International Journal of Agriculture and Biosciences 2026 xx(x): xx-xx.
https://doi.org/10.47278/journal.ijab/2026.054

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

Individual Sperm Protein Profile and Its Correlation with Kinematics Motility in Pesisir Bulls

Pajri Anwar [1,4], Cece Sumantri [2], Iis Arifiantini [3] and Asep Gunawan [2*]

[1]Graduate School of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor, Indonesia
[2]Department of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor, Indonesia
[3]Division of Veterinary Reproduction and Obstetrics, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia
[4]Department of Animal Science, Faculty of Agriculture, Islamic University of Kuantan Singing, Teluk Kuantan Riau, Indonesia
*Corresponding author: pajri20pajri@apps.ipb.ac.id; agunawan@apps.ipb.ac.id

ABSTRACT

The success of Pesisir cattle breeding programs depends on sperm quality and the presence of functional proteins associated with motility and fertility, which may serve as reproductive biomarkers for sperm quality. This study evaluated individual sperm kinematic parameters, mapped protein profiles, and examined their relationships with sperm motility and molecular weight. Ten Pesisir bulls (aged <2-4 years) were assessed. Sperm motility was analyzed using Computer-Assisted Sperm Analysis (CASA; IVOS-Hamilton) for accurate and objective measurements. Protein molecular weights were determined via one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1D SDS-PAGE) using a PM2700/3-Color Broad Range Protein Marker (5-270 kDa). The results showed inter-individual variation in the kinematic parameters, all within the normal range. A total of 6-14 protein bands were identified in the sperm of Pesisir bulls. Total and progressive motility exhibited strong positive correlations ($r=0.97$; $P<0.05$) with all kinematic parameters, including VCL, VSL, VAP, DCL, DSL, and DAP ($r>0.70$). Protein bands in the 21-25 kDa and 50-75 kDa ranges were significantly correlated with motility parameters and the number of protein bands detected. In conclusion, Pesisir bull sperm exhibited normal motility kinematics, and 6-14 protein bands were identified as potential markers for semen quality assessment.

Keywords: Reproduction, CASA, Protein function, Molecular Weight, Electrophoresis.

Article History
Article # 25-772
Received: 03-Dec-25
Revised: 28-Jan-26
Accepted: 17-Feb-26
Online First: 03-Mar-26

INTRODUCTION

Pesisir cattle are a native Indonesian breed and an important genetic resource that has developed in the coastal regions of West Sumatra. The breed is officially recognized by the government as a genetic resource that contributes to the biodiversity of the national livestock population. Pesisir cattle are known for their high disease resistance, strong adaptability to tropical climates, and smaller body size than other local cattle breeds. These traits make them a valuable genetic resource, particularly for addressing environmental stress challenges in tropical regions (Widyas et al., 2022). Pesisir cattle are typically managed under a semi-extensive grazing system, in which animals are allowed to graze freely in the morning and are returned to enclosures in the evening. This system enables the utilization of naturally available forage in the surrounding environment, reflecting the breed's ecological compatibility with local land use and the availability of natural resources. These characteristics have made Pesisir cattle a preferred choice among smallholder farmers in West Sumatra. However, the current management system has not yet been fully effective in promoting an optimal increase in the population of Pesisir cattle (Iskandar & Sartika, 2019). A similar management system has also been applied to several other Indonesian local cattle breeds, including Aceh (Handayani & Safrida, 2023), Kuantan (Yendraliza et al., 2020), Bali (Hidayat et al., 2023), and Sumbawa cattle (Romjali et al., 2024).

Cite this Article as: Anwar P, Sumantri C, Arifiantini I and Gunawan A, 2026. Individual sperm protein profile and its correlation with kinematics motility in pesisir bulls. International Journal of Agriculture and Biosciences xx(x): xx-xx. https://doi.org/10.47278/journal.ijab/2026.054

A Publication of Unique Scientific Publishers

Enhancing the population and genetic quality of Pesisir cattle can be achieved through controlled breeding programs that are supported by appropriate reproductive technologies. Advances in reproductive science have led to the use of Computer-Assisted Sperm Analysis (CASA) integrated with omics technologies, particularly proteomics, as a bull selection approach to identify proteins involved in reproductive mechanisms and functions. CASA technology is used to evaluate sperm motility and kinematic parameters through the automated recording and movement analysis of spermatozoa. CASA software analyzes sperm motion in real time, producing accurate quantitative data covering a range of motility parameters (Van de Hoek et al., 2022; Pichardo-Matamoros et al., 2023). Kinematic parameters, such as curvilinear velocity (VCL), straight-line velocity (VSL), and linearity (LIN), are measured to provide a precise assessment of sperm quality (Hidayatullah et al., 2021). Sperm quality evaluation is a critical step in predicting fertility potential and supporting breeding programs for Pesisr cattle. This method has been applied in various studies to predict the success of reproductive technologies through more efficient sperm selection (Yoshiakwa-Terada et al., 2024).

Sperm quality parameters, particularly motility, are insufficient when used in isolation to accurately predict the male fertility. Therefore, such specific configurations or approaches require further validation to ensure their accuracy and reliability in identifying Pesisir bulls with superior reproductive qualities. Advanced validation of sperm motility kinematic analysis can be performed through protein profiling using the 1D SDS-PAGE method, a technique that separates proteins based on their molecular weight and enables the identification of proteins associated with reproductive function. Electrophoretic analysis of semen has been shown to predict protein functions potentially related to reproductive capability (Azizah et al., 2023; Musa & Abdulkareem, 2023).The integration of this method with motility kinematic analysis can be used to verify the results as part of a comprehensive selection process for superior Pesisir bulls. This combined approach provides a broader perspective on the reproductive biological system and facilitates a deeper understanding of the molecular mechanisms underlying the function of sperm proteins. The identification of key proteins through 1D SDS-PAGE analysis of sperm has the potential to serve as a reference for determining reproductive function parameters (Baharun et al., 2023), including those in seminal plasma (Iskandar et al., 2022a; Maulana et al., 2024). Several previous studies have reported a positive correlation between sperm motility and the presence of functional proteins involved in reproduction (Diansyah et al., 2025); (Mappanganro et al., 2025). The present study aimed to evaluate sperm kinematic parameters for each individual, map individual-specific protein profiles, and establish correlations between sperm motility kinematics and sperm protein molecular weights in Pesisir bulls. This study is expected to provide a scientific foundation for developing more targeted and efficient breeding strategies to enhance the productivity of Pesisir cattle.

MATERIALS & METHODS

Animals and Ethical Clearance

This study was conducted at the Balai Pembibitan Ternak Unggul Hijauan Pakan Ternak (BPTUHPT) Padang Mangatas, a technical implementation unit under theministry of Agriculture of the Republic of Indonesia. Ten Pesisir bulls aged between less than two years and four years, were used as experimental animals, each providing one fresh semen sample. All bulls were maintained under uniform environmental and management conditions, including standardized feeding, housing, hygiene, and regular health monitoring, in accordance with the Standard Operating Procedures (SOP) of the BPTUHPT Padang Mangatas. Prior to sample collection, all animals were declared clinically healthy by a licensed veterinarian following a thorough clinical examination. The study was approved by the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, IPB University, Indonesia, under the ethical clearance certificate number 221/KEH/SKE/VII/2024.

