



Identification of Reproductive Performance in Landrace Boar: The Sperm Quality and Molecular Weight of Protein

WILMIENTHE MARLENE MESANG NALLEY^{1*}, THOMAS MATA HINE¹, ALOYSIUS MARAWALI¹, SOLVI MARIANA MAKANDOLU¹, MARIA MARSEFAR RAJA KOTA¹, ATHHAR MANABI DIANSYAH², ANNISA RAHMI³, ABDULLAH BAHARUN³

¹Faculty of Animal Husbandry, Marine and Fisheries, Nusa Cendana University, Kupang, Indonesia; ²Faculty of Animal Science, Hasanuddin University, Jl. Perintis Kemerdekaan 10 Tamalanrea Makassar, South Sulawesi, Indonesia; ³Department of Animals Science, Faculty of Agriculture, Djuanda University, Jl. Tol Ciawi No.1, Ciawi, Bogor, Indonesia.

Abstract | The study aimed to investigate the role of specific proteins in improving the quality of Landrace boar semen, focusing on how these proteins may influence semen characteristics related to reproductive performance. Using the 1D-SDS-PAGE technique, relevant proteins were separated and identified. Semen from six Landrace boars was evaluated macroscopically (volume, pH) and microscopically (concentration, motility, viability, and abnormality), while protein content was analyzed using 1D-SDS-PAGE. Statistical analysis using ANOVA revealed variations in semen quality across the boars. Notably, semen volume in ID6 was significantly lower ($p < 0.05$) compared to the others, though pH did not significantly differ ($p > 0.05$) across all boars. Similarly, no significant differences ($p > 0.05$) were found in sperm motility, concentration, viability, or abnormality. In frozen semen, ID1 had significantly higher sperm motility and viability ($p < 0.05$) than ID5 and ID6, suggesting differences in preservation potential. Additionally, frozen semen production potential was highest in boars ID1, ID2, and ID6. Proteomic analysis identified three common protein bands in the semen plasma, with molecular weights (MW) of 10-15 kDa, 75-100 kDa, and 250 kDa, and in sperm, with MWs of 10-15 kDa, 50 kDa, and 75 kDa. The Pearson's correlation showed a strong positive correlation ($p < 0.05$) was found between sperm motility and MWSP, and between sperm viability and MWSP, particularly in ID5 and ID6. These findings suggest that the variation in sperm quality across Landrace boars is associated with the presence of critical proteins, which can significantly impact reproductive performance. Proteins such as OPN12, HSP90, and GRP94, identified in the analysis, are known to play key roles in sperm motility, viability, and fertility, offering insights into their influence on sperm quality and infertility in Landrace boars.

Keywords | Landrace boar, Sperm quality, 1D-SDS-PAGE, Protein, Molecular weight, Reproductive performance

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***Correspondence** | Wilmienthe Marlene Mesang Nalley, Faculty of Animal Husbandry, Marine and Fisheries, Nusa Cendana University, Kupang, Indonesia; Email: nalleymw@yahoo.co.id

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The pig population in Indonesia has experienced a drastic decline, which can cause depression in the demand for pork. In 2021, the pig population in Indonesia reached 7,178,088 and decreased by 429,474 (5.98%) in 2022. East Nusa Tenggara (NTT) is Indonesia's number one pig producer, which can contribute as much as 29.30% (2.103.259 heads). One of the leading causes is African Swine Fever (ASF), which has significantly reduced the pig population and caused enormous losses for farmers. The rapid spread of ASF, together with the disease's ability to cause mass mortality, so that superior seeds in Landrace pigs cannot be saved (Shel-Ewert *et al.*, 2023), therefore making the recovery of the pig population in NTT a problematic task.

A further impact of ASF faced by pig farming in NTT is inbreeding depression due to lack of access to superior boar and restrictions in reproductive management have led to high inbreeding among pigs. This inbreeding can result in a decrease in genetic quality, which ultimately affects the reproductive quality and overall performance of the boar. The impact is seen in decreased litter size, increased piglet mortality, and decreased growth ability and resistance to disease (Kostiyunina *et al.*, 2022). Furthermore, in the study of Tsheten *et al.* (2022) it was shown that pigs with inbreeding cases had a significant association of 0.231% in decreased motility.

