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Electrophoretic Protein Profiles of Seminal Plasma and their Correlation with Fresh Semen Quality in Indonesia Toraya Buffalo (*Bubalus bubalis carabanesis*) Bulls

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ABSTRACT

The current study aimed to evaluate the relationship between seminal plasma proteins and sperm quality in Toraya buffalo bulls. Semen samples were obtained from eight Toraya buffalo bulls aged 4-10 years. Semen collection was conducted using an artificial vagina, followed by assessment through macroscopic and microscopic analysis. The concentration of seminal plasma protein was measured utilizing the bicinchoninic acid (BCA) protein assay, followed by protein analysis through 1D SDS-PAGE based on protein molecular weight. The sperm quality of Toraya buffaloes showed an average semen volume of 2.08±1.12mL, sperm motility of 73.87±5.30%, sperm concentration of 883.12±381.35×10^6/mL, viability of 81.47±3.79%, abnormality of 7.36±3.24%, membrane integrity of 83.14±5.19%, and acrosome integrity of 92.98±2.56%. The parameter intact acrosome in sperm quality showed a positive correlation (0.73) with sperm viability with a significant (P<0.05). The total motile sperm per ejaculate was 1326.61±714.99x10^6, and the total straw per ejaculate was 54.06±28.59. The average seminal plasma protein concentration in Toraya buffalo was 161.41±12.41µg/mL. The Pearson correlation results of seminal plasma protein correlated with intact acrosomes with a significant P<0.05. The analysis of seminal plasma protein bands using 1D SDS-PAGE founded the presence of 12-19 protein bands with molecular weight ranging from11-155kDa. This study concluded a significant correlation between the seminal plasma protein concentration and sperm acrosome integrity in Toraya buffalo bulls. The identification of 12-19 protein bands that correlate with these factors is a promising finding and can be utilized to determine the reproductive quality and fertility of the bulls.

Key words: Fresh semen, Seminal plasma, SDS Page, Sperm quality, Toraya buffalo.

INTRODUCTION

The Toraya buffalo (*Bubalus bubalis carabanesis*) is one of Indonesia's indigenous buffaloes, and it has a unique color and pattern compared to swamp buffalo. Toraya buffalo has been established as a local Indonesian buffalo breed by Ministerial Decree Number 2845/Kpts/LB430/8/2012. The Toraya buffalo is endemic to the South Sulawesi region, particularly in the regions of Tana Toraja and North Toraja. This buffalo holds significant cultural importance for the Toraja people, contributing to their nutritional needs. Toraya buffalo is recognized by its unique reddish-white and black color pattern, complemented by white eye color. The species displays varying head colors, including grey, black, and white. Notably, their horns curve from the side towards the back, and their ears angle towards the sides. Understanding these distinctive features contributes to insights into the species' genetics, evolution, and ecological adaptations, facilitating conservation efforts (Maulana et al. 2023).

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Seminal plasma constitutes a complex fluid comprising diverse macromolecules sourced from the testes, epididymis, and accessory sexual glands, playing a vital role in sustaining sperm cell viability. Seminal vesicles and ampullae are responsible for the production and secretion of seminal plasma proteins (Moura et al. 2006). Recent advancements in reproductive technologies highlight the nutritive-protective attributes of seminal plasma for suspended sperm cells. Certain components within seminal plasma play crucial roles in sperm metabolism, function, survival, and transportation within the female reproductive tract (Juyena and Stelleta, 2012). Seminal plasma proteins contribute to sperm abilities such as motility, cell membrane integrity, protection against reactive oxygen species (ROS), oviduct reservoir formation, sperm capacitation, acrosome reaction, and interaction with the pellucid zone (Pardede et al. 2020).

