

Protein expression profile patterns of pesisir bulls' seminal plasma in young and productive age groups through 1D-SDS-PAGE

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Abstract. In this study, we comparatively evaluated sperm quality parameters and semen plasma protein profiles in young Pesisir bulls (< 2 years old) and productive adult bulls (4 years old). This study aimed to identify the protein profiles in semen plasma obtained from young and productive adult bulls (n = 6) using a 1D SDS-PAGE analysis approach. Sperm kinematics were evaluated using Computer-Assisted Sperm Analysis (CASA). The results showed no significant differences in semen quality between age groups, except for the Velocity Average Path (VAP) and total sperm motility, which were higher in adult bulls. Visualization of the protein bands on the gel revealed nine protein bands in semen plasma, classified based on molecular weight ranges: 10, 15–19, 20, 25, 26–37, 38–50, 51–74, 75, and 110 kDa, present in all age groups. In conclusion, protein profiling analysis using 1D SDS-PAGE successfully identified nine protein bands consistently associated with high motility levels in young and productive-aged bulls. These findings significantly expand our understanding of the reproductive aspects of pesisir cattle and provide opportunities for applying the molecular characteristics of semen plasma proteins as fertility markers in pesisir bulls.

1 Introduction

Pesisir cattle is one of Indonesia's native livestock breeds and possesses important genetic value compared to other Indonesian livestock breeds. Pesisir cattle have a high physiological and ecological adaptation to tropical environments and are more resilient than other cattle breeds. One of the distinctive characteristics of Pesisir Cattle is its relatively small body weight and size. Additionally, its ability to withstand limited forage availability makes this breed highly promising for development across Indonesia's pesisir regions. This makes Pesisir Cattle a highly potential candidate for development as a superior breeding stock in

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Sumatra.

To support the potential development of the pesisir cattle population, in-depth research is needed on their reproductive performance, including semen characteristic analysis and semen protein profiling. Proteins in semen plasma play an active role in protecting spermatozoa from damage and maintaining their viability and are correlated with semen characteristics. Proteins in semen plasma constitute the majority of biochemical components of semen fluid and play a specific role in regulating sperm function [1]. Some studies have indicated that seminal plasma proteins are associated with sperm motility and fertility. Bu [2], reported that the CRISP3 protein content in semen plasma positively correlates with pigs' reproductive performance.

Meanwhile, Diansyah [3], identified TEXT101, BSP1, BSP3, SPADH2, and PRSS55 proteins in semen plasma associated with motility. Potential proteins in the semen plasma offer opportunities as biological markers for fertility and their application in reproductive technology. These findings could provide an essential foundation for developing pesisir cattle in West Sumatra.

Molecular approaches based on omics technology, particularly proteomics, offer deep insights into the protein profiles involved in biological reproductive mechanisms, notably semen plasma [4];[5]. Analysis of semen quality and plasma protein profiles using proteomics through 1D SDS-PAGE can provide initial information on protein expression patterns and various physiological processes related to sperm function. The 1D SDS-PAGE method supports precise studies of semen proteins [6].

Scientific information on the genetic and molecular aspects of pesisir cattle is minimal, especially regarding reproductive quality and proteomic profiles, which have not been extensively explored. Studies on biomolecular characteristics, such as semen plasma protein profiles, are crucial to support efforts to improve genetic quality and reproductive efficiency in pesisir cattle.

2 Materials and Methods

2.1 Ethics Committee Clearance

The location of this research was the Center for Superior Livestock Breeding and Forage (BPTUHPT), Padang Mangatas, using six bulls of Pesisir cattle aged <2 and 4 years. All cattle were raised according to a uniform management system using BPTUHPT Padang Mangatas SOPs. Ethical clearance for this study was granted by the Animal Ethics Committee of the Faculty of Veterinary Medicine and Biomedical Sciences, IPB University (no. 221/KEH/SKE/VII/2024).

2.2 Collection and Assessment of Semen

All bulls selected for this study were confirmed to be clinically healthy after a veterinarian's examination. Sperm kinematic analysis was performed using Androscope (CASA Portable; Minitube, Germany). Semen collection was performed by an experienced veterinarian using a specialized electroejaculator for cattle (Minitube, Germany) to ensure safety and effectiveness. Androscope software then processed the recordings to track sperm trajectories and quantitatively calculate various kinematic parameters, such as VCL, VSL, VAP, ALH, and BCF, as well as total motility and progressive sperm rates.

2.3 Separation of sperm and semen plasma in Pesisir bulls

The separation of semen plasma was achieved by subjecting samples to centrifugation at

6500 rpm for 30 minutes, a process repeated a second time. A microscopic evaluation was then performed to ensure the plasma was devoid of sperm. Finally, each sample was aliquoted into a 1 mL microtube for storage at -20 °C until required for analysis.

