Protein Profiles of Seminal Plasma and their Correlation with Semen Quality in Aceh Bulls (*Bos indicus*)

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Abstract. *The Indonesia Artificial Insemination Center wants the production of frozen semen from Aceh bulls as soon as possible to take advantage of the desired genetics. Genetic selection and breeding soundness examination are required to be accepted in the artificial insemination industry. Therefore, seminal plasma protein analysis and semen quality evaluation have prospective use in the selection of superior bulls. This study aims to characterize the protein profile of seminal plasma in Aceh bulls and to determine its correlation with semen quality. A total of four Aceh bulls (24–34 months) belonging to the Livestock Breeding and Forage Center of Indrapuri were selected randomly. Semen ejaculation was used for the evaluation of semen quality (semen volume, colour, consistency, and pH, mass motility, sperm motility, sperm viability, and sperm morphology). The remaining volume of semen was centrifuged to obtain seminal plasma. Total seminal plasma protein concentration (μg/μL) was calculated using the Bradford method, and then the seminal plasma protein was electrophoresed on SDS PAGE and visualized with Coomassie Blue. The results of protein visualization found 16 protein bands with different molecular weights, ranging from 11 to 180 kilo Dalton. In general, the protein band of 15.24 kilo Dalton was more prominent in Aceh bulls. In addition, the seminal plasma protein concentration showed a positive correlation with sperm motility, sperm viability, and sperm morphology. In conclusion, the seminal plasma protein of Aceh bulls is positively correlated with several semen quality variables and may be a useful as an additional parameter for determining semen quality or bulls fertility.*

Introduction

In Indonesia, there are several local beef cattle whose reproductive performance and production must be improved to be used sustainably for food security. One of the cattle whose performance needs to be improved is the Aceh cattle. Aceh cattle are one of the local beef cattle in Indonesia that have been genetically identified, and research is increasingly being carried out on reproductive efficiency to increase production (Abdullah et al. 2012; Sutarno et al. 2019; Panjaitan et al. 2021). Efforts to increase the reproductive efficiency of Aceh cattle have been carried out through several studies, ranging from follicle dynamics studies (Siregar et al. 2016; Armansyah et al. 2017), application of oestrous synchronization, and artificial insemination (AI) (Hafizuddin et al. 2012; Ramli et al. 2016). However, the reproductive efficiency of Aceh bulls has not been widely reported. Male reproductive efficiency continues to be developed through several studies finding a tool or method of male fertility assessment (Kaya and Memili 2016; Karunakaran and Devanathan 2017; Druart and de Graaf 2018; Hafizuddin et al. 2020). Male fertility is influenced by several factors, including physical condition, semen quality, and biochemical content of seminal plasma (Assumpção et al. 2005; Almadaly et al. 2016).

Semen characteristics, especially the sperm motility variable and sperm morphology, are the main criteria used in the assessment of semen quality. According to Rodrigues et al. (2013) and Boe-Hansen et al. (2015), if the seminal plasma as the microenvironment of spermatozoa cells is evaluated, it has a high potential for assessing semen quality or male fertility. This potential can explain how the characteristics of sperm are related to protein expression in the reproductive tract, both at maturation and during the transport of spermatozoa.

Several studies in mammals have reported the protein profile in seminal plasma and its effect on semen characteristics. Research in ram showed that seminal plasma protein was correlated with sperm motility (Rodrigues et al. 2013), and sperm capacitation (Carballada and Esponda 1998). Nonetheless, there have also been studies reporting negative effects of seminal plasma protein on semen quality (Iwamoto et al. 1995; Schöneck et al. 1996; La Falci et al. 2002). Thus, it is necessary to identify seminal plasma protein and its correlation with several semen quality variables as the main variable for assessing fertility in bulls.

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Materials and methods

Animals

This study used four Aceh bulls (24–34 months) who were randomly selected at the Livestock Breeding and Forage Centre of Indrapuri, Ministry of Agriculture, The Republic of Indonesia. Bulls have a good body condition score with criteria between 3 and 4 on a score scale of 5.

Semen collection

Semen was collected using an artificial vagina, previously dried and cleaned alongside the rubber and reservoir tubes in order to prevent contamination (Sutriana et al. 2022).

Semen quality examination

Semen macroscopic examination

Semen samples were obtained from each animal and analyzed as previously reported by Hafizuddin et al. (2021) unless stated in other references. After collection, the quality was macroscopically evaluated based on volume, colour, consistency, and pH.

