P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2025.060

Seminal Plasma Protein Profiles Based on Molecular Weight and Their Relationship with Sperm Quality in Kokok Balenggek Roosters

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Article History: 25-091 Received: 05-Apr-25 Revised: 10-Jun-25 Accepted: 12-Jun-25 Online First: 26-Jun-25

ABSTRACT

This study aimed to characterize the seminal plasma protein profiles of Kokok Balenggek roosters and investigate their relationship with semen quality parameters. Semen samples were collected from 15 roosters and analyzed for motility, viability, abnormality, and intact plasma membrane (IPM). Seminal plasma proteins were separated by SDS-PAGE, and molecular weights were estimated. The results showed that the 51kDa protein band exhibited strong positive correlations with sperm motility, viability, and IPM, and a significant negative correlation with sperm abnormality. These findings suggest that the 51kDa protein, possibly clusterin, plays a crucial role in sperm function and may serve as a potential fertility biomarker in indigenous poultry. The study highlights the importance of seminal plasma protein profiling in improving reproductive performance and selection strategies for local poultry breeds. Further studies are recommended to identify the specific proteins involved and their biological functions.

Key words: Kokok Balenggek roosters, SDS-PAGE, Seminal plasma proteins, Sperm quality

INTRODUCTION

Male fertility plays a crucial role in the reproductive efficiency of poultry, especially in breeding systems that rely on artificial insemination (AI). The success of such programs depends largely on the quality of semen, including parameters such as motility, viability and morphological integrity—critical indicators of fertilizing potential (Yahaya et al. 2013; Irfan et al. 2018; Song et al. 2024). These sperm characteristics are essential for maximizing fertilization rates and improving the productivity of poultry breeding programs. In local Indonesian chickens such as the Kokok Balenggek, understanding and improving male reproductive performance are vital for both conservation and genetic development.

Kokok Balenggek roosters (KBR) are an indigenous breed from West Sumatra, Indonesia, known for their multilevel crowing and distinct phenotypic characteristics (Maharani et al. 2021; Dinh 2022). Due to their adaptability and unique genetic traits, KBRs are considered a valuable local germplasm. However, variability in semen quality—including differences in motility, viability, and abnormality rates—has been observed across KBR phenotypes (Ananda et al. 2024). Evaluations of fresh semen samples have shown considerable individual variation, reinforcing the need for identifying superior males to support reproductive performance (Jaswandi et al. 2023; Husmaini et al. 2024).

Although several studies have investigated the reproductive physiology of Kokok Balenggek roosters—including semen quality, fertility, and hatching performance—molecular-level exploration remains limited. Recent investigations have evaluated sperm motility and viability in various storage media, revealing individual variation in sperm longevity under cold conditions (Ananda et al. 2025a). These findings, along with evidence of improved sperm preservation through semen supplementation (Ananda et al. 2023), underscore the need for deeper molecular-level investigations—such

Cite This Article as: Gusdinal H, Jaswandi, Rusfidra, Kaiin EM, Maulana T and Ananda, 2025. Seminal plasma protein profiles based on molecular weight and their relationship with sperm quality in Kokok Balenggek roosters. International Journal of Veterinary Science x(x): xxxx. https://doi.org/10.47278/journal.ijvs/2025.060

as protein profiling—to better understand the mechanisms influencing sperm quality in this indigenous breed.

Seminal plasma, the acellular fluid portion of semen, is a vital component for sperm survival, comprising proteins, enzymes, ions and other molecules secreted by the testis and accessory sex glands (Susilowati et al. 2021; Viana et al. 2022; Iskandar et al. 2023). These proteins perform diverse functions including the protection of sperm from oxidative stress, maintenance of membrane integrity, and facilitation of capacitation (Karunakaran et al. 2019; Mappanganro et al. 2025). Specific protein fractions have been associated with improved sperm quality traits across species, with proteins in the 46–50kDa range linked to higher viability and motility (Karunakaran et al. 2019).