The composition of proteins in the seminal plasma of mammals differs among species, affecting functions of sperm such as their freezeability (Asadpour et al. 2007). Additionally, seminal plasma proteins play a role in protecting sperm from damage and maintaining their longevity, as evidenced by correlations observed between semen characteristics and seminal plasma proteins (Sharma et al. 2015). In different species, these proteins play roles in energy metabolism, cellular signalling, the production of sperm, and the movement of sperm cells, directly influencing fertility outcomes (Druart et al. 2019; Iskandar et al. 2023). Notable proteins like spermadhesin, ribonuclease, 14-3-3 protein zeta/delta, acrosin inhibitor, prosaposin, and peptide YY have been linked to sperm motility, serving as potential utility as indicators of semen quality (Codognoto et al. 2018). Bulls expressing higher levels of seminal plasma proteins are correlated with high fertility (Peddinti et al. 2008).

The seminal plasma protein profiles of Iraqi buffalo bulls revealed nine types of protein bands ranging from 10-68 kDa associated with good semen quality (AbdulKareem and Musa 2021). Fu et al. (2019) reported a total 864 protein were identified from seminal plasma buffalo were analyzed using a bottom-up approach, with high abundant protein were albumin (Alb), clusterin (Clu), zinc alpha 2 glucoprotein (Azgp1), glia-derived nexin (serpine 2) and serotransferrin (Tf). Iskandar et al. (2023) reported a total of 94 proteins were identified in the seminal plasma of Bali bulls ranged from <11-110kDa. Proteins spermadhesin 1 (SPADH1), C-type natriuretic peptide (NPPC), clusterin (CLU), apoliprotein A-II (APOA2), inositol-3-phosphate synthase 1 (ISYNA1), and sulfhydryl oxidase 1 (QSOX1) were identified as important for fertility in *Bos javanicus* analyzed using LC-MS/MS (liquid chromatography–mass spectrometry).

Bull fertility, reliant on the sperm's fertilizing ability, is typically evaluated through fertility trials, a costly and time-consuming process. As of now, there is no single objective test indicating sperm fertilizing ability. Recent emphasis has been placed on investigating genetic markers for bull and semen quality (Singh et al. 2014). The identification of seminal proteins could serve as a valuable resource for forthcoming assessments of spermatozoa and fertility predictions (Abdulkareem and Musa 2021). Biomarkers proteins from bull play a critical role within the biological system of bulls (Iskandar et al. 2023).

Although many studies have highlighted an association between proteins in seminal plasma and fertility in bulls across different domestic species, including buffalo bulls (El-shamaa et al. 2016) and bovine bulls (Asadpour et al. 2007), there remains a lack of data on how these proteins impact semen traits and fertility in Toraya buffalo bulls. Thus, this research aims to examine how seminal plasma proteins correlate with the semen quality of Toraya buffalo bulls, desiring to identify a possible marker for selecting fertile bulls.

MATERIALS AND METHODS

Animals and ethical clearance

Fresh semen was obtained from eight Toraya buffalo bulls classified as Saleko, Bonga, Lottong Boko, and Sambo batu with ages 4-10 years and average body weight of 400-500kg (Fig. 1).

The bulls were maintained according to the standard ethical protocol of animal care by Artificial Insemination Centre as the superior bulls. The bulls were kept in a barn with individual stalls, each bull was fed 10% fresh forage and 1% concentrated in the morning and evening. Semen samples were collected in mid-morning (06.00-10.00 a.m.) after an extended period of the routine collection twice a week. The Animal Ethics Commission of the National Research and Innovation Agency approved this study's animal models and experimental designs with certificate number 050/KE.02/SK/03/2023.

Semen collection and evaluation

The process of collecting semen involved using an artificial vagina while a female buffalo served as a teaser. Once collected, the semen was immediately stored at 34^{o}C and examined through macroscopic and microscopic evaluation. Macroscopic evaluation included assessing its volume, pH, consistency, and color. Microscopic examination covered areas such as motility, viability, sperm membrane integrity, sperm concentration, and sperm abnormality. The examination used a phase contrast microscope (Olympus CX23) and sperm concentration was determined through a photometer (SDM 6, Minitube, Germany).

