Luteinizing Hormone Receptor Gene Polymorphism of Pasundan Cattle in Ciamis

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Abstract. Pasundan cattle is a local livestock that has lived and well adapted in West Java. In 2015, the population declined by 20.96% caused by the high selling prices and changes in land functions. The impact of declining of the population can causes genetic degradation indirectly. One of the efforts to increase the population is selection of cattle based on luteinizing hormone receptor gene polymorphism that can used as molecular selection for reproductive traits. Thirty seven heads of Pasundan cattle with artificial insemination calving records was used in this study. The exon 11 of LHR gene was amplified and subsequently analysed by sequencing method. The sequencing result were found three genotypes of CC, CT and TT in SNP g.1337C>T. The frequency of T allele (0.527) shows more dominant over C allele (0.473). The chi square test showed that Pasundan cattle were in Hardy-Weinberg equilibrium. The heterozygosity value of Ho (0.513) indicating that the heterozygosity value of the LHR gene belongs to high category (>50%). The high genetic diversity of the Pasundan cattle population indicates that there has not been selection program yet. The results of this study can be used as a reference to continue the selection program improving genetic quality in the reproductive traits of Pasundan cattle.

1. Introduction
Pasundan cattle live spread throughout the South Coast of West Java and buffer zone area of protected forests along the North Priangan region[1]. In 2013, the population of Pasundan cattle reaches about 50,000 heads [6]. In 2015, the population decreased by 20.96%, the most common in the buffer zone area. It causes by highly selling price and changing of land function. The impact of declining of the population can causes genetic degradation indirectly, such as decrease of genetic quality, effective population, inbreeding increment and purity degradation [4]. Conservation of cattle pasundan important to be implemented, to overcome the threat of the population base area experiencing some limitations that can lead to extinction [1].

Reproduction traits plays an important role in increasing livestock populations. Reproduction of Pasundan cattle potency needs to be studied more deeply through genotypic studies which is a modern method of selecting superior offspring. According to [9] one approach to increase the informativeness of genetic information is to identify the markers for genes that have direct effects on specific aspects of fertility such as the luteinizing hormone/choriogonadotropin receptor (LH/CGR). LHR is one of reproductive genes related to reproduction. These gene affect the endocrine system that plays a major role in controlling reproductive function regulated through the pituitary-gonad hypothalamus. Mutations in the LHR genes, affect the regulation of gonadotropin hormones that are potentially linked to reproductive phenotypes such as early puberty and calving interval [12].
2. Materials and Methods

2.1. Procedure
Thirty seven heads of Pasundan cattle with artificial insemination calving records was used in this study. The sample is a productive female cattle in Cattle Breeding and Artificial Insemination Development Center (BPPIBTSP) Ciamis, West Java.

2.2. DNA extraction
DNA extraction refers to the reference of [14]. Blood samples were collected from a coccygeal vein into a vacutainer tube containing K3EDTA. Blood samples were stored under refrigerator temperature (4 – 5 °C) until DNA isolation is performed. DNA isolation was performed using Genomic DNA mini kits (Geneaid Biotech Ltd., Taiwan) according to the procedures available from the manufacturer. The obtained DNA product was stored at -20 °C until the polymerase chain reaction (PCR) process is performed.

2.3. DNA amplification
The PCR assay for LHR gene was using with a primer set (forward 5' CAA ACT GAC AGT CCC CCG CTT T_3' and reverse 5' CCT CCG AGC ACT ATG ATG AG GAG GC_3') proposed by [10] and [3]. The extracted DNA samples were taken as 2μl, then added 0.5μl primer F, 0.5μl primer R, 5 μl PCR mix and 2 μl destillation water. Mixtures of these were incubated using a thermocycler PCR machine. A mixture of PCR LHR gene was performed, following the protocol: initial denaturation at 94 °C for 5 min. Continued with 37 cycles starting at 94 °, 60 ° and 72 °C (45 seconds respectively) followed by the final extension at 72 °C for 10 minutes. Digested PCR products were electrophoresed on 1% agarose gels and then visualized under a UV transilluminator. After PCR products were visualized, volume of mixtures converted into 30 μl for sequencing analysis. The data of sequences obtained were processed and analyzed by using FinchTV and MEGA7.

3. Results and Discussion

3.1. DNA sequence
The sequencing of LHR gene in Pasundan cattle yields 303 bp sequence and only one SNP is found. The single nucleotide polymorphism found lies in the position of SNP g.1337C> T. This position coincides at the point of restriction by HhaI enzyme in RFLP method [3, 10]. Based on GenBank (NC_037338.1), Pasundan cattle undergo a mutation at that base position. There are three genotypes found, labelled CC, CT and TT by identified its chromatogram using FinchTV. The type of mutation occurring in the LHR gene of Pasundan cattle is a transition mutation that changes between the pyrimidine base (C) and pyrimidine (T). This SNP was analysed as non-synonymous mutation that altered amino acid Arginine to Tryptophan.

Sequencing analysis is used initially to be expected to find many SNPs. In a previous study, using two different primer pairs, [8] found other SNPs from LHR gene analysis results in exon 11. There were found three SNPs at position g.1401G> T, g.1470C> T and g.1581G> T in Bos taurus.