Protein profiling using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has become a reliable technique for characterizing seminal plasma proteins based on their molecular weight. This method has been widely used in livestock—including bulls, goats, and buffaloes—to identify protein bands associated with semen quality (Marco-Jiménez et al. 2008; Peñaranda et al. 2010; Diansyah et al. 2022). Although proteomic studies have emerged in commercial poultry (Li et al. 2020), comprehensive research on protein expression and its association with sperm quality in indigenous chickens such as KBR remains scarce.

This study aimed to characterize the seminal plasma protein profiles of Kokok Balenggek roosters using SDS-PAGE based on molecular weight and to evaluate their relationship with sperm quality parameters including motility, viability, abnormality, and membrane integrity. The findings are expected to contribute to the identification of potential fertility biomarkers and support breeding and conservation strategies for this valuable Indonesian breed.

MATERIALS AND METHODS

Ethical clearance

All procedures involving animals in this study were approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Andalas, Indonesia (Approval No: 473/UN.16.2/KEP-FK/2024). All semen collection and sample handling followed institutional and national ethical standards.

Animals and semen collection

Fifteen Kokok Balenggek roosters (aged 1.5–2 years; average body weight 1.8±0.2kg) were used in this study. The roosters were individually housed and maintained under standardized feeding and environmental conditions at the Faculty of Animal Science, Universitas Andalas. Semen was collected via the dorsal-abdominal massage method as described by Arifiantini (2012). Each individual was sampled three times at 3-day intervals, resulting in a total of 45 ejaculates. Following each collection, semen was evaluated macroscopically (volume, color, pH, and consistency) and microscopically for sperm motility, viability, abnormality, and plasma membrane integrity using standard laboratory procedures.

Seminal plasma collection and pooling

After semen evaluation, each ejaculate was centrifuged at 3,000rpm for 45 minutes at 4°C to separate the seminal plasma from sperm cells. The supernatant (seminal plasma) from each rooster was collected and stored separately at −20°C. This procedure was repeated for all three collection rounds. Once the third collection was completed, the stored seminal plasma samples from all individuals and time points were pooled to obtain a representative sample for protein quantification and electrophoretic analysis.

Protein quantification and SDS-PAGE analysis

Protein concentration of the pooled seminal plasma was determined using the Bradford Protein Assay Kit (E-BC-K168-S, Elabscience®) and absorbance was measured at 595nm using a UV-1800 spectrophotometer (Shimadzu, Japan). Seminal plasma protein profiles were analyzed using SDS-PAGE on 10% polyacrylamide gels (Q-PAGE™ Precast Gels, SMOBIO®). A 20µL aliquot of the protein sample was loaded into each well. Protein bands were visualized using Fast Coomassie Blue Staining Solution (E-IR-R129, Elabscience®), and molecular weights were estimated using the ExcelBand™ 3-color Broad Range Protein Marker (PM2700, SMOBIO®). Densitometric analysis was conducted using ImageJ software. The molecular weights of protein bands were calculated based on the relative migration distance (Rf) using a third-degree polynomial regression equation:

$$\log(MW) = -10.538x^3 + 20.455x^2 - 14.499x + 5.2615$$

with a coefficient of determination (R^2=0.9994), indicating high reliability of the calibration curve (Fig. 1).

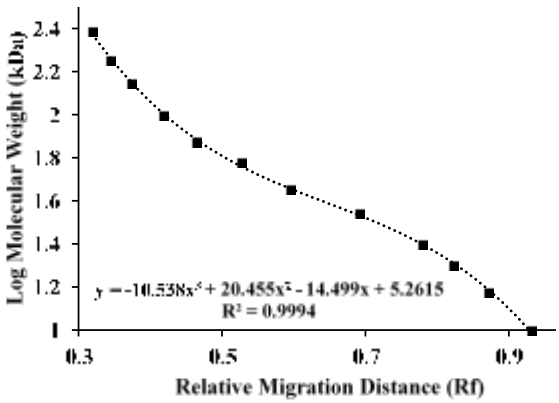

Fig. 1: Regression curve for estimating the molecular weight (MW) of PM2700 protein marker bands.

