgtgacgg**ct cctcttgagc ctctggtg**gc ctcccacaag aggaatattc acatgtctgt

3601 aaattttaca tgtgtgaaag accagctgca gcatccttca gcgcgggaca gcctgtttaa

3661 catgactctt ctcgtagcgc cctcactgct tttgtatctg aacgacacaa gtgctcaggc

3721 ttttcacagg tggcattccc tccaccctaa aaggaagcct tcacagggtc ctgaccagag

3781 gagagagcta tctgcctacc ctgtgggaga agaagctgct gagggtgtaa gatcgtcccg

3841 tcaccgcaga gaccaggaga gtgtcagctc tgaattgaag aagcctctgg ttccagcttc

3901 agtcaatctg agtgaatact tcaaacagtt tctttttccc cagaatgaat gtgagctcca

3961 tgactttaga cttagcttta gtca**gctgaa gtgggacaac tgga**ttgtgg ccccacacaa

Figure 1. Sequence target of GDF9 in goat GenBank acc no EF446168.2 exon 2 (underline: primer).

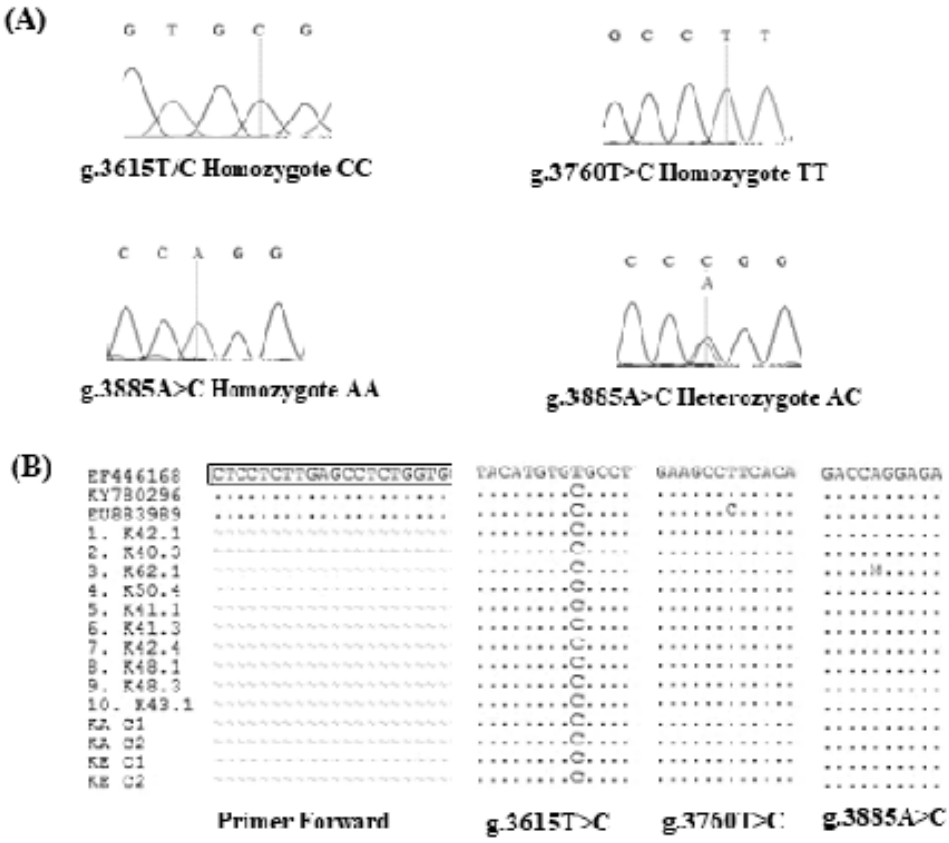

Figure 2. SNPs variants of Local goat compare to the GenBank data. (A) Chromatogram at SNP position. (B) Alignment three GenBank of Capra hircus and local goat (No.1-10=Bligon Goat, KA=Kacang Goat, KE=Kejobong Goat).