Sperm motility examination, 10µL of semen was diluted with 40µL of buffer, homogenized, and evaluated under a microscope at 400x magnification. Sperm concentration was calculated using a photometer (SDM 6, Minitube, Germany) according to standard procedures (Hasbi et al. 2023). A mixture of 10µL fresh semen and 1mL saline solution was transferred to a cuvette and put into a photometer to calculate the concentration in units of 10^9 sperm cells per mL with a wavelength of 546nm.

To determine sperm viability and abnormalities was performed using the eosin-nigrosine staining method. The assessment started by dropping 10µL of semen, which was then mixed with 40µL eosin-nigrosine (1:4) on the slide glass; both were homogenized, smeared, and dried at 37°C. The smear s were viewed under a microscope with a magnification of 400x. Live (viable) sperm were not stained (transparent), while the dye solution stained non-viable sperm. Live and dead sperm were count from 200 sperm cells. Sperm morphology was evaluated by viewing 200 sperm cells under a microscope with 400x magnification, distinguishing normal and abnormal sperm.

Fig. 1: Type spotted color pattern of coat Toraya buffalo bulls: (A) Bonga tenge, (B) Lottong boko, and (C) Saleko (Source: personal images).

The assessment of sperm membrane integrity was conducted using the hypoosmotic swelling (HOS) test. Incubating semen in a hypoosmotic solution (made up of 0.735g of sodium citrate, 1.351g of fructose, and 100mL of distilled water) where 30µL of semen was mixed with 300µL of HOS (in a 1:10 ratio) and examined under a microscope at 400x magnification. The assessment was carried out by looking for tail coiling (intact) or straightening (not intact) in 10 fields of view, with a total of 200 cells evaluated in each assessment.

The sperm acrosome status was evaluated by using the fluorescence staining method, which involved FITC-PNA (Sigma St. Luis MO) combined with propidium iodide (P.I.). Smears of semen samples were air-dried at room temperature and fixed in 96% ethanol for 10 minutes. Then, a 30µL (100µg/mL) solution of peanut agglutinin (PNA) lectin was applied to the smears and left to incubate at 37°C for 30min. Afterward, 5µL (1µg/µL) of P.I (Sigma, St. Louis, MO) was added to the smears and incubated for 5min, followed by rinsing with phosphate-buffered saline (PBS). The acrosome status was evaluated using a fluorescent microscope with a wavelength of 380-420nm, with 200 sperm cells observed in each treatment. Those sperm cells that displayed a green fluorescent acrosome were considered intact, while those exhibiting a reddish color were categorized as having damaged acrosomes.

Estimation of frozen semen production

The evaluation of semen production estimation was based on two factors, namely, the total motile sperm and the quantity of frozen semen straws. To determine the number of motile sperm per ejaculate, the volume of semen was multiplied by the motility and the concentration of sperm. The amount of frozen semen straw produced was calculated by dividing the number of motile sperm by the insemination dose, which is $25x10^6$ sperm in 0.25mL or $100x10^6$ sperm/mL.

Identification of seminal plasma protein

The semen was centrifugated at 3000rpm for 30min, and then the supernatant was put into the straw and saved in liquid nitrogen (Iskandar et al. 2023). Protein characterization used 1D SDS-PAGE based on protein molecular weight (MW). The concentration of proteins in seminal plasma was measured using the bicinchoninic acid (BCA) protein assay (Thermo Scientific™, USA) to analyze the protein composition based on molecular size. SDS-PAGE analysis was utilized to show the protein bands on the gels.

Protein were separated using 1D SDS-PAGE (SurePAGE™, 4-20% gradient gel, M00656; Genscript) and Protein Standard (Broad Multi Color Pre-Stained, M00624; GenScript), encompassing a molecular weight range of approximately 5-270kDa. Electrophoresis was carried out at 200V and 100mA for 40min. Subsequently, the gel was stained with Coomassie Brilliant Blue (R-250; Bio-rad, USA) for protein visualization.

Statistical analysis

The data on fresh semen quality were analyzed using a descriptive method. Two-way ANOVA (Minitab software 20 version) was applied to the different data groups. The relationship between semen quality and seminal plasma protein was assessed using Pearson's correlation analysis.