Data Analysis

Data on fresh semen quality parameters (sperm motility, viability, abnormality and plasma membrane integrity) from individual Kokok Balenggek roosters were analyzed using one-way analysis of variance (ANOVA) to determine whether significant differences existed among individuals. If differences were found ($P<0.05$), Duncan's Multiple Range Test (DMRT) was used for post-hoc comparisons. Correlations between seminal plasma protein concentration and semen quality parameters were assessed using Pearson's correlation coefficient (Table 3). Similarly, the relationships between the number of protein bands (Table 5) and the molecular weights of specific protein bands (Table 6) with sperm quality traits were analyzed

using Pearson's method. Correlation significance was interpreted at two levels: P<0.05 (statistically significant) and P<0.01 (highly significant). All statistical analyses were conducted using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Semen characteristic of Kokok Balenggek Roosters

The macroscopic and microscopic characteristics of fresh semen collected from Kokok Balenggek roosters are summarized in Table 1. The average semen volume was 525.89±320.03µL, with a rooster-specific odor. The semen had an average pH of 6.99±0.30, milky white color, and thick consistency. Microscopic evaluation showed a mass movement score of ++, average motility of 78.56±14.05%, and sperm concentration of 1675±1095.89 million/mL. The average sperm viability was 86.13±6.04%, with an abnormality percentage of 12.22±3.35%. The percentage of sperm intact plasma membrane was 83.57±6.21%.

Table 1: Macroscopic and Microscopic Characteristics of Fresh Semen of Kokok Balenggek Roosters

Semen Evaluation Parameters		Mean±SD
Macroscopic Characteristics	Volume (µL)	525.89±320.03
	Odor	Rooster-spesific
	pH	6.99±0.30
	Color	Milky White
	Consistency	Thick
Microscopic Characteristics	Mass Movement	++
	Motility (%)	78.56±14.05
	Concentration (million/mL)	1675±1095.89
	Viability (%)	86.13±6.04
	Abnormality (%)	12.22±3.35
	Intact Plasma Membrane (%)	83.57±6.21

Table 2 presents the semen quality parameters of Kokok Balenggek roosters, including sperm motility, viability, abnormality, plasma membrane integrity (PMI), and seminal plasma protein concentration (PC). The average sperm motility ranged from 33.30 to 88.30%, while viability varied between 66.30 and 90.30%. Sperm abnormality ranged from 10.00 to 18.00%, and IPM values were observed between 63.30 and 88.70%. The protein concentration in seminal plasma ranged from 0.65 to 2.24mg/mL. Statistical analysis using Duncan's Multiple Range Test (DMRT) indicated significant differences (P<0.05) among individual roosters for motility, viability, and PMI. Superscripts in each column denote statistically significant groupings based on the test results.

Correlation between seminal plasma protein concentration and microcopic quality parameters of Kokok Balenggek Rooster Sperm

Table 3 shows the correlation coefficients between seminal plasma protein concentration and various semen quality parameters in Kokok Balenggek roosters. The correlation between protein concentration and sperm motility was positive but weak (r=0.174). A moderate positive correlation was observed between protein concentration and both sperm viability (r=0.287) and intact plasma membrane (r=0.286). In contrast, a negative correlation was found between protein concentration and sperm abnormality (r=–0.345).

