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The Potency of the BMP 15 Gene as a Marker of Reproductive Traits in Sumba Ongole (*Bos indicus*) Cattle

Cynthia Dewi Gaina[1], Maxs U.E. Sanam[2], WMM Nalley[3], Imanuel Benu[3], Filphin Adolfin Amalo[4]

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ABSTRACT

Background: As one of the important genetic resources for Ongole beef cattle, the study aimed to identify any potential polymorphisms in the Bone Morphogenetic Protein 15 gene in a group of Sumba Ongole (SO) cattle.

Methods: Blood samples from 161 SO cattle were obtained and examined. The initial stage was the isolation of deoxyribonucleic acid (DNA). The next procedures were electrophoresis and extraction, followed by annealing temperature optimization, amplification and sequencing. The Basic Local Alignment Search Tool (BLAST) was used to examine the sequencing findings. The annealing temperature was 58°C for 15 seconds, with an amplification gain of 350 base pairs (bp).

Result: The result showed that there were six single nucleotide polymorphisms (SNPs) of the BMP 15 gene in this region. Six mutations were SNP c.57 G>A, SNP c.120 C>T, SNP c.338 G>T, SNP c.360 C> A, SNP c.361 G>T, SNP c.365 A>T. The polymorphic informative content (PIC) value (0.25<=PIC<=0.5) was reached from low (0.02) to moderate high (0.45 and 0.47). A total of 2 SNPs in the BMP 15 gene with moderate-high PIC value was c.360 C>A and c.365 A>T. In conclusion, we reported for the first time the polymorphism of the BMP 15 gene which is required for further investigation as to whether this polymorphic could be responsible for reproductive trait variations in Ongole cattle.

Key words: BMP 15, Bos indicus, Cattle, Gene polymorphism, Reproductive, Sumba ongole.

INTRODUCTION

During folliculogenesis, the oocyte is the primary source of oocyte-secreted components that promote granulosa cell differentiation *via* paracrine signaling mediated by steroid hormones (de Castro *et al*., 2016; Smołucha *et al*., 2020; Belli andShimasaki, 2018; Chantepie *et al*., 2020; D'Occhio *et al*., 2020). Bone morphogenetic protein-15 (BMP-15) is one of the oocyte-secreted factors with an X-linked gene locus (Heath *et al*., 2017; Otsuka *et al*., 2011). Being a part of the transforming growth factor-beta (TGF-β) superfamily, it is categorized as a fecundity gene(Chen *et al*., 2017; Tang *et al*., 2019; Yin *et al*., 2019). It assists granulosa cells grow and differentiate, which influences ovulation rates, follicular growth and oocyte maturation (Gong *et al*., 2021; Tang *et al*., 2019; Chantepie *et al*., 2020. According to earlier research, developing mouse oocytes (Dube *et al*, 1998), as well as the ovaries and cumulus cells of calves and cows, release BMP-15; however, more so than in cumulus cells, its expression is favored in oocytes (Hosoe *et al*., 2011; Pennetier *et al*., 2004).

The involvement of BMP 15 and other candidate genes responsible for reproductive features that are particularly important for economic output has been found in small ruminants. Sheep and goats, like the Small Tail Han sheep, Chinese goats (Wang *et al*., 2011), Iranian Baluchi sheep (Moradband *et al*., 2011), Xinjiang Cele Black sheep (Niu *et al*., 2021) and Markhoz goats (Ghoreishi *et al*., 2019; Pourali *et al*., 2020) are susceptible to having low birth rates because of mutations in the BMP 15 gene (Belli and Shimasaki, 2018). However, the significance of the BMP 15 gene in the fertility of *Bos indicus* cattle has received little attention. Until now, the BMP 15 gene was found to be polymorphic in Peranakan Ongole (PO) and Bengali cattle which is categorized as one of Zebu cattle (*Bos indicus*). However, this result could

[1]Laboratory of Veterinary Clinic, Reproduction, Pathology and Nutrition, Faculty of Medicine and Veterinary Medicine, Universitas Nusa Cendana, Kupang, Indonesia.
[2]Laboratory of Animal Diseases and Veterinary Public Health, Faculty of Medicine and Veterinary Medicine, Universitas Nusa Cendana, Kupang, Indonesia.
[3]Faculty of Animal Husbandry, Marine and Fisheries, Universitas Nusa Cendana, Kupang, Indonesia.
[4]Laboratory of Veterinary Anatomy, Physiology, Pharmacology and Biochemistry, Faculty of Medicine and Veterinary Medicine, Universitas Nusa Cendana, Kupang, Indonesia.