2.4 Plasma Semen protein isolation in Pesisir bulls

The isolation of proteins from semen plasma was achieved with the PRO-PREP extraction kit (iNtRON Biotechnology, Korea), after which their concentration was measured by a bicinchoninic acid (BCA) assay (Thermo Scientific, USA).

2.5 Protein analysis using the 1D SDS-PAGE method

Proteins were characterized according to their molecular weight (MW) using one-dimensional Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (1D SDS-PAGE). The analysis was conducted on a Mini-PROTEAN® Dodeca™ Cell system (Bio-Rad) with SurePAGE™ precast gels (4-20%, 10 x 8 x 0.46 cm cassette) and a 10–250 kDa all-blue prestained protein standard (Bio-Rad). Electrophoresis was performed using a 4% stacking gel at a voltage of 200 V and a current of 100 mA for 40 min. After Electrophoresis, the gel was analyzed using the Image Lab™ software and documented using an imaging system. The devices used included Gel Doc™ EZ and ChemiDoc™ MP Imagers, which were integrated into the software. In addition, pre-printed gels containing trihalo compounds can quickly detect protein fluorescence without conventional staining processes.

2.6 Statistical Analysis

Sperm kinematic data were analyzed descriptively and are presented as mean ± standard deviation. Paired T-tests were performed using SPSS version 22 to compare the two age groups. Protein identification and naming based on molecular weight were obtained from the relevant references.

3 Results

3.1 Sperm kinematics of Pesisir bulls

A comparison of sperm kinematics between the young and productive age groups of Pesisir bulls revealed no significant differences (Table 1). Total sperm motility in the young and adult age groups ranged from $75.56 \pm 12.88\%$ to $91.53 \pm 4.06\%$. Progressive motility ranged from $63.11 \pm 23.40\%$ to $84.81 \pm 5.82\%$ ($p > 0.05$). The age groups were found to be comparable across all measured variables, with no statistical differences noted in sperm kinematic parameters (VCL, VSL, VAP, DCL, DSL, DAP, ALH, BCF, HAC, LIN, STR) or seminal plasma protein concentration (SPPC). The younger age group was relatively more consistent, averaging 147.78 ± 0.26 . The young age group showed highly variable SPPC values, as indicated by the high standard deviation of 115.64 ± 56.03 [$\mu\text{m}/\text{mL}$]. These results suggest that young and productive ages do not affect sperm kinetics or SPPC. In contrast, average path velocity (VAP), total motility, and progressive motility were significantly higher in adult bulls than their younger counterparts.

Table 1. Kinematics of Pesisir bulls' sperm

Kinematic Sperm	Mean ± SD		P Value
	Younger Bulls	Productive Bulls	
Motility [%]	75.56 ± 12.88	91.53 ± 4.06	0.22
Progressive M	63.11 ± 23.40	84.81 ± 5.82	0.32
VCL [µm/s]	188.33 ± 57.44	179.81 ± 50.97	0.89
VSL [µm/s]	76.31 ± 14.79	80.33 ± 38.44	0.85
VAP [µm/s]	89.31 ± 18.18	92.96 ± 37.80	0.89
DCL [µm]	56.71 ± 12.97	55.03 ± 10.63	0.89
DSL [µm]	23.46 ± 2.42	24.36 ± 11.93	0.89
DAP [µm]	27.32 ± 3.00	28.56 ± 10.90	0.85
ALH [µm]	3.95 ± 1.26	3.57 ± 0.87	0.78
BCF [Hz]	14.13 ± 1.73	16.81 ± 3.64	0.19
HAC [rad]	0.51 ± 0.15	0.43 ± 0.16	0.69
LIN [VSL/VCL]	0.39 ± 0.04	0.44 ± 0.10	0.39
STR [VSL/VAP]	0.77 ± 0.04	0.81 ± 0.08	0.26
SPPC [µm/mL]	147.78 ± 0.26	115.64 ± 56.03	0.42

Note: (VCL): Velocity curve linear, (VSL): Velocity straight line, (VAP): Velocity average path, (DCL): Distance curve linear, (DSL): Distance straight line, (DAP): Distance average path, (ALH): Amplitude of lateral head, (BCF): Beat cross-frequency, (HAC): Horizontal Amplitude of Head, (LIN): Linearity, (STR): Straight, (SPPC): Seminal Plasma Protein Concentration; differences between groups were considered statistically not significant at $P > 0.05$.

3.2 Molecular weight of pesisir bulls' seminal plasma protein

The results showed that the molecular weight distribution of proteins in the plasma semen of Pesisir bulls ranged from 10 to 112 kDa and was expressed in each age group. The protein electrophoresis profile of plasma from pesisir bulls was visualized, with samples P3, P4, and P5 representing the young age group and P10, P11, and P12 representing the productive age group (Figure 1).