Table 3: Correlation Between Seminal Plasma Protein Concentration and Semen Quality

Parameters	Seminal Plasma Protein Concentration
Sperm Motility	0.174
Sperm Viability	0.287
Sperm Abnormality	-0.345
Sperm Intact Plasma Membrane	0.286

Distribution of seminal plasma protein bands based on molecular weight in Kokok Balenggek Roosters

Fig. 2 presents the protein profiles of seminal plasma from Kokok Balenggek roosters as separated by SDS-PAGE using a 12% polyacrylamide gel. The protein bands were resolved based on molecular weight, ranging from 10 to 245kDa. Each lane represents an individual rooster sample, labeled BR1 to BL2. The figure reveals clear variability in the number and intensity of protein bands among individuals, suggesting differences in the protein composition of seminal plasma across the sampled roosters. This variation could be related to individual reproductive traits or physiological conditions.

Table 2: Semen Quality Parameters of Kokok Balenggek Roosters Evaluated Based on Sperm Motility, Viability, Abnormality, Plasma Membrane Integrity, and Seminal Plasma Protein Concentration

Rooster ID	Sperm Motility (%)	Sperm Viability (%)	Sperm Abnormality (%)	Sperm IPM (%)	Seminal Plasma PC (mg/mL)
BR1	88.30±2.89^{a}	90.30±4.25^{a}	12.70±2.57^{ab}	87.80±3.33^{a}	1.12
BR2	80.00±10.00^{a}	87.30±3.06^{ab}	11.70±4.73^{ab}	85.20±3.82^{a}	1.43
BR4	85.00±0.00^{a}	84.30±1.76^{b}	12.30±4.04^{ab}	81.80±2.47^{a}	0.73
BR5	85.00±5.00^{a}	88.30±0.76^{ab}	11.80±2.57^{ab}	85.30±0.29^{a}	1.04
BR6	68.30±7.64^{b}	87.20±3.06^{ab}	10.30±3.06^{a}	84.50±3.50^{a}	1.12
BR7	85.00±5.00^{a}	89.70±2.25^{ab}	12.50±3.12^{ab}	87.20±2.25^{a}	2.24
BR8	81.70±2.89^{a}	86.80±2.08^{ab}	12.20±3.55^{ab}	84.50±2.65^{a}	1.84
JK1	76.70±5.77^{ab}	86.50±2.29^{ab}	11.70±3.62^{ab}	83.50±1.80^{a}	1.89
KN1	76.70±10.41^{ab}	86.50±3.50^{ab}	11.80±2.84^{ab}	84.20±3.82^{a}	0.90
BL1	85.00±5.00^{a}	88.70±1.53^{ab}	11.20±3.69^{a}	86.50±1.32^{a}	0.97
BR10	80.00±5.00^{a}	86.70±2.52^{ab}	13.70±3.06^{ab}	84.70±2.75^{a}	0.76
JK2	85.00±5.00^{a}	88.30±2.93^{ab}	10.80±2.84^{a}	85.20±2.52^{a}	0.69
JK3	33.30±5.77^{c}	66.30±4.86^{c}	18.00±3.75^{b}	63.30±5.35^{b}	0.69
KN2	85.00±5.00^{a}	86.30±3.75^{ab}	15.00±3.55^{a}	83.50±4.27^{a}	0.71
BL2	83.30±5.77 ^{a}	88.70±1.89^{ab}	15.50±3.69^{a}	86.30±2.25^{a}	0.71
Mean±SD	78.56±14.05	86.13±6.04	12.22±3.35	83.57±6.21	1.12±0.50

IPM (intact plasma membrane); PC (protein concentration); Means in a column with different superscripts differ significantly (P<0.05).

