

Identification and Characterization of Protamine 3 (*prm3*) Gene in Aceh Bull Testis

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ABSTRACT

The incidence of infertility in breeding male cows highly contributes to economic loss in breeders. To date, there is still a lack of a precise and accurate molecular biomarker to predict the fertility rate on each ejaculation. Therefore, it is important to accurately identify genes related to the fertility of breeding male cows to improve the efficiency of cattle reproduction. In cows, the *prm1*, *prm2*, *prm3*, and *tmp2* genes encode basic chromosome proteins located in a compact gene cluster observed in rats and humans. Recently, there is only a few studies and information on *prm3*. Therefore, explanations on its function, gene profile, characteristics, regulation pattern, its correlation to sperm motility, and fertility in male cows are limited. This study aims to identify and characterize protamine 3 derived from Aceh bull testis, using PCR and DNA sequence analysis. This study was conducted on 10 Aceh bull testes obtained from a slaughterhouse. The steps taken were DNA isolation, *prm3* gene amplification, DNA sequencing, and analysis of nucleotide sequences. The results showed that the nucleotide sequence of the *prm3* gene in Aceh bull had a high level of homology with the *prm3* gene from *Bos Taurus* and *Bos indicus* after being compared with data from The *GenBank* which was around 98-99%. From this study, it can be concluded that the *prm3* gene from Aceh cow is identical to the *prm3* gene from *Bos taurus* and *Bos indicus* at the level of DNA sequence.

Keywords: Aceh cow, *prm3* gene amplification, DNA sequencing, fertility

1. INTRODUCTION

Aceh cow is a type of beef cattle that is mostly maintained by the people in Aceh province. Aceh cows have been designated as the native Indonesian local cattle and also as the National germplasm through the Decree of the Minister of Agriculture of The Republic of Indonesia, number: 2907/Kpts.OT.140/06/2011. Although the cross-breed cattle have a higher carcass weight, local beef cattle are still the main choice for local breeders because of their high resistance to extreme environments [1].

Aceh cow is designated as genetic resources of Indonesian local livestock that must be protected and preserved to prevent extinction. However, based on the report from the Aceh Province Animal Health

Department, the number of Aceh cow population has decreased from 2002, which amounted to 711.143 to 671.086 cows in 2010, and decreased again in 2015 to 580.287 cows [2]. Based on the facts above, it is necessary to make efforts to increase the Aceh cow population in various ways. According to Putra *et al.* [3], the efforts that must be made to increase the Aceh cow population is to increase their reproducibility. In this effort, the most urgent need is the availability of high-quality heifers and bulls.

Reproduction of a good quality bull is not only assessed by several general examinations such as the bull's body phenotype, or the concentration, morphology, and motility of spermatozoa cells. Because the quality of

genetic material plays a big role in the success of conception, although the quality of genetic material cannot be assessed by the general tests mentioned above [4]. Until now, there has not been much research on the genetic components associated with the reproducibility of Aceh bull. One of the genetic materials that is closely related to reproducibility is the Protamine 3 gene (*prm3*) [5].

Generally, protamine is a protein that binds to DNA in the nucleus of spermatozoa so that the DNA strands can be less than 5% of the volume of somatic cell nuclei [4]. Protamine gene classification consists of protamine 1 (*prm1*), protamine 2 (*prm2*), and protamine 3 (*prm3*) [5]. According to Suganuman *et al.* [6], a low level of protamine gene expression has a relationship with spermatozoa DNA damage in male mice. The results of research by Gangluyi *et al.* [5], showed that high *prm1* mRNA expression was closely related to the good quality of semen produced by bulls.

Information regarding the function of the *prm1* and *prm2* genes has been widely studied, but research on the function and regulation of the *prm3* gene is still rare [5]. Therefore, the researchers identified and characterized the *prm3* gene in the testes of Aceh bull which was associated with the reproducibility level of Aceh bull. The information studied was the size of the *prm3* gene in the testes of Aceh bull as a result of the PCR amplification method, phylogenetic analysis, and analysis of amino acid levels in the protamine 3 protein.

2. MATERIALS AND METHODS

This research was carried out with several activities at different places: the field activity (data collection and testicular samples), laboratories (total DNA extraction from the testes of Aceh bulls, *prm3* gene amplification), and sequencing of the *prm3* gene nucleotide base sequence at First Base Singapore through the services of PT. Genetics Science Indonesia. Testicular samples were obtained from 10 healthy male Aceh bulls from the Slaughterhouse (RPH) of Banda Aceh.

