

# Identification and Characterization of Protamine1 Gene in Aceh Cattle

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**Abstract.** Naturally, the fertility of Aceh cattle has declined and caused huge losses in the development of Aceh cow germplasm. Several genetic-based studies have been carried out in Aceh cattle, including characteristics of genetic diversity using microsatellite DNA and its relationship to body weight in Aceh cows, but there is no research on genomics that is closely related to fertility, especially fertility in aceh cows. Previous studies have revealed a correlation between fertility with the expression of several genes involved in spermatogenesis such as Protamines (*PRM 1, 2, and 3*) and Transition Nuclear Proteins (TNPS 1 and 2). This study aims to identify the *PRM1* gene that plays an important role in spermatogenesis and the fertility status of Aceh cattle. This research was carried out using 10 Aceh cattle testes taken from slaughterhouses, followed by DNA isolation, amplification of the *PRM1* gene, DNA sequencing, and DNA base sequence analysis. The result showed that the nucleotide sequences of aceh cattle *PRM1* gene did not differ from the *PRM1* gene from *Bos taurus* and *Bos indicus* in the GeneBank. Analysis of protein sequences showed that *PRM1* in Aceh cattle sperm is rich in the amino acid arginine, which is 36.92%. From this study, it can be concluded that the *PRM1* gene from Aceh cattle is identical to the *PRM1* gene from *Bos taurus* and *Bos indicus* both at the DNA sequence level and at the protein sequence.

Keywords: Aceh cattle, *PRM 1* gene, quality of sperm.

## 1 Introduction

Aceh cattle are one type of livestock that are widely developed by the people of Aceh as a livelihood. Aceh cattle through the decision of the Minister of Agriculture of the Republic of Indonesia Number 2907/Kpts.OT.140/06/2011 has been established as a native Indonesian cattle family and as national germplasm that needs to be conservative [1]. Some of the advantages of Aceh cattle are that they have high fertility, are more resistant to adverse environmental conditions, quickly adapt to new environments, and breed quickly [2]. One of the adverse effects due to the high exploitation of Aceh cows is the decline in genetic quality [3].

The main problem faced in the development of Aceh cattle is the low quality of seedlings caused by the occurrence of inbreeding and management of livestock maintenance that is not good [3]. The existence of negative selection makes an important contribution to the decline in genetic quality [4]. A number of genetic-based studies have been carried out in Aceh cows, including characterization of genetic diversity using microsatellite DNA [5], and characterization of genetic diversity of microsatellite DNA and its relationship to body weight in

Aceh cows [6], but there are no studies on genomics that are closely related to fertility, especially fertility of Aceh bulls.

Naturally, the fertility power of Aceh cattle decreases, causing huge losses to the Aceh cattle germplasm development program. The use of good quality semen is very important for the success of reproductive technology such as artificial insemination and embryo transfer. According to Ferraz *et al.* [7], infertility in bulls is characterized by a reduced ability of spermatozoa to fertilize oocytes and support early embryonic development. The technique commonly used to evaluate cow sperm quality is to look at the concentration, morphology, and motility of spermatozoa. This simple evaluation has not been able to predict the actual level of cattle fertility, due to the quality of genetic material contained in sperm. Fatehi *et al.* [8] states that sperm DNA damage does not affect oocyte fertilization or completion of the embryo division sequence, but inhibits embryo formation due to apoptosis.

During spermiogenesis, haploid spermatids undergo a series of changes in chromatin composition and density [9]. Meanwhile, in the round spermatid, the bond between deoxy nucleic acid (DNA)-histone will be replaced by

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transition proteins, whereas in elongated spermatid, the transition protein will be replaced by protamine [10]. Transition proteins are the main proteins found in chromatin in condensed spermatids which are believed to play an important role in the replacement of histone and chromatin condensation during spermatogenesis in mammals [11].

During fertilization, spermatozoa play an important role in the development of the embryo by providing oocyte activation factors, centrosomal components, and a paternal chromosome. Protamine (PRM) is a basic nucleus protein with small molecular weight contained in the head of spermatozoa [12,13], which is important in the packaging of sperm DNA [14] and plays an important role in spermatogenesis and quality of sperm [15]. The results of the study by Ganguli *et al.* [16] showed that the high *PRMI* mRNA expression was closely related to the good quality of semen produced by bulls.