Table 4: Distribution of Seminal Plasma Protein Bands by Molecular Weight in Kokok Balenggek Roosters

MW (kDa)	Rooster ID															Protein Absence*
	BR 1	BR 2	BR 4	BR 5	BR 6	BR 7	BR 8	JK 1	KN 1	BL 1	BR 10	JK 2	JK 3	KN 2	BL 2	
≥ 245	+	+	-	-	-	+	-	+	+	+	+	-	-	+	-	8/15(53.3)
244-180	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	14/15(93.3)
179-140	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	4/15(26.7)
139-100	+	+	-	+	-	-	-	-	-	-	+	-	-	-	+	5/15(33.3)
99-75	-	-	-	-	+	-	-	+	+	-	-	-	+	-	-	4/15(26.7)
74-60	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15/15(100)
59-45	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	14/15(93.3)
44-35	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	1/15(6.7)
34-25	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	2/15(13.3)
24-20	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	3/15(20.0)
19-15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/15(0)
14 10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/15(0)
≤ 9	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-	4/15(26.7)
Σ Bands	6	6	3	4	6	5	4	6	7	6	5	3	5	4	4	74/195(37.9)

*n(%); MW (molecular weight); + (protein expressed); - (protein non-expressed).

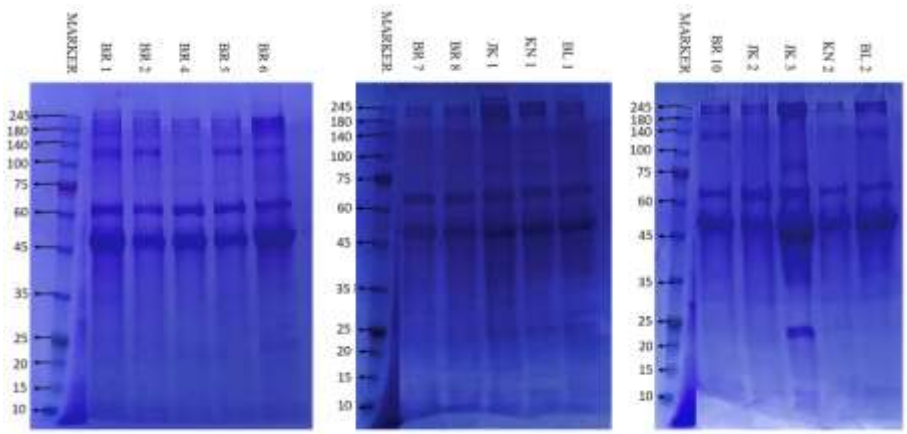

Fig. 2: SDS-PAGE analysis of seminal plasma protein profiles from Kokok Balenggek roosters. Protein bands were separated based on molecular weight using a 10% polyacrylamide gel. Lanes labeled BR1–BL2 represent individual Kokok Balenggek roosters. The molecular weight PM2700 marker (leftmost lane of each gel) ranges from 10 to 245kDa.

Table 4 complements the visual data from the gel electrophoresis by detailing the distribution of protein bands across different molecular weight ranges for each rooster. The most frequently expressed protein bands were observed in the 244–180kDa and 59–45kDa ranges, each present in 93.3% (14/15) of the samples. In contrast, protein bands in the lowest molecular weight ranges (14–10kDa and ≤9kDa) were absent in all samples. The table also shows the total number of bands per individual, ranging from 3 to 7, with a total of 195 protein observations and a 37.9% absence rate.

Table 5 further explores the relationship between the number of protein bands and semen quality parameters. Although weak, a positive correlation was found between the number of protein bands and both sperm viability (r=0.039) and membrane integrity (r=0.059), while negative correlations were observed with sperm motility (r=-0.194) and abnormality (r = -0.194). These findings suggest a possible functional relevance of seminal plasma proteins in influencing sperm quality, though further investigation is needed to confirm their biological significance.