One technology that is expected to help overcome the problem of pig reproduction, including in the NTT region, is semen cryopreservation. This technology allows long-term storage of boar semen for use in artificial insemination programs. However, the success rate of boar semen cryopreservation is still low, especially in terms of maintaining sperm motility and viability after the freezing process (Ozimic *et al.*, 2023). This problem is a significant obstacle in improving boar genetics through artificial insemination. In general, sperm quality evaluation typically focuses on assessing key physical parameters, such as motility, viability, and morphology, but often remains limited in exploring the molecular factors that influence the regulatory functions within sperm (Baharun *et al.*, 2021). This gap highlights the need for more comprehensive analyses that include proteomic and molecular profiling to better understand the underlying biological mechanisms affecting sperm quality and function. To improve semen quality and the success of cryopreservation, the approach through proteomic analysis is gaining more attention (Iskandar *et al.*, 2023). This approach allows the identification of specific proteins that play a role in the reproductive process, including those related to sperm motility, membrane stability, and resistance to freezing. One method often used to study protein profiles is 1D-SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis), which separates

proteins based on their molecular weight (Diansyah *et al.*, 2022a). This method is particularly suitable due to its ability to profile essential proteins linked to fertility, making it valuable for studying boar semen quality and overcoming infertility challenges.

Thus, this study aimed to investigate the role of specific proteins in improving the quality of Landrace boar semen, focusing on how these proteins may influence semen characteristics related to reproductive performance. It is hoped that this study shows that specific proteins involved in sperm motility, membrane stability, and resistance to freezing play a crucial role in enhancing the quality of Landrace boar semen, particularly in improving the success rate of semen cryopreservation. By identifying and analyzing these proteins through proteomic techniques, it is expected that the preservation of semen quality post-freezing will be significantly improved. This, in turn, will contribute to better reproductive performance and restore the pig population in NTT, improving both its genetic diversity and reproductive health.

MATERIALS AND METHODS

ETHICAL APPROVAL AND EXPERIMENTAL ANIMALS

The study was conducted at Happy Farm Tilong East Nusa Tenggara. This study used 6 Landrace boars kept according to the SOP to maintain boars and supervised by a veterinarian, fulfilling every animal welfare principles. This study used an experimental method. Designed by comparing each boar and 5 replications (semen collection). All procedures were approved by the Animal Ethics Commission at the Faculty of Animal Husbandry, Marine and Fisheries, Nusa Cendana University (075/1.KT/KEPPKP/IV/2024). All boars kept in individual barn and were given feed and vitamins with drinking water *ad libitum*.

SEMEN COLLECTION

Semen collection was done twice a week using an artificial vagina (Diansyah *et al.*, 2022b). The artificial vagina was prepared by filling the space between the casing and inner with approximately 55 °C warm water (Lukusa and Kabuba, 2020). The lubricant (Vaseline) was applied to the inner liner at the open end of the artificial vagina before collection. At the other end of the artificial vagina, a graduated glass tube was inserted for semen collection. Before collection, the prepuce of the boar was wiped clean with distilled water to reduce contamination. The estrous doe was restrained in a collection pen. The semen collector held the artificial vagina alongside the doe flank with the open end facing toward the buck and downwards at an angle of 45 °C. When the boar mounted the doe, the erect penis was diverted into the artificial vagina. After ejaculation, the graduated glass tube was separated from the artificial vagina, and then semen volume was recorded and transferred into Laboratory.

The semen was evaluated macroscopically (volume and pH) and microscopically (concentration, motility, viability, and abnormality). The volume was measured using a graduated cylinder, and the pH was measured using pH indicator paper. The pH of sperm was measured in the range of 6.0 to 8.0. The sperm concentration and motility were measured using an AndroScop® (compact mobile computer-assisted sperm analysis/CASA system based on AndroVision®, Minitube, Germany) (Raafi *et al.*, 2021). The sperm viability and abnormality were determined using eosin-nigrosin staining (Surahman *et al.*, 2021).