Based on the problems above, this study was conducted aiming to identify and characterize the *PRMI* gene in Aceh cattle as well as to find out the differences in the *PRMI* gene sequence in several Aceh cattle with other types of cattle. The results of this study are expected to be the basis for the selection of males with good fertility so that they can be applied in reproduction technology to support central and local government programs in developing Aceh cow germplasm.

## 2 Materials and Methods

### 2.1. Time and Place of Research

This research activity includes field activities and laboratory activities. Field activities were carried out through taking data and testes of Aceh cattle in the Slaughterhouse (RPH) of Keudah in Banda Aceh City. Laboratory activities for total DNA extraction from Aceh cattle testes, amplification of the *PRMI* gene, were carried out at the Research Laboratory of the Faculty of Veterinary Medicine at Syiah Kuala University, sequencing of the nucleotide sequence of the *PRMI* gene was carried out at First Base Singapore through the services of PT Genetics Science Indonesia.

### 2.2 Research Samples

This study uses 10 testes of Aceh cattle which are local cows from Aceh Province. Sampling is carried out immediately after the cattle are slaughtered, then sample preparation is carried out to separate the cauda epididymis from other parts of the testis. The sample is put in a sterile tube, then stored in an icebox to be taken to the Research Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University. Samples were stored at -20 °C until DNA extraction was carried out.

### 2.3 Research Materials

The materials used in this study include DNA isolation kits using PureLink® Genomic DNA Kits from Invitrogen, PCR master mix reaction using Platinum TM

Hot Start PCR Master Mix, Ultrapure DNase / RNase Free Distilled Water from Invitrogen, SYBR® Safe DNA Gel Stain, 100bp DNA ladder from Cleaver Scientific, Buffer Tris-Borate EDTA 10x. Primary sequences of partial specific PRM genes that were designed using the Primary-Blast Program on the NCBI website: <https://www.ncbi.nlm.nih.gov>, with the reference gene GU394047.1.

## 2.4 Procedure

### 2.4.1. Total DNA extraction from the Aceh Cattle Semen

Total DNA extraction from Aceh cattle testes using PureLink® Genomic DNA Kits, the extraction procedure is carried out following the work in the manual issued by the factory that manufactures the kit. The crushed testicle sample is put into a 25 mg microtube, then 180 µL PureLink® Genomic Digestion Buffer and 20 µL Proteinase K are added to the tube. The microtubes are then shaken using a mini-mixer and incubated at 55 °C for 4 hours or until lysis, then spindown for a few seconds centrifuged for 3 minutes at maximum speed to pick up cell pellets. Then 20 µL RNase A was added to the cell lysate, vortexed and incubated at room temperature for 2 minutes, then 200 µL PureLink® Genomic Lysis / Binding Buffer was added as much as 200 µL, and vortexed again. Then to lysate added 200 µL 96-100% ethanol.

Furthermore, 640 µL was transferred into PureLink® Spin Column, then centrifuged at a speed of 10,000 xg, then carried out the Washing step by adding 500 µL Wash Buffer I and centrifuged at a speed of 10,000 xg for 3 minutes. The spin column was transferred to the new collection tube, then added 500 µL Wash Buffer II and centrifuged at 10,000 xg for 3 minutes. Furthermore, PureLink® Spin Column was placed in a 1.5 µL microtube, and added with 50 µL PureLink® Genomic Elution Buffer, then incubated at room temperature, then centrifuged at maximum speed for 5 minutes. The results of DNA isolation can already be used for amplification.

### 2.4.2. Analysis of Gene Expression with Realtime PCR (qPCR)

Total DNA extracted was carried out by amplification of the *PRMI* gene, by PCR reaction using Platinum TM Hot Start PCR Master Mix (Invitrogen, California, United States), with a total reaction of 25 µL reaction mix consisting of 12.5 µL Platinum TM Hot Start PCR Master Mix, 0.5 µL forward primer, 0.5 µL reverse primer, and 5 µL DNA template (10 ng), then added with 6.5 µL ddH<sub>2</sub>O.

The PCR program was arranged as follows: initial denaturation at 94 °C for 2 minutes; denaturation temperature at 94 °C for 30 minutes, annealing at 59 °C for 30 seconds, elongation at 72 °C for 45 seconds, continued extension at 72 °C for 5 minutes, and post-elongation for 5 minutes at 72 °C reaction amplification carried out as many as 35 cycles.

