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Abstract. A prolific trait was essential to determine the litter size in goats including Indonesian Local Ettawah Goats (ILEG), and one of the genes that influence the prolific trait was the GDF9 gene. The purpose of this study was to describe the amino acid expression of the GDF9 gene sequencing in ILEG compared with Genbank accession number GU784823.2. Capra hircus GDF9. This research method was an experiment which includes the sampling of 21 does who had given birth more than once with low, medium and high prolific categories. Furthermore, samples were PCR with exon 1 GDF9 gene, and sequencing results were carried out by amino acid alignment with BioEdit software. The results showed that there were 3 variants in the exon 1 GDF9 gene in ILEG which was located at the location of 27, 61 and 85 amino acid residues. At the 27th residue there was a change in the proline amino acid to alanine (CCT to GCT), at the 61st residue there was no change in the amino acid Leucine, but there was a change in the base N compound (CTA to CTC), and at 85 residues there was a change in acid residue amino Alanine becomes Glycine (GCT to GGT).

1. Introduction

Indonesian Local Ettawah Goats (ILEG) is one of the many Indonesian local goats that are maintained in Indonesia. In 2017, the goat population of 18,410 tails was the highest population compared to the population of sheep, cattle, and buffalo in Indonesia [1]. The distinctive features of ILEG goats are folded and long ears and about 80% fur color dominated by black and white and $\pm$ 20% brown and white [2,3]. The history of ILEG was a goat from a crossing of a local Indonesian goat with an Indian goat brought by Dutch colonizers to Indonesia. ILEG is a dual-purpose animal with more than one child per birth [2]. To exploit the potential of increasing the prolific potency in PE goats, and these properties must be studied molecularly using genes related to the nature of litter size or the nature of prolification. Several genes including GDF9 [4]. GDF9 could influence prolific traits of goats was a gene group of TGF beta superfamily which plays a role in the process of folliculogenesis and prolification and triggers the secretion of progesterone in luteal cells [5,6,7]. The process of folliculogenesis was essential in follicular development.

The GDF9 gene strongly influences increased ovulation rate. Polymorphism in the GDF 9 gene was associated with litter size [8,9]. Prolification trait was essential in determining the amount of litter size in goats. Research on ILEG is important, especially regarding the GDF9 gene which plays a role

in the prolification process was still rare. Determination of this genetic information could help farmers in determining the does that had a high prolification rate so that it could increase the amount of litter size. An increase in the number of litter sizes would increase the number of goat populations which was urgent for breeding.

2. Materials and Methods

The samples used in this study were 21 does of ILEG who had to kid more than once with the following data: 1) kidding group 1 - <2 heads per kidding (sample codes GR12, GR18, GR10, GR110, I47, BWC17, BWA4 and AGI24), 2) kidding group 2 - <2.5 heads per kidding (sample codes AGI1, AGI4, AGI5, AGI6, AG123, AG122, AG117, AG110, and IB135), 3) kidding groups 2.5 - 3 heads per kidding (sample codes LWI2, LWI10, AGI18). Prolific data was obtained from farmer interviews because no recording in their farm and breeders could remember the number of kidding to each does, they own with common ownership of 5-10 animals.

The blood samples were isolated using the salting out method. The PCR program used was predenaturation at 95 ° C for 5 minutes, the second stage with 34 cycles, each cycle consisting of denaturation at 95 ° C for 30 seconds, annealing at 61 ° C for 30 seconds and extension at 72 ° C for 30 seconds and the final extension stage at 72 C for 10 minutes. Primary GDF9 exon 1 used in the N-base sequence: GDF9 exon 1 Forward 5'GGAAGAAGACTGGTATGGGGAAATG 3' and Reverse 5' CTGCTCCTACACACCTGCCGC 3'.

Sequencing data were obtained by sending PCR product to 1st BASE, PT Genetika Science Jakarta. The results obtained from sequencing data were in the form of curved lines consisting of 4 colors that determined nucleotide bases. The black color indicates the base Guanine (G), the color green indicates the base of Adenine (A), the blue color is base Cytosine (C), and the color red indicates the base of Thymine (T).

Sequence results from 1 GDF9 exon gene segment from ILEG were analyzed using Bioedit and MEGA (Molecular Evolutionary Genetics Analysis) Version 6.0 to determine the point of mutation that occurred or SNP (Single Nucleotide Polymorphism). Furthermore, different sequences were analyzed by BLAST (Basic Local Alignment Search Tool) compared with Genbank accession number GU784823.2. Capra hircus GDF9.