Corresponding Author: Cynthia Dewi Gaina, Laboratory of Veterinary Clinic, Reproduction, Pathology and Nutrition, Faculty of Medicine and Veterinary Medicine, Universitas Nusa Cendana, Kupang, Indonesia. Email: cynthia.gaina@staf.undana.ac.id

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probably be different from other Ongole breeds, which are extensively grazed under a harsh environment in a tropical semi-arid region, just like in Sumba Island. PO cattle are crossbreds of Java cattle and Sumba Ongole (SO) cattle, with SO cattle being one of Indonesia's native beef cows, introduced straight from India by the Indonesian government and extensively grazed on Sumba Island for genetic development (Gaina *et al*., 2020). They are well-known for their protein content and for being very adaptable to severe conditions, as seen on Sumba Island (Gaina *et al*., 2019; Sutarno and Setyawan, 2015). While we know that variations in a number of genes might affect reproductive ability, the role of the BMP 15 gene and its precise biological function in SO cattle has not been investigated.

Considering the significance of the BMP 15 gene, this prospective study was designed to analysis a molecular characterization of the BMP 15 gene in SO cattle and to find its possible polymorphisms that might affect reproductive features in a population of SO cattle as one of the valuable genetic resources of Ongole beef cattle. This discovery may serve as a valuable genetic marker for identifying the BMP 15 gene in other Ongole cattle, as the GenBank data for the BMP 15 gene only be found in cattle of a different species, namely *Bos taurus* cattle.

MATERIALS AND METHODS

Collecting samples and DNA

In order to collect blood samples from 161 SO cattle on Sumba Island, NTT, a venoject needle was attached to a vacutainer Ethylenediamine tetraacetic acid (EDTA) tube. Three to five milliliters of blood were collected and kept at 4°C for use in later research, including DNA extraction, amplification and analysis. Spectrophotometry and agarose gel electrophoresis were used to evaluate the purity and concentration of the DNA sample. The genotype identification of BMP 15 gene was carried out in the Laboratory of Veterinary Clinics, Reproduction, Pathology and Nutrition, Faculty of Medicine and Veterinary Medicine, Universitas Nusa Cendana, Kupang, From March 2020-September 2020.

PCR primers and amplification

This research made use of polymerase chain reaction (PCR), electrophoresis and DNA sequencing. These were the thermal cycling conditions: Denaturation at 95°C for 1 minute, annealing at 58°C for 15 seconds and extension at 72°C for 10 seconds; this is repeated 35 times. The final period of elongation lasted 1 minute at 72°C. All DNA samples were amplified using the BMP 15 primer for 35 cycles and the resulting amplicons were seen on a 1.5% agarose gel in 0.5 Tris-borate-EDTA (TBE) solution using a 100 bp ladder as a size standard.

Amplicons were detected on 1.5% agarose gel in 0.5 Tris-borate-EDTA (TBE) buffer using a 100 bp ladder as a molecular weight marker from all DNA samples amplified with BMP 15 primer for 35 cycles. In addition, PCR amplification was performed using 25 µl reactions containing 2 µl of DNA template, 10 µl of nuclease-free water, 0.5 µl of forward and reverse primers and 12.5 µl of MyTaq HS red Mix 2x. Forward primer 5′-GCTCTGG AAT CAC AAG GGG-3′ and reverse primer 5′-AGA GAT GGG GAG CGA TGAT-3′ were used to amplify the BMP 15 gene (Damayanti *et al*., 2013).

Sequencing and data analysis

The PCR products were sequenced in 1st Base Malaysia. The results showed that this gene had a different genotype. BioEdit was used to examine the sequencing findings. Sequencing the amplified nucleotide is the last step. The results of the DNA sequencing were examined for nucleotide mutations using MEGA version 6.0 (Tamura *et al*., 2013). The GenBank database hosted by NCBI was searched using the BLAST tool for reference and homologous sequences.

RESULTS AND DISCUSSION

Utilizing a pair of primers that specifically targeted the BMP 15 gene's coding region, the gene was amplified successfully in a polymerase chain reaction (PCR). Data points to a DNA fragment size of 350 bp as the amplification fragment. The results of electrophoresis in Fig 1 showed that the primers used are specific to the BMP 15 gene, with only one DNA band formed for all samples.