Table 5: Correlation Coefficients Between the Number of Protein Bands and Semen Quality Parameters in Kokok Balenggek Roosters

Parameters	Number of Protein Bands
Sperm Motility	-0.194
Sperm Viability	.039
Sperm Abnormality	-.194
Sperm Intact Plasma Membrane	.059

Correlation between molecular weight of seminal plasma proteins and sperm quality parameters

Table 6 displays the correlation coefficients between the molecular weight (MW) of seminal plasma proteins and various sperm quality parameters in Kokok Balenggek roosters. A significant positive correlation was observed between the 51kDa protein and sperm motility (r=0.866, P<0.01), viability (r=0.918, P<0.01), and intact plasma membrane (IPM) (r=0.922, P<0.01), while its correlation with sperm abnormality was significantly negative (r=–0.768, P<0.01). Conversely, proteins with molecular weights of 42kDa and 24kDa showed strong negative correlations with sperm motility, viability, and IPM, and a

Table 6: Correlation Between Molecular Weight of Seminal Plasma Protein and Sperm Characteristics

Protein MW (kDa)	Sperm Motility	Sperm Viability	Sperm Abnormality	Sperm IPM
252	0.291	0.317	-0.121	0.337
222	-0.152	-0.068	0.113	-0.047
152	0.113	0.240	-0.324	0.233
132	0.259	0.275	0.127	0.289
85	-0.644**	-0.452	0.030	-0.460
66	0.272	0.367	-0.338	0.386
51	0.866**	0.918**	-0.768**	0.922**
42	-0.929**	-0.964**	0.715**	-0.965**
26	0.072	0.107	-0.250	0.127
24	-0.727**	-0.551*	0.145	-0.570*
9	0.099	0.109	-0.257	0.133

MW (molecular weight); IPM (intact plasma membrane); **Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level.

positive correlation with sperm abnormality. Notably, the 42kDa protein exhibited the strongest inverse correlations across all quality parameters, including motility ($r=-0.929$, $P<0.01$), viability ($r=-0.964$, $P<0.01$), and IPM ($r=-0.965$, $P<0.01$), along with a positive correlation with abnormality ($r=0.715$, $P<0.01$). Other proteins such as those at 85kDa and 24kDa also showed significant correlations with specific parameters, while higher molecular weight proteins (e.g., 252, 222 and 152kDa) demonstrated weak or non-significant correlations.

DISCUSSION

This study investigated the seminal plasma protein profiles of Kokok Balenggek roosters and their relationship with semen quality parameters. The macroscopic and microscopic characteristics of fresh semen showed that Kokok Balenggek roosters generally produce semen of acceptable quality, with mean motility (78.56±14.05%), viability (86.13±6.04%), and intact plasma membrane (IPM) integrity (83.57±6.21%) comparable to values reported in other indigenous breeds (Jaswandi et al. 2023; Ananda et al. 2024). However, considerable variation was observed among individuals, particularly in motility and abnormality levels (Table 2), suggesting that genetic and physiological factors may influence male reproductive performance in this local breed.

The average concentration of seminal plasma protein in Kokok Balenggek roosters found in this study (1.12±0.50mg/mL) is relatively lower than the reported range in other chicken breeds, which typically ranges from 3 to 10mg/mL (Li et al. 2020). For instance, BJY roosters have shown protein concentrations around 6mg/mL, highlighting possible breed-specific differences. Although lower in concentration, the proteins in Kokok Balenggek seminal plasma may still perform essential roles in sperm protection and fertility regulation. Proteomic studies in chickens have demonstrated that seminal plasma proteins not only supply nutrients but also regulate immune protection, capacitation, and oxidative defense mechanisms (Santiago-Moreno and Blesbois 2020). Functional annotation using Gene Ontology (GO) analysis has revealed that many of these proteins are involved in proteolysis, ion homeostasis, and immune modulation, underscoring their multifunctional roles in maintaining sperm function.

The concentration of seminal plasma proteins in this study showed weak to moderate correlations with sperm viability and IPM ($r=0.287$ and $r=0.286$, respectively) and a moderate negative correlation with sperm abnormality ($r=-0.345$) (Table 3). Although not statistically significant, these findings suggest that higher protein content in seminal plasma may contribute to better sperm quality, particularly through membrane protection and stabilization. Similar findings were reported in Bali bulls, where seminal plasma proteins were shown to be involved in sperm capacitation, acrosome reaction, and membrane integrity (Iskandar et al. 2023). Likewise, Azizah et al. (2023) reported that protein fractions identified based on molecular weight in Madura bulls could serve as potential biomarkers of semen quality.