DETERMINATION OF SEMEN PROTEIN CONCENTRATION AND 1D-SDS-PAGE ANALYSIS

The seminal plasma and sperm cells were separated immediately after collection by washed three times using phosphate buffered saline (PBS) and centrifugation (1800 rpm for 30 min). Following the manual's instructions, the sperm pellet was extracted using PRO-PREP™ protein extraction solution (iNtRON Biotechnology, Korea). The total soluble protein concentration of the sample was measured before analysis by 1D-SDS-PAGE (Diansyah *et al.*, 2023a). The colorimetric detection and quantification of total protein were conducted using the bicinchoninic acid (BCA) method, employing the Pierce™ BCA Protein Assay Kit (Thermo Scientific™, USA). 1D-SDS-PAGE analysis was performed to determine the protein profile based on MW, which is represented as bands on the gels. Protein separation was carried out using 1D-SDS-PAGE (Sure PAGE™), Bis Tris, 10×8 cm, 12 wells, 4-20% gradient gel (GenScript) (Sure PAGE, Genscript Biotech Corp, Hongkong) with Broad Multi Color Pre-Stained Protein Standard (M00624; GenScript) with a MW range of ~5-270 kDa and Tris-MOPS-SDS Running Buffer (M00138; GenScript) with a voltage of 140 V and current of 75 mA for 55 min. The gel was then stained using Instant Blue® Coomassie Protein Stain (ab119211; abcam). The differential by conducting ratio analysis with the aid of ImageJ software (Baharun *et al.*, 2023).

CRYOPRESERVATION

Semen cryopreservation was carried out following the procedure of Diansyah *et al.* (2023b) and Foeh *et al.* (2017). Filling and sealing were performed and gradual cooling (approximately 1 °C/min) from 15 to 5 °C. Then, pre-freezing is carried out by placing the straw ± 10 cm above the surface of liquid nitrogen for 10 - 15 minutes. The following process is to insert the straw into the goblet and dip it into liquid nitrogen at a temperature of -196°C, which is then stored in a storage container.

THAWING

The straw is taken from the liquid nitrogen container and thawed in a water bath for 30 seconds at 37 °C (Kajabova *et al.*, 2020). The straw was cleaned and cut near the cotton-plugged end (Diansyah *et al.*, 2021). One drop of semen was dripped onto a glass slide, and post-thawing motility, viability, abnormality were assessed under a microscope (Rahmat *et al.*, 2024).

STATISTICAL ANALYSIS

The data on fresh and frozen semen quality were analyzed using a descriptive method, with the results presented as mean ± standard error for all parameters. Pearson's correlation (Sigma Plot software version 14.0) was used to assess the relationships between semen characteristics, seminal plasma, and sperm protein. This test was chosen due to its effectiveness in evaluating the strength and direction of linear relationships between continuous variables. Additionally, a one-way ANOVA (SPSS software version 26, IBM® Corp., Armonk, NY) was applied to compare the means of sperm quality parameters across different groups.

RESULTS AND DISCUSSION

THE SPERM QUALITY OF LANDRACE BOARS

The sperm quality of Landrace boars can be seen in Table 1, 2, and 3. Table 1 shows the fresh semen quality of Landrace boar on different ID numbers. The statistical analysis showed that the volume in ID1 did not differ significantly

Table 1: The fresh semen quality of Landrace Boars on different ID number.

Parameter	ID Boar (Means ± SD)						Means ± SD
	1	2	3	4	5	6	
Volume (mL)	207.50±15.00 ^a	227.50±39.26 ^a	210.00±7.07 ^a	193.76±15.48 ^a	200.00±14.14 ^a	267.50±11.90 ^b	217.71±17.14
pH	6.55±0.17 ^a	6.55±0.17 ^a	6.55±0.17 ^a	6.48±0.15 ^a	6.55±0.17 ^a	6.4±0.01 ^a	6.51±0.17
Sperm motility (%)	81.88±2.39 ^a	80.00±4.08 ^a	79.38±1.25 ^a	80.00±2.04 ^a	78.38±2.36 ^a	80.75±2.99 ^a	80.07±2.52
Sperm concentration (×10 ⁶ /mL ⁻¹)	248.75±14.52 ^a	253.75±20.79 ^a	233.75±12.97 ^a	225.75±16.13 ^a	230.00±25.13 ^a	221.75±10.31 ^a	235.63±16.64
Sperm viability (%)	91.52±1.81 ^a	92.39±4.42 ^a	88.72±2.14 ^a	89.65±2.81 ^a	88.27±1.25 ^a	89.56±2.23 ^a	90.02±2.44
Sperm abnormality (%)	3.43±0.64 ^a	3.85±0.20 ^a	4.11±0.66 ^a	3.91±0.48 ^a	4.32±0.09 ^a	4.08±0.55 ^a	3.95±0.44

Note: Different superscripts in the same row were significantly differences ($p < 0.05$).