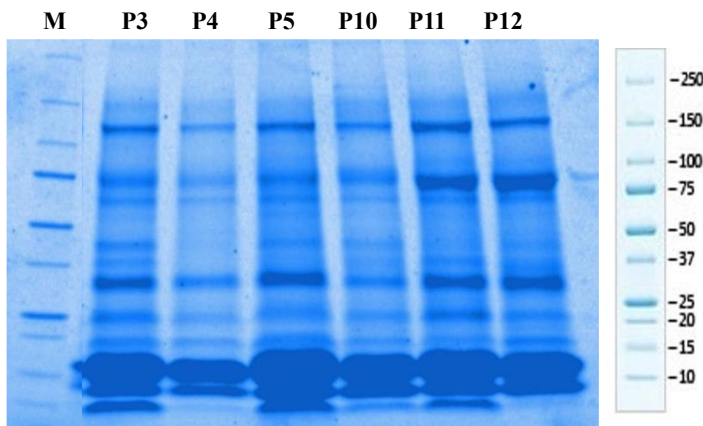


Fig. 1. Seminal plasma protein profiles in Pesisir bulls. M: Marker; Bull numbers indicate protein bands from the young age group (P3, P4, and P5) and the productive age group (P10, P11, and P12).

Analysis via 1D SDS-PAGE identified a series of protein bands with molecular weights ranging from 10 to 112 kDa (specifically at 10–14, 16, 20, 25, 26–37, 38–50, 51–74, 75, and 112 kDa), all of which were present in both the young and productive Pesisir cattle (Table 2). 1D SDS-PAGE electrophoresis profile analysis identified nine candidate proteins expressed in 100% of the pesisir bulls, both from the young and productive age groups.

The observed protein bands were found to correspond to several proteins, including Spermadhesin Z13 (SPADH2, 10–14 kDa), A-Kinase Anchor Protein 3 (AKAP3, 16 kDa), Seminal Plasma Protein BSP-30 kDa (BSP5, 20 kDa), Metalloproteinase Inhibitor 2 (TIMP2, 25 kDa), serine protease 55 (PRSS55, 26–37 kDa), clusterin (38–50 kDa), Isyana1 (51–74 kDa), albumin (75 kDa), and Centrosomal Protein (112 kDa).

Table 2. Protein profiles of pesisir bulls' seminal plasma (analyzed by 1D SDS-Page)

MW (kDa)	Candidate Protein	Bull Number						References
		P3	P4	P5	P10	P11	P12	
112	Centrosomal Protein	+	+	+	+	+	+	[7]
75	Albumin	+	+	+	+	+	+	[10]
51-74	Isyana1	+	+	+	+	+	+	[8]
38-50	Clusterin	+	+	+	+	+	+	[8]
26-37	Serine protease 55 (PRSS55)	+	+	+	+	+	+	[3]
25	Metalloproteinase Inhibitor 2 (TIMP2)	+	+	+	+	+	+	[8]
20	Seminal Plasma Protein BSP-30 kDa (BSP5)	+	+	+	+	+	+	[1]
16	A-Kinase Anchor Protein 3 (AKAP3)	+	+	+	+	+	+	[9]
10-14	Spermadhesin Z13 (SPADH2)	+	+	+	+	+	+	[3]

Note: Bull numbers indicate protein bands from the young age group (P3, P4, and P5) and the productive age group (P10, P11, and P12); MW: Molecular weight; +: protein expressed; -: protein non-expressed.

4 Discussion

The inherent variability in the sperm movement characteristics of Pesisir bulls did not translate into significant differences between the age groups studied. Kinematic parameters that are indicative of a sperm's fertilization capability typically include the velocity curve linear (VCL), velocity straight line (VSL), and velocity average path (VAP). Among these, VAP is most closely associated with fertility [10]. The average values recorded were $155.37 \pm 67.19 \mu\text{m/s}$ for VCL, $61.86 \pm 32.84 \mu\text{m/s}$ for VSL, and $73.13 \pm 35.77 \mu\text{m/s}$ for VAP. To successfully penetrate the ovum, the sperm must achieve $\text{VCL} > 70 \text{ m/s}$, $\text{VSL} > 45 \text{ m/s}$, and $\text{VAP} > 45 \text{ m/s}$ [11].

Kinematic values of Pesisir bull sperm, referring to Inanc *et al.* (2018), on average, can fertilize the egg. However, if evaluated per male, the male P6 VSL and VAP values were below these criteria. Sperm hyperactivity can be identified by measuring the amplitude of the lateral head (ALH). ALH values $> 7 \mu\text{m/s}$ are considered an indication of hyperactivation [11]. The ALH values in this study were $3.95 \pm 1.26 \mu\text{m/s}$ for the younger age group and $3.57 \pm 0.87 \mu\text{m/s}$ for the productive age group. These results indicate that spermatozoa are normal and do not exhibit hyperactivity.

