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## The *MspI* Polymorphism of the *BMP-15* Gene in Indonesian PO Cattle.

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### ABSTRACT

Bone Morphogenetic Protein 15 (BMP-15) was a member of the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily known to regulated ovarian functions in mammals. The aim of this research was to determine the *BMP-15* gene polymorphisms of PO cattle. Random blood samples were collected from 30 animals. DNA was extracted from blood by QIAamp DNA blood mini kit (Qiagen). A 400 bp gene fragment was amplified by PCR (Polymerase Chain Reaction) using *BMP-15* gene cattle specific primers (forward primer : 5'-AGTTTGTACTGAGCCGGTCT -3' and reverse primer : 5'-CTGACACACGAA GCGGAGT -3'). Restriction fragment length polymorphisms (RFLPs) in the amplified fragments were studied using *MspI* restriction enzyme. In this population, AA and AB genotypes have been identified with the 53.67 and 43.33 % frequencies, respectively. A and B alleles frequencies were 0.78 and 0.22, respectively. AA genotype is the dominant genotype and the A allele is the dominant allele. It can be concluded that *MspI* locus of *BMP-15* gene of PO cattle was polymorphic.

**Keywords:** *BMP-15* gene, *MspI*, PO cattle, polymorphism

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## INTRODUCTION

Folliculogenesis process is associated with the development of a follicles group at various stage through the primordial, primary, preantral, and antral stages [1]. Proliferation and differentiation of granulosa cells is the character of folliculogenesis [2]. These process are controlled by steroid hormones [3] and paracrine that secreted by oocytes, granulosa and theca cells [4]. The oocyte plays an important role in regulating and promoting folliculogenesis by the production of oocyte growth factors that predominantly act on supporting granulosa cells via paracrine signaling [5]. Two important oocyte-secreted factor (OSFs) are growth differentiation factor (GDF-9) and bone morphogenetic protein-15 (BMP-15) [6].

The BMP-15 is a specific proteins that is secreted by growing oocytes in rat, mouse and human [2, 7-8]. The BMP-15, also known as GDF-9B [9], is a member of transforming growth factor- $\beta$  (TGF $\beta$ ) superfamily [10]. BMP 15 has an important role in female fertility [10] because has a potent stimulator of granulosa cells proliferation [2, 11], regulator of follicular development and ovulation rate in cattle [12,13]. In bovine oocytes, BMP-15 express from respective primary and primordial follicle [14] and was significantly higher in the adult ovaries than in calf ovaries [1]. In mice BMP-15 was detected in growing oocyte from the secondary follicle stage [15]. By contrast, immunization against BMP-15 in sheep have been causing premature luteinisation and thereby limiting the numbers follicle recruited for ovulation [16]. BMP-15 level appears to be potential to predicting oocyte quality and embryo development [17]. In human, insufficiency [18] or mutation [19] of BMP 15 may be a factor contributing in POF (Premature Ovarian Failure). Mutation of BMP-15 gene is thought to result in reduced level of mature protein or altered binding to cell-surface receptor [20]. BMP-15 imunization increased ovulation rate in ewes [21, 22]. In transgenic mice was found that BMP-15 play role in follicle growth and preventing follicle maturation [23].

Genetic studies have identified correlation between BMP-15 genotype and litter size of goat [24, 25]. Moreover, Wang et al [25] show that heterozygous of BMP-15 had greater liter size than BB homozygous in Chinese goat. However, heterozygous BMP15-knockout mice have no phenotypic defects, while homozygous mice have a decreased fertility due to defects in ovulation and early embryonic development [26]. Polymorphism of the BMP-15 gene was associated with both increased ovulation rate in litter size, sterility in sheep and goat [21, 24-27]. Studies in genetic mutations have elucidated the role of BMP-15 proteins in regulating the primary to secondary follicle transition. Mutations in BMP-15 gene result in growth arrest at the primary stage ( 22).

PO cattle are one of the popular cattle for meat production in Indonesia, particularly in East Java. The status of these genes in PO cattle has not been explored yet. The objective of this study was to determine the occurrence polymorphism in the *BMP-15* gene of PO cattle using PCR-RFLP method.