Semen Collection and Evaluation

Ten fresh semen samples were collected from healthy, productive Pesisir bulls using an amini tube electroejaculator between 06:00 and 10:00 WIB, following standard operating procedures and under the supervision of an experienced veterinarian. Macroscopic and microscopic evaluations were performed immediately after collection. Sperm kinematics were analyzed using a computer assisted semen analyzer (CASA; IMV-Hamilton), with diluted samples placed on pre-warmed (± 37°C) slides and observed under a video-integrated microscope. The CASA system quantified total motility, velocity curve linear (VCL), velocity straight line(VSL), velocity average path (VAP), distance curve linear (DCL), distance straight line (DSL), distance average path (DAP), amplitude of lateral head (ALH), beat cross frequency (BCF), horizontal amplitude of head (HAC), linearity (LIN), and straightness (STR). For protein profiling via one-dimensional sodium dodecyl sulfate–polyacrylamide gel electrophoresis (1D SDS-PAGE), spermatozoa were separated from the seminal plasma, and the total protein concentration was determined prior to electrophoresis.

Sample Processing and Protein Preparation

Semen collected from each pesisir bull was centrifuged at 6,500 rpm for 30min at room temperature to separate the seminal plasma (upper fraction) from the sperm cell pellet (lower fraction). Fractions were carefully separated using a micropipette to avoid cross-contamination, and sperm pellets were transferred into sterile 1mL microtubes with a new pipette for each sample before storage at –20°C. For protein extraction, frozen sperm pellets were thawed at room temperature and processed using PRO-PREP™ solution (iNtRON Biotechnology, Korea) according to the manufacturer's protocol. Each pellet was mixed with 400µL of PRO-PREP solution, incubated for 20min at –20°C, and centrifuged at 13,000rpm for 5min at 4°C. The resulting supernatants

were transferred to sterile tubes, and the total protein concentrations were determined colorimetrically using the bicinchoninic acid (BCA) method with the Pierce™ BCA Protein Assay Kit (Thermo Scientific™, USA). Protein samples were analyzed using one-dimensional sodium dodecyl sulfate–polyacrylamide gel electrophoresis (1D SDS-PAGE). For sample preparation, sperm protein samples were mixed with PBS to obtain a total volume of 30 µL. Then, 15 µL of the mixture was transferred into a 1.5mL microtube, supplemented with 5 µL blue loading dye, homogenized for 5s, and heated at 95°F for 5min. Subsequently, 20 µL of each prepared sample was loaded into the wells of 1D SDS-PAGE gel.

Protein Analysis Using 1D SDS-PAGE

Protein characterization was performed using one-dimensional sodium dodecyl sulfate–polyacrylamide gel electrophoresis (1D SDS-PAGE) based on the molecular weight (MW). Analyses were conducted using SurePAGE™ 4–20% gradient gels (M00656; GenScript) and a broad multi-color pre-stained protein standard (M00624; GenScript). Electrophoresis was performed using a 4% stacking gel at 200V and 100 mA for 40min. Following electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250 (Bio-Rad, USA) for 3 h, with the staining process repeated twice to enhance band visualization. After electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250 (Bio-Rad, USA) for 3h, and the staining procedure was repeated thrice to enhance protein band visualization. The resulting protein bands were compared with the ExcelBand™ 3-Color Broad Range Protein Marker PM2700 (SMOBIO® Technology, Inc., Taiwan), covering a molecular weight range of 5–270 kDa, to determine the molecular weights of the sperm protein bands.

Statistical Analysis

Sperm motility kinematic parameters and protein concentrations in Pesisir bulls were expressed as mean ± SD. The protein bands identified in the spermatozoa of each individual were analyzed descriptively. Correlations among sperm motility kinematics, sperm protein concentration, and protein molecular weight were evaluated using correlation analysis, and the results were visualized as heatmaps. Statistical significance was set at $P<0.05$ or $P<0.01$. All statistical computations were performed using SPSS software version 26 (IBM® Corp., Armonk, NY, USA).

RESULTS & DISCUSSION

Kinematic Motility of Sperm of Pesisir Bulls

Assessment of sperm kinematics is a valuable tool for evaluating semen quality and selecting breeding bulls. The Computer-Assisted Sperm Analysis (CASA) system enables the precise quantification of sperm motility characteristics (Fig. 1), offering greater predictive accuracy for fertility potential than conventional methods. In the present study, Pesisir bulls demonstrated exceptional motility, with values reaching 95.66% (Table 1), a profile consistent with previous reports for other indigenous breeds, such as Bali bulls (Diansyah et al., 2025; Sarsaifi et al., 2015), Brahman bulls (Rego et al., 2015) and Kangayam bulls (Elamurugan et al., 2023). Despite these similarities, substantial variation in fertility among bulls persists, underscoring the limitations of relying solely on the concentration and motility metrics. Protein profiling has emerged as a critical complementary approach that enables more accurate diagnosis of subfertility or infertility (Susandani et al., 2021). In this context, 1D SDS-PAGE is an effective method for identifying candidate sperm proteins with potential

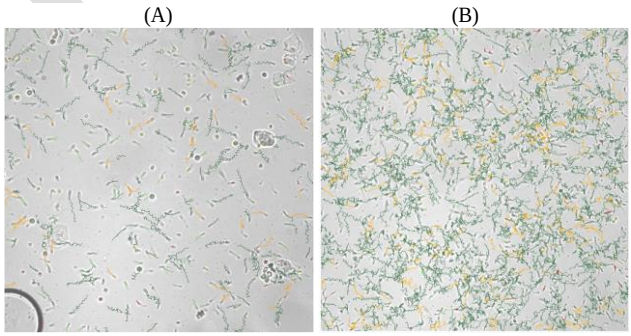

Fig. 1: Sperm motility in pesisir bulls was evaluated using a Computer Assisted Sperm Analysis (CASA) system. A: Fresh semen age 2 year (P8); B. Fresh semen age 4 year (P12). Motility colour symbol: Fast motility, Slow motility, Circle motility Local motility, mmotile.

Table 1: Individual profile of kinematic parameters of sperm motility in Pesisir bulls

Kinematics Motility	Age (year) and code bulls										
	1.33	1.67	1.75	1.83	2.00	2.00	2.75	3.75	3.75	4.00	Mean% ± SD
	P5	P4	P3	P7	P1	P8	P2	P11	P12	P10	
Total motility [%]	61.49	78.43	86.76	87.78	5.30	91.62	82.31	87.55	95.66	91.37	76.83 ± 26.88
Progressive motility [%]	36.86	70.69	81.78	80.90	2.33	75.97	73.58	78.80	91.20	83.44	67.56 ± 27.15
VCL [µm/s]	122.96	211.29	230.73	237.93	49.19	132.42	202.45	214.31	203.85	121.26	172.64 ± 62.09
VSL [µm/s]	60.73	90.16	78.05	101.66	4.75	59.09	93.5	120.91	75.60	44.47	72.89 ± 32.80
VAP [µm/s]	68.98	104.00	94.96	117.47	12.69	66.78	106.75	129.07	96.15	53.67	85.05 ± 34.73
DCL [µm]	42.03	61.46	66.63	65.04	20.03	45.59	55.73	64.94	56.34	43.8	52.16 ± 14.54
DSL [µm]	21.00	25.84	23.54	26.37	1.64	20.37	26.25	38.01	19.18	15.9	21.81 ± 9.27
DAP [µm]	23.98	29.79	28.19	31.42	4.92	23.08	30.22	40.56	25.84	19.27	25.73 ± 9.31
ALH [µm]	2.50	4.50	4.84	4.63	1.41	2.63	4.03	3.61	4.42	2.68	3.53 ± 1.15
BCF [Hz]	12.13	15.21	15.05	17.11	3.25	18.00	14.72	20.65	13.40	16.37	14.59 ± 4.65
HAC [rad]	0.35	0.54	0.65	0.57	0.13	0.44	0.48	0.47	0.57	0.26	0.45 ± 0.16
LIN [%]	0.40	0.42	0.35	0.42	0.17	0.44	0.46	0.56	0.37	0.40	0.40 ± 0.10
STR [%]	0.73	0.81	0.77	0.8	0.38	0.82	0.82	0.89	0.73	0.80	0.76 ± 0.14

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=Straightness.

biomarker value. Supporting this, Mappanganro et al. (2025) demonstrated that proteins with specific molecular weights are strongly positively correlated with motility, viability, and acrosome integrity, underscoring the importance of integrating proteomic analysis into semen quality assessment to improve the robustness of fertility prediction in breeding programs.