RESULTS

Fresh semen quality and estimation of frozen semen production

The fresh semen in this study was used from eight Toraya buffalo bulls with an average age of 6.75±2.43 years and had a variety of spotted coat patterns. The ejaculates of Toraya buffalo collected in this experiment had normal characteristics and were appropriate for cryopreservation (Table 1 and 2). The sperm quality of Toraya buffaloes showed an average semen volume of 2.08±1.12mL, sperm motility of 73.87±5.30%, and sperm concentration of $883.12±381.35×10^6$/mL (Table 2). The average percentage values of sperm viability, abnormality, membrane integrity, and acrosome integrity were 81.47±3.79, 7.36±3.24, 83.14±5.19, and 92.98±2.56%, respectively (Table 1). The estimated frozen semen production of Toraya buffalo in this study has a total motile sperm per ejaculate of $1326.61±714.99x10^6$ and a total straw per ejaculate of 54.06±28.59 (Table 2).

Seminal plasma protein Toraya buffalo

The results of the seminal plasma protein concentration analysis are shown in Table 3. Buffalo bull ID Lotong bokko (P4) had a protein concentration of 181.47±24.43µg/mL, significantly ($P<0.05$) higher than compared to Saleko 4 (P8) of 140.11±25.53µg/mL, while the average seminal plasma protein of Toraya buffalo in this study was 161.41±12.41µg/mL. Pearson correlation results of seminal plasma protein in this study showed that seminal plasma protein had a significant ($P<0.05$) negative correlation (-0.77) with intact acrosome.

Table 1: Fresh semen quality of Toraya buffalo bulls

Buffalo Bulls	Age (Year)	Sperm Quality Parameters				
		Motility (%)	Viability (%)	Abnormality (%)	Membrane integrity (%)	Acrosome (%)
Bonga 1	7	80	82.70	3.2	83.40	93.97
Bonga 2	10	70	79.50	2.9	83.00	93.50
Sambo Batu	4	65	78.03	11.38	80.09	91.22
Lotong Bokko	10	70	79.40	9.13	84.27	90.37
Saleko 1	8	80	84.20	9.55	83.90	90.82
Saleko 2	6	70	83.89	9.91	90.14	95.02
Saleko 3	5	80	76.35	7.81	87.61	91.16
Saleko 4	4	70	87.76	5	72.72	97.75
Mean±SD	6.75±2.43	73.87±5.30	81.47±3.79	7.36±3.24	83.14±5.19	92.98±2.56

Table 2: Estimation productivity of the frozen semen of Toraya buffalo bulls

Buffalo Bulls	Parameters				
	Semen Volume (mL)	Fresh semen motility (%)	Sperm Concentration ($\times10^6$/mL^{-1})	Total motile sperm/ejaculate	Total straw/ejaculate
Bonga 1	1.30	80	1600	1664	66.56
Bonga 2	2.50	70	1151	2014.25	80.57
Sambo Batu	1.60	65	950	988	39.52
Lotong Bokko	1.30	70	650	591.5	23.66
Saleko 1	4.50	80	750	2362.5	94.5
Saleko 2	2.50	70	778	1361.5	54.46
Saleko 3	2.00	80	890	1424	56.96
Saleko 4	1.00	70	296	207.2	8.29
Mean±SD	2.08±1.12	73.87±5.30	883.12±381.35	1326.61±714.99	54.06±28.59

Table 3: Seminal plasma protein of Toraya buffalo

Buffalo bulls	Protein Concentration (µg/mL)
Bonga 1 (P1)	166.87±15.11^{ab}
Bonga 2 (P2)	161.04±7.20^{ab}
Sambo Batu (P3)	170.29±10.78^{ab}
Lotong Bokko (P4)	181.47±24.43^{a}
Saleko 1 (P5)	163.52±2.27^{ab}
Saleko 2 (P6)	153.70±6.45^{ab}
Saleko 3 (P7)	154.30±0.53^{ab}
Saleko 4 (P8)	140.11±25.53^{b}
Mean±SD	161.41±12.41

This means that in a column with different superscripts, a and b differ significantly at P<0.05.

