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PROTEOMIC SIGNATURES ASSOCIATED WITH BREEDING SOUNDNESS STATUS AND CRYOPRESERVATION RESILIENCE IN PERANAKAN ETAWAH BUCKS

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ABSTRACT

Breeding soundness examination (BSE) is widely used to evaluate male reproductive suitability, but its relationship with resilience to sperm cryopreservation and sperm molecular characteristics remains insufficiently understood in Peranakan Etawah (PE) bucks. This study compared post-thaw sperm quality and sperm proteomic profiles between PE bucks classified as satisfactory (SAT; n=10) and unsatisfactory (UNS; n=10) using a modified BSE framework. BSE classification was based on body condition score, locomotion score, scrotal circumference, progressive motility, and sperm morphology. Frozen–thawed semen was evaluated for motility recovery and morphology preservation, while sperm proteomic profiles were analyzed using LC–MS/MS followed by differential abundance, multivariate, functional enrichment, and correlation analyses. SAT bucks showed higher total BSE score, locomotion score, progressive motility, motility recovery, and morphology preservation than UNS bucks. Proteomic analysis identified distinct sperm protein signatures between groups, with SAT-associated proteins primarily involved in flagellar organization, axonemal structure, membrane regulation, energy metabolism, and oxidative stress response, including CFAP43, AKAP4, AQP3, ALDOC, CS, FLOT1, SPAG6, DNAI1, and GSTM3. In contrast, UNS-associated proteins, including MSN, ALB, and MMP9, were linked to altered membrane remodeling, the extracellular protein environment, and reduced sperm functional or structural resilience. Correlation analysis showed that selected SAT-associated proteins were positively associated with progressive motility and morphology preservation, whereas UNS-associated proteins were negatively associated with these outcomes. These findings indicate that integrating BSE classification, post-thaw semen assessment, and sperm proteomic profiling provides a more comprehensive framework for evaluating reproductive soundness in PE bucks. The identified sperm protein signatures represent exploratory candidates for future validation in fertility and cryopreservation resilience studies.

Keywords: Peranakan Etawah buck, Breeding soundness examination, Sperm, Cryopreservation resilience, Proteomics.

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1. INTRODUCTION

Peranakan Etawah (PE) goats are an important local genetic resource in Indonesia and are widely maintained in smallholder production systems for meat, milk, and breeding purposes. In goat breeding programs, bucks' reproductive performance strongly influences herd productivity because a single male can contribute genetically and reproductively to multiple females. Therefore, accurately evaluating buck reproductive potential is essential for breeding selection, semen utilization, and reproductive management in PE goat populations (Husen et al., 2025; Warmadewi et al., 2026).

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Breeding soundness examination (BSE) is commonly used as a practical and structured approach for evaluating the reproductive suitability of male livestock. In small ruminant males, BSE generally integrates physical condition, locomotor ability, scrotal development, and semen quality parameters to support reproductive decision-making (Harighi et al., 2022). Body condition score and locomotion reflect general health and mating ability, while scrotal circumference is associated with testicular development and sperm production capacity. Semen-based parameters, particularly progressive motility and sperm morphology, provide direct information regarding sperm functional competence and structural integrity (Alam et al., 2024). Together, these parameters allow bucks to be classified by reproductive soundness status.

Despite its practical value, BSE remains largely dependent on phenotypic and semen-based observations. This approach is useful for routine reproductive screening, but it may not fully explain the molecular variation underlying sperm function, semen freezability, and post-thaw resilience. This limitation is particularly relevant in semen cryopreservation programs, where individual variation in post-thaw semen quality is frequently observed even when semen is processed under comparable laboratory conditions (Tanga et al., 2021; Suo et al., 2024; Said et al., 2025). Bucks with acceptable reproductive characteristics before freezing may exhibit varying degrees of sperm motility recovery and morphological preservation after thawing, suggesting that conventional evaluation alone may not capture the biological mechanisms underlying tolerance to cryopreservation.