The kinematic analysis of Pesisir bull spermatozoa in this study revealed considerable variation, likely influenced by individual factors, as previously reported for fresh semen from Pasundan (Baharun et al., 2023) and Madura bulls (Azizah et al., 2023). Kinematic parameters associated with fertilization capacity are commonly evaluated using velocity curve linear (VCL), velocity straight line (VSL), and velocity average path (VAP), with VAP being the most relevant indicator of fertility potential (Nagy et al., 2015). In this study, the mean total motility was 76.83 ± 26.88%, while the mean progressive motility was 67.56 ± 27.15%. The mean values for VCL, VSL, and VAP were 172.64 ± 62.09, 72.89 ± 32.80, and 85.05 ± 34.73µm/s, respectively. According to Inanc et al. (2018), the threshold values required for sperm to penetrate the oocyte are VCL>70 µm/s, VSL>45 µm/s, and VAP>45 µm/s. Based on these thresholds, the average kinematic values observed for Pesisir bull sperm in this study indicated that all samples had the potential to fertilize oocytes. The amplitude of lateral head displacement (ALH) serves as an indicator of hyperactivation, with values above 7 µm/s considered indicative of this state (Marquez & Suarez, 2007). The ALH value obtained in this study was 3.25 ± 1.26 µm/s, indicating that the sperm were in a normal, non-hyperactivated condition. According to Donnellan et al. (2022), VCL is significantly associated with fertility in bulls, with highly fertile bulls exhibiting markedly higher VCL values than less fertile bulls. These findings underscore the relevance of VCL as a predictive parameter for assessing reproductive success.

Protein Concentration in Sperm of Pesisir Bulls

Based on these findings, the sperm protein concentration profiles of ten Pesisir bulls aged 1.33–4.00 years exhibited considerable variation. The detected protein concentrations ranged from 25.81 to 174.78µg/mL, with a mean value of 98.74µg/mL and a standard deviation of ± 60.20µg/mL (Table 2).

Table 2: Profile of sperm protein concentration in pesisir bulls

Pesisir Bulls	Age (year)	Sperm Protein Concentration (SP conc) (µg/mL)
P5	1.33	73.58
P4	1.67	25.81
P3	1.75	170.01
P7	1.83	134.06
P1	2.00	148.36
P8	2.00	65.27
P2	2.75	154.74
P11	3.75	108.79
P12	3.75	174.78
P10	4.00	101.31
Mean ± SD	2.48 ± 0.94	98.74 ± 60.20

The analysis revealed marked inter-individual variation in sperm protein concentrations among Pesisir bulls, ranging from 25.81 to 174.78µg/mL. The highest concentration was recorded in bull P12 (3.75 years) at 174.78µg/mL, followed by P3 (1.75 years) at 170.01µg/mL and P2 (2.75 years) at 154.74µg/mL. In contrast, bull P4 (1.67 years) exhibited the lowest concentration at 25.81µg/mL. While some mature and older bulls tended to have higher protein concentrations, several younger bulls also exhibited either high or very low levels, as seen in P3 and P4, which were of comparable age but showed sharply contrasting concentrations. The overall mean sperm protein concentration was 98.74 ± 60.20µg/mL. These findings are consistent with those of Mappanganro et al. (2025), who reported seminal plasma protein concentrations of 171.95 ± 0.72µg/mL in Bali bulls, but lower than those reported by Maulana et al. (2024) for buffalo seminal plasma (181.47 ± 24.43µg/mL). Collectively, the present results indicate that sperm protein concentrations vary substantially among individuals; however, the mean value falls within the normal range. Sperm proteins are critical for motility, capacitation, and fertility, and fluctuations in protein abundance during maturation prime sperm for fertilization (Kiyozumi et al., 2023). Several key proteins, including zincoproteins, play essential roles in the adhesion, motility, and metabolic regulation of spermatozoa (Zigo et al., 2022).

Electrophoretic Profiles of Sperm in Pesisir Bulls

A predictive approach has been developed to estimate bull fertility based on the seminal protein content. Previous studies have reported correlations between specific protein profiles and fertility outcomes (Mappanganro et al., 2025). Bull semen contains a diverse array of proteins that play pivotal roles in reproductive function, particularly in supporting sperm motility. Based on 1D SDS-PAGE analysis, the proteins present in Pesisir bull semen exhibited molecular weights ranging between 10 and 150 kDa (Table 3) and were visualized as distinct bands on the gel (Fig. 2).

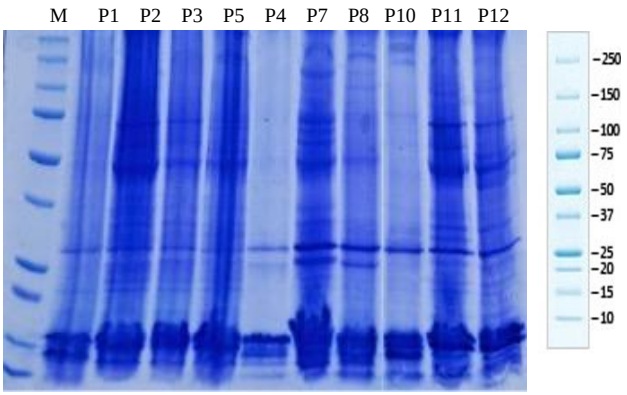

Fig. 2: Electrophoresis Profile Protein Sperm Pesisir bulls, M : Marker, P1-P12 : Sperm Protein Samples from pesisir bulls.

1D SDS-PAGE analysis of Pesisir bull sperm revealed protein bands spanning diverse molecular weights (MW), reflecting interindividual and age-related variations in protein expression. Low-MW bands at 10, 11–14, 15, 16, and 31 kDa were consistently detected across all age groups, indicating conserved structural or physiological roles. Bands at 20, 21–24, and 50 kDa occurred in seven individuals, while the 33 and 75 kDa bands appeared in six.

Table 3: Electrophoretic individual profile of Sperm Protein in Pesisir Bulls

MW (kDa)	Candidate protein	References	Code Pesisir Bulls										Protein Persence (%)
			PS1	PS2	PS3	PS5	PS4	PS7	PS8	PS10	PS11	PS12	
150	IGF-1	DM	-	-	-	-	-	+	+	-	+	+	4/10(40)
100	AKAP 4	RA	-	-	-	-	-	+	+	-	+	+	4/10(40)
51-75	SPAM1	RA	-	+	+	+	-	+	-	-	+	+	6/10(60)
50	TUBB1A	RA	-	+	+	+	-	+	+	-	+	+	7/10(70)
37	ZPBP1	WB	-	-	-	-	-	+	-	-	+	+	3/10(30)
33	PRSS55	DM	-	+	+	+	-	-	-	-	+	+	5/10(50)
31	SPACA1	SF	+	+	+	+	+	+	+	+	+	+	10/10(100)
25	PEBP4	SF	-	+	+	+	-	+	-	+	+	+	8/10(80)
21-24	PEBP1	SF	-	+	+	+	-	+	-	+	+	+	7/10(70)
20	PARK7	WB	+	+	+	+	-	+	-	-	+	+	7/10(70)
17	PRDX5	WB	+	+	+	+	+	+	+	+	+	+	10/10(100)
15	SPADH2	DM, WB	+	+	+	+	+	+	+	+	+	+	10/10(100)
11-14	BSP1	DM, WB	+	+	+	+	+	+	+	+	+	+	10/10(100)
10	SPADH1	WB	+	+	+	+	+	+	+	+	+	+	10/10(100)
ΣBands			6	11	11	11	5	13	8	7	14	14	

Note : PS1-PS12= Protein Sperm 1-12, MW= molecular weight. MR: (Mappanganro et al., 2025); DM : (Diansyah et al., 2025); RA: (Rosyada et al., 2023); SF: (Satrio et al., 2024); WB:(Westfalewicz et al., 2021).