3. Result and Discussion

PCR with GDF9 exon one gene which was carried out in this study using 21 samples with three various prolifications.

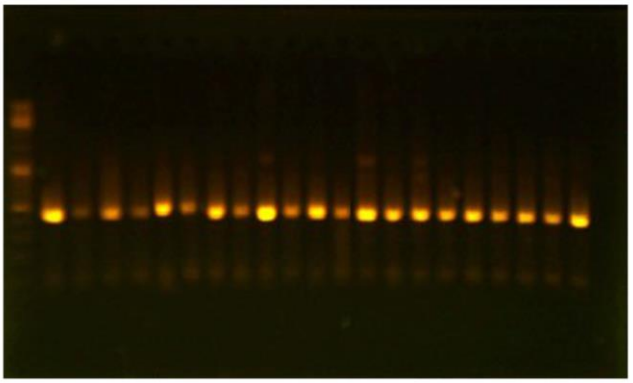

Figure 1. Electroforesis results of PCR product of GDF9 exson 1 gene

Figure 1 showed that all PCR products with exon 1 GDF9 gene were amplified at 400 bp, while research used local Chinese sheep (White Goat) using the same amplified gene at a size of 461 bp [10] and samples with Egyptian sheep amplified at 710 bp [11]. The results of the study found novelty in

the polymorphism of GDF9 exon one gene in Indonesian Local Ettawah Goat based on Genbank accession number GU784823.2 Capra hircus GDF9. The details of polymorphism can be seen in Table 1.

GDF2 gene consists of 2 exons namely exon 1 and exon 2 with amino acid residues of 453 amino acids. The peptide signal is in positions 1-32 and the amino acid chain in positions 33-453. Based on the data in Table 1 shows that there are three variants in the exon 1 GDF9 gene which was located at the locations of amino residues 27, 61 and 85. At the 27th residue, the amino acid proline was changed to alanine (CCT-GCT), at the 61st residue was unchanged in the amino acid Leucine (CTA-CTC) and at 85 residues there was changed in the residual amino acid Alanine to Glycine (GCT-GGT). The description of the polymorphism of the Indonesian Local Ettawah Goat was found in Figure 2.

Tabel 1. GDF9 exon 1 gene polymorphism on ILEG based on Genbank accession number GU784823.2 Capra hircus GDF9

Varian GDF	Perubahan basa	Lokasi basa ke-	Lokasi residu asam amino ke-	Perubahan asam amino
G1	C-G	129	27	Proline - Alanine (CCT-GCT)
G2	A-C	233	61	Unchanged Leucine (CTA-CTC)
G3	C-G	304	85	Alanine-Glycine (GCT-GGT)

In Figure 2, it could be seen that the alignment of GDF9 exon one protein in Indonesian Local Ettawah Goat was almost the same as Genbank accession number GU784823.2 Capra hircus Gdf9 except in three protein residue regions. Changes in the three protein residue areas at positions 27, 61 and 85, while other protein residue areas (450 residual regions) are the same conserve areas as protein sequences from Genbank accession number GU784823.2 Capra hircus GDF9. Two locations of the 27th and 85th amino acid residues that were individual mutations and one amino acid location at the 61st residue did not change in amino acids (Leucine), but there was a change in N-base compounds to CTA from CTC (Figure 3). Individual mutations that occurred in the 27th and 85th amino acid residues were only 9.52% (from AGI11 and AGI24 individuals who had multiple and single kidding) and 14.29% of the total samples analyzed from individuals who have multiple and single births.