($p > 0.05$) from ID2, ID3, ID4, and ID5 (207.50 mL vs. 227.50 mL vs. 210.00 mL vs. 193.76 mL vs. 200.00 mL). However, ID 6 was significantly ($p < 0.05$) lower than other IDs. The pH in the ID1 did not differ significantly ($p > 0.05$) from other ID numbers (6.55 vs. 6.55 vs. 6.55 vs. 6.48 vs. 6.55 vs. 6.4). Likewise, sperm motility (81.88% vs. 80.00% vs. 79.38% vs. 80.00% vs. 78.38% vs. 80.75%), sperm concentration ($248.75 \times 10^6/\text{mL}^{-1}$ vs. $253.75 \times 10^6/\text{mL}^{-1}$ vs. $233.75 \times 10^6/\text{mL}^{-1}$ vs. $225.75 \times 10^6/\text{mL}^{-1}$ vs. $230.00 \times 10^6/\text{mL}^{-1}$ vs. $221.75 \times 10^6/\text{mL}^{-1}$), sperm viability (91.52% vs. 92.39% vs. 88.72% vs. 89.65% vs. 88.27% vs. 89.56%), and sperm abnormality (3.43% vs. 3.85% vs. 4.11% vs. 3.91% vs. 4.32% vs. 4.08%) did not differ significantly ($p > 0.05$).

Table 2: The frozen semen quality of Landrace boars on different ID numbers.

ID Boar	Parameters		
	Sperm motility (%)	Sperm viability (%)	Sperm abnormality(%)
1	39.82±1.91 ^a	62.14±2.95 ^a	5.62±1.15 ^a
2	36.56±1.59 ^a	60.17±1.17 ^a	6.09±1.78 ^a
3	36.31±2.33 ^a	58.84±1.69 ^a	6.15±1.08 ^a
4	38.74±1.81 ^a	61.31±1.99 ^a	6.09±0.64 ^a
5	31.52±1.40 ^b	55.82±1.12 ^b	6.57±0.79 ^a
6	29.11±2.49 ^b	52.32±1.86 ^b	5.66±0.71 ^a
Means±SD	35.34±1.92	58.43±1.80	6.03±1.02

Note: Different superscripts in the same row were significantly differences ($p < 0.05$).

Table 3: Frozen semen production potential of Landrace boars on different ID numbers.

ID Boar	Volume (mL)	Motility of Fresh Semen %	Sperm Concentration ($\times 10^6/\text{mL}^{-1}$)	Total motile spermatozoa/ ejaculate	Total straw/ ejaculate
1	207.50±15.00 ^a	81.88±2.39 ^a	248.75±14.52 ^a	42262.87 ^a	845.26
2	227.50±39.26 ^a	80.00±4.08 ^a	253.75±20.79 ^a	46182.50 ^a	923.65
3	210.00±7.07 ^a	79.38±1.25 ^a	233.75±12.97 ^a	38965.66 ^a	779.31
4	193.76±15.48 ^a	80.00±2.04 ^a	225.75±16.13 ^a	34992.69 ^a	699.85
5	200.00±14.14 ^a	78.38±2.36 ^a	230.00±25.13 ^a	36054.80 ^a	721.10
6	267.50±11.90 ^a	80.75±2.99 ^a	221.75±10.31 ^a	47899.39 ^a	957.99
Means ± SD	217.71±17.14	80.07±2.52	235.63±16.64	41059.65	821.19

Table 2 shows the frozen semen quality of Landrace boar on different ID numbers. The statistical analysis showed that the sperm motility and viability in ID1 did not differ

significantly ($p > 0.05$) from ID2, ID3, and ID4. However, ID1 was significantly ($p < 0.05$) higher than ID5 and ID6 (39.82% and 62.14% vs. 36.56% and 60.17% vs. 36.31% and 58.84% vs. 38.74% and 61.31% vs. 31.52% and 55.82% vs. 29.11% and 52.32%). The sperm abnormality in the ID1 did not differ significantly ($p > 0.05$) from other ID numbers (5.62% vs. 6.09% vs. 6.15% vs. 6.09 vs. 6.57% vs. 5.66%).