The SDS-PAGE analysis revealed that the number of protein bands ranged from 3 to 6 per individual (Table 4, Fig. 2), indicating variation in the seminal plasma protein profile among roosters. However, the number of protein bands was not significantly correlated with any semen quality parameter (Table 5). These results suggest that while the diversity of proteins is present, their functional role may not be directly reflected in total band count alone. This is consistent with previous findings in Toraya buffalo and Pasundan cattle, where only certain molecular weight fractions—but not the total number of bands—were associated with semen quality (Baharun et al. 2023; Maulana et al. 2024).

A more detailed analysis of individual protein bands based on molecular weight showed several significant correlations with semen quality (Table 6). Notably, the protein band at 51kDa exhibited strong positive correlations with sperm motility ($r=0.866$), viability ($r=0.918$) and IPM ($r=0.922$), and a significant negative correlation with sperm abnormality ($r=-0.768$), all at the $P<0.01$ level. These findings suggest that proteins of approximately 51 kDa may play a crucial role in sperm function, possibly related to capacitation or membrane stability. This result aligns with findings in Pasundan bulls, where 35–50kDa proteins were only found in high-quality semen (Baharun et al. 2023) and also corroborates reports in Simental bulls showing that 62–48kDa protein fractions were associated with sperm motility and morphology (Baharun et al. 2021).

The strong correlation between the 51kDa protein band and key semen quality parameters suggests its biological importance in sperm function. Based on previous studies, one plausible identity for this band is clusterin, a highly conserved glycoprotein present in the seminal plasma of mammals, with an apparent molecular weight ranging from 50 to 80kDa depending on glycosylation (Carlsson et al. 2004; Han et al. 2012). Clusterin is known for its multifunctional roles, including regulation of apoptosis, lipid transport, and protection against oxidative stress—functions that are critical for maintaining sperm viability and membrane integrity (Salehi et al. 2013; Hassan et al. 2022). In mammals, clusterin is synthesized in the Sertoli cells and secreted into the seminiferous tubule fluid, where it interacts with maturing spermatozoa and prevents protein aggregation. Although direct molecular studies in birds are limited, the evolutionary conservation of clusterin suggests that its protective functions may also apply to avian species (Janiszewska and Kratz 2020). The high metabolic activity and susceptibility of avian sperm to oxidative stress further support the hypothesis that clusterin plays a cytoprotective role in maintaining sperm function in roosters. Therefore, the presence of a protein band around 51kDa in Kokok Balenggek seminal plasma may reflect clusterin or clusterin-like activity, and its strong association with sperm quality supports its potential as a fertility biomarker in avian reproduction. Supporting this interpretation, Jaswandi et al. (2024) identified several proteins—such as CLU (36–44kDa), PGAM2 (25kDa), and GPI (61–74kDa)—that contributed to motility and viability in cryopreserved spermatozoa of local Indonesian rams. These findings reinforce the potential role of 51kDa and 35kDa protein bands in Kokok Balenggek seminal plasma as functional biomarkers of sperm quality through similar molecular mechanisms.

Moreover, specific proteins such as chicken liver trypsin inhibitor-1 (ClTI-1) have been proposed as potential biomarkers for male fertility in chickens, based on their association with individual fertility outcomes (Thélie et al. 2019). This supports the notion that the 51kDa band identified in this study may similarly serve as a fertility biomarker in Kokok Balenggek roosters. Recent investigations have also identified extracellular vesicles (EVs) in rooster seminal plasma, primarily under 100nm in size, that resemble mammalian exosomes and may participate in sperm maturation and intercellular communication (Cordeiro et al. 2021). Taken together, these findings support the broader concept that avian seminal plasma is a biologically active fluid, integrating proteins and vesicular components to support sperm function, fertilizing ability, and male reproductive success.

