Molecular Identification of Bone Morphogenetic Protein-15 (BMP-15) gene of Sumba Ongole cattle

Cynthia Dewi Gaina[1*], Maxs U.E. Sanam[1], WMN Nalley[2], Imanuel Benu[2]

[*1]*Faculty of Veterinary Medicine, University of Nusa Cendana, Kupang, Indonesia*
[2] *Faculty of Animal Science, University of Nusa Cendana, Kupang, Indonesia*
[*]*Corresponding author. Email: cynthia.gaina@staf.undana.ac.id*

ABSTRACT
This research aims to identify the *BMP-15 gene* in *Sumba Ongole* (SO) *cattle* (*Bos indicus*) which were reared extensively in Sumba Island. This *gene* has been known for its function in regulating reproductive performance in cows. The blood sample was collected from 161 SO *cattle*. The first step was DNA isolation from blood and the second step was electrophoresis and extraction. It then continued with optimization of annealing temperature, amplification, and sequencing. The results of sequencing were analyzed using a software program, Basic Local Alignment Search Tool (BLAST). The annealing temperature optimization results obtained temperature of 58 °C was the proper temperature to amplify DNA. All samples studied with *BMP-15 gene* amplification gained results of 350 bp. Results of BLAST program analysis and Mega 6.01 program showed a close genetic relationship, characterized by the result sequence of *BMP-15* of SO *cattle* possessing proximity to other ruminants, such as *Bos taurus* and *Capra hircus* breed. Based on this study, it could be concluded that the *BMP-15 gene* amplified successfully of 350 bp and has the adjacency of nucleotide sequence with the *BMP 15 gene* in some other types of ruminants.

Keywords: *BMP-15, genes, Sumba, Ongole, Cattle*

1. INTRODUCTION

Bone morphogenetic protein 15 (*BMP-15*) is a superfamily member of the transforming growth factorβ (TGFβ) that is expressed by oocytes. Bone morphogenetic protein 15 (*BMP-15*) is specific protein factor that originate from the oocyte and its locus is X-linked gene [1,2]. It plays major role in stimulating the proliferation and differentiation of granulose cells that affect ovulation rate, folliculogenesis, oocyte maturation, and fecundity which is related to its classification as fecundity gene [3]. During folliculogenesis, it acts as a modulator effect on FSH action. Differentiation of granulose cell is regulated by mitosis granulose sell which is controlled by steroid hormone and paracrine that is secreted by oocytes, granulose cells and theca cells [3,4]. During folliculogenesis, the oocyte plays the main role in producing oocyte secreted factors that act on supporting granulose cells through signaling paracrine. Two important oocyte-secreted factors are growth differentiation factor (GDF-9) and bone morphogenetic protein-15 (*BMP-15*). The *BMP-15* is a specific protein that is secreted by growing oocytes in mice [5] and is secreted by the ovary and cumulus cells of calves and cows, while its expression in the oocytes was higher compared to in the cumulus cells [6,7]. In humans, it is located in the X chromosome. Previous studies have mentioned the genetic variations in *BMP-15* gene in goat and cow, for example in Chinese goat [8] and Iranian Baluchi Sheep [9]. The SO cattle are categorized as *Bos indicus* cattle that are one of Indonesia's native beef cows that have been known for its benefit as a source of protein and highly adaptive to extreme climates, just like in Sumba Island [10,11]. Many genes are known to affect reproductive performance traits, but the role of the *BMP-15* gene, specifically in SO cattle has not been explored yet. The biological roles of Bone Morphogenetic Protein 15 (*BMP-15*) are not completely understood even if genes regulate the function of granulose cells of the ovary.

Due to the rare information about this gene characteristic, this study aimed to identify the *BMP-15* gene in the SO cattle.

2. MATERIALS AND METHODS

2.1 Samples and DNA Collection

Blood samples were randomly collected from SO cattle which were reared extensively in Sumba Island, NTT. The total numbers of SO cattle used in this study was 161 random samples. Blood sampling was done with a venoject needle on the jugular vein and the coccygeal vein. The venoject needle was connected to the vacutainer tube containing EDTA. The required blood was about 3-5 ml and stored at 4^0C for further analysis, such as DNA extraction, DNA amplification and data analysis. The quality and quantity of isolated DNA were measured by spectrophotometer methods and agarose gel electrophoreses. This work was approved by the Animal Research Ethic Committee, Faculty of Veterinary Medicine, Nusa Cendana University with the series number KEH/FKH/NEPH/2019/003.