## MATERIALS AND METHODS

### Animals and DNA extraction

Blood samples were randomly collected from 30 PO cattle from Pasuruan, East Java and were transferred to the laboratory using cooling chain and stored at -20°C for further analysis. Genomic DNA was isolated by QIAamp DNA blood mini kit (Qiagen). The quality and quantity of isolated DNA was measured by spectrophotometer methods and agarose gel electrophoreses.

### PCR primers and amplification

The bovine BMP-15 primer sequence were forward primer, 5'-AGTTTGTACTGAGCCGGTCT -3' and reverse primer 5'-CTGACACACGAA GCGGAGT -3', adopted from Zhang [28]. The PCR was performed in a 20 ul reaction, containing 10 pmol of each primer, 50 ng of genomic DNA, 10 ul of PCR master mix (Intron Biotech). The PCR cycling conditions was an initial denaturation step of 94 °C (2 min); followed by 34 cycles of 94 °C (30 s), 58°C (45 s) and 72°C (60 s), final extension at 72°C for 5 min. Electrophoresis of PCR products was performed in 2 % agarose gel containing ethidium bromide and visualized by a UV trans-illuminator.

### Restriction Fragment Length Polymorphism (RFLP) analysis

The result amplicon of 400 bp was digested with *MspI* restriction enzyme, the restriction site at C\*CGG. The digestion reaction was carried out in 10 ul of mixture reaction which consist of 3 ul of PCR product, 1.2 ul buffer, 0.5 ul free ionized water and 3.5 Unit *MspI* restriction enzyme. The reaction mixture was incubated in 37°C for 3 hour. Electrophoresis of digested PCR products was performed in 7.5% non-denaturing polyacrylamide gels, stained by silver nitrate staining method. All the PCR-RFLP fragments resulted was used for analysis of polymorphism.

## RESULT AND DISCUSSION

The analysis of *BMP-15* gene polymorphism was carried out using PCR-RFLP method. Genomic DNA of PO cattle was successfully amplified by pair of primer that cover entire coding sequence of *BMP-15* gene. Genomic DNA of all samples was successfully amplified using primer for *BMP-15* gene. The result show that amplification fragment size is a DNA fragment with 400 bp (Fig. 1).

The digestion of PCR product with restriction enzyme *MspI* produced 2 alleles (A and B) and 2 genotypes (AA and AB) (Fig. 2). This result shows that the polymorphism were detected in *BMP-15* gene of PO cattle. AA genotype showed the two band pattern (110 and 220 bp) and AB genotype showed the three band pattern (110, 220 and 260 bp).

In population of PO cattle, 2 genotypes (AA, AB) were detected. The homozygous genotype AA and heterozygous AB was detected in 17, and 13 cattle animals respectively.

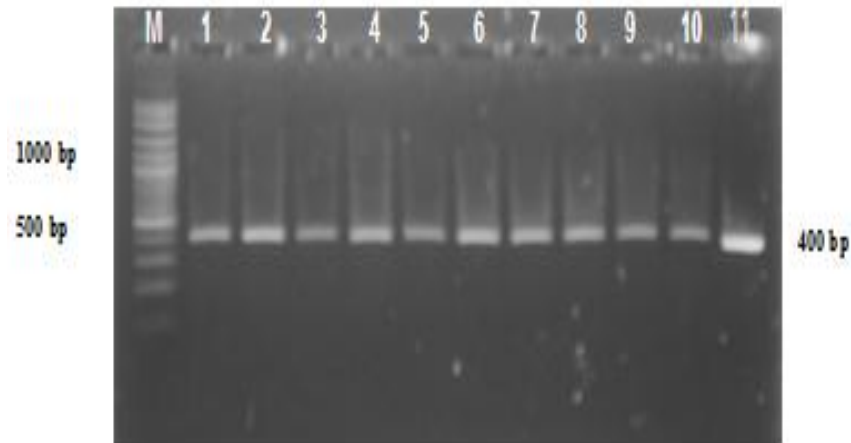


Fig. 1: Electrophoretic profile for the *BMP-15* gene fragments amplified by PCR in a 2 % agarose gel. M represented a marker with 100 bp ladder. Lane 1-11 represented a strand with 400 bp.