High-MW bands (100–150 kDa) were observed in only four individuals, suggesting more specific or condition-dependent expression, whereas the 37 kDa band had the lowest detection rate (three individuals), indicating a limited or individual-specific role (Table 3). The protein profile demonstrated variability in both the number and MW range of bands, with 6–14 bands per individual (10–150 kDa). These findings are comparable to previous reports of 10–14 bands (15–165 kDa) in Bali cattle semen (Mappanganro et al., 2025) and eight bands in buffalo sperm (Said et al., 2024).

Sperm proteins within the 51–75 kDa range, identified as Sperm Adhesion Molecule 1 (SPAM1), and 25 kDa proteins, including Phosphatidylethanolamine-Binding Protein 4 (PEBP4), were detected in six and eight Pesisir bulls, respectively, indicating broadly consistent expression across the population. PEBP4 is implicated in the regulation of sperm motility via flagellar function control (Satrio et al., 2024), whereas SPAM1 mediates sperm–zona pellucida binding and facilitates membrane fusion (Kasimanickam et al., 2019). During capacitation, the SPAM1 complex specifically relocates to the peri-acrosomal region, which is a key step in sperm functional maturation Gomez-Torres et al. (2021). These proteins represent potential molecular targets for enhancing fertility and refining reproductive protocols in cattle.

In addition, the tubulin alpha chain (TUBB1A) was detected in 50 kDa bands and identified in seven individuals. This protein is essential for sperm cytoskeleton formation, particularly in supporting the microtubule structures that compose cilia and flagella, thereby contributing to sperm motility and its structural integrity (Rosyada et al., 2023). Microtubule- and tubulin-associated proteins are also critical for maintaining flagellar stability and regulating sperm motility (Chawan et al., 2020). Structural protein imbalances or dysfunctions, such as those in leucine-rich repeat-containing protein 23 (LRRC23), which is involved in the assembly of radial spoke 3 in the flagellum, have been associated with impaired sperm motility and potential infertility (Hwang et al., 2023).

Within the 20–24 kDa molecular weight range, two major proteins were identified: protein/nucleic acid deglycase DJ-1 (PARK7) and phosphatidylethanolamine-binding protein 1 (PEBP1), each detected in seven individuals. PARK7 functions as a key antioxidant, protecting sperm cells from oxidative stress, which directly affects sperm quality and viability (Westfalewicz et al., 2021). PEBP1, in contrast, contributes to sperm maturation and plays a role during capacitation, a critical stage preceding fertilization (Satrio et al., 2024). In addition, serine protease 55 (PRSS55), with an approximate molecular weight of 33 kDa, was identified in five individuals. PRSS55 is essential for the formation and maintenance of cellular structures, particularly acrosomal vesicles and the sperm plasma membrane, both of which are crucial for fertilization because of their roles in the acrosome reaction and zona pellucida penetration (Diansyah et al., 2025). These findings underscore the importance of structural and functional proteins, including TUBB1A, PARK7, PEBP1, and PRSS55, in supporting the quality and reproductive function of pesisir bull sperm.

Specifically, a 37 kDa protein identified as Zona Pellucida-Binding Protein 1 (ZPBP1) was detected in only three older individuals, indicating inter-individual variation in expression that may be associated with sperm maturation or fertility (Westfalewicz et al., 2021). Additionally, SPACA1, a 31 kDa protein involved in acrosome formation and supporting the capacitation process, was identified (Satrio et al., 2024). The analysis of SPACA1 and ZPBP1 provides a more comprehensive understanding of sperm biological function and fertility potential (Maulana et al., 2025). Notably, the essential role of SPACA1 in acrosome formation and capacitation underscores its importance in facilitating sperm penetration of the zona pellucida during fertilization (Chen et al., 2021).

High-molecular-weight protein bands (100–150 kDa) were identified in four Pesisir bulls, including A-kinase Anchoring Protein 4 (AKAP4) and Insulin-like Growth Factor-1 (IGF-1), each detected in all four individuals. The presence of these proteins reflects their functional roles in supporting fertility-related physiological processes. IGF-1, detected at 150 kDa, stimulates spermatogenesis (Diansyah et al., 2022). AKAP4 protein, previously identified a 99.6 kDa in Madura bull sperm, binds ATP, which is essential for maintaining optimal sperm motility (Rosyada et al., 2023). This function is further supported by Blommaert et al. (2019), who reported that both mature AKAP4 and its precursor form (proAKAP4) are actively

expressed during spermatogenesis and play a critical role in flagellar structure formation.

Low-molecular-weight proteins in the 10–17 and 31 kDa ranges were detected in all Pesisir bulls. These include spermadhesin 1 (SPADH1; 10 kDa), Peroxiredoxin-5 (PRDX5; 17 kDa), Binder of Sperm Protein 1 (BSP1; 11–14 kDa), and SPACA1 (31 kDa). These proteins are associated with key functions in sperm transport and fertilization processes. BSP1 plays a critical role in sperm-oviduct interactions, influencing sperm binding capacity and modulating chemotaxis, which are essential for successful fertilization (Pardede et al., 2021). SPADH1 significantly modulates sperm binding to the zona pellucida, which is crucial for sperm penetration and fertilization (Westfalewicz et al., 2021). In addition to sperm cells, SPADH1 is also present in seminal plasma, supporting reproductive success by enhancing motility and stimulating the acrosome reaction (Iskandar et al., 2022b). Moreover, studies in young Nellore bulls have indicated that SPADH1 abundance correlates with reproductive parameters, reinforcing its potential as a biomarker for assessing male fertility (Ramirez-Lopez et al., 2023). Consistently, Kasimanickam et al. (2019) reported higher SPADH1 expression in the sperm and seminal plasma of bulls with superior fertility. Overall, these findings highlight the crucial biological roles of these proteins in motility, capacitation, acrosome reaction, fertilization, and protection against oxidative stress. Variation in the number of individuals expressing specific proteins further supports their potential use as molecular markers in sperm quality assessment and the selection of superior Pesisir bulls.

Overall, protein band analysis using a 1D SDS-PAGE approach successfully identified several proteins across specific molecular weight ranges, including SPADH1, PRDX5, BSP1, SPAM1, PEBP4, PARK7, PRSS55, SPACA1, ZPBP1, TUBB1A, AKAP4, and IGF-1, which play important roles in regulating sperm quality, motility, capacitation, fertilization efficiency, and reproductive function in pesisir bulls. Variation in expression among individuals further underscores the potential of these genes as molecular markers for sperm quality assessment and the selection of superior bulls.

Correlation between Sperm Motility Kinematic Parameters, Protein Concentration and Protein Band Profiles in Pesisir Bull Sperm

The analysis results are presented as a heatmap illustrating the degree of association between variables (Fig. 3). The findings revealed that total motility and progressive motility exhibited very strong positive correlations (r=0.97; P<0.05) with nearly all kinematic parameters, including VCL (r=0.77 and 0.71), VSL (r=0.72 and 0.71), VAP (r=0.72 and 0.71), DCL, DSL and DAP (r>0.70). Progressive motility showed the highest correlation with VSL (r=1.00; P<0.05), indicating that increased sperm linear velocity substantially contributes to forward progression, which is a key determinant of fertilization potential. These results are consistent with those of (Gai et al., 2022), who reported that progressive motility is a critical predictor of fertilization success because of its direct association with the sperm's ability to reach and penetrate the oocyte zona pellucida. In sperm motility analysis, High curvilinear velocity (VCL) was also significantly associated with enhanced fertility, corroborating the findings of Donnellan et al., (2022), who reported that fertile bulls exhibit markedly higher VCL than subfertile individuals, underscoring VCL as a valuable reproductive performance indicator.

