Melatonin receptor 1A (MTNR1A) gene polymorphisms in the local goat population in South Sulawesi region of Indonesia

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Abstract. Some important traits are involved in controlling the reproductive trait of goats, one of which is the Melatonin Receptor 1A (MTNR1A) gene. The MTNR1A gene is known with seasonal reproductive activity and related to lambing frequency in the goat. The purpose of this research was to determine the genetic polymorphisms of the MTNR1A gene in Kacang, and Peranakan Ottawa (PE) goat population reared traditionally in South Sulawesi region of Indonesia. In total 253 heads of goat consist of 137 heads of Kacang and 116 Peranakan Ottawa from South, Sulawesi region was used as research samples for blood collection. The blood samples were collected from the jugular vein, which was then continued for DNA extracted by using a DNA extraction kit. The MTNR1A genotype was identified by PCR-RFLP technique using restriction enzyme RsaI. The result showed that there was genetic diversity in the MTNR1A gene in Kacang and PE population with the obtained of two alleles R and r. The common allele was R with frequency 0.93 in Kacang population while in PE population was 0.89. The r allele was 0.06 and 0.10 in Kacang and PE population, respectively. The most common genotype found in the population was RR (0.95), while Rr was only 0.05 and rr genotype did not found in this population. Observed heterozygosity value was 0.05. According to the Hardy-Weinberg test, this population was in equilibrium for MTNR1A gene. In conclusion, this finding indicated that there was genetic diversity exists in local goat population, and future research needed to find any association with this genetic variation with reproductive performance, the data obtained from this study could be used for the strategic program in goat breeding to increase reproductive performance of local goat especially fertility traits.

1. Introduction
Kacang and Peranakan Ettawa (PE) breeds are common local goats bred by farmers in South Sulawesi Province. Kacang goat is generally bred for meat purposes, while Peranakan Ettawa for dual purposes (producing meat and milk). In terms of reproductive performance, both types were prolific, with average litter size 1.8 and 1.59 for Peranakan Ettawa and Kacang goat, respectively [1, 2].

Reproductive traits are one of the economic traits in farm animals that can be used in a selection program. These traits were controlled by many genes. A number of important genes are involved in controlling the reproductive trait of goats, one of which is the Melatonin Receptor 1A (MTNR1A) gene. The MTNR1A gene is known a correlation with seasonal reproductive activity and related to lambing frequency in the goat [3].

Polymorphism of the MTNR1A gene has been reported by several studies to be related to the expression of seasonal reproduction in goats [4-6]. Polymorphism of the MTNR1A gene showed an
association between genotype RR and year-round estrus in goats, while genotype Rr showed an association with seasonal estrus in goats [5].

The purpose of this research was to determine the genetic polymorphisms of the MTNR1A gene in Kacang, and Peranakan Ettawa (PE) goat population reared traditionally in South Sulawesi region of Indonesia. Thus, we analyzed the genotype and allele frequencies in two goat breeds, the Peranakan Ettawa goat representative of high fertility goat while Kacang representative of good adaptability goat in tropical South Sulawesi environment.

2. Materials and Methods

2.1. Blood sampling and DNA extraction
In total 253 heads of goat consist of 137 heads of Kacang and 116 Peranakan Ettawa from South Sulawesi region was used as research samples for blood collection. The blood samples were collected from the jugular vein, which was then continued for DNA extracted by using a DNA extraction kit.

2.2. MTNR1A genotyping
The MTNR1A genotype was identified by PCR-RFLP technique using restriction enzyme Rsal. Fragment of the MTNR1A gene was amplified with forward and reverse primer sequences based on [7] with predicted amplicon length 824 bp.

The PCR condition starting with an initial denaturation at 94 °C for 5 mins, followed by 35 cycles each of 94 °C for 30 s, annealing temp at 60 °C for 45 s, and ending with final extension cycle at 72 °C for 5 min in the Eppendorf PCR machine. PCR products were then checked on the 2% agarose gel with 1x TBE buffer and visualized on the gel documentation system. Genotypes were determined based on the length of RFLP DNA fragments product which is restricted to the Rsal enzyme.

2.3. Data analysis
Allele and genotype frequencies, H-W equilibrium, observed and expected heterozygosity were calculated by using PopGene32 program [8].