In contrast, bands at 42kDa and 24kDa were significantly negatively correlated with motility and viability, and the 42kDa band showed a strong positive association with abnormality and a negative correlation with IPM. This pattern may reflect the presence of stress-related or degradation-associated proteins that accumulate under suboptimal sperm conditions. Similar conclusions were drawn by Li et al. (2020), who profiled the seminal plasma proteome in chickens and found that proteins related to oxidative stress and immune activation negatively affected sperm function. From a biological perspective, the proteins positively correlated with semen quality are likely involved in antioxidant defense, membrane stabilization, or capacitation control (Viana et al. 2022; Iskandar et al. 2023). Conversely, proteins that negatively correlate with sperm traits may reflect oxidative damage or premature capacitation, both of which are detrimental to sperm viability and fertilizing capacity.

This study also carries important practical implications. Identifying specific proteins associated with sperm quality can aid in the selection of superior males for breeding programs, particularly in indigenous breeds like Kokok Balenggek, which are often underutilized despite their adaptive potential. The 51 kDa band, in particular, may serve as a fertility indicator in future AI-based selection strategies. Similar proteomic approaches have been applied to Pesisir cattle, where differentially expressed proteins between high- and low-fertility bulls were identified using LC-MS/MS and bioinformatic analyses, highlighting the potential of seminal plasma proteomics for fertility biomarker discovery in local genetic resources (Ananda et al. 2025b). Nevertheless, this study has some limitations. The use of pooled seminal plasma samples may mask individual variation in protein expression, limiting the ability to attribute protein effects to specific roosters. Additionally, SDS-PAGE provides only an estimation of protein size and abundance; functional validation through proteomic and immunological assays is necessary to confirm protein identity and activity. Future research should focus on proteomic mapping of individual seminal plasma samples and assessing the functional roles of identified proteins in fertilization.

Conclusion

This study demonstrated that specific seminal plasma proteins, particularly a 51kDa band, are strongly associated with sperm motility, viability, membrane integrity, and reduced abnormalities in Kokok Balenggek roosters. These findings suggest the potential of this protein—possibly clusterin—as a fertility biomarker in local poultry. Despite lower protein concentrations compared to other breeds, the seminal plasma of Kokok Balenggek appears biologically active in supporting sperm function. This research highlights the importance of molecular characterization in indigenous breeds and encourages further proteomic studies to enhance reproductive selection and conservation strategies.

DECLARATIONS

Funding: The authors received no specific funding for this research.

Acknowledgement: The authors would like to express their sincere gratitude to the Laboratory of Animal Biotechnology, Faculty of Animal Science, Universitas Andalas, for providing laboratory facilities and technical support during this research. Special thanks are extended to the Research Center for Applied Zoology, National Research and Innovation Agency (BRIN), for assistance in protein analysis. The authors also acknowledge UPT Peternakan, Universitas Andalas, for providing animal housing facilities for Kokok Balenggek roosters. Appreciation is also given to all field and laboratory technicians whose contributions were invaluable.

Conflict of Interest: The authors declare no conflict of interest.

Data Availability: All data generated or analyzed in this study are presented in the manuscript.

Ethics Statement: All procedures involving animals in this study were approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Andalas, Indonesia (Approval No: 473/UN.16.2/KEPFK/ 2024). All procedures were conducted in accordance with institutional and national ethical guidelines.

Author's Contribution: HG conducted the experiment, collected data, performed laboratory analysis, and drafted the manuscript. J supervised the study, contributed to the methodology and statistical analysis, and critically revised the manuscript. R provided input on protein analysis and SDS-PAGE interpretation. EMK and TM supported laboratory procedures and data interpretation related to proteomics. A conceptualized and designed the study, provided resources and facilities, guided data interpretation, and finalized the manuscript. All authors reviewed and approved the final version of the manuscript.

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

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