Table 3 shows the frozen semen production potential of Landrace boars with different ID numbers. ID1, ID2, and ID6 have the highest potential (845.26 straws, 923.65 straws, and 957.99 straws). However, ID3, ID4, and ID5 have the lowest potential (779.31 straws, 669.85 straws, and 721.10 straws).

THE MOLECULAR WEIGHT OF PROTEINS ON LANDRACE BOAR SEMEN

The profile molecule weight of proteins on Landrace boar plasma seminal and sperm can be seen in Figure 1 and 2. Figures 1 and 2 show that three protein bands are expressed in the semen plasma of all ID boars, namely proteins with molecular weights (MW) ranging from 10-15 kDa, 75-100 kDa, and 250 kDa (Figure 1). The expression of protein bands in sperm is displayed in all ID boars with molecular weights (MW) ranging from 10-15 kDa, 50 kDa, and 75 kDa (Figure 2).

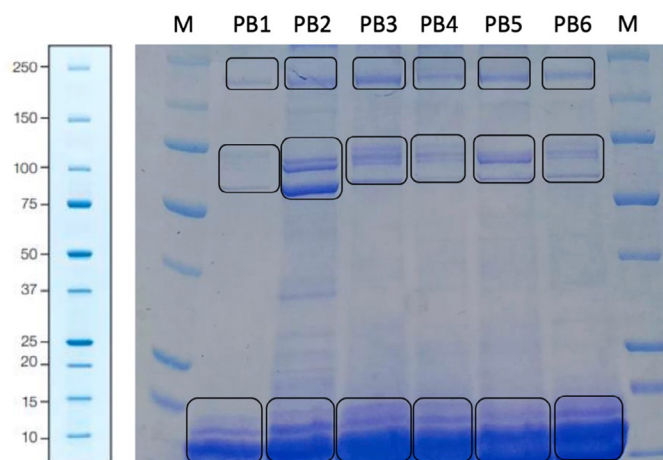


Figure 1: The profile protein of plasma seminal based on molecular weight (MW) using SDS-PAGE.

THE CORRELATION OF SPERM QUALITY WITH MOLECULAR WEIGHT OF PROTEIN ON LANDRACE BOARS

The correlation of sperm quality with molecular weight of protein on Landrace boar can be seen in Table 4. Table 4 shows the correlation of sperms quality molecular weight of protein on Landrace boars (ID5 and ID6). Statistical analysis showed that correlation was significantly ($p < 0.05$) positive and very strong ($r = 0.924$) for sperm viability and motility. Likewise, the correlation of sperm motility with

MWSP and sperm viability with MWSP was significantly ($p < 0.05$) positive and strong ($r = 0.550$ and $r = 0.556$). However, the correlation of sperm abnormality with MWSP did not significantly ($p > 0.05$) positive and fair.

Table 4: The correlation of sperm quality with molecular weight of protein on Landrace boar.

	Sperm Motility	Sperm Viability	Sperm Ab-normality	MWSP
Sperm Motility	1	0.924*	0.044	0.550*
Sperm Viability	0.924*	1	0.19	0.556*
Sperm Abnormality	0.044	0.19	1	0.174
MWSP	0.550*	0.556*	0.174	1

Note: *Significant correlation ($p < 0.05$); very strong (0.76-0.99); strong (0.51-0.75); fair (0.26-0.50); weak (0.00-0.25); **MWSP:** Molecular Weight of Sperm Protein.

The main problem in the pig farming industry, especially in areas such as NTT, is the low reproductive quality due to inbreeding, disease attacks such as ASF, and the inability of semen cryopreservation technology to maintain sperm viability after the freezing process. Various studies have shown that genetic and environmental factors significantly affect semen quality, so a more comprehensive approach is needed to understand the mechanisms underlying the decline in sperm quality, especially in cryopreservation (Diansyah *et al.*, 2021). One approach used is proteomic analysis, which can identify essential proteins that play a role in the reproductive process. In this study, 1D-SDS-PAGE was used to analyze proteins in seminal plasma and sperm of Landrace boar, which provided important information regarding proteins that play a role in the decline in sperm motility and viability after freezing.