Genotype frequency and allele frequency of BMP15 gene fragment

The BMP 15 is one of the reproductive genes that regulate ovarian folliculogenesis, which is vital for cattle production since it impacts breeding profitability (Belli and Shimasaki, 2018; D'Occhio *et al*., 2020; Tang *et al*., 2019). Since the importance of the BMP 15 gene, the biological roles of BMP 15 in Ongole cattle must be investigated, as mutations in small ruminants have been linked to high ovulation rates and fertility (Ghoreishi *et al*., 2019; Heath *et al*., 2017; Pourali *et al*., 2020). BMP 15 supplementations during in vitro fertilization in mouse group increased oocyte quality and blastocyst rate significantly (Velásquez *et al*., 2019). The BMP 15 gene's DNA sequence was amplified using the PCR technique, with the PCR result being 350 bp in length (Fig 1). Another obtained amplicon of the BMP 15 gene based on the NCBI information has a length of 1185 bp, which is possibly caused by breed difference with the accession number (NM_001031752) for *Bos taurus* BMP 15 Gene mRNA. Six polymorphic SNPs were discovered during the sequencing of the BMP 15 gene in SO cattle. They were SNP c.57 G>A, SNP c.120 C>T, SNP c.338 G>T, SNP c.360 C> A, SNP c.361 G>T and SNP c.365 A>T.

The SNPs in this study were polymorphic, with an allele frequency less than 0.99 or greater than 0.01. As a result, it is possible to conclude that the BMP 15 gene in Sumba SO cattle is polymorphic. When a change in the DNA sequence happens in a population at a rate of 1% or greater, it is called a polymorphism (Brookes, 1999). The greatest allele frequency was identified in SNP c.120 C > T (0.95) and SNP

c.361 G>T (0.95) (Table 1). It suggests that this BMP 15 polymorphism may have spontaneously developed with a neutral or advantageous impact because of the increased prevalence in the population.

Previous studies have reported that polymorphism of the BMP 15 gene with a high ovulation rate that causes high litter size in Anglo-Nubian goats (Abdel-Rahman *et al*., 2013), Batang goats (Hidayat, 2008) and white goats (Ran *et al*., 2010). In Olkusha sheep, a single mutation of the BMP 15 gene was responsible for high prolificacy resulting in a high ovulation rate and litter size with excellent maternal abilities, which are valuable in sheep breeding (Smo³ucha *et al*., 2020). It is also shown that mutations in heterozygous ewes affect fecundity by increasing the ovulation rate, while homozygous ewes are infertile due to the blockage of follicle development (Zhang *et al*., 2009).

The BMP 15 gene, however, was not correlated with the efficacy of artificial insemination in polymorphic Peranakan Ongole (PO) cattle from Pasuruan in East Java (Damayanti *et al*., 2013). It was also reported that some heterozygous *Bos taurus* breeds in Luxi, Qinchuan, Nanyang, Jinnan and Bohai Black cattle were found to be polymorphic, but fecundity was not increased in these individuals' cattle (Zhang *et al*., 2009). The result of this finding implies that six mutations of the BMP 15 gene could provide opportunities as a molecular marker for improving reproductive performance in SO cattle breeding, although the observation of BMP 15 gene polymorphism could be connected to the particular type of animals. More study is needed to determine whether this mutation affects the phenotype of Ongole cattle.

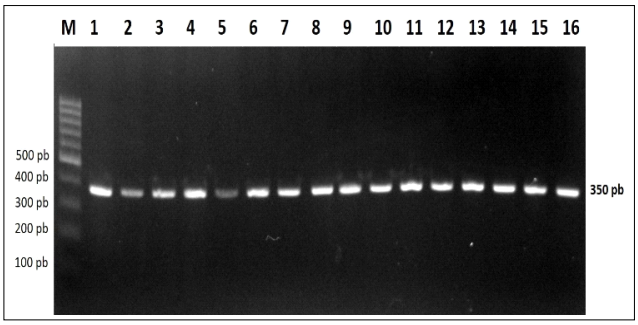

Fig 1: M: DNA ladder (100-10,000 bp), BMP 15 gene product (lanes 1-16) and PCR product (band size 350 bp) on 1.5% agarose gel electrophoresis with 1x TBE buffer.

Heterozygosis of BMP 15 gene and hardy-weinberg equilibrium

According to the findings, the observed heterozygosis (Ho) was lower than the expected heterozygosis (He). Based on the result, the value of Ho is less than He, which may imply an active selection process and a high likelihood of inbreeding in this SO population. Furthermore, it suggests that the difference between the Ho and the He might be utilized to detect a genetic imbalance in the observed SO cattle. This might imply that there has previously been selection action and that there is no random mating (Kuralkar *et al*., 2016; Surekha *et al*., 2018). All of the SNPs in SO cattle (Table 2) have Ho values below 0.5, indicating limited diversity, as reported by Vissers *et al.* (2003). Adaptation efforts by SO cattle to the severe climatic circumstances on Sumba Island are thought to have resulted in the mutation seen in SO cattle as it is stated by (Gaina *et al*., 2022; Vani *et al*., 2022). Heterozygosis values are a method of gauging a population's genetic diversity that can inform a selection program's choices (Marson *et al*., 2005).