2.1. DNA Extraction

The DNA extraction of testicular tissue was carried out using PureLink® Genomic DNA Kits. In general, the DNA extraction method goes through several stages, namely lysis, binding, washing, elution step. All procedures are carried out according to the instructions stated on the PureLink® Genomic DNA Kits guideline.

2.2. DNA Amplification with Polymerase Chain Reaction (PCR)

The DNA amplification to detect the *prm3* gene was carried out using the Biorad CFX96 Real-Time PCR Detection System machine. The PCR tube was inserted into 12.5 µl of Platinum™ Hot Start PCR Master Mix (Invitrogen, California, United States), 1 µl of reverse primer, 1 µl of forward primer, 5 µl of DNA template, and 5 µl of ddH₂O.

The mixture was put into the PCR machine with the following time and temperature rules: pre-denaturation of 95°C for 2 minutes, denaturation of 95°C for 30 seconds, annealing 59.0°C for 45 seconds, extension to 72°C for 45 seconds, post-extension 72°C for 5 minutes, all the stages are carried out in 30 cycles.

2.3. Electrophoresis of PCR Product

The PCR products were identified by the electrophoresis method using the *BioRad* electrophoresis machine. Visualization of the electrophoresis results was performed using the *Biorad GelDoc Viewer*.

2.4. Sequencing

For sequencing of nucleotide sequences, the PCR products that have been obtained are sent to *First Base* Singapore through PT. *Genetika Science Indonesia*. Sequencing is done using the *Dye* terminator sequencer method, which reads the nucleotide sequence from the 3' to 5' direction. The sequenced data is displayed as an electropherogram, which is then analyzed using the *Blast* program.

2.5. Data Analysis

The data from the *prm3* gene fragment sequences from the Aceh bull testes were processed using the Clustal W program from the *Mega 10.0* software, then homology analysis with the sequences contained in *GenBank* using the Basic Local Alignment Search Tool (BLAST) software program. The analysis result data is presented descriptively in the form of tables and figures down.

3. RESULTS AND DISCUSSION

3.1. Analysis of PCR Product

This study shows that the *prm3* gene can be isolated and amplified using a *prm3* gene-specific partial primer. The *prm3* primer sequence used in the amplification

process was adopted from the research of Ferraz *et al.* (2013) with forward primer design (F): 5'-GAAGAAGCTCGTGGCCTGT-3', and *prm3* reverse primary (R): 5'-TCAGGAGTGTGTCTGCTTGG-3' [5].

The *prm3* gene was successfully amplified with a size of 250 bp (Figure 1). The size of the *prm3* gene from the amplification result could be identified using the electrophoresis method.

3.2. Sequencing Analysis

Sequencing analysis showed that the *prm3* gene from Aceh cows had the same *prm3* gene in *Bos taurus*. The mentioned-information above has been presented in the phylogenetic tree (Figure 2), and the analysis result of the amino acid content encoded by the *prm3* gene is presented in Table 1.

Based on the phylogenetic tree analysis, it can be concluded that *prm3* gene from 10 Aceh bulls has a high homology level (98-99%) among the samples in group. This is indicated by the distance between the genes that are not too far apart.

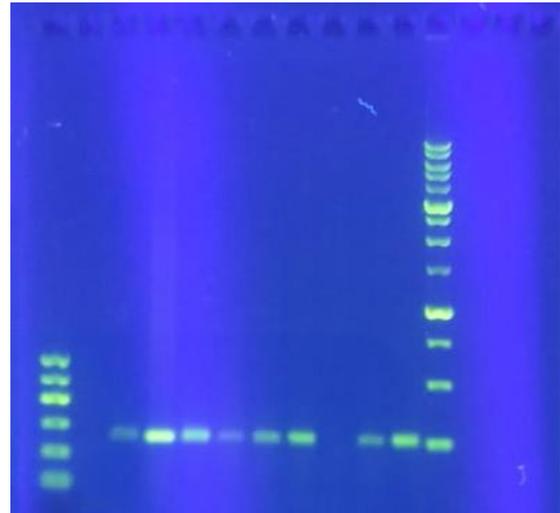


Figure 1 Electrophoresis result of amplification product from *prm3* gene in Aceh bull testis.