3.2. Genotypic and allelic frequencies
The LHR gene in Pasundan cattle is polymorphic. [7] states an allele is polymorphic if one of its allele frequencies is less than 0.99. The frequency of LHR gene genotype in Pasundan cattle shows that the frequency of CT genotype (0.513) is generally the highest in the observed population or the dominant allele. The genotype frequency of CC (0.216) is the lowest and the genotype TT (0.270) which is also not much different. The frequency of T allele shows higher than C allele (Table 1).
3. Frequency genotype and allele of LHR gene in pasundan cattle

| Frequency | Genotype Freq. (n) | Allele Freq. | N |
|-----------|-------------------|--------------|---|
| Observed  |                   |              |   |
|           | CC 0.216 (8)      | CT 0.513 (19)| TT 0.270 (10) |
| Expected  | 0.224             | 0.498        | 0.278 |

At the mixing of *Bos taurus* × *Bos indicus* cattle in Europe, identification of the LHR gene in the PCR-RFLP method with the *HhaI* enzyme also produced three genotypes, CC, CT and TT. The frequency of the TT genotype originates from 0.366 – 0.888 and CC belongs to 0.151 – 0.574. The frequency of C alleles is higher than the T allele [10]. In Turkish dairy cows (*Bos taurus*), the frequencies of CC, CT and TT genotypes are 0.928, 0.047 and 0.023, respectively. The frequency of C allele (95.20) is higher than the T allele (4.80) [2]. In addition, feed on Nellore (*Bos indicus*) cattle in Brazil, high frequency of TT genotype (0.540), low frequency of CC genotype (0.030) and T allele frequency (0.755) higher than C allele (0.245) [11].

Based on these findings, each type of cow has a different genetic diversity. The genetic diversity of Pasundan cattle (*Bos sondaicus*) is close to the diversity possessed by Nellore cattle as *Bos indicus* which has a higher T allele frequency than C alleles. This may be due to morphological and physiological similarities, and Pasundan cattle also have *Bos indicus* blood which is a living and adapts well in the tropical climate.

### 3.3. Genetic parameters

Genetic diversity can be determined by the value of heterozygosity, Hardy-Weinberg Equilibrium and Polymorphism Information Content (PIC) as genetic parameters shown in Table 2.

| Table 1. Frequency genotype and allele of LHR gene in pasundan cattle |
|-------------------------|---------------------|----------------|---|
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|-----------|-------------------|--------------|---|
| Observed  |                   |              |   |
|           | CC 0.216 (8)      | CT 0.513 (19)| TT 0.270 (10) |
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Heterozygosity observation (Ho) of LHR gene in Pasundan cattle have value that is 0.513, while heterozygosity expectation (He) is 0.498. The value of Ho is greater than He. The heterozygosity value above 0.5 (> 50%) indicates the variation of a gene in a population is high [7]. This is because at the beginning of the procurement of Pasundan cattle, BPPIBSPT Ciamis bring cattle pasundan from various breeders so that the pattern of random mating and not the occurrence of inbreeding.

The value of chi-square Hardy-Weinberg Equilibrium shows there’s no significant, thus the diversity of cattle population pasundan still in a balanced state. This condition indicates that the selection program has not been implemented in Pasundan cattle. In this case the application refers to the diversity of LHR genes.

The PIC value is one of the parameters that indicates the level of information of a genetic marker in livestock [5]. The value of PIC Pasundan cattle obtained is 0.374. According to [15], the PIC value is high if the value of PIC > 0.50, if PIC 0.25 – 0.50 is categorized as moderate and when PIC < 0.25 is categorized as low. Based on these parameters, the PIC value of LHR gene in Pasundan cattle is included in the moderate category. It is also found in Sudanese cattle that have relatively similar PIC values [13]. It is assumed that the LHR gene in *Bos indicus* is still diverse. Although not in a high category, this gene is still potential to serve as assist in selection (marker assisted selection).

Other studies conducted by [8] indicating that the combinations of the three SNPs g.1401G>T, g.1470C>T and g.1581G>T in *Bos taurus* were significantly associated with variations in the fertility
characteristics of cattle, particularly calving intervals and days to first service. In this case the diversity of LHR genes as genetic markers in Pasundan cattle has potency and applicable as marker assist selection (MAS).

Luteinizing hormone is a hormone that plays an important role in ovulation. In ovulation, increasing of LH will cause the ovum cells out of the ovary to be fertilized by sperm. Ovulatory conditions that are not optimal can cause no ovulation that affects pregnancy failure in cattle. This has an impact on the decrease of reproduction performance, which is increasing the amount of service per conception, prolonged the length of days open and calving interval. Economically it will increase its cost in the business of beef cattle, both longer maintenance for the expenditure of feed and maintenance costs to improve the condition of livestock under normal circumstances.

4. Conclusion
LHR gene in the Pasundan cattle shows diversity with the discovered of three types of genotypes. Based on the genetic parameters obtained, the LHR gene has high heterozygosity, the population belongs to Hardy-Weinberg equilibrium, and the moderate PIC value that still have potency as marker assist selection. So the results of this study can be used as a reference to continue the selection program improving genetic quality in the reproductive traits of Pasundan cattle.

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