2.2 PCR Primers and Amplification

The blood sample from 161 SO cattle which reared extensively in Sumba Island, were collected and were analyzed. The method used in this research was polymerase chain reaction (PCR), electrophoresis, and DNA Sequencing. Thermal cycling conditions were done as follows: Pre-denaturation at 95°C for 1 minute, followed by 35 cycles of denaturation at 95°C for 15 s, annealing at 58°C for 15 s and extension at 72°C for 10s. The final extension step was at 72°C for 1 min. All DNA samples were amplified using BMP15 primer for 35 cycles and amplicons were visualized on 1.5% agarose gel in 0.5 TBE buffer containing a 100 bp ladder as a molecular weight marker. The BMP 15 gene forward primer was 5'-GCTCTGG AAT CAC AAG GGG-3' and the reverse primer was 5'-AGA GAT GGG GAG CGA TGAT-3' [12].

2.3 Sequencing and Data Analysis

PCR products representing different genotypes of each gene were sequenced in 1st Base Malaysia. The sequence results were analyzed by BioEdit [13]. Results of the DNA sequencing were analyzed using Mega version 6.0 [14] in order to find nucleotide mutation. The software Basic Alignment Local Search Tool (BLAST) was used to sequence find the NCBI GenBank database for reference and homologous sequences.

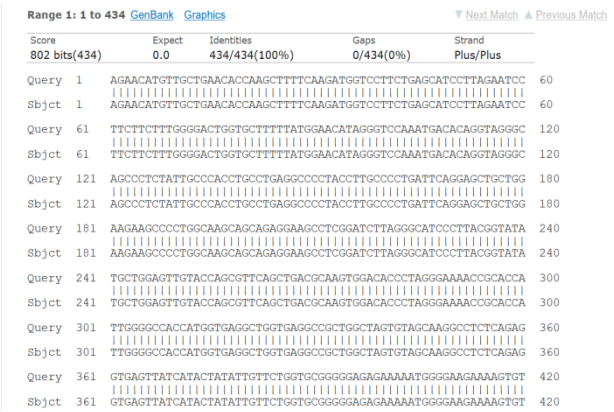

Figure 1. Sequencing of the BMP 15 gene in the SO cattle in gene bank

3. RESULT AND DISCUSSION

The BMP 15 gene was successfully amplified in the PCR process using a pair of primers that cover the entire coding sequence of the BMP 15 gene. The *DNA* tube *sequenced* must *first* be broken into smaller pieces and *amplified.* The results show that amplification fragment size is a DNA fragment with 350 bp. Amplification of the *BMP-15* gene using a thermocycler machine with an annealing temperature of 58° C for 30 seconds. The PCR products were visualized with 1% TBE agarose gel. The results showed that the amplification fragment has a good specificity, which directly proceeds to sequence analysis. Visualization of PCR products is shown in Figure 2.

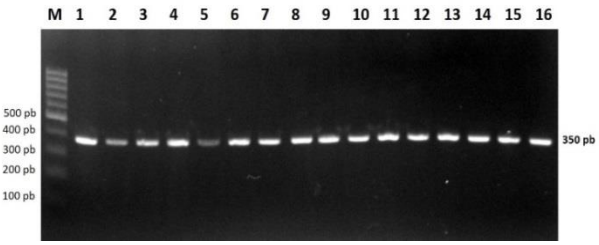

Figure 2. The product was electrophoresis on 1.5% agarose gel 1x TBE buffer M: DNA ladder (100-10000 bp), Lane 1-16 product for *BMP-15* gene of adult cattle, PCR product of band size 350bp.

Sequenced fragments of the *BMP-15* gene were assembled into one sequence of 350 bp that resulted in 100% identical with that was in the existing data in GenBank derived compared with several types of other breeds, including *Bos Taurus* and *Capra hircus. Bos taurus* partial bmp15 gene for bone morphogenetic protein 15 precursor, exon 1 and *Capra hircus* partial CDS bmp15 gene. The results in this study showed the various length of base pairs that are submitted to the BLAST NCBI data base under AJ489251.1. The sequencing of amplified product of *BMP-15* gene from cow, out of them appeared 98% compatibility with standard *Bos taurus* breed bone morphogenetic protein 15 (*BMP-15*) of nucleotide from the gene of gene bank results.