Figure 2 The phylogenetic tree of *prm3* gene in Aceh bull

Table 1. The amino acid encoding by the *prm3* gene

Total	Ala	Cys	Asp	Glu	Phe	Gly	His	Ile	Lys	Leu	Met	Asn	Pro	Gln	Arg	Ser	Thr	Val	Trp	Tyr	
3658276 PRM 3 10 H5 PRM 3	13.95	1.16	3.49	6.98	1.16	29.07	0.00	0.00	0.00	10.47	0.00	0.00	5.81	2.33	18.60	2.33	1.16	3.49	0.00	0.00	86
3658274 PRM 3 9 F5 PRM 3	13.95	1.16	2.33	6.98	1.16	30.23	0.00	0.00	0.00	10.47	0.00	1.16	5.81	2.33	18.60	1.16	1.16	3.49	0.00	0.00	86
3658272 PRM 3 8 D5 PRM 3	13.95	1.16	2.33	6.98	1.16	30.23	0.00	0.00	0.00	10.47	0.00	1.16	5.81	2.33	18.60	1.16	1.16	3.49	0.00	0.00	86
3658270 PRM 3 7 B5 PRM 3	13.95	1.16	2.33	6.98	1.16	30.23	0.00	0.00	0.00	10.47	0.00	1.16	5.81	2.33	18.60	1.16	1.16	3.49	0.00	0.00	86
3658268 PRM 3 6 H4 PRM 3	13.95	1.16	3.49	6.98	1.16	30.23	0.00	0.00	0.00	10.47	0.00	0.00	5.81	2.33	18.60	1.16	1.16	3.49	0.00	0.00	86
3658266 PRM 3 5 F4 PRM 3	13.95	1.16	3.49	6.98	1.16	30.23	0.00	0.00	0.00	10.47	0.00	0.00	5.81	2.33	18.60	1.16	1.16	3.49	0.00	0.00	86
3658264 PRM 3 4 D4 PRM 3	13.95	1.16	3.49	6.98	1.16	30.23	0.00	0.00	0.00	10.47	0.00	0.00	5.81	2.33	18.60	1.16	1.16	3.49	0.00	0.00	86
3658262 PRM 3 3 B4 PRM 3	13.95	1.16	3.49	6.98	1.16	30.23	0.00	0.00	0.00	10.47	0.00	0.00	5.81	2.33	18.60	1.16	1.16	3.49	0.00	0.00	86
3658260 PRM 3 2 H3 PRM 3	13.95	1.16	2.33	6.98	1.16	30.23	0.00	0.00	0.00	10.47	0.00	1.16	5.81	2.33	18.60	1.16	1.16	3.49	0.00	0.00	86
3658258 PRM 3 1 F3 PRM 3	13.95	1.16	3.49	6.98	1.16	30.23	0.00	0.00	0.00	10.47	0.00	0.00	5.81	2.33	18.60	1.16	1.16	3.49	0.00	0.00	86
XM 027528043.1 PREDICTED Bos indicus x Bos taurus protamine3	12.79	1.16	3.49	6.98	1.16	30.23	0.00	0.00	0.00	10.47	0.00	0.00	5.81	2.33	18.60	1.16	2.33	3.49	0.00	0.00	86
XM 019987362.1 PREDICTED Bos indicus protamine 3 (PRM3)	13.95	1.16	2.33	6.98	1.16	30.23	0.00	0.00	0.00	10.47	0.00	1.16	5.81	2.33	18.60	1.16	1.16	3.49	0.00	0.00	86
BC108198.1 Bos taurus protamine 3	13.95	1.16	3.49	6.98	1.16	30.23	0.00	0.00	0.00	10.47	0.00	0.00	5.81	2.33	18.60	1.16	1.16	3.49	0.00	0.00	86
NM 001078053.2 Bos taurus protamine 3	13.95	1.16	3.49	6.98	1.16	30.23	0.00	0.00	0.00	10.47	0.00	0.00	5.81	2.33	18.60	1.16	1.16	3.49	0.00	0.00	86

