Genetic marker exploration of fertility genes IGF 1 and IGF 2 at Ongole Cross breed cattle with naturally twin birth

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Abstract. The phenomenon of beef cattle with twin births naturally is still unknown. The aim of this research is to explore genetic marks of IGF1 and IGF 2 genes as regulators of fertility in beef cattle with twin births naturally. Ongole Cross (OC) breed cattle, each 10 cows with single or twin births naturally in Beef Cattle Research Station, take blood to make plasma samples according to the Boyan method, than make DNA isolation samples according to the QIAamp method, to be PCR analysis according to the Perkin-Elmer method and to be sequencing according to the Phenol-Chloroform method, in the Tropical Disease Center (TDC) Hospital, Airlangga University. The primers used are IGF 1 forward 5’-CCTCTGGGGGCGAATGGGTG-3’, reverse 5’-CGACTTGGCGGGGCTTGAGAG-3’ and IGF 2 forward 5’-TCTGTGGGCGGCAGGAGGAGTGTG-3’, reverse 5’-AGTCTCCAGCAGGAGGAGATGA-3’. Parameters observed: allele band, genotypic diversity and nucleotide acids composition of IGF genes. Allele band variation data is processed with Nt programe software to determine its genotype, nucleotide gene sequens data is processed using the GENETIC MAC version 8 program to determine gene mutation construction of IGF, then presented descriptively. The results showed: the spread of IGF 1 allele band in cows with single birth at position 875–1200 bp and twin birth at position 875–1485 bp; spreading of IGF 2 allele band in cows with single birth at position 450-590 bp and twin birth at position 455–1110 bp; genotype diversity of IGF 1 genes at cows with single birth was homozygous AA with allele size 920–1200 bp and twin birth was heterozygote AB with allele size A 875-1150 bp and B allele 1250–1485 bp; genotype diversity of IGF 2 genes in cows with single birth was homozygous AA with allele size 460–560 bp and twin birth was heterozygote AB with allele size A 450-590 bp and B allele 940–1110 bp; at certain positions and nucleotide acids arrangement in both IGF 1 and IGF 2 genes of cows with single birth, was different with twin birth. It was concluded, the genetic marka of IGF 1 and IGF 2 genes of OC breed cattle, were different among cows with single birth and cows with twin birth.

1. Introduction

Identification of genotype specific genes that control the production capability or economically valuable traits in cattle, is thing to do in order to produce superior germ cattle [1]. Development of biomolecular technology for polymorphic molecular markers mapping have been able to identify quantitative trait (QTL) genetic variation of several genes, so that we can choose a cattle to be a parent with trait genes specific are desired to be passed on to offspring.

The incidence of twin birth in cattle, one of the main factors is determined by genetic [2]. There is a strong genetic correlation (0.75–0.90) between twin birth and ovulation rate [3]. The trait of multiple ovulation in cattle that allows to occurrence of twin birth, has been identified in several genes on
chromosomes 1, 5, 7, 10, 19, 20 and 23 [4], so that trait of multiple ovulation or twin birth in cattle is not determined by single gene, but by many genes [5].

Genotypes that have genetic control to twin birth in beef cattle, such as insulin-like growth factor 1 gene or IGF 1 gene that located on chromosome 5 (BTA5) at nucleotide position 66,532,877 - 66,604,734 [3] and the IGF 2 gene. [6]. The IGF 1 gene stimulates mitogenesis of granulosa cell and ovaries to respond to gonadotropins in growth and steroidogenesis of ovarian cell culture, also important role in regulation of folliculogenesis which is thought to be involved in the multiple ovulation process in cattle [7]. Hypothesis of Sawa et al [8] which found an increase of hormone IGF 1 concentration in blood serum (extra ovary) and in follicle fluid (intra ovarian) states, the IGF 1 gene is thought to role stimulating ovulation rate until during fertilization process which results pregnancy and twin pregnant in cattle; also, work and function of IGF 1 in fact together and support each other with function of the IGF 2 gene.