Semen cryopreservation is an essential technology for the conservation, storage, distribution, and wider utilization of superior genetic material. However, freezing and thawing expose spermatozoa to cold shock, osmotic stress, membrane destabilization, oxidative damage, and structural disruption, which may reduce sperm motility and alter sperm morphology after thawing (Bodu et al., 2025). In goat semen production, individual differences in cryotolerance remain a major challenge because they can affect the efficiency of artificial insemination and the utilization of selected bucks. Therefore, evaluating sperm motility recovery and morphology preservation after cryopreservation in relation to BSE status may provide a more informative assessment of buck reproductive potential.

Sperm proteomic analysis offers a complementary molecular approach for understanding the biological basis of sperm quality and cryopreservation resilience. Sperm proteins are involved in key processes such as flagellar movement, axonemal organization, membrane stability, energy metabolism, oxidative stress response, and fertilization-related functions (Damayanti et al., 2026; Sahiruddin et al., 2026). Changes in sperm protein abundance may therefore reflect differences in sperm functional capacity and cellular adaptation to cryopreservation-induced stress. Previous studies in livestock have shown that sperm proteomic profiles are associated with semen quality, fertility-related traits, and freezing tolerance (Jia et al., 2022; Keller et al., 2026). However, information linking BSE-based reproductive classification with frozen–thawed semen quality and sperm proteomic signatures in PE Bucks remains limited.

This knowledge gap is important because PE buck selection is still largely based on conventional reproductive evaluation, while the molecular characteristics that distinguish satisfactory and unsatisfactory bucks are poorly understood. Integrating BSE classification with cryopreservation outcome assessment and sperm proteomic profiling may provide a more comprehensive framework for identifying reproductive soundness-associated protein signatures. Such an approach may improve the biological interpretation of BSE status and support the selection of bucks with better semen quality and cryopreservation resilience.

Therefore, this study aimed to compare sperm motility recovery, sperm morphology preservation, and sperm proteomic profiles between PE bucks classified as satisfactory and unsatisfactory based on a modified BSE framework. This study was designed to determine whether BSE status is associated with post-thaw sperm resilience and distinct sperm protein signatures, thereby providing an integrated phenotypic and molecular interpretation of reproductive soundness in PE bucks.

2. MATERIALS AND METHODS

2.1. Experimental Design and Ethical Approval

This study was designed to compare breeding soundness status, sperm motility recovery, sperm morphology preservation, and sperm proteomic profiles in Peranakan Etawah (PE) bucks. Twenty clinically healthy PE bucks (aged 18–24 months) were used and classified into two groups based on a modified breeding soundness examination (BSE) framework: satisfactory (SAT; n=10) and unsatisfactory (UNS; n=10). The BSE framework included body condition score, locomotion score, scrotal circumference, progressive motility, and sperm morphology.

All bucks were maintained under comparable feeding, housing, and management conditions to minimize non-experimental variation. Physical and scrotal examinations were performed before semen collection. Semen collection was carried out twice a week using an artificial vagina. The artificial vagina is filled with warm water at a temperature of about 35-40°C (Lukusa & Kabuba, 2020). Before collection, lubricant (Vaseline) was applied

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to the artificial vagina, and a semen collection scale tube was installed at the lower end. During mounting, the erect penis was guided into the artificial vagina, and the collected semen was immediately evaluated for volume and transferred to the semen processing laboratory for further analysis (Rahmat et al., 2026). Fresh semen was evaluated for progressive motility and sperm morphology and used as the basis for BSE classification (Perrett et al., 2021). Semen samples were then processed for cryopreservation. Semen cryopreservation was carried out following the procedure of Diansyah et al. (2023). Filling and sealing were performed, and the specimen was equilibrated at 3-5°C for 2-3 hours in the refrigerator. Then, pre-freezing was carried out by placing the straw ±10 cm above the surface of liquid nitrogen for 10- 5 minutes; frozen–thawed semen was used to evaluate sperm motility recovery and sperm morphology preservation. Sperm pellets obtained from frozen–thawed semen were further processed for LC–MS/MS-based proteomic analysis. This design was used to determine whether BSE status was associated with cryopreservation-related sperm resilience and sperm protein abundance profiles.