3. Results and Discussion
The amplification of the MTNR1A gene in local goat population shows PCR product approximately 824 bp in length. The amplification results consistent with previously reported by [7]. The PCR product was then restricted with Rsal enzyme and presented in Figure 1. Restriction with Rsal enzyme produced five fragments (23, 53, 70, 267 and 411 bp). The site in position 53 was polymorphic, the presence of restriction site produces two fragments of 53 and 267 bp (R allele), while the absence will produce one fragment 320 bp (r allele).

Two alleles (R and r) and two genotypes (RR and Rr) were observed for MTNR1A gene. The highest allelic frequencies value for R (97.45) was found in Kacang goat. Based on genotype frequency, rr genotype for MTNR1A was not identified in both breed population. Allele and genotype frequencies of the MTNR1A gene in local goat population presented in Table 1.

Several studies reported an association between variations in the MTNR1A gene with reproductive traits such as year-round estrus and seasonal anovulatory activity in Small Tailed Han and Dorset Sheep [3, 9]. In the goat, RR genotype was reported to have an association with year-round estrus in Jining Grey and Boer goats, while Rr genotype was reported to have an association with seasonal estrus in local Chinese goat in China [5]. Unlike goats in sub-tropical areas, Kacang and Peranakan Ettawa were goats that have long been adapted in the tropical environment then do not know seasonal breeding, almost all year can mating in any season. These conditions are likely to cause common alleles identified in the MTNR1A gene was the R allele, and the common genotype was RR in both Kacang and Peranakan Ettawa goat populations in South Sulawesi province.

The R allele is probably fixed in both populations, it can be seen from the high frequency of the R allele in both populations. Fixation of this allele can be caused by genetic drift and natural selection [10].
Figure 1. Gel visualization for MTNR1A genotypes by PCR-RFLP analysis. Lane M = DNA marker (100 bp DNA Ladder), RR genotype with = genotype RR and Rr

Table 1. Allele and genotype frequencies of the MTNR1A gene in local goat population

| Breed Population | N   | Genotype Frequency (%) | Allele Frequency (%) |
|------------------|-----|------------------------|----------------------|
|                  | RR  | Rr                     | rr                   |
| Kacang           | 137 | (94.89)                | 7 (5.11)             |
| Peranakan Ettawa | 116 | (78.44)                | 25 (21.56)           |
| Total            | 253 | (87.35)                | 32 (12.65)           |

Note: N = Sample size

The genetic diversity of the MTNR1A gene could be seen from observed and expected heterozygosity (Table 3). The observed heterozygosity value for Kacang and Peranakan Ettawa goat were 0.05 and 0.21, respectively. These results indicate that the genetic diversity of MTNR1A in local goat population was very low (Ho < 0.2). The allele diversity index was categorized low less than 0.3 [11].

Table 2. Observed and Expected frequencies of the MTNR1A gene in local goat population

| Genotype | Observed Freq. (O) | Expected Freq. (E) | $\chi^2$ (Chi-Square) | p-value (0.05:1) |
|----------|--------------------|--------------------|-----------------------|------------------|
| RR       | 221                | 211.98             | 1.11*                 | 0.29             |
| Rr       | 32                 | 30.03              |                       |                  |
| rr       | 0                  | 0.98               |                       |                  |
| Total    | 253                | 253                |                       |                  |

Based on Chi-square test results showed that the distribution of genotype frequency and an allele of the MTNR1A gene in local goat population (Kacang and Peranakan Ettawa) were according to the Hardy-Weinberg equilibrium.

Table 3. Heterozygosity value of MTNR1A gene in local goat population

| Breed Population | n   | Heterozygosity |
|------------------|-----|----------------|
|                  |     | $H_o$     | $H_e$     | Nei*     | Average |
| Kacang           | 137 | 0.0511   | 0.0500   | 0.0498   |         |
| Peranakan Ettawa | 116 | 0.2155   | 0.1931   | 0.1923   |         |
| Total            | 253 | 0.1265   | 0.1187   | 0.137    | 0.1185  |

Note: Ho = Observed Heterozygosity, He = Expected Heterozygosity, *Nei heterozygosity

Genetic variation data from the MTNR1A gene can be used as a source of information in improving the genetic quality of local goats in South Sulawesi province. Many information about genetic diversity can be used in the selection or breeding program [12,13], especially reproductive traits and breeding strategies to increase farmer’s income and welfare.
4. Conclusion
In conclusion, this finding indicated that there was genetic diversity exists in local goat population, and future research needed to find any association with this genetic variation with reproductive performance, the data obtained from this study could be used for the strategic program in goat breeding to increase reproductive performance of local goat especially fertility traits.

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