The results of our observations to South Sumatra, West Sumatra, Central Java, East Java, South Kalimantan and NTB the provinces, showed that number of cows who have twin birth naturally is very small and chance of recurrence is also very small. Through twin birth will be more calves than single birth [9], although the natural incidence in cows is very low [10]. This research aims to determine how allele bands, genotypic diversity and nucleotid acid composition of the IGF 1 and IGF 2 genes in Ongole Cross breed cattle as genetic markers of twin birth trait regulate.

2. Method

This study used 10 Ongole Cross breed cows that had twins naturally and 10 Ongole Cross breed cows that never had twin birth. Approximately 3 mL of blood samples were collected from jugular vein in the EDTA tubes and then stored in refrigerated temperature (4℃) before processed at Reproduction Laboratorium in Beef Cattle Research Station to get their plasma.

2.1. DNA isolation

Blood samples that have been collected, then proceed to the stage of DNA isolation at the Tropical Disease Center Biomolecular Laboratory of Airlangga University using the QIAamp DNA extraction kit (QIAGEN) according to the protocol provided.

2.2. Detection of IGF 1 and IGF 2 genes by PCR and RFLP techniques

The extracted DNA sample was then continued for PCR and PCR-RFLP tests using IGF1 and IGF2 primers. The primers pairs for IGF1 and IGF2 identification were:

a. IGF 1 forward : 5’ CCTCTGCGGGGCTGAGTTGGT-3’
   reverse : 5’-CGACTTGGCGGGCTTGAGAGGC-3’

b. IGF 2 forward : 5’-TCTGTGCGGCGGGGAGCTGGT-3’
   reverse : 5’-AGTCTCCAGCAGGGCCAGGTCG-3’

The PCR reactions used are as follows: pre heating/pre denaturation 93-95°C for 300 seconds, denaturation 94–95°C for 30 seconds for 30 cycles, annealing 55–60°C for 30 seconds, extension 72°C for 60 seconds and post PCR 4°C for 60 seconds. The results of PCR amplification in the form of band electrophoresis in 2% agarose gel and ethidium bromide were photo with polaroid film. The electrophoresis of bands which were assumed as gene alleles, is evaluated together with its positive and negative controls. Migration of allele bands in sample of each cow is assumed as allele diversity of genes.

2.3. Detection of IGF 1 and IGF 2 genes by sequencing technique

Allele variations obtained from the PCR-RFLP results were then verified by DNA sequencing techniques in the laboratory of Tropical Disease Center Biomolecular, Faculty of Medicine, Airlangga University.

Observed variables: allele bands, genotypic variability and sequence of nucleotide acids of IGF 1 and IGF 2 genes. Data of allele band variations were processed with Nt program software to determine
its genotype, while data sequence of nucleotide IGF 1 and IGF 2 genes were processed using the GENETIC MAC version 8 program to determine mutation constructions of gemon from IGF 1 and IGF 2, then presented descriptively.

3. Results and discussion

3.1. Allele bands of IGF 1 and IGF 2 genes

Distribution allele bands of IGF 1 and IGF 2 genes of Ongole Cross breed cows with twins birth nor single birth history, its data are listed in figures 1 (IGF 1 gene) and 2 (IGF 2 gene). It appears that: 1). distribution of allele bands between IGF 1 gene and IGF 2 gene, also between cows with twin birth history (a) and single birth history (b), are different from one another; 2). there is a change in distribution IGF 1 and IGF 2 genes allele band of cows with single to twin birth history; 3). distribution allele band of IGF 1 gene cows (figure 1) with single birth history, is in a position between 875 and 1,200 bp (red circle). However, with genetic traits of twin birth, it appears that distribution of allele band widened to a position about 1,485 bp (yellow circle) ; and 4). likewise in IGF 2 gene (figure 2), where there is an expansion distribution of its allele band from 450 to 590 bp (red circle) became until 1,110 bp (yellow circle).