The results of this study, as shown in Table 1 and Table 2, show that the quality of fresh semen in various boars did not show significant differences in parameters such as motility, viability, and sperm abnormalities. However, there was a significant decrease in the quality of frozen semen, especially in bulls with IDs 5 and 6. This decrease in viability and motility highlights internal factors specific to these individuals that may affect cryopreservation success, suggesting that physical analysis of semen alone may not fully account for these variances.

Previous studies support the finding that intrinsic biological factors, such as membrane composition, antioxidant capacity, and seminal plasma proteins, can influence sperm resilience to freezing. For instance, research has shown that boars with higher levels of antioxidant enzymes in their seminal plasma tend to exhibit better post-thaw motility, likely due to an enhanced ability to counteract oxidative damage during the cryopreservation process (Willforss *et al.*, 2021; Li *et al.*, 2023). Additionally, proteins in seminal

plasma, particularly those involved in stress responses or cell adhesion, can impact structural stability and functional integrity in frozen-thawed sperm (Gomes *et al.*, 2020). These findings suggest that the observed quality decline in bulls 5 and 6 could stem from variations in such intrinsic properties, indicating that their seminal plasma may lack certain protective factors that help maintain sperm quality during freezing.

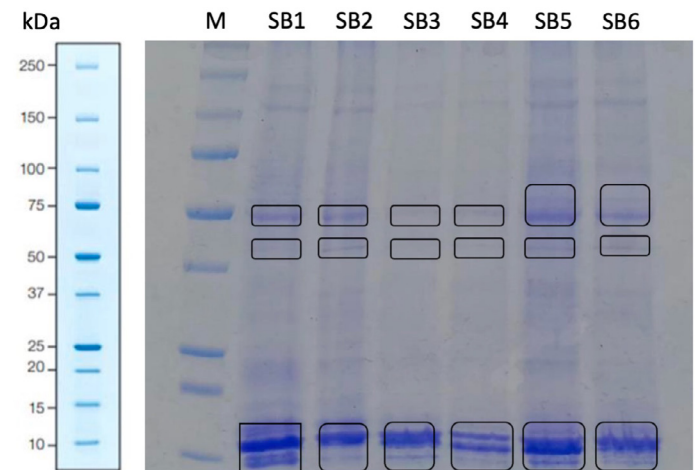


Figure 2: The profile protein of sperm based on molecular weight (MW) using SDS-PAGE.

In proteomic analysis using 1D-SDS-PAGE, as seen in Figures 1 and 2, several proteins with different molecular weights (MW) were found to be expressed in the seminal plasma and sperm of Landrace boars. One protein consistently detected in all boars' IDs was a protein with an MW of 10-15 kDa, which was indicated as OPN12 (Osteopontin 12) (Luedtke *et al.*, 2002). This protein's function has not yet been sufficiently clarified (Moura *et al.*, 2018). Novak *et al.* (2010) identified OPN12 but failed to correlate it with in vivo fertility. Thus, the protein may be a factor in the infertility of the reproductive performance of boars.

Interestingly, the protein with MW 90 kDa was only expressed in Landrace boar with ID 5 and 6, which had low sperm motility and viability after freezing (Table 2). This protein was indicated as HSP90 (Heat Shock Protein 90), a stress protein involved in sperm resistance to freezing ability. Michos *et al.* (2021) reported that HSP90 is associated with decreased sperm freezing capability due to cold stress. Increased HSP90 expression in boar sperm correlates with decreased sperm ability to survive cryopreservation, as Valencia *et al.* (2017) reported. HSP90 protects other proteins from denaturation due to stress, but on the other hand, increased HSP90 can disrupt the standard sperm mechanism, leading to decreased sperm viability and motility.

Furthermore, HSP90 is known to interact with GRP94 and BiP (GRP78), other chaperone proteins that play a role in cell protein homeostasis. The interaction between HSP90

and GRP94 can trigger the activation of the IGF-I and IGF-II pathways, which are known to increase the secretion of stress hormones through the IGF pathway mechanism (Michos et al., 2021). These increased stress hormones can cause physiological and pathological changes in sperm cells, decreasing semen quality after cryopreservation. Berwin et al. (2001) also reported that GRP94 secreted to the outside of the cell has the potential to damage the sperm cell membrane, thereby reducing overall sperm viability. This phenomenon could explain why Landrace males with ID 5 and 6 had low levels of sperm viability and motility after freezing, as seen in this study.