Semen microscopic examination

Spermatozoa mass motility

A drop of 5 µL of raw semen was deposited on a pre-warmed glass slide (≈37°C), and the edge of the drop was observed at low magnification (10 × objective) on the thermally controlled stage of a phase contrast microscope. Observations at the edges of the drop provide for assessment of the rapid flogging of black waves and whirlpools on a grey background which is termed as the wave motion or mass sperm motility. This mass sperm motility was scored subjectively from 0 (no motion) to 5 (numerous rapid waves) on a scale with steps equal to 1 (David et al. 2015)

Spermatozoa motility

Spermatozoa motility was evaluated by dripping the sample on a glass slide, then one drop of physiological NaCl was added, followed by observation through a microscope with 40 x 10 magnification. The number of motile sperm was calculated based on the movement, categorized as fast progressive (A), slow progressive (B), circular (C) and vibration (D) (Syafruddin et al. 2020). The percentage was determined using the following formula:

$$\% \text{ Motility} = \frac{A}{A+B+C+D} \times 100\%.$$

Spermatozoa viability

The examination of viability was performed by introducing one drop of spermatozoa on a glass slide, followed by the addition of one staining eosin-nigrosin drop. A smear preparation was made and fixed on a spiritus lamp, then evaluated using a microscope of 40 x 10 magnification. The dead cells absorb a red pigmentation, while the live spermatozoa tend not to absorb any colour, leading to a white appearance. The spermatozoa were then counted and divided by the total visible and presented as a percentage value (Padrik et al. 2010).

$$\% \text{ Live} = \frac{\text{Total of live spermatozoa}}{\text{Total live and dead spermatozoa}} \times 100\%.$$

Spermatozoa morphology

This observation was performed by dripping spermatozoa and eosin-nigrosin on the object glass, fixed on a spiritus lamp, and observing in a microscope with 40 x 10 magnification. The morphological examination identified deformities that are categorized as primary (small/large head size, double head or double tail, and abnormal head shape) and secondary abnormalities (head rupture, tail breaking at the neck or middle, and folded tail) (Klimas et al. 2012). The minimum spermatozoa observed were 200 cells, and the calculations were conducted using the following formula:

$$\% \text{ Normal sperm morphology} = \frac{\text{Normal sperm morphology}}{\text{Normal sperm morphology} + \text{Abnormality}} \times 100\%.$$

Identification of seminal plasma proteins

SDS-PAGE of 4 biological replicates of seminal plasma (90 µg/per lane) was performed using self-cast 12.5% separating polyacrylamide gels according to the method of Laemmli (1970). After that, electrophoresis gels were stained overnight (0.05% CBB R-250, 50% methanol, 10% acetic acid) and destained with 5% methanol and 7% acetic acid. Seminal plasma protein concentration (µg/µL) was calculated using the Bradford method (Bradford 1976), and then the seminal plasma protein was electrophoresed on SDS PAGE and visualized with Coomassie blue.

Data analysis

Semen characteristics data and seminal plasma protein profiles were presented descriptively. Meanwhile, the correlation between seminal plasma protein and semen characteristics was determined using the Pearson test.

Results

Semen characteristics

The mean semen characteristics in Aceh bulls are summarized in Table 1.

Seminal plasma protein profile

The present data showed that the mean protein concentration in the semen of Aceh bulls was 1.83 µg/µL, and the range was 1.72–1.97 µg/µL. Furthermore, 16 protein bands with different molecular weights were found, ranging from 11 to 180 kDa. In general, the protein band of 15.24 kDa was more prominent in the Aceh bull. A typical SDS-PAGE profile of Aceh bull seminal plasma is shown in Figure 1.

Correlation of seminal plasma protein with semen quality

Based on the results of the Pearson test, the concentration of seminal plasma protein had a positive correlation with semen volume (r = 0.166), sperm motility (r = 0.877), sperm viability (r = 0.716) and sperm morphology (r = 0.646). Meanwhile, mass motility had a negative correlation (r = –0.877) (Table 2).

Table 1. Semen characteristics in Aceh bulls

Parameter	Unit	Bulls (n=4)	Range
Semen volume	mL	2.90 ± 0.53	2.5–3.5
Colour	-	Cream	-
Consistency	-	Moderate	-
Semen pH	-	6.50 ± 0.17	6.4–6.7
Mass motility	score	2.67 ± 0.58	2–3
Sperm motility	%	76.67 ± 2.89	75–80
Sperm viability	%	86.33 ± 4.65	82.50–91.50
Sperm morphology	%	91.50 ± 5.57	86.50–97.50

Table 2. The correlation coefficient (r) between seminal plasma protein with semen characteristics

Parameters	Correlation coefficient (r)				
	Semen volume	Mass motility	Sperm motility	Sperm viability	Sperm morphology
Seminal plasma protein	0.166	–0.877	0.877	0.716	0.646