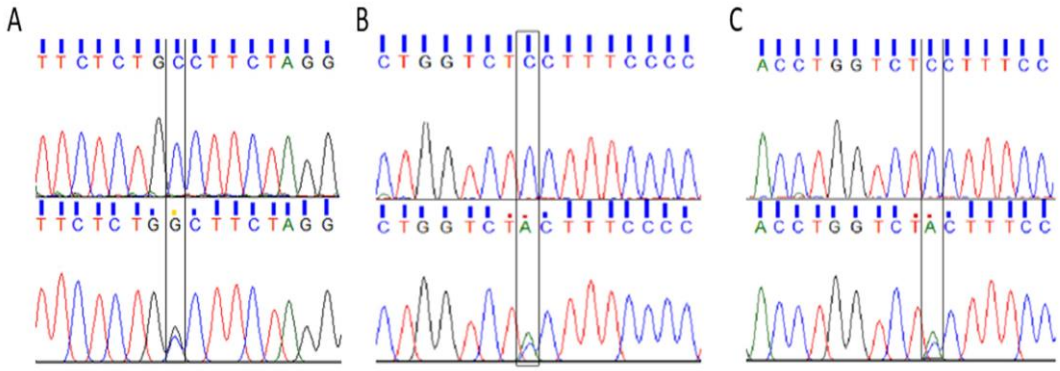

Figure 2. SNPs Variants of Indonesian Local Ettawah Goat C129G (A), C233A (B) dan C304A(C).

In Figure 2 we could see nucleotide base changes C129G (A), C233A (B) and C304A (C). The modification of base cytosine (C) to guanine (G) and base cytosine (C) to adenine (A) is a change in

the base of pyrimidine to purine that is the change in base of the double bond to double bond or vice versa is called the transversion mutation. Transversion mutations also occurred in Indian goat livestock research with Gdf9 gene, there was a change in nucleotide position number 959 from A → C, and at 1189 nucleotide position from G → A 12, and changes were also found in Gaddi Goat in position C1893T and C1962A [13].

Based on the phylogenetic tree using the neighbor-joining of Figure 3 showed that AGI24, AGI11, and GRI10 were incorporated into one cluster. Samples of LW2, LWI10, GRI8, GRI2, I47, BWC17, BWA4, IB35, AGI23, AGI22, AGI18, AGI17, AGI10, AGI8, AGI6, AGI5, AGI1, and AGI4 were located in the same group. This showed that mutations only occur randomly in goat populations with various prolific. Also, the change of the amino acid from the proline to alanine at position 27 and the change in the amino acid alanine to glycine at position 85, was a change in non-polar amino acids. Proline, alanine, and glycine are non-polar amino acids, in the absence of changes in amino acid configurations it was likely not to affect the prolific nature. According to Assam Hill Goat result that prolification of that animal was not influenced by the GDF9 gene and genetic factors that control the amount of litter size were not related to mutations that occur in the GDF9 gene [14]. The GDF9 gene was associated with role ovarian function and GDF9 in goats is different when compared to sheep [15]. Naturally goats had a low ovulation rate compared to sheep [10]. This low ovulation rate caused the goat's prolific trait to be lower when compared to sheep.

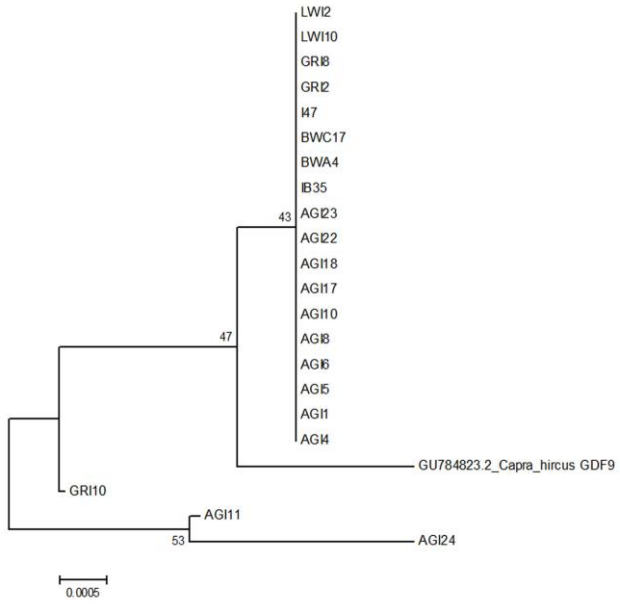

Figure 3. Analysis result of ILEG Phylogenetic by Neighbor joining analysis

4. Conclusion

The conclusion of this study was the DNA and protein sequences of Indonesian Local Ettawah Goats with GDF9 exon one gene were almost similar to Genbank accession number GU784823.2 Capra hircus GDF9. Polymorphism novelty at the 233 base location changed in Adenine bases without changing the protein residues produced. The occurrence of mutations was an individual who had nothing to do with the prolific trait.

Acknowledgments

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