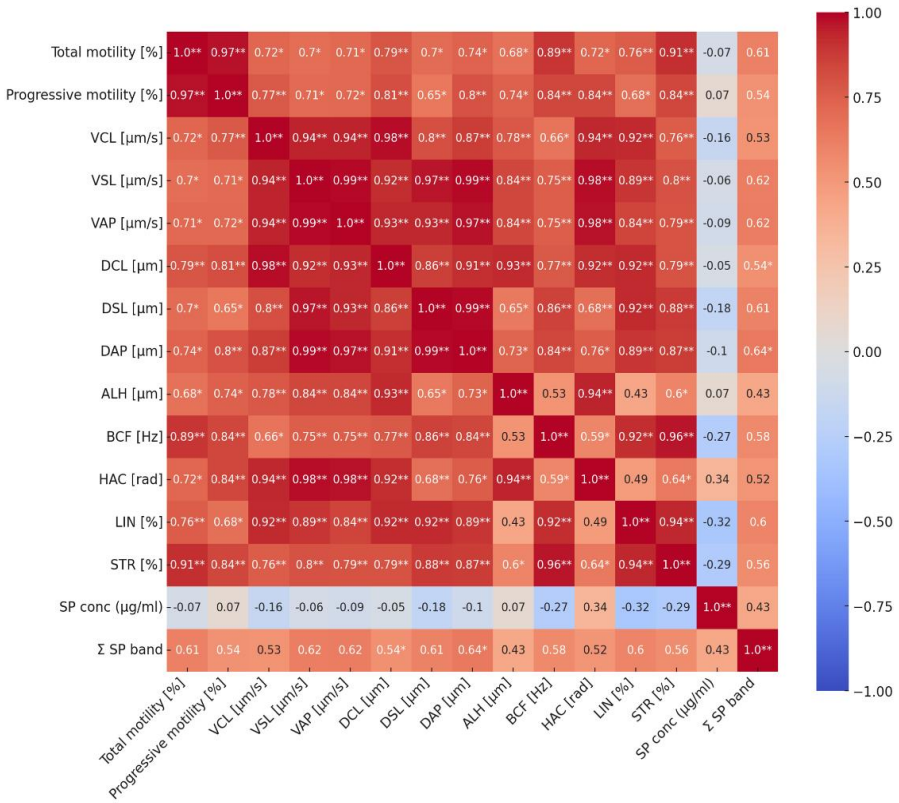

Fig. 3: Visualization of a heatmap of Pearson's correlation coefficients between sperm kinematics, sperm proteins, and total protein bands in Pesisir bulls. The scale is based on colours ranging from red (positive) to blue (negative); *=significant correlation (p<0.05); **=highly significant correlation P<0.01; SP conc=Sperm Protein Concentration; Σ SP bands=Total Sperm Protein Band.

Sperm kinematic parameters, including VCL, VSL, VAP, DCL, DSL and DAP, generally exhibited very strong positive correlations with one another, with r values ranging from 0.86 to 1.00 (P<0.05), indicating a close association between sperm trajectory, velocity, and displacement. In contrast, parameters such as LIN (linearity) and STR (straightness) showed moderate correlations with velocity and trajectory metrics (r=0.43–0.94; P<0.05). Barquero et al. (2021) reported that sperm kinematic parameters, including speed, trajectory, and movement patterns, play critical roles in assessing sperm motility and directly influence reproductive success. Furthermore, parameters such as VCL, VAP, ALH, and BCF have been identified as potential predictors of fertilization success (Donnellan et al., 2022). Consistently, Bucci et al. (2019) emphasized that a comprehensive evaluation of sperm kinematics provides deep insights into sperm biological function and fertilization potential, thereby serving an essential role in diagnosing and managing male infertility.

Meanwhile, sperm protein concentration (SP conc) exhibited negative correlations with most motility and kinematic parameters, including DAP (r=-0.18), ALH (r=-0.07), and most strongly with BCF (r=-0.27). These results suggest that increased concentrations of certain sperm proteins are generally associated with reduced in sperm motility. Excessive protein levels may indicate cellular stress or structural damage, particularly in the mitochondria, which are the primary organelles responsible for energy production. Mitochondrial dysfunction can impair ATP synthesis, thereby reducing the efficiency of sperm motility (Chakraborty & Saha, 2022). In Pesisir bulls, the observed sperm protein concentration averaged 98.74 ± 60.20µg/mL, remaining within the normal physiological range and comparable to values reported for Bali bull and buffalo semen, highlighting that these levels do not necessarily compromise reproductive potential Mappanganro et al. (2025); (Maulana et al., 2024).

The total number of sperm protein bands (Σ SP band) exhibited moderate positive correlations with total motility (r=0.54) and progressive motility (r=0.53), and very strong correlations with sperm velocity parameters such as VCL (r=0.62) and VAP (r=0.62). These findings suggest that the diversity of protein expression in sperm is positively associated with sperm motility, highlighting its potential as a biomarker for semen quality assessment. Consistently, previous studies have reported links between reproductive proteins and sperm motility. Dordas-Perpinyà et al. (2024) demonstrated that proAKAP4 protein levels positively correlated with sperm motility and velocity in donkeys. Similarly, Mappanganro et al. (2025) identified proteins of 50, 46, and 25 kDa that showed strong positive associations with motility, viability, and acrosome integrity in Bali bull sperm, including sorbitol dehydrogenase (SORD) and glutathione peroxidase 6 (GPX6), which are known to play key roles in sperm motility. Collectively, these findings indicate that an integrated evaluation of sperm motility, kinematics, and protein profiling provides a robust framework for predicting semen quality and fertilization success.

Correlation between Sperm Protein Molecular Weight and Sperm Motility Kinematics in Pesisir Bulls

Pearson's correlation analysis between the presence of protein bands across molecular weight (MW) ranges and sperm motility parameters, as well as the total number of protein bands in Pesisir bulls, revealed variable patterns, suggesting potential biological roles for specific proteins in sperm motility (Fig. 4). Among the 14 protein bands identified in the electrophoretic profiles, only a subset exhibited significant correlations with the sperm motility parameters.

Fig. 4: Visualization of a heatmap of Pearson's correlation coefficients between sperm proteins MW (kDa) and kinematics motility in pesisir bulls. The scale is based on colours ranging from red (positive) to blue (negative); *=significant correlation (P<0.05); **=highly significant correlation P<0.01.

Protein bands within the 50–75 kDa molecular weight (MW) range exhibited significant and consistent positive correlations with all sperm motility parameters. The highest correlations were observed for total motility (r=0.69; P<0.05) and progressive motility (r=0.60; P<0.05), as well as sperm velocity parameters, including VCL (r=0.61; P<0.05), VSL (r=0.64; P<0.05), and VAP (r=0.54; P<0.05). Moreover, protein bands in this MW range showed a very strong correlation with the total number of detected protein bands (bands; r=0.91; P<0.05), indicating that the higher abundance or presence of proteins in this range corresponds to increased quality and complexity of the sperm proteomic profile. Consistent with these findings, Mappanganro et al. (2025) reported that protein bands in the 46–50 kDa MW range displayed very strong positive correlations with sperm motility (r=0.96) and viability (r=0.99) in Bali cattle. The involvement of proteins around 50 kDa is further supported by Rosyada et al. (2023), who identified two tubulin isoforms, TUBA1A and TUBB4B, in the sperm of Madura cattle, these proteins are key components of microtubules essential for cilia and flagella formation. Similarly, Maulana et al. (2024) demonstrated that proteins in the 50–60 kDa range in buffalo semen play critical roles in catalytic mechanisms, particularly in the regulation of mitochondrial energy metabolism. Additionally, Said et al. (2024) reported that β-N-acetyl-glucosaminidase (55–50 kDa) is conservatively involved in metabolic and fertilization processes in buffaloes and exhibits a strong negative correlation (r=-0.98) with sperm abnormalities in Toraya buffalo.