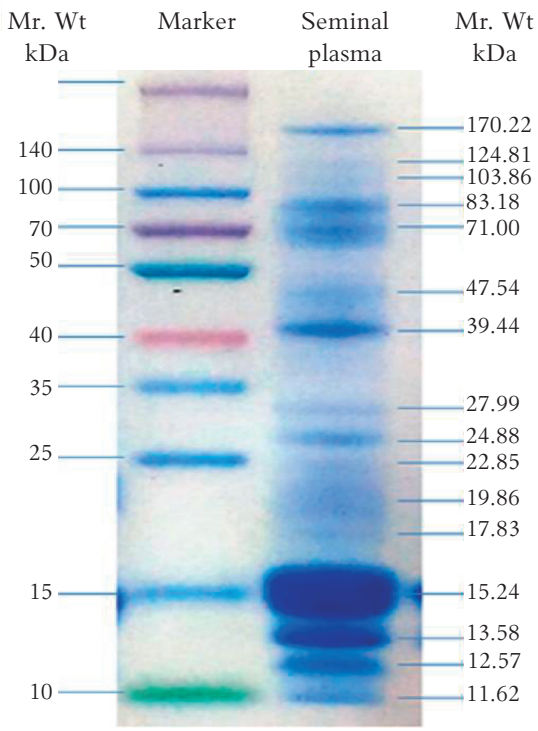

Fig. 1. SDS-PAGE of seminal plasma proteins in Aceh bulls
Mr. Wt = molecular weight; kDa = kilodalton

Discussion

Semen characteristics

The semen volume reported in this study was lower than that reported by Isnaini et al. (2019) and Vince et al. (2017). The mass motility was higher than that reported by Gopinathan et al. (2018), whereas the motility of sperm, viability of sperm, and morphology of sperm were similar to the results of other studies in bull (Rego et al. 2015; Vince et al. 2017). Based on the comparison of these data, the semen collection and evaluation in this study are still within the range of general bull semen characteristics.

Seminal plasma protein profile

The results of this study found low molecular weight protein bands similar to previous studies on bulls by Jobim et al. (2004). The study identified proteins in seminal plasma with low molecular weight (10–30 kDa). Our studies found proteins with small sizes of 11.62, 12.57, 13.58, 15.24, 17.83, 19.86, 22.85, 24.88, and 27.99 kDa. Previous studies on ram found that the concentration and number of protein bands with low molecular weight (11, 13, and 22.5 kDa) were found more in the highly fertile group than in the fertile and sub fertile groups (Almadaly et al. 2016).

The medium molecular weight protein in this study was similar to that found in buffalo bull seminal plasma. In that study, it was found that the protein of medium size was 45 and 55 kDa (Asadpour et al. 2007). Overall, the 16 protein bands found in this study were similar to the results of a recent proteomic study on seminal plasma (Soleilhavoup et al. 2014).

Low and medium molecular weight proteins such as BSP1, BSP3, BSP5, sperm-adhesin, albumin, TIMP, AK1, and PEBP1 have previously been reported in bulls that were significantly more in the highly fertile group (Kasimanickam et al. 2019)

Correlation of seminal plasma protein with semen quality

There was a positive relationship between seminal plasma protein concentration and several semen quality criteria observed in this study. Testing the spermatozoa motility variable is currently the most informative test, because the results of the study consistently show that spermatozoa motility is correlated with male fertility. In addition, the percentage of morphologically normal sperm is also a variable that is often used in the assessment of male fertility. These two variables are the main criteria used in the breeding soundness examination (BSE) (Chenoweth and McPherson 2016). Therefore, microscopic evaluation of sperm motility and morphology are likely to continue to be the two most important predictors of fertility in terms of utilization and fertility variation explained by these variables (Almadaly et al. 2016).

Several previous studies have reported the relationship between semen quality and seminal plasma protein, such as the relationship between seminal plasma and ram sperm motility (Rodrigues et al. 2013), and the relationship between seminal plasma proteins and the percentage of morphologically normal sperm in bull (Boe-Hansen et al. 2015).

Previous studies have been reported in bull by Killian et al. (1993), who stated that seminal plasma protein has positive features with bull male fertility.

Other studies that have found a positive correlation of seminal plasma protein with semen quality and male fertility have been reported in rams (Rodrigues et al. 2013; Almadaly et al. 2016), boars (Novak et al. 2010a), bulls (Karunakaran and Devanathan 2017), and stallions (Novak et al. 2010b). Based on these data, our study has similarities with previous studies. This can support the efforts to characterize seminal plasma proteins as biomarkers of semen quality and bull fertility.

Conclusion

The seminal plasma protein of Aceh bulls is positively correlated with several semen quality variables and may be useful as an additional parameter for determining semen quality or bull fertility. Characterization of deeper protein in seminal plasma is needed in the search for male fertility biomarkers.

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Conflict of interests

The authors declare that they have no conflict of interest.

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