The young and productive age groups of male Pesisir cattle did not differ significantly in their sperm kinematic parameters. This study categorized males as productive males,

including young individuals in the transition phase to productivity. However, parameters such as motility speed, progressive motility, and total motility tended to be higher in productive adult bulls than in the younger age group. Westfalewicz, reported that, within the age range of 2–4 years, there were no significant differences in sperm motility. However, sperm from adult bulls have a higher level of maturity, which is positively correlated with an increased fertilization capacity.

The SPPC values were statistically comparable between the young and old age groups. High SPPC concentrations in semen plasma were associated with high sperm motility rates in both age groups, ranging from 75.56% to 91.53%. Meanwhile, Mappanganro [5] reported that the plasma protein concentration in Bali cattle semen reached 171.95 µg/mL with a motility rate of 77.14%. According to Iskandar [6], the expression levels of proteins in semen plasma can be positively or negatively correlated with fertility and sperm quality.

In this study, one-dimensional SDS-PAGE analysis of proteins in pesisir cattle semen plasma successfully identified various types of proteins with varying sperm kinematic characteristics. Proteins with a molecular weight range of 10–14 kDa were identified, with a 13.4 kDa protein band categorized as Spermadhesin Z13 (SPADH2). This protein is associated with an average path velocity (VAP) of 60.41–69.20 µm/s [3], lower than the VAP values found in this study. Ramirez-Lopez [12], identified that the spermadhesin-1 protein, which is highly abundant in bull semen plasma, can be used as a reproductive parameter in livestock selection. Additionally, the spermadhesin-1 protein has been identified as an essential protein in semen plasma that plays a role in sperm coagulation, motility, and survival.

Based on the results, proteins with molecular weights (MW) of 10–14, 16, 20, 25, 26–37, 38–50, 51–74, 75, and 112 kDa were found in all age groups, young, and productive. Both groups showed high protein concentration and motility and were categorized as groups with good sperm quality. The protein band expression pattern differed from the low-quality sperm group and could be used for male selection. Kaya [13], found that sperm protein differences between high and low-fertility bulls can be biomarkers to assess semen quality and predict fertility. Sperm Binding Protein (BSP5) is the protein identified at a molecular weight of 20 kDa [3]. Proteins with a molecular weight of 16 kDa are categorized as AKAP3 protein [9]. Both proteins, which are explicitly found in the sperm tail, are involved in regulating sperm motility.

Furthermore, Diansyah [3], reported through LC-MS/MS analysis that Seminal Plasma Protein A3 (BSP3), with a molecular weight of approximately 16.1 kDa, was successfully characterized, and this protein plays an essential role in supporting sperm motility. In the present study, approximately nine protein bands were successfully characterized in the semen plasma of Pesisir cattle, with molecular weights spanning from 10 to 112 kDa. This represents a more complex protein profile than that previously reported by Baharun [14], who identified only three leading bands (15, 30, and 65 kDa) in Bali cattle. However, the number of identified protein bands was lower than in the present study. These findings represent the first report documenting the plasma protein band profile of Pesisir cattle semen (Figure 1).

Furthermore, scanning results using Image Lab™ software revealed the presence of a 22 kDa protein band, identified as TIMP2 protein [9]. A protein band at 75 kDa was also detected and identified as albumin [6]. This particular protein is known to be the most abundant in the semen plasma of Bali cattle. Furthermore, the presence of specific proteins in semen plasma, such as Isyana1, Clusterin, and CRISP3, has been associated with regulating reproductive performance through immunomodulatory mechanisms [2]. The 112 kDa protein band identified in this study belongs to the centrosomal protein family [7], which is correlated with sperm motility. The CEP128 protein significantly influences sperm flagella structure, plays a role in spermatogenesis in the testes, and functions in the male reproductive

system [15]. Furthermore, Zhang [15] added that genetic variants of CEP128 indicate the involvement of this protein in regulating gene expression and the phosphorylation of signaling pathways that play a crucial role in spermatogenesis. Further verification is needed to confirm the specificity of the protein in fresh semen as a fertility indicator, which could serve as a basis for selecting superior pesisir cattle in Indonesia.

5 Conclusion

Protein profile analysis using 1D SDS-PAGE successfully identified nine protein bands consistently associated with high motility levels in young and productive bulls. These findings deepen our understanding of the reproductive aspects of pesisir bulls and provide opportunities for the molecular characterization of semen plasma proteins as markers of bull fertility.

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