A similar pattern was observed for protein bands in the 21–25 kDa range, which exhibited significant correlations with sperm velocity parameters (VCL r=0.61; VSL r=0.64; VAP r=0.54; P<0.05) and a very strong correlation with the total protein band number (r=0.91; P<0.05). Proteins identified in this range include PEBP1 and PEBP4, which are involved in the regulation of sperm motility and contribute to capacitation processes (Satrio et al., 2024). These findings suggest that mid-range proteins play a role in enhancing sperm movement quality, particularly linearity and motility efficiency. Additionally, bands at 33 and 37 kDa demonstrated moderate to strong associations with motility parameters. The 37 kDa band showed significant correlations with VCL and VSL (r=0.61; P<0.05), as well as with VAP (r=0.54) and total band number (r=0.91), indicating its involvement in optimizing sperm trajectory. The 33 kDa band displayed a moderate correlation with total motility (r=0.23) and a strong correlation with band number (r=0.70). These proteins have been reported in Toraja buffalo sperm (Maulana et al., 2025) and Iraqi Buffalo Bulls (Musa & Abdulkareem 2023), highlighting their important roles in sperm motility and contributing to bull fertility.

Protein bands with low molecular weights (≤17 kDa; including 10, 11–14, 15, 17, and 20 kDa) and very high molecular weights (≥100 kDa; including 100–150 and 150 kDa) exhibited weak correlations with both sperm motility parameters and total protein band number. Correlation coefficients close to zero indicated that these protein bands were relatively uniformly expressed across all Pesisir bulls, thereby not reflecting individual-specific variation. This suggests that proteins within these molecular weight ranges cannot yet be used as individual-specific biomarkers; rather, they are generally involved in multiple semen quality parameters. Therefore, protein bands with molecular weights ≤17 kDa may be considered general markers of semen quality based on the sperm protein profiles in Pesisir bulls. These findings are consistent with those reported by Mappanganro et al. (2025), who demonstrated that low-molecular-weight (15 kDa) and high-molecular-weight (130–165 kDa) proteins were consistently expressed across all Bali bulls. Similar expression patterns were also reported by Baharun et al. (2025) in Donggala bulls and Baharun et al. (2021) in Simmental bulls, where proteins within these molecular weight ranges were detected in all examined individuals. Notably, proteins in the 5–30 kDa range belong to the binder of sperm protein (BSP) family, including BSP1, BSP3, and BSP5, which are involved in sperm motility and capacitation processes (Said et al., 2024); and (Westfalewicz et al., 2021). In addition, proteins from the BSP family, including the BSP-30 kDa protein, have been identified in the seminal plasma of high-fertility dairy bulls (Viana et al., 2018), and in the sperm and seminal plasma of high-fertility Holstein bulls (Kasimanickam et al., 2019).

Sperm proteins with medium molecular weights (21-24 kDa and 50-71 kDa) play a key role in supporting sperm motility and movement quality in Pesisir bulls, whereas low and very high molecular weight proteins tend to be non-specific and do not contribute directly to motility. Mapping these proteins provides a potential basis for developing reproductive function biomarkers and proteomics-based bull selection strategies.

Conclusion

Sperm motility kinematics in Pesisir bulls were within the normal range, with 14 proteins identified according to their functions. Protein bands in the 21-75 kDa range, particularly 50-75 kDa, showed strong correlations with sperm motility kinematics. By integrating kinematic data with electrophoresis analysis, this study provides a more informative and predictive framework for bull selection, enabling an evidence-based approach that combines motility parameters with the molecular validation of sperm proteins.

DECLARATIONS

Funding: This research was financially supported by the Ministry of Education, Culture, Research, and Technology through the Beasiswa Pendidikan Indonesia (BPI) scholarship program. The funding was administered by the Center for Higher Education Funding and Assessment (PPAPT), Ministry of Higher Education, Science, and Technology of the Republic of Indonesia, and the Indonesia Endowment Fund for Education (LPDP), Ministry of Finance of the Republic of Indonesia.

Acknowledgement: The authors wish to express their sincere gratitude for the financial support of this study

provided by the Indonesia Endowment Fund for Education (LPDP) through the Indonesia Education Scholarship (BPI) scheme, as well as the Center for Higher Education Funding and Assessment (PPAPT), and also to the Tua Sekato Artificial Insemination Center and BPTU HTP Padang Mangatas, West Sumatra.

Conflict of Interest: The authors declare that no competing interests or relationships exist that might affect the conduct or reporting of this study.

Data Availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement: The study was approved by the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, IPB University, Indonesia, under ethical clearance certificate number 221/KEH/SKE/VII/2024.

Author's Contribution: Conception and design of the study: P.A., A.G., C.S., and I.A. Acquisition of data: P.A., A.G., C.S., and I.A. Analysis and/or interpretation of data: P.A., A.G., I.A. Drafting the manuscript: P.A., A.G., C.S., and I.A. Critical review/revision P.A., A.G., C.S., and I.A. All authors read and approved the final manuscript.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

Publisher's Note: All claims stated in this article are exclusively those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product that may be evaluated/assessed in this article or claimed by its manufacturer is not guaranteed or endorsed by the publisher/editors.

REFERENCES

Azizah, N., Susilowati, S., Utomo, B., Kusumaningrum, D.A., Kostaman, T., Muttaqin, Z., & Arrazy, A.F. (2023). Seminal plasma protein profiles and testosterone levels as biomarker semen quality of candidate Madura bulls. *Journal of Advanced Veterinary and Animal Research*, *10*(3), 429–436. https://doi.org/10.5455/javar.2023.j696

Baharun, A., Arifiantini, R.I., Karja, N.W.K., & Said, S. (2021). Seminal plasma protein profile based on molecular weight and their correlation with semen quality of Simmental bull. *Journal of the Indonesian Tropical Animal Agriculture*, *46*(1), 20–28. https://doi.org/10.14710/JITAA.46.1.20-28

Baharun, A., Iskandar, H., Maulana, T., Rahmi, A., Handarini, R., Pramartaa, I.Q., Pamungkas, F.A., Samsudewa, D., Kaiin, E.M., Agung, P.P., Gunawan, M., Duma, Y., Arifiantini, R.I., & Said, S. (2025). Sperm protein profiles and their correlation with DNA integrity and protamine deficiency in Donggala bulls (Bos indicus): Implications for fertility assessment. *Veterinary World*, *18*(8), 2357–2366. https://doi.org/10.14202/vetworld.2025.2357-2366

Baharun, A., Rahmi, A., Handarini, R., Maulana, T., Said, S., Iskandar, H., Darussalam, I., Nalley, W.M.M., & Arifiantini, R.I. (2023). Semen quality and frozen semen production in Pasundan bulls: A molecular weight perspective on seminal plasma and spermatozoa protein. *Journal of Advanced Veterinary and Animal Research*, *10*(4), 730–737. https://doi.org/10.5455/javar.2023.j728

Barquero, V., Roldan, E.R.S., Soler, C., Vargas-Leitón, B., Sevilla, F., Camacho, M., & Valverde, A. (2021). Relationship between fertility traits and kinematics in clusters of boar ejaculates. *Biology*, *10*(7). https://doi.org/10.3390/biology10070595

Blommaert, D., Sergeant, N., Delehedde, M., Jouy, N., Mitchell, V., Franck, T., Donnay, I., Lejeune, J.P., & Serteyn, D. (2019). Expression, localization, and concentration of A-kinase anchor protein 4 (AKAP4) and its precursor (proAKAP4) in equine semen: Promising marker correlated to the total and progressive motility in thawed spermatozoa. *Theriogenology*, *131*, 52–60. https://doi.org/10.1016/j.theriogenology.2019.03.011