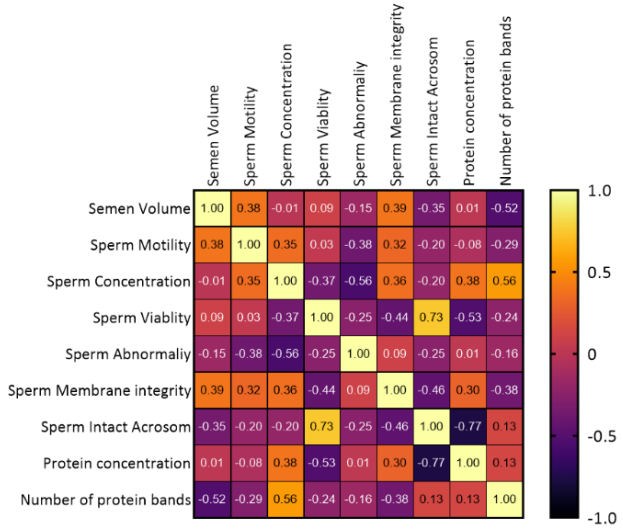

Fig. 2: Heatmap visualization of Pearson's correlations seminal plasma protein concentration and semen quality in Toraya Buffalo. The scale is based on colors from yellow (positive) to black and blue (negative); * had a significant correlation P<0.05.

In addition, the sperm intact acrosome had a significant (P<0.05) positive correlation (0.73) with sperm viability (Fig. 2). The results of 1D-SDS page analysis showed the seminal plasma protein bands of Toraya buffalo in this study were found of 12 to 19 protein bands with protein molecular weights of 11, 15, 16, 21, 24, 30, 33, 35,40, 43, 46, 49, 50, 60,69, 97, 112, 130, and 155kDa, respectively (Table 4). The seminal plasma protein sample of Bonga (P2) bull had the highest absence protein band (19 protein bands) compared to other samples, and the sample of Saleko (P5) had 12 protein bands with seven proteins, namely centrosomal protein, dystroglycan, MCP, serpine 2, osteopontin, BSP30, and seminal ribonuclease were not expressed.

DISCUSSION

The quality of fresh semen from Toraya buffalo in this study supports the previous findings reported by Kaiin et al. (2017) and Riwu et al. (2023) in Toraya buffalo aged 6-9 years. The semen volume of 2.20±0.32mL, progressive motility of 76.88±2.30%, viability of 86.85±1.80%, membrane integrity of 84.72±1.66%, abnormality of 2.49±0.36%, and a concentration of 1112±143.50x106/mL. Parmar et al. (2020) reported in Jafarabadi buffalo semen motility of 79.53±0.34%, viability of 84.26±0.50%, membrane integrity of 85.46±0.31%, abnormality of 6.06±0.33%, and acrosome integrity of 92.06±0.20%. The average result of sperm abnormality analysis in this study was lower than the findings reported by Herbowo et al. (2019) in swamp buffalo bulls (age 7-10 years), which were 15.34±0.53%.

Our results support the previous findings in Aceh swamp buffalo (Eriani et al. 2018) and Khundi river buffalo reported by Kaka et al. (2016) and Abdulkareem and Musa (2021) reported in Iraqi river buffalo bulls with good fertility, semen volume of 2.63±0.22mL, sperm cell concentration of 835.43±124.58x10^6/mL, sperm viability of 75.46±1.34%, abnormality of 5.26±0.24%, membrane integrity of 79.4±1.22% and acrosome integrity of 81.8±1.09%.