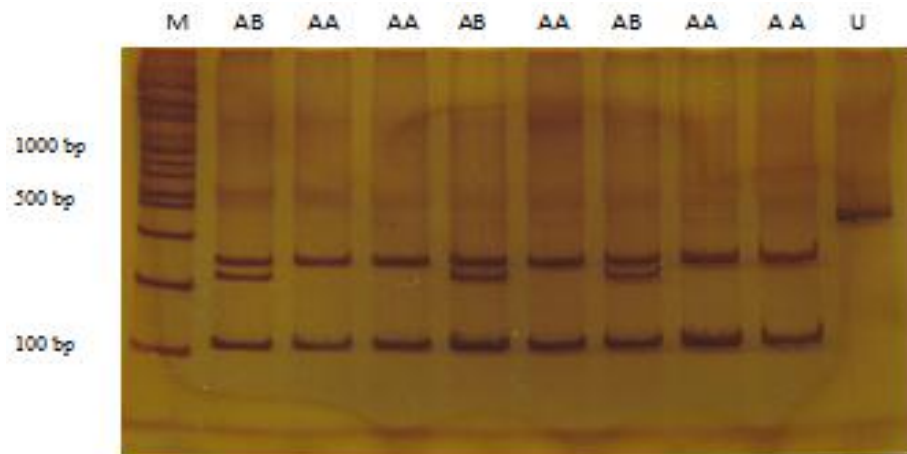


Fig. 2: Restriction pattern of 400 bp fragment of *BMP-15* gene after digesting with *MspI* on 7.5% on non-denatured polyacrylamide gel after silver nitrate staining. M: DNA ladder 100 bp (Fermentas). U = Uncut PCR product

The frequencies of *BMP-15* alleles and genotypes are presented in Table 1. Allelic frequencies of *BMP-15* gene was estimated to be 78 % and 22 % for A and B, respectively.

The *BMP-15* gene is the most documented gene which associated to ovulation rate, expressed in the ovary and located in the X-chromosome in the human, mouse and sheep genomes [28]. In recent year, researches in DNA polymorphism have been widely studied in the geen of cattle. A polymorphism of *BMP-15* gene was found to be associated to the ovarian respon [29], litter size of chine goat [25]. In this study distribution of allele frequencies of the PO cattle *BMP-15* gene showed that the frequency of allele A was higher than allele B (Table 1). The level *BMP-15* gene polymorphism that found in this study were lower than those reported by Zhang et al [28] working with same allele in Luxi bovine and Nanyang bovine. The frequency of the homozygous genotype AA was higher than genotype BB in the studied PO cattle (Table 1). Similar result by Zhang *et al.* [28] reported in the Luxy and Nanyang bovine that who observed homozygous genotype was higher than heterozygous. In sheep, ewes that are heterozygous carriers of loss-of-function *BMP-15* mutations have an increased fertility [9; 22]. However, heterozygous *BMP15* knockout mice

have no phenotypic defects. Another study using Iranian goat found that there is no polymorphism in *BMP-15* gene [30]

**Table 1: Distribution of BMP-15 alleles and genotypes frequency**

Gene	n	Genotype		Allele	
		AA	AB	A	B
<i>BMP-15</i>	30	17 (56.67 %)	13 (43.33 %)	0.78	0.22

n = number of animal

The results imply that there is polymorphism in the *MspI* locus of the Indonesian PO cattle *BMP-15* gene. Polymorphism of the *BMP-15* gene is associated with prolificacy and sterility in *Rasa aragonesa* [31]. *BMP-15* gene mutation affecting ovulation rate and litter size of Lacauna sheep [32]. In human, *BMP-15* gene mutation is associated with ovaria dysgenesis [33]. Further research is needed to examine whether *BMP-15* gene polymorphism of Indonesia PO cattle has effect on fertility.

### CONCLUSION

This study concluded that there is polymorphism in the *MspI* locus of the PO cattle *BMP-15* gene. Two genotype observed include AA and AB genotypes with the genotype frequencies of 53.67 and 43.33 % . AA genotype is the dominant genotype and the A allele is the dominant allele.

### DECLARATION OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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