Figure 1. Differences distribution allele bands of IGF 1 gene cows with twin birth (a) and cows with single birth (b).

Figure 2. Differences distribution allele bands of IGF 2 gene cows with twin birth (a) and cows with single birth (b).
Expansion distribution of allele bands in IGF 1 and IGF 2 genes Ongole Cross breed cows with twin birth history, presumably because of a change in certain arrangement/sequence of nucleotide acid pairs in its IGF gene [11], so its genotype changes and cows have genetic potential to mate twin.

### Table 1. The sequence of nucleotide acids that make up the IGF 1 and IGF 2 genes

| Gene     | Genetic Bounds | Size (bp) | Nucleotide acids configuration |
|----------|----------------|-----------|--------------------------------|
| IGF 1    | twin upper     | 1.475 – 1.495 |                                   |
|          |                |           |                                 |
|          | twin lower     | 1.140 – 1.160 |                                 |
|          | single upper/lower | 1.190 – 1.210 |                                 |
| IGF 2    | twin upper     | 1.100 – 1.120 |                                 |
|          |                |           |                                 |
|          | twin lower     | 580 – 600     |                                 |

3.2. Genotype diversity of IGF 1 and IGF 2 genes
The data of genotyping results in figures 1 and 2, show that: 1). IGF 1 gene at Ongole Cross breed cows with twin birth history, was genotype heterozygous AB, because it show a polymeric pattern (more than one bands) with A allele measuring between 875 and 1,150 bp (figure 1, a, red circle) and B allele between 1,250 and 1,485 bp (figure 1, a, yellow circle) ; while in cows with single birth history, IGF 1 gene was genotype homozygous AA, because it show a monomeric pattern (single band) with A allele measuring between 920 and 1,200 bp (figure 1, b, red circle) ; 2). almost the same thing happened to its IGF 2 gene. IGF 2 gene in cows with twin birth history were genotype heterozygous AB, because it show polymeric pattern (more than one bands) with A allele measuring between 450 and 590 bp (figure 2, a, red circle) and B allele between 940 and 1,110 bp (figure 2, a, yellow circle) ; while in cows with single birth history, IGF 1 gene was genotype homozygous AA,
because it show monomeric pattern (single band) with A allele measuring between 460 and 560 bp (figure 2, b, red circle ); and 3). in order to determine strength of genotypic differences to controlling genetic traits of twin birth, it can be predicted from its large frequency of AB genotypes appearance in cows with twin birth history at a large replication observation

3.3. The sequence nucleotide acids of IGF 1 and IGF 2 genes
An example sequencing results of IGF 1 and IGF 2 genes, show in table 1. It appears that sequence of nucleotide acids : 1), between IGF 1 and IGF 2 genes, both in cows with twin and single birth history, in certain parts were different and in other parts were same. This difference is thought to cause several differences in the distribution of allele bands and diversity genotypes of its genes. This reseach result found occurrence of nucleotide thymine (T) replacement with cytosine (C), or otherwise for Bos taurus that twin birth [11]. However, this research result showed that in Bos taurus occcur replacement in other nucleotide acids; and 2), in both IGF 1 and IGF 2 genes, the upper end of nucleotide acid sequence from cow with twin birth, some of nucleotide acids have same structure with cow with single birth. Likewise, at cow with single birth, the upper end of its nucleotide acid, some its nucleotide acid have same arrangement with the lower initial limit arrangement of some nucleotide acids from cows with twin birth. There are still similarities arrangement of some nucleotide acids in certain parts of IGF 1 and IGF 2 genes, indicating that appearance of twin trait in cows is the result of changes in arrangement of certain nucleotide acids at certain locations of IGF 1 and IGF 2 genes cows with single birth.

4. Conclusion
The genetic marka of IGF 1 and IGF 2 genes at Ongole Cross breed cows, were different among cows with twin with single birth history.

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