Table 1: The allelic and genotypic frequencies of the BMP 15 gene in SO.

SNPs	Genotype frequency			Allele frequency	
c.57 G>A	GG	GA	AA	G	A
	18	3	0		
	0.86	0.14	0	0.93	0.07
c.120 C>T	CC	CT	TT	C	T
	20	1	0		
	0.95	0.05	0	0.98	0.02
c.338 G>T	GG	GT	TT	G	T
	18	0	3		
	0.8	0	0.14	0.86	0.14
c.360 C>A	CC	CA	AA	C	A
	13	1	7		
	0.62	0.045	0.33	0.64	0.36
c.361 G>T	GG	GT	TT	G	T
	20	0	1		
	0.95	0	0.05	0.95	0,05
c.365 A>T	AA	AT	TT	A	T
	8	0	13		
	0,38	0	0.619	0.38	0.62

Table 2: Allelic and genotypic frequency values of the BMP 15 in SO (*Bos Indicus*) cattle.

SNPs	Genotype frequency			He	Ho	X^2	pic
c.57 G>A	GG	GA	AA				
	18	3	0	0.13	0.14	0.005^{ns}	0.13
c.120 C>T	CC	CT	TT				
	20	1	0	0.05	0.05	$0,001^{ns}$	0.05
c.338 G>T	GG	GT	TT				
	18	0	3	0.24	0.00	$0,020^{ns}$	0.24
c.360 C>A	CC	CA	AA				
	13	1	7	0.46	0.05	0.127^{ns}	0.46
c.361 G>T	GG	GT	TT				
	20	0	1	0.09	0.00	0.002^{ns}	0.09
c.365 A>T	AA	AT	TT				
	8	0	13	0.47	0.00	0.383^{ns}	0.47

Note: Ho= Observed heterozygosis; He= Expected heterozygosity; χ^2= Hardy-weinberg equilibrium; Not significant (ns) at α 5% (χ^2obs ≤ 5.59); n=161 heads.

A molecular selection marker in cattle is essential for evaluating genetic diversity. Following the. criteria of (Botstein *et al*., 1980), the polymorphic informative content (PIC) value was reached from low (0.02) to moderate-high (0.45 and 0.47). The SNPs with moderate-high PIC values were c.360 C>A and c.365 A>T. In addition to the heterozygosity value, the PIC value can not only be used to determine genetic markers but can also be used to determine the presence and absence of polymorphic alleles. Thus, based on this finding, the BMP 15 gene showed polymorphism, which could be potentially used in molecular selection for the breeding program. Further research in a bigger population is necessary to corroborate those findings, though, as SNPs identified on the BMP 15 gene's entire genomic structure may have a greater impact on production in SO cattle.

Although, Godara *et al*. (2012) stated that BMP 15 gene is not a major gene that affects fecundity in Marwari Goats like in Some of the Chinese native cattle (Zhang *et al*., 2009), this gene was discovered to be a major gene that affected the prolificacy of Jining Grey goats and other ruminants. Several mutations in this gene have been linked to prolificacy and fertility in ruminants, both small and large ruminants (Niu *et al*., 2021; S. Bibinu *et al*., 2016). With a p-value close to the significant level (p =0.08), the detected SNP on BMP15 (BMP15-1: G > A) demonstrated an association with the litter size with some BMP 15 mutations have previously been shown to influence litter size and ovulation rate in small ruminants (El-Halawany *et al*., 2018). This finding implies that BMP 15 gene might have a beneficial effect on fertility traits in SO cattle like in other small ruminants as it was polymorphic. However, further investigation is required to determine whether this mutation exists and whether it affects phenotype in the SO cattle. Moreover, the findings of this study also add support to the idea that variations in BMP15 function among species may be responsible for phenotypic differences between them.

CONCLUSION

In summary, for the first time, the BMP 15 gene was isolated and characterized in Sumba Ongole cattle. This finding showed that the BMP 15 gene in SO cattle is polymorphic. Six mutations were identified, where c.360 C>A and c.365 A>T have moderate-high PIC in this SO cattle population. The BMP 15 is a potential unique genetic marker utilized in cow breeding for marker-assisted selection due to its potential role in regulating bovine reproductive capability. This study implies that identifying the BMP 15 gene in SO cattle is a first step toward improving reproductive traits.

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Ethical approval

The Animal Research Ethics Committee, Faculty of Veterinary Medicine, Universitas Nusa Cendanaapproved this study (KEH/FKH/NEPH/2019/003).

Conflict of interest

No conflict of interest to be declared.

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