Table 4: Electrophoretic profiles of seminal plasma proteins of Toraya buffalo bulls

MW (kDa)	Candidate protein	References	Bull Number								Overall presence of protein n (%)
			P1	P2	P3	P4	P5	P6	P7	P8	
155	Pacifastin	AK	+	+	+	+	+	+	+	+	8/8 (100)
130	T-cadherin	AK	+	+	+	+	+	+	+	-	7/8 (87.5)
112	Centrosomal protein	AK	+	+	+	+	-	+	-	+	6/8 (75)
97	Dystroglycan 1	ZC	+	-	+	+	-	-	+	+	5/8 (62.5)
69	Albumin	Fu	+	+	+	+	+	+	+	+	8/8 (100)
60	ISYANA1	IH	+	+	+	+	+	+	+	+	8/8 (100)
50	Clusterin	Fu	+	-	+	+	+	+	+	+	7/8 (87.5)
49	BSP-A3	KL	+	+	+	+	+	-	-	+	6/8 (75)
46	MCP	SH	+	+	+	-	-	-	-	-	3/8 (37.5)
43	SERPINE2	Fu	+	+	+	+	-	-	+	+	6/8 (75)
40	Clusterin	Fu	+	+	+	+	+	+	+	+	8/8 (100)
35	Osteopontin	KL; RG	+	+	+	-	-	-	-	-	3/8 (37.5)
33	AZGP1	Fu	+	+	+	-	+	+	+	+	7/8 (87.5)
30	BSP-30	IB	+	+	+	-	-	+	+	+	6/8 (75)
24	TIMP2	Fu	+	+	+	-	+	+	+	+	7/8 (87.5)
21	BSP5	GM	+	+	+	+	+	+	+	+	8/8 (100)
16	Seminal ribonuclease	GM	-	-	+	-	-	-	-	-	1/8 (12.5)
15	PDC-109	GM	+	+	+	+	+	+	+	+	8/8 (100)
11	Apolipoprotein A-II	IH	+	+	+	+	+	+	+	+	8/8 (100)
		∑ Bands	18	16	19	13	12	13	14	15	

Note: MW (molecular weight); + (Protein expressed); - (Protein non-expressed); BSP5, Heparin-binding protein; TIMP2 Metalloproteinase inhibitor 2; Serine protease inhibitor (SERPINE2), Seminal plasma protein BSP-30 kDa (BSP5), Zinc-alpha-2-glycoprotein (AZGP1), Spermadhesin 1 (PDC-109), membrane cofactor protein (MCP), SH=Simpson and Holmes (1994), AK=Abdulkareem and Musa (2021), IH=Iskandar et al. (2023), Fu=Fu et al. (2019), GM=Gomes et al. (2020), IB=Ibraghimov-Beskrovnaya et al. (1992), ZC=Zoca et al. (2023), KL=Kelly et al. (2006), RG=Rego et al. (2014).

Based on the data in this study, the semen quality of Toraya buffalo bulls has met the minimum requirement for frozen semen production with motility of 70% and abnormality of less than 20% (BSN 2021). The assessment of sperm motility is a factor in determining whether the ejaculate is suitable for further processing and utilization in artificial insemination programs. This study attributes variations in the semen characteristics of each bull to the extent of sexual excitement and the frequency of semen collection. Various factors contribute to semen attributes, encompassing age, breed, body condition, scrotal size and weight, reproductive health, collection method and frequency, nutrition, season, and overall management (Baruti et al. 2018; Parmar et al. 2020).

Assessing sperm abnormalities is vital for determining fertilization success and sperm-ovum capacitation, which affects the sperm's ability to penetrate the zona pellucida (Felton-Taylor et al. 2020). Membrane integrity plays a crucial role in metabolic processes related to sperm motility and viability, essential for successful fertilization and overall sperm function in artificial insemination. Furthermore, alongside analyzing the quality of fresh semen, the production of frozen semen is essential for determining the number of frozen semen straws produced by each Toraya buffalo bull.