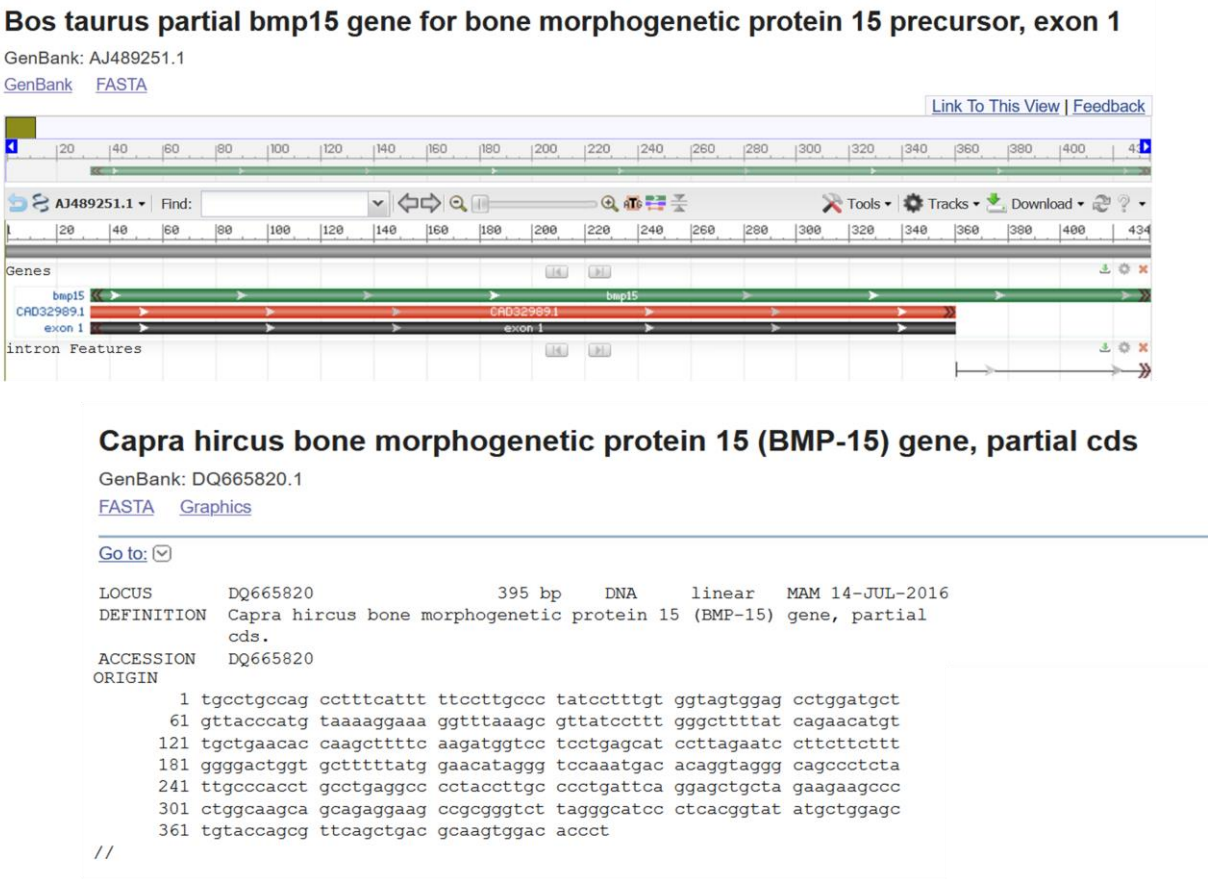

Figure 3. Sequencing of the BMP 15 gene in *Bos Taurus* cattle

4. CONCLUSION

It could be concluded that *BMP-15* gene was polymorphic. This gene could be possibly used for selection towards increasing SO cattle productivity. Identification of single nucleotide polymorphism (SNP) on the whole structure of the *BMP-15* gene may potentially contribute to the selection of the SO cattle. Sequenced fragments of the *BMP-15* gene were assembled into one sequence of 350 bp that resulted in 100% identical with that was in the existing data in GenBank derived compared with several types of other breeds, including *Bos Taurus* and *Capra hircus. Bos taurus* partial bmp15 gene for bone morphogenetic

ACKNOWLEDGMENTS

The study was funded by Anbiocore project in 2019. The authors also would like to thank the Department of Animal Husbandry in East Sumba and local farmers for the assistance during field study.