Bucci, D., Spinaci, M., Galeati, G., & Tamanini, C. (2019). Different approaches for assessing sperm function. *Animal Reproduction*, *16*(1), 72–80. https://doi.org/10.21451/1984-3143-AR2018-0122

Chakraborty, S., & Saha, S. (2022). Understanding sperm motility mechanisms and the implication of sperm surface molecules in promoting motility. *Middle East Fertility Society Journal*, *27*(1). https://doi.org/10.1186/s43043-022-00094-7

Chawan, V., Yevate, S., Gajbhiye, R., Kulkarni, V., & Parte, P. (2020). Acetylation/deacetylation and microtubule associated proteins influence flagellar axonemal stability and sperm motility. *Bioscience Reports*, *40*(12), 1–13. https://doi.org/10.1042/BSR20202442

Chen, P., Saiyin, H., Shi, R., Liu, B., Han, X., Gao, Y., Ye, X., Zhang, X., & Sun, Y. (2021). Loss of SPACA1 function causes autosomal recessive globozoospermia by damaging the acrosome-acroplaxome complex. *Human Reproduction*, *36*(9), 2587–2596. https://doi.org/10.1093/humrep/deab144

Diansyah, A.M., Santoso, S., Herdis, H., Yusuf, M., Priyatno, T.P., Maulana, T., Toleng, A.L., Dagong, M.I.A., Said, S., Iskandar, H., Nurlatifah, A., Lestari, P., Affandy, L., & Baharun, A. (2025). Identification of reproductive performance in Bali-polled bulls using computer-assisted semen analysis and plasma seminal proteomics. *Veterinary World*, *18*(1), 102–109. https://doi.org/10.14202/vetworld.2025.102-109

Diansyah, A.M., Yusuf, M., Toleng, A.L., Dagong, M.I.A., & Maulana, T. (2022). The Expression of Plasma Protein in Bali-polled Bulls Using 1D-SDS-PAGE. *World's Veterinary Journal*, *12*(3), 316–322. https://doi.org/10.54203/SCIL.2022.WVJ40

Donnellan, E.M., Lonergan, P., Meade, K.G., & Fair, S. (2022). An ex-vivo assessment of differential sperm transport in the female reproductive tract between high and low fertility bulls. *Theriogenology*, *181*, 42–49. https://doi.org/10.1016/j.theriogenology.2022.01.011

Dordas-Perpinyà, M., Sergeant, N., Yánez-Ortiz, I., Mevel, V., Catalán, J., Bruyas, J.F., Briand-Amirat, L., & Miró, J. (2024). ProAKAP4 as a motility long-lasting marker in Catalan donkey spermatozoa. *Animal Reproduction Science*, *262*(September 2023), 1–11. https://doi.org/10.1016/j.anireprosci.2024.107427

Elamurugan, K., Selvaraju, M., Napolean, R.E., Ruthrakumar, R., Periyannan, M., & Gopikrishnan, D. (2023). CASA Based Assessment of Kangayam bull semen processed with different extenders and Conception rate following AI. *Indian Journal of Animal Reproduction*, 44(2), 75–82. https://doi.org/10.48165/ijar.2023.44.02.14

Gai, J., Dervisevic, E., Devendran, C., Cadarso, V.J., O'Bryan, M.K., Nosrati, R., & Neild, A. (2022). High-Frequency Ultrasound Boosts Bull and Human Sperm Motility. *Advanced Science*, *9*(11), 1–10. https://doi.org/10.1002/advs.202104362

Gomez-Torres, M.J., Huerta-Retamal, N., Robles-Gómez, L., Sáez-Espinosa, P., Aizpurua, J., Avilés, M., & Romero, A. (2021). Arylsulfatase a remodeling during human sperm in vitro capacitation using field emission scanning electron microscopy (Fe-sem). *Cells*, *10*(2), 1–6. https://doi.org/10.3390/cells10020222

Handayani, S., & Safrida, S. (2023). Characteristics of cattle farm breeders in Aceh Province. *IOP Conference Series: Earth and Environmental Science*, *1183*(1), 012037. https://doi.org/10.1088/1755-1315/1183/1/012037

Hidayat, Z. (2023). Nutritional status and smallholder farmer characteristic of Bali cattle-oil palm integration system in the rural dryland area of Bangka Island, Indonesia. *Pakistan Journal of Agricultural Sciences*, *60*(04), 603–613. https://doi.org/10.21162/PAKJAS/23.90

Hidayatullah, P., Mengko, T.L.E.R., Munir, R., & Barlian, A. (2021). Bull Sperm Tracking and Machine Learning-Based Motility Classification. *IEEE Access*, *9*, 61159–61170. https://doi.org/10.1109/ACCESS.2021.3074127

Hwang, J.Y., Chai, P., Nawaz, S., Choi, J., Lopez-Giraldez, F., Hussain, S., Bilguvar, K., Mane, S., Lifton, R.P., Ahmad, W., Zhang, K., & Chung, J.-J. (2023). LRRC23 truncation impairs radial spoke 3 head assembly and sperm motility underlying male infertility. *ELife*, *12*, 1–25. https://doi.org/10.7554/elife.90095.3

Inanc, M.E., Uysal, O., & Ata, Y. (2018). Cryopreservation and evaluation of Akkaraman ram semen with 7-dehydrocholesterol. *Ankara Üniversitesi*

Veteriner Fakültesi Dergisi, *65*(2), 187–192. https://doi.org/10.1501/vetfak_0000002845

Iskandar, H., Sonjaya, H., Arifiantini, R.I., & Hasbi, H. (2022a). Bull Sperm and Seminal Plasma Proteins and Their Relationship With Fertility: a Review. *Online Journal of Animal and Feed Research*, *12*(5), 292–301. https://doi.org/10.51227/ojafr.2022.40

Iskandar, H., Sonjaya, H., Arifiantini, R.I., & Hasbi, H. (2022b). The Quality of Fresh and Frozen Semen and its Correlation with Molecular Weight of Seminal Plasma Protein in Bali Cattle. *Tropical Animal Science Journal*, *45*(4), 405–412. https://doi.org/10.5398/tasj.2022.45.4.405

Iskandar, I., & Sartika, W. (2019). Study of the application of technical aspects of Pesisir cattle in several regions of West Sumatera to maintain the existence of native Indonesian beef cattle. *IOP Conference Series: Earth and Environmental Science*, *287*(1), 012036. https://doi.org/10.1088/1755-1315/287/1/012036

Kasimanickam, R.K., Kasimanickam, V.R., Arangasamy, A., & Kastelic, J.P. (2019). Sperm and seminal plasma proteomics of high- versus low-fertility Holstein bulls. *Theriogenology*, *126*, 41–48. https://doi.org/10.1016/j.theriogenology.2018.11.032

iyozumi, D., Shimada, K., Chalick, M., Emori, C., Kodani, M., Oura, S., Noda, T., Endo, T., Matzuk, M.M., Wreschner, D.H., & Ikawa, M. (2023). A small secreted protein NICOL regulates lumicrine-mediated sperm maturation and male fertility. *Nature Communications*, *14*(1), 1–12. https://doi.org/10.1038/s41467-023-37984-x

Mappanganro, R., Sonjaya, H., Baco, S., Hasbi, H., & Gustina, S. (2025). Seminal plasma protein profiles based on molecular weight as biomarkers of sperm fertility in horned and polled Bali bulls. *Veterinary World*, *18*(1), 122–132. https://doi.org/10.14202/vetworld.2025.122-132

Marquez, B., & Suarez, S.S. (2007). Bovine sperm hyperactivation is promoted by alkaline-stimulated Ca 2+ influx. *Biology of Reproduction*, *76*(4), 660–665. https://doi.org/10.1095/biolreprod.106.055038