The potential for semen production is influenced by the number of ejaculations, which is assessed through semen volume analysis, sperm concentration, and motility. In this study, the average number of motile sperm per ejaculate was higher than the findings Abdulkareem and Musa (2021) reported in Iraqi buffalo. Their study reported $414.013\pm60.69x10^6$ motile sperm per ejaculate in bulls with good fertility and $369.55\pm34.24x10^6$ in bulls with poor fertility. The increased estimation of frozen semen production observed in this study compared to the previous one by Abdulkareem and Musa (2021) may be attributed to the superior quality of fresh semen, particularly in motility

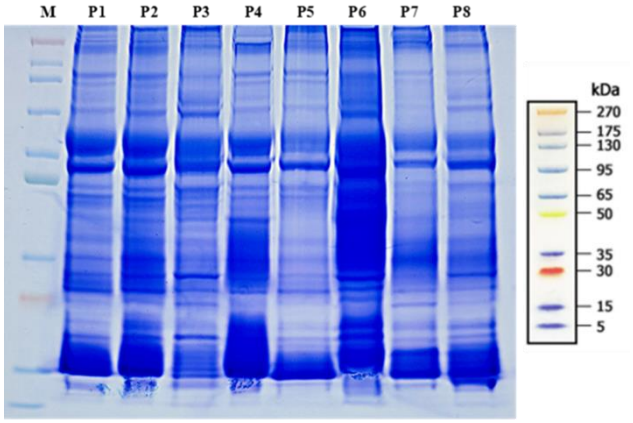

Fig. 3: Seminal plasma protein profile of Toraya buffalo bulls. kDa: kilodaltons; M: marker; P1: Bonga1; P2: Bonga2; P3: Sambo batu; P4: Lotong Boko; P5: Saleko1; P6: Saleko2; P7

and sperm concentration, in Toraya buffalo bulls. This quality is believed to be associated with the levels of protein expression in seminal plasma (Fig. 3).

Iskandar et al. (2023) reported that protein expression in seminal plasma could positively or negatively correlate with spermatozoa fertility or quality. Seminal plasma contains proteins, lipids, organic acids, enzymes, and minerals, all of which play crucial roles in spermatozoa metabolism and can influence spermatozoa fertility. Indeed, semen quality is the primary determinant of bull fertility in buffaloes. The constitution of buffalo semen significantly influences sperm membrane integrity and, consequently, fertility outcomes (El-Sheshtawy et al. 2008).

In this study, the average concentration of seminal plasma proteins was lower than the findings reported by Dixit et al. (2016) in Bharadai buffalo (4.63±0.16mg/dL). This is the first study to report that the relationship between seminal plasma protein levels and the quality of

semen from Toraya buffalo. The results obtained in this study are consistent with previous research conducted by Harshan et al. (2009), who identified 19 bands ranging from 3 to 250kDa, and Asadpour et al. (2007), who reported 25 bands spanning from 14.4 to 80.5kDa. Additionally, the study detected approximately 18 molecular weights of protein bands ranging from 12 to 127kDa in buffalo seminal plasma, which is in line with the findings of this investigation.

Dixit et al. (2016) reported that seminal plasma from various buffalo breeds contained 24 bands, ranging from 6 to 200kDa. Conversely, Abdulkareem and Musa (2021) reported fewer protein bands, with nine bands ranging from 10 to 68kDa in Iraqi buffalo bulls. Baruti et al. (2018) found seven bands (25, 28, 38, 55, 65, 71, and 161kDa), while Almadaly et al. (2005) identified ten protein bands in the seminal plasma of fertile and sub-fertile Egyptian buffalo bulls. El-Shamaa et al. (2016) revealed that high-fertile bulls predominantly exhibited 65 and 54-59kDa-proteins, comprising 60-70% of the bands, whereas low-fertile bulls showed 58 and 45-49kDa-proteins, constituting 60 and 80% of the bands, respectively.

The correlation between the molecular weight of seminal plasma proteins and fertility was attributed to the coating of these proteins on the external surface of sperm cells post-semen collection. Almadaly et al. (2005) Iskandar et al. (2023) reported that seminal plasma with protein bands ranging from 25 to 104kDa indicated high fertility in bulls. Ivanova et al. (2019) reported that protein bands of 26 and 55kDa were prevalent in the seminal plasma of highly fertile buffalo bulls, whereas those in the range of 15-16kDa were more frequently identified in the seminal plasma of low-fertility buffalo bulls.