REFERENCES

[1] E.M. Clarke, E.A. Emerson, Design and synthesis [1] Otsuka, F., McTavish, K. J., & Shimasaki, S. (2011). Integral role of GDF‐9 and BMP‐15 in ovarian function. *Molecular reproduction and development*, *78*(1), 9-21.

[2] Otsuka, F., Yao, Z., Lee, T. H., Yamamoto, S., Erickson, G. F., & Shimasaki, S. (2000). Bone morphogenetic protein-15: identification of target cells and biological functions. *Journal of Biological Chemistry*, *275*(50), 39523-39528.

[3] Knight, P. G., & Glister, C. (2006). TGF-β superfamily members and ovarian follicle development. *Reproduction*, *132*(2), 191-206.

[4] de Castro, F. C., Cruz, M. H. C., & Leal, C. L. V. (2016). Role of growth differentiation factor 9 and bone morphogenetic protein 15 in ovarian function and their importance in mammalian female fertility—A review. *Asian-Australasian journal of animal sciences*, *29*(8), 1065.

[5] Dube, J. L., Wang, P., Elvin, J., Lyons, K. M., Celeste, A. J., & Matzuk, M. M. (1998). The

bone morphogenetic protein 15 gene is X-linked and expressed in oocytes. *Molecular endocrinology*, *12*(12), 1809-1817

[6] Hosoe, M., Kaneyama, K., Ushizawa, K., Hayashi, K. G., & Takahashi, T. (2011). Quantitative analysis of bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9) gene expression in calf and adult bovine ovaries. *Reproductive Biology and Endocrinology*, *9*(1), 1-8.

[7] Pennetier, S., Uzbekova, S., Perreau, C., Papillier, P., Mermillod, P., & Dalbiès-Tran, R. (2004). Spatio-temporal expression of the germ cell marker genes MATER, ZAR1, GDF9, BMP15, and VASA in adult bovine tissues, oocytes, and preimplantation embryos. *Biology of reproduction*, *71*(4), 1359-1366.

[8] Wang, Y., Yuanxiao, L., Nana, Z., Zhanbin, W., & Junyan, B. (2011). Polymorphism of exon 2 of BMP15 gene and its relationship with litter size of two Chinese goats. *Asian-Australasian Journal of Animal Sciences*, *24*(7), 905-911.

[9] Moradband, F., Rahimi, G., & Gholizadeh, M. (2011). Association of polymorphisms in fecundity genes of GDF9, BMP15 and BMP15-1B with litter size in Iranian Baluchi sheep. *Asian-Australasian Journal of Animal Sciences*, *24*(9), 1179-1183.

[10] Gaina, C. D., Sanam, M. U. E., Nalley, W. M. M., Benu, I., & Saputra, A. (2019). Hematological profile of sumba ongole cattle extensively reared in semiarid land, Sumba, NTT based on age and sex. In *IOP Conference Series: Earth and Environmental Science* (Vol. 387, No. 1, p. 012022). IOP Publishing.

[11] Gaina, C. D., Sanam, M. U., Nalley, W. M., Benu, I., & Saputra, A. (2020). Blood AST and ALT profile of Sumba Ongole cattle. *ARSHI Veterinary Letters*, *4*(1), 17-18.

[12] Damayanti, E. U., & Rahayu, S. (2013). Analisa Polimorfisme Gen BMP-15 (Bone Morphogeninetic Protein) Sapi PO (Bos Indicus) Dan Hubungannya Dengan Keberhasilan Inseminasi Buatan. *Biotropika: Journal of Tropical Biology*, *1*(5), 216-220.

[13] Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 951/98/NT. In *Nucleic acids symposium series* (Vol. 41, No. 41, pp. 95-98). [London]: Information Retrieval Ltd., c1979-c2000.

[14] Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA 6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution*, *30*(12), 2725-2729.