Table 1. Amino acids analysis of GDF9 gene from representative local goat and GenBank data

SNP	Genbank	Codon	Amino Acid	Mutation type	Genotype
g.3615T>C	EF446168.2	GUG	Valine	Non	TT
	KY780296	GCG	Alanine	synonimous	CC
	EU883989	GCG	Alanine		CC
	SampleK42.1	GCG	Alanine		CC
	Sample K62.1	GCG	Alanine		CC
	KA C1 (Kacang)	GCG	Alanine		CC
	KE C1 (Kejobong)	GCG	Alanine		CC
g.3760T>C	EF446168.2	CCU	Proline	synonimous	CC
	KY780296	CCU	Proline		CC
	EU883989	CCC	Proline		CC
	Sample K42.1	CCU	Proline		CC
	Sample K62.1	CCU	Proline		CC
	KA C1 (Kacang)	CCU	Proline		CC
	KE C1 (Kejobong)	CCU	Proline		CC
g.3855A>C	EF446168.2	CCA	Proline	synonimous	CC
	KY780296	CCA	Proline		AA
	EU883989	CCA	Proline		AA
	Sample K42.1	CCA	Proline		AA
	Sample K62.1	CCC	Proline		AC
	KA C1 (Kacang)	CCA	Proline		AA
	KE C1 (Kejobong)	CCA	Proline		AA

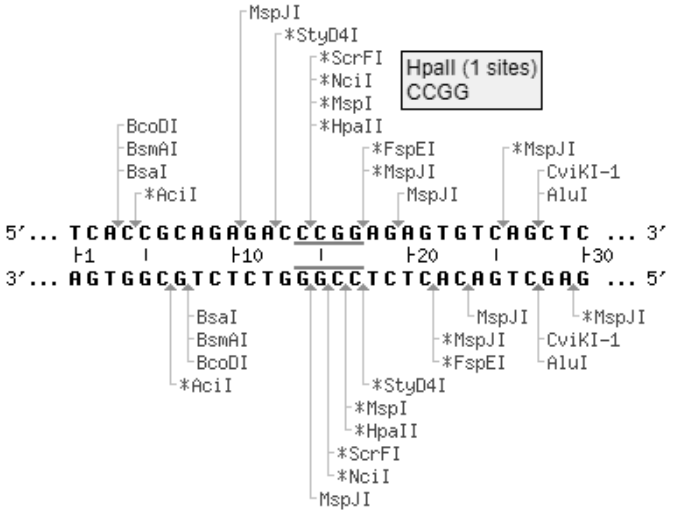

Figure 3. Restriction enzymes g.3855A>C.

Table 2. Restriction enzyme mapping

Sampel	MspI (C^CG_G)		HpaII (C^CG_G)		Genotip
	Site	Fragment	Site	Fragment	
K42.1	0	456	0	456	AA
K40.3	0	456	0	456	AA
K62.1	1	456, 306, 149	1	456, 306, 149	AC
K50.4	0	456	0	456	AA
K41.1	0	456	0	456	AA
K41.3	0	456	0	456	AA
K42.4	0	456	0	456	AA
K48.1	0	456	0	456	AA
K48.3	0	456	0	456	AA
K43.1	0	456	0	456	AA
KA C1	0	456	0	456	AA
KA C2	0	456	0	456	AA
KE C1	0	456	0	456	AA
KE C2	0	456	0	456	AA

The restriction enzyme price list shows that the price of the HpaII enzyme is $ 67 for 2000 units and the MspI for 5000 units is $ 67 (New England Biolabs, 2021). The criteria for selecting restriction enzymes is ease of use: only 1 µl is required for each reaction, and standard enzyme concentrations are sold at 2000-20,000 units / ml (2-20 units / µl). A specific intersection. Problems can occur if the intersection is not identified and you are better aware of other intersections. The number of pieces is not too large and the band size is not too short, exceeding 100 bp (Gerstein, 2001). Based on the restriction enzyme selection criteria and considering price factors, the MspI enzymes met these criteria.

Conclusions

Based on the research that has been done, it can be concluded that there are 3 SNPs, namely g.3615T>C, g.3760T>C, and g.3855A>C. The restriction enzymes identified were found in target sequence SNP g.3855A>C, namely HpaII and MspI. The recommended restriction enzymes for further use are MspI.

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