Buffalo bulls' seminal plasma contains essential substances that play a significant role in the biological properties of sperm, particularly in relation to their ability for effective cryopreservation. Elnagar et al. (2022) reported that the association between the presence of certain molecular weight proteins in seminal plasma and the fertility of bulls. Similarly, research by Sharma et al. (2014) demonstrated a relationship between the proteins in seminal plasma and the quality of semen in Bhadawari buffalo bulls from India. Seminal proteins present in sperm, such as osteopontin (55kDa), fertility-associated antigens (FAA), proteins that bind to the ovum (26kDa), proteins that bind to heparin (31kDa), and phospholipase A2 (from bovines, 18 and 16kDa), have been correlated with bull fertility (Manjunath and Thérien, 2002).

The presence or absence of specific proteins in seminal plasma can alter sperm functions, influencing semen fertility. Seminal plasma proteins are pivotal in modulating sperm protection (Fu et al. 2019), and this regulatory mechanism may be associated to either high or low fertility in bulls (Höfner et al. 2020; Iskandar et al. 2022). The characterization of seminal plasma proteins holds significant potential for predicting and enhancing fertility.

A specific protein identified in the bovine seminal plasma, referred to as PDC-109, which is made up of two acidic proteins known as BSP-A1 and BSP-A2, has been shown to enhance sperm movement according to Gomes et al. (2020). Furthermore, seminal plasma contains binder sperm proteins (BSPs), which have molecular weights varying between 15 to 30kDa and play a role in the function of sperm. These BSPs include varieties like BSP1, BSP3, and BSP5.

Dystroglycan 1 has been identified in the sperm head of dairy bulls. These dystroglycan proteins become associated with the sperm during the process of ejaculation (Zoca et al. 2023). The role of Dystroglycan 1 in sperm function remains unclear, particularly in the case of alpha-dystroglycan. This ambiguity arises partly because alpha-dystroglycan, being an extracellular/surface protein, is more frequently measured compared to beta-dystroglycan (Ibraghimov-Beskrovnaya et al. 1992).

Seminal plasma proteins, particularly those within the molecular weight range of 50 to 60kDa, are significant influence on catalytic mechanisms, particularly in modulating mitochondrial energy metabolism. Two such proteins, Osteopontin (OPN) and BSP A3, found in bovine seminal plasma, have been shown to be vital in supporting various sperm functionalities, including capacitation, adherence to the oviductal epithelium, and maintaining motility (Kelly et al. 2006; Rego et al. 2014; Preedaa et al. 2020).

Additionally, the 46kDa seminal plasma protein, also known as membrane cofactor protein (MCP), is an important seminal plasma component that is membrane-associated (Simpson and Holmes 1994). This protein is found in the seminal plasma of both fertile and vasectomized male, which suggests that its presence is not solely dependent on the presence of sperm. Further confirmation is needed to ensure the specificity of proteins in fresh semen for use as fertility markers. This could form the basis for selecting excellent Toraya buffalo bulls in Indonesia.

Conclusion

This study found a significant correlation between the seminal plasma protein concentration and sperm acrosome integrity in Toraya buffalo bulls. The identification of 12-19 protein bands that correlate with these factors is a promising finding and can be utilized to determine the reproductive quality and fertility of the bulls. However, to select superior Toraya buffalo bulls with greater accuracy, further analysis using LCMS/MS is required to identify specific proteins as biomarker candidates.

Author's contributions

Conceptualization: Tulus Maulana and Syahruddin Said; Data curation: Tulus Maulana and Hikmayani Iskandar; Investigation; Tulus Maulana, Raden Iis Arifiantini, Jakaria, and Asep Gunawan, Methodology: Tulus Maulana and Syahruddin Said; Resources: Tulus Maulana, Hasbi, and Hikmayani Iskandar; Supervision: Syahruddin Said, Raden Iis Arifiantini, Jakaria, and Asep Gunawan; Writing – original draft: Tulus Maulana; Writing – review & editing: Tulus Maulana, Hasbi, Hikmayani Iskandar, Syahruddin Said, Raden Iis Arifiantini, Jakaria, and Asep Gunawan.

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Conflict of interest

The authors declare no conflict of interest.

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