Maulana, T., Indonesia, I.A., & Hasbi, H. (2024). Electrophoretic Protein Profiles of Seminal Plasma and their Correlation with Fresh Semen Quality in Indonesia Toraya Buffalo (Bubalus bubalis carabanesis) Bulls. *International Journal of Veterinary Science*, *July*. https://doi.org/10.47278/journal.ijvs/2024.170

Maulana, T., Said, S., Arifiantini, R.I., Jakaria, J., & Gunawan, A. (2025). Proteomic analysis of Toraya buffalo seminal plasma and sperm: uncovering insights to optimize reproductive success. *Frontiers in Veterinary Science*, *12*(April), 1–11. https://doi.org/10.3389/fvets.2025.1492135

Musa, K.S., & Abdulkareem, T.A. (2023). Protein Profiles in Seminal Plasma of Iraqi Buffalo Bulls (Bubalus bubalis) Associated with Fresh and Cryopreserved Semen Quality. *IOP Conference Series: Earth and Environmental Science*, *1262*(7). https://doi.org/10.1088/1755-1315/1262/7/072095

Nagy, A., Polichronopoulos, T., Gáspárdy, A., Solti, L., & Cseh, S. (2015). Correlation between bull fertility and sperm cell velocity parameters generated by computer-assisted semen analysis. *Acta Veterinaria Hungarica*, *63*(3), 370–381. https://doi.org/10.1556/004.2015.035

Pardede, B.P., Maulana, T., Kaiin, E.M., Agil, M., Karja, N.W.K., Sumantri, C., & Supriatna, I. (2021). The potential of sperm bovine protamine as a protein marker of semen production and quality at the National Artificial Insemination Center of Indonesia. *Veterinary World*, *14*(9), 2473–2481. https://doi.org/10.14202/vetworld.2021.2473-2481

Pichardo-Matamoros, D., Sevilla, F., Elizondo-Salazar, J., Jiménez-Sánchez, C., Roldan, E.R.S., Soler, C., Gacem, S., & Valverde, A. (2023). Exploration of semen quality analyzed by casa-mot systems of brahman bulls infected with BLV and BHV-1. *Scientific Reports*, *13*(1), 1–13. https://doi.org/10.1038/s41598-023-45981-9

Ramirez-Lopez, C.J., Barros, E., Vidigal, P.M.P., Silva Okano, D., Duarte Rodrigues, J.N., Lopes Gomes, L., Montes-Vergara, J.C., Petro Hernandez, V.G., Baracat-Pereira, M.C., Guimarães, S.E.F., & Guimarães, J.D. (2023). Relative Abundance of Spermadhesin-1 in the Seminal Plasma of Young Nellore Bulls Is in Agreement with Reproductive Parameters. *Veterinary Sciences*, *10*(10). https://doi.org/10.3390/vetsci10100610

Rego, J.P.A., Moura, A.A., Nouwens, A.S., McGowan, M.R., & Boe-Hansen, G.B. (2015). Seminal plasma protein profiles of ejaculates obtained by internal artificial vagina and electroejaculation in Brahman bulls. *Animal Reproduction Science*, *160*, 126–137. https://doi.org/10.1016/j.anireprosci.2015.07.015

Romjali, E., Prihandini, P.W., Tresia, G.E., & Hasinah, H. (2024). Morphometric characteristics of local cattle in Sumbawa district. *AIP Conference Proceedings*, *2957*(1), 70060. https://doi.org/10.1063/5.0183954

Rosyada, Z.N.A., Pardede, B.P., Kaiin, E.M., Gunawan, M., Maulana, T., Said, S., Tumbelaka, L.I.T.A., Solihin, D.D., Ulum, M.F., & Purwantara, B. (2023). A proteomic approach to identifying spermatozoa proteins in Indonesian native Madura bulls. *Frontiers in Veterinary Science*, *10*(December), 1–10. https://doi.org/10.3389/fvets.2023.1287676

Said, S., Maulana, T., Iskandar, H., Kaiin, E.M., Khaerunnisa, I., Putra, W.P.B., Hasan, F., & Arifiantini, R.I. (2024). Sperm protein profile and their correlation with frozen semen quality of indigenous Indonesian buffalo bulls. *Journal of Advanced Veterinary and Animal Research*, *11*(4), 846–855. https://doi.org/10.5455/javar.2024.k836

Sarsaifi, K., Vejayan, J., Wahid Haron, A., Yusoff, R., Hani, H., Rasoli, M., Ariff Omar, M., & Mazni Othman, A. (2015). Protein profile and functionality of spermatozoa from two semen collection methods in Bali bulls. *Livestock Science*, *172*, 96–105. https://doi.org/10.1016/j.livsci.2014.12.004

Satrio, F.A., Karja, N.W.K., Setiadi, M.A., Kaiin, E.M., Pardede, B.P., & Purwantara, B. (2024). Age-dependent variations in proteomic characteristics of spermatozoa in Simmental bull. *Frontiers in Veterinary Science*, *11*(6). https://doi.org/10.3389/fvets.2024.1393706

Susandani, O., Suprayogi, T.W., Damayanti, R., & Ma'ruf, A. (2021). Factors Affecting Fresh Semen Quality in Pasundan Cattle at UPTD BPPIBTSP Ciamis. *Journal of Applied Veterinary Science And Technology*, *2*(2), 37. https://doi.org/10.20473/javest.v2.i2.2021.37-42

Van de Hoek, M., Rickard, J.P., & de Graaf, S.P. (2022). Motility Assessment of Ram Spermatozoa. *Biology*, *11*(12), 1–26. https://doi.org/10.3390/biology11121715

Viana, A.G.A., Martins, A.M.A., Pontes, A.H., Fontes, W., Castro, M.S., Ricart, C.A.O., Sousa, M.V., Kaya, A., Topper, E., Memili, E., & Moura, A.A. (2018). Proteomic landscape of seminal plasma associated with dairy bull fertility. *Scientific Reports*, *8*(1), 1–13. https://doi.org/10.1038/s41598-018-34152-w

Westfalewicz, B., Słowińska, M., Judycka, S., Ciereszko, A., & Dietrich, M.A. (2021). Comparative proteomic analysis of young and adult bull (Bos taurus) cryopreserved semen. *Animals*, *11*(7). https://doi.org/10.3390/ani11072013

Widyas, N., Widi, T.S.M., Prastowo, S., Sumantri, I., Hayes, B.J., & Burrow, H.M. (2022). Promoting Sustainable Utilization and Genetic Improvement of Indonesian Local Beef Cattle Breeds: A Review. *Agriculture (Switzerland)*, *12*(10). https://doi.org/10.3390/agriculture12101566

Yendraliza, Y., Rodiallah, M., Astuti, T., & Elfawati, E. (2020). Reproduction Status and Population Dynamic of Kuantan Cattle in the Kuantan Singingi Regency. *Jurnal Ilmu Ternak Dan Veteriner*, *25*(4), 162–172. https://doi.org/10.14334/jitv.v25i4.2541

Yoshiakwa-Terada, K., Takeuchi, H., Tachibana, R., Takayama, E., Kondo, E., & Ikeda, T. (2024). Age, sexual abstinence duration, sperm morphology, and motility are predictors of sperm DNA fragmentation. *Reproductive Medicine and Biology*, *23*(1), 1–8. https://doi.org/10.1002/rmb2.12585

Zigo, M., Kerns, K., Sen, S., Essien, C., Oko, R., Xu, D., & Sutovsky, P. (2022). Zinc is a master-regulator of sperm function associated with binding, motility, and metabolic modulation during porcine sperm capacitation. *Communications Biology*, *5*(1), 1–12. https://doi.org/10.1038/s42003-022-03485-8