### 2.4.3. PCR product electrophoresis

PCR products were electrophoresed by the electrophoretic device from Biorad and visualization of electrophoresis results using GelDoc Viewer from Biorad.

### 2.4.4. Sequencing

PCR products are sent to First Base Singapore through the services of PT Genetics Science Indonesia, for nucleotide sequence sequencing. Sequencing is done by the Dye terminator sequencer method, which will read the nucleotide sequence from the 3' to the 5' direction. The sequencing data is displayed in the form of an electropherogram, which can be analyzed with the BioEdit or Blast program.

### 2.4.5. PCR Data Analysis

Data analysis of Aceh cattle *PRM1* gene fragment sequences results using the Clustal W program from Mega 10.0 software and homology analysis with sequences contained in GenBank using the Basic Local Alignment Search Tool (BLAST) software program. Data analysis results are displayed descriptively in the form of Figures and Tables.

## 3 Results and Discussion

### 3.1 Results of PCR Analysis

The results showed that the *PRM1* gene isolated from the testes of Aceh cattle could be amplified by the specific

Tabel-1. Alignment of 200 nucleotide base *PRM1* gene isolates compared to access code M18395.1, and Aceh cattle *PRM1* gene isolates.

Comparison sequences:		
Sekuen Penelitian :	CCAAGGCGGTATCCCCTGCTCTGAGCATCCAGGCCGAATCCACCCAGCA	ACCATGGCCAGATACCGATGCTGCCTCACCCA [ 720]
Comparison sequences:	-----	-----ACCATGGCCAGATACCGATGCTGCCTCACCCA [ 720]
Sekuen Penelitian :	TAGCGGGAGCAGATGCCGCGCCGCCCGCCGAAGAAGATGTCGCAGACGAAGGAGGCGCTTTGGTCGGAGGCGCAGGAGGA	[ 800]
Comparison sequences:	TAGCGGGAGCAGATGCCGCGCCGCCCGCCGAAGAAGATGTCGCAGACGAAGGAGGCGCTTTGGTCGGAGGCGCAGGAGGA	[ 800]
Sekuen Penelitian :	GAGGTGAAGAGGGTCCATCCTTGGGGCCAGGGCCAGGGAGCTGGGGCGGGCTGGGGCTTTGGGCTGTGCTGAAGTGTG	[ 880]
Comparison sequences:	GAGGTGAAGAGGGTCCATCCTTGGGGCCAGGGCCAGGGAGCTGGGGCGGGCTGGGGCTTTGGGCTGTGCTGAAGTGTG	[ 880]
Sekuen Penelitian :	CTCGTGTCTCTGGTTCTCTGCAGTGTGCTGCCGTCGCTACACCGTCATAAGGTGTACAAGACAGTAACCACACAGTAGC	[ 960]
	CTCGTGTCTCTGGTTCTCTGCAGTGTGCTGCCGTCGCTACACCGTCATAAGGTGTACAAGACAGTAACCACACAGTAGC	[ 960]

▲ 889

689 ▼

Protamine is a basic nucleus protein with a small molecular weight that is sperm-specific and rich in arginine. Protamine genes are transmitted in the post meiosis phase, which is then stored as inactive messenger ribonucleoproteins (mRNPs) until the differentiation of spermatid cells takes place. At this stage, the corresponding mRNAs are then translated so that the newly synthesized protamine polypeptide enters the nucleus to replace the somatic histone position on the chromosomes during spermiogenesis [17].

primers of the partial *PRM1* gene. Sequence primers used in this study is a primer designed using Primer-Program Blast on the NCBI website: <https://www.ncbi.nlm.nih.gov>, with gene reference GU394047.1. The primary sequence in this study is PRM\_1 forward: 5- ACC ATG GCC AGA TAC CGA TG- 3, PRM\_1 reverse: 5- GAC ACG AGG ACA CTT CAG CA- 3. The PRM 1 gene that can be amplified with PRM1\_F and PRM1\_R primers is 200 bp, as shown in Figure 1.