However, if we re-identify there are three small clusters between the samples (which are likely to be more similar), namely sample numbers 1, 3, 4, 5, and 6, which are in the same small cluster and the sample number 9, 8, 7, and 2 are in another small cluster, while sample number 10 separated from another two small clusters. However, if we look back at the phylogenetic tree above, the *prm3* gene from 10 samples of Aceh bull still has a high relationship with the *prm3* gene in *Bos taurus* and *Bos indicus*. Protamine is the type of protein that is most commonly found in the sperm nucleus of various mammal species [7,8] and consists of short chains of amino acids (5-10 types of amino acids) [8]. The *prm3* gene mRNA translation activity was detected in the spermiogenesis stage, and the *prm3* gene began to encode small intronless proteins in the cytoplasm of elongated spermatid [9]. When compared with other protamine gene types, the *prm3* gene has the lowest gene expression in the bovine testis [5]. The mechanism of *prm3* that affects fertility and motility of bovine semen has not been widely studied Kumar *et al.* [10], while Grzmil *et al.* [11], stated that low levels of protamine 3 protein in rat spermatozoa do not interfere with fertility but reduce motility levels. Therefore, more studies should be performed to identifying the function of this kind of protein in bull reproductivity. Other researchers noted that the *prm3* gene encodes a protein in many mammalian species but it seems that this protein is not involved in the chromatin condensation process of spermatozoa [12]. Therefore, more studies should be performed to identify the function of this protein in bull reproductivity.

4. CONCLUSION

Based on the result, it can be concluded that *prm3* gene in Aceh cow testis is 250bp, has a high level of homology with *Bos taurus* and *Bos indicus*, dan contains a high level of glycine (30.23%). The regulatory function of the *prm3* gene and protamine 3 proteins in bull still needs further investigation.

AUTHORS' CONTRIBUTIONS

This research was designed by TZH and MA. The field and laboratory work was conducted by SS, RR, RSZ, TNS, and SW. The article was written by RSZ, TZH, and MA.

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REFERENCES

- [1] A. Rasyid, Y. Adinata, Yunizar, L. Affandy, Karakteristik Fenotipe dan Pengembangan Sapi Aceh Di Provinsi Nanggroe Aceh Darussalam, MADURANCH: Jurnal Ilmu Peternakan, 2(1) (2017) 1-11.
- [2] Z. Yahya, Dasrul, S. Wahyuni, M. Akmal, M.A.N. Abdullah. The characteristics of semen and chemical composition of the seminal plasma of Aceh cattle maintained in BIBD saree Aceh Besar, Agripet. 16(2) (2016) 121-130.
- [3] W.P.B. Putra, Sumadi, T. Hartatik, The genetic correlation of growth traits in Aceh cattle at indrapuri district Aceh Province. Agripet. 14(1) (2014) 37-41.
- [4] A.M.M. Ferrasz, R. Simoes, F.O. Barros, M.P. Millazzoto, J.A. Visintin, M.E.O. Assumpcao. Gene Expression Profile of Protamines and Transition Nuclear Proteins in Bovine Testis. Braz. J. Vet. Res. Anim. 50(4) (2013) 316-322.
- [5] I. Gangluyi, K. G. Gaur, S. Kumar, D.K. Mandal, M. Kumar, U. Singh, S. Kumar, A. Sharma, Differential expression of protamine 1 and 2 genes in mature spermatozoa of normal and motility impaired semen producing crossbred Frieswal (HFxShaiwal) bulls. Res. Vet. Sci. 94(2) (2013) 256-262.
- [6] R.R. Sukanuma, Yanagimachi, dan M.L. Meistrich, Decline in fertility of mouse sperm with abnormal chromatin during epididymal passage as revealed by ICSI. *Hum. Rep. J.* 20(11) (2005) 3101-3108.
- [7] R. Oliva R, G.H. Dixon, Vertebrate protamine genes and the histone-to-protamine replacement reaction. *Prog. Nuc. Acid Res. Mol. Bio.* 40 (1991) 25-94
- [8] R. Balhorn, The protamine family of sperm nuclear proteins. *Gen. Bio.* 8 (2007) 227
- [10] S. Kumar, U. Singh, I. Gangluyi, R. Deb, R. Singh, S. Mann, G. Sengar, D.K. Mandal, M. Kumar, A. Sharma. Protamine 3 expression in crossbred bull spermatozoa may not be a marker for differentiating good and poor quality semen. *African J. Biotechnol.* 12(4) (2014) 1999-2003.
- [11] P.D. Grzmil, K.C. Boinska, I. Kleene, G. Adham, Schluter, M. Kamper, B. Buyandelger, A. Meinhardt, S. Wolf, W. Engel, PRM3, The fourth gene in the mouse protamine gene cluster, encodes a conserved acidic protein that affects sperm motility. *Bio. Rep. J.* 78 (2008) 958-967.
- [12] C.J. Martin, G. Monsterrat, R.R.S. Eduardoo, Protamine 3 shows evidence of weak, positive selection in mouse species (genus *Mus*)-but it is not a protamine. *Bio. Rep.* 84(2) (2011) 320-326.