The results in Table 4 indicate a very strong and statistically significant positive correlation ($r = 0.924$, $p < 0.05$) between sperm viability and motility in Landrace boars, highlighting a close association between these two essential quality parameters. Additionally, the positive correlations between sperm motility and molecular weight of sperm protein (MWSP) ($r = 0.550$, $p < 0.05$) and sperm viability and MWSP ($r = 0.556$, $p < 0.05$) suggest that certain proteins in seminal plasma are linked to improved sperm quality characteristics. However, these associations may be influenced by confounding factors, such as genetic variability, environmental conditions, and diet, which can affect seminal protein expression and sperm resilience during cryopreservation. For example, while HSP90 a 90 kDa protein found exclusively in boars with IDs 5 and 6 correlates with reduced cryopreservation success, additional influences, such as specific genetic expressions and stress adaptation mechanisms, might also impact sperm resilience.

The findings on OPN12, combined with the exclusive expression of HSP90 in boars with poorer cryopreservation outcomes, provide important insights into the role of molecular proteins in cryo-tolerance. The presence of HSP90 alongside chaperone proteins such as GRP94 and BiP (GRP78) indicates a complex interaction that could impair normal sperm function under stress, reducing viability and motility post-thaw. Furthermore, OPN12 may contribute to infertility factors by interfering with standard cellular processes needed for post-thaw functionality. These insights underscore the significance of OPN12, HSP90, GRP94, and related proteins as potential biomarkers for assessing cryopreservation resilience in boars. A better understanding of their functions could lead to improvements in cryopreservation protocols, allowing artificial insemination programs to select breeding candidates with greater resilience to freezing, thereby enhancing breeding efficiency.

Overall, this study's results underline the importance of a deeper understanding of the proteins expressed in Landrace boar semen, especially in the context of cryopreservation. Proteins such as OPN12, HSP90 and GRP94 can be potential targets for further research to improve cryopres-

ervation techniques and molecular markers for the selection of boars that are more resistant to the freezing process. Further research linking protein expression to semen quality may help develop strategies to improve the efficiency of pig breeding programs through artificial insemination.

This study has several limitations, such as the limited number of boar samples, so the results may not fully represent the entire Landrace pig population. In addition, the proteomic technique used, namely 1D-SDS-PAGE, has limitations in separating more complex proteins, so using more sophisticated methods, such as 2D-gel electrophoresis or mass spectrometry, could provide more detailed results. This study also did not measure other important parameters, such as sperm DNA fragmentation or acrosome capacity, that could affect the success of cryopreservation. Although there is an indication that proteins such as OPN12, HSP90 and GRP94 play a role in the decline in sperm quality after freezing, the causal relationship has not been confirmed and requires further experimentation. Therefore, future studies with larger samples and more in-depth analysis techniques are needed to strengthen these findings and improve the quality of boar semen cryopreservation.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, this study demonstrates that significant variations in sperm quality among Landrace boars, influenced by proteins such as OPN12, HSP90, and GRP94 identified via 1D-SDS-PAGE proteomic analysis, can impact reproductive performance and guide boars selection for breeding programs in East Nusa Tenggara. The findings suggest that these proteins may serve as molecular markers for evaluating fertility potential and resilience to cryopreservation, offering breeding programs a tool to enhance reproductive outcomes by selecting boars with optimal protein profiles. However, further research is essential to clarify the roles of these proteins in sperm viability, motility and infertility, with future studies potentially focusing on expression levels across larger populations or interventions targeting these proteins to improve cryo-tolerance. This research underscores the promise of proteomics as a diagnostic tool, though its effectiveness may be limited without accounting for additional genetic and environmental factors. By advancing the understanding of protein markers in semen quality, these findings contribute valuable insights to fertility management strategies and pave the way for more targeted and efficient breeding practices.

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NOVELTY STATEMENTS

This study provides new insights into the role of specific proteins in improving the quality of Landrace boar semen, identifying proteins specific such as OPN12, HSP90, and GRP94 that correlate with sperm motility, viability, and preservation potential. Using 1D-SDS-PAGE, it links proteomic variations to reproductive performance, offering novel biomarkers for semen quality and fertility in Landrace boars.

AUTHOR'S CONTRIBUTIONS

All authors equally contributed and approved the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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