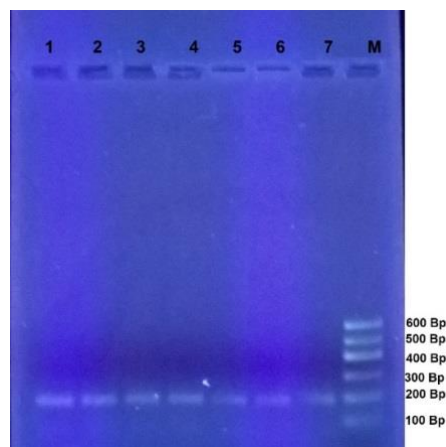


Figure-1. Electrophoresis results from Aceh cattle *PRM1* gene; 7 data displayed from 10 samples analyzed. (1-7 Samples, M: 100bp DNA Marker)

### 3.2 Results of Sequencing Analysis

*PRM1* gene sequencing results showed that Aceh cows had the same *PRM1* gene character as Bos Taurus.

Based on the analysis of the 65 amino acid sequences of the Aceh cattle *PRM1* gene after being compared with the Bos Taurus *PRM1* gene from GenBank data, 24 amino acids or 36.92% were obtained, including Arginine.

Arginine, also known as L-arginine, is a semi-essential amino acid that acts as a substrate for the production of nitric oxide by immune and endothelial cells of the body [18]. The results showed that the administration of arginine can improve semen quality in bulls [19].

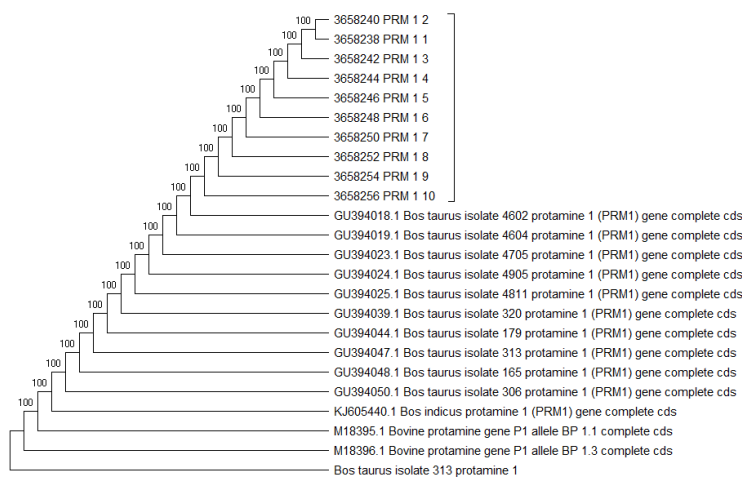
**Table 2.** The 65 amino acid sequences of the *PRMI* gene isolate compared to the access code M18395.1, with the Aceh cow *PRMI* gene isolate.

Comparison sequences (M18396.1):	T	MARYRCCLTH	SGSRCRRRRR	RRCRRRRRRF	GRRRRRRGEE	GPSLGAGARE	LGRGWGFGLC	*SVLV
Research sequences :	T	<b>MARYRCCLTH</b>	<b>SGSRCRRRRR</b>	<b>RRCRRRRRRF</b>	<b>GRRRRRRGEE</b>	<b>GPSLGAGARE</b>	<b>LGRGWGFGLC</b>	<b>*SVLV</b>

232
283

**Table 3.** Composition of 65 amino acid *PRMI* gene isolates compared to access code M18395.1, with Aceh cattle *PRMI* gene isolates.

	Ala	Cys	Glu	Phe	Gly	His	Leu	Met	Pro	Arg	Ser	Thr	Val	Trp	Tyr	Total
Comparison sequences (M18396.1):	4.62	7.69	4.62	3.08	15.38	1.54	7.69	1.54	1.54	36.92	6.15	3.08	3.08	1.54	1.54	<b>65</b>
Research sequences :	4.62	7.69	4.62	3.08	15.38	1.54	7.69	1.54	1.54	36.92	6.15	3.08	3.08	1.54	1.54	<b>65</b>



**Figure 2.** Results of phylogenetic construction of the Aceh cattle *PRMI* gene with comparative isolates from GenBank, using the Neighbor-Joining method with the Kimura 2 Parameter model and 1000x bootstrap.

The results showed that protamine gene disorders cause disruption of chromatin density, sperm DNA damage, and male infertility. In addition, the presence of heterozygous single nucleotide polymorphism in the *PRMI* gene causes infertility in men [20]. Decreasing the number and localization of *PRMI* causes defects in the structure of sperm chromatin, thereby reducing the fertility of bulls [14].

#### 4 Conclusion

From this study, it can be concluded that the *PRMI* gene from Aceh cattle is identical to the *PRMI* gene from *Bos taurus* and *Bos indicus* both at the DNA sequence level and at the protein sequence.

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