2.2. Parameters Studied

2.2.1. BSE and Group Classification: Breeding soundness examination was used as the primary framework to classify PE bucks into SAT and UNS groups. The modified BSE system was based on selected physical and semen-based reproductive soundness indicators, including body condition score, locomotion score, scrotal circumference, progressive motility, and sperm morphology. This scoring framework was adapted from reproductive soundness evaluation principles for small ruminant males, in which physical condition, locomotor ability, scrotal development, and semen quality are considered key components of male reproductive evaluation (Whitesell et al., 2020; Harrison et al., 2022).

We calculated a modified total BSE score from five indicators, with a maximum total score of 100 points. Body condition score and locomotion score contributed 10 points each, scrotal circumference contributed 20 points, progressive motility contributed 30 points, and sperm morphology contributed 30 points. We assigned greater weight to semen-based parameters because progressive motility and sperm morphology directly reflect sperm functional competence and structural integrity.

Table 1 presents the detailed scoring criteria and cut-off values used in this study. Bucks were classified as SAT when they obtained a total BSE score of ≥70 and fulfilled both major semen-based criteria, namely progressive motility ≥70% and sperm morphology ≥80% morphologically normal spermatozoa. Bucks were classified as UNS when they obtained a total BSE score of <70, failed to meet the progressive motility criterion, failed to meet the sperm morphology criterion, and/or failed to meet both major semen-based criteria. We used the final BSE classification as the primary grouping variable for subsequent comparisons of sperm motility recovery, sperm morphology preservation, and sperm proteomic profiles.

Table 1: Modified breeding soundness examination scoring system for PE bucks

Indicator	Criteria	Score
Body condition score	BCS=3.0-3.5	10
	BCS=2.0-2.5; BCS=4.0	5
	BCS ≤1.5; BCS≥4.5	0
Locomotion score	Score 5: normal gait, able to stand and walk normally	10
	Score 3-4: mild locomotor impairment but still able to support mating activity	5
	Score 1-2: severe locomotor impairment affecting reproductive activity	0
Scrotal circumference	≥25.0 cm	20
	22.0-24.9 cm	10
	<22.0 cm	0
Progressive motility	≥70%	30
	50-69%	15
	<50%	0
Sperm morphology	≥80% morphologically normal spermatozoa	30
	70–79% morphologically normal spermatozoa	15
	<70% morphologically normal spermatozoa	0
Total BSE score	Maximum total score	100

2.2.2. Physical and Scrotal Examination: Physical and scrotal examinations were performed before semen collection. Body condition score was assessed using a 1–5 scale, where 1 indicated emaciated, 2 thin, 3 moderate to ideal, 4 fat, and 5 obese. Locomotion was evaluated using a modified 1–5 scale based on each buck's ability to stand, walk, and support reproductive activity, with higher scores indicating better locomotor function. Scrotal circumference was measured at the widest part of the scrotum using a flexible measuring tape and recorded in centimeters (Ouchene-Khelifi & Ouchene, 2021).

2.2.3. Fresh Semen Assessment: Fresh semen assessment focused on progressive motility and sperm morphology. Immediately after collection, semen samples were gently homogenized and maintained at 37°C before

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evaluation. Progressive motility was evaluated using a computer-assisted sperm analysis system (SpermVision™, Minitube GmbH, Tiefenbach, Germany). For CASA analysis, fresh semen was diluted in a physiological medium to obtain an appropriate sperm concentration for motility assessment. A 10 µL aliquot of diluted semen was loaded onto a pre-warmed chamber slide and examined at 37°C. Progressive motility was expressed as the percentage of spermatozoa showing forward progressive movement. Several microscopic fields were analyzed for each sample according to the CASA system settings (Diansyah et al., 2025a).

Sperm morphology was assessed using eosin–nigrosin staining. Briefly, 10 µL of fresh semen was mixed with 20 µL of eosin–nigrosin stain on a clean glass slide, gently homogenized, and smeared to produce a thin and uniform film. The smear was air-dried and examined under a trinocular microscope (Olympus CX33, Olympus Corporation, Tokyo, Japan) at 400× magnification. A minimum of 200 spermatozoa per sample were evaluated in duplicate smears, and the mean value was used for analysis. Morphological defects were classified as head, midpiece, and tail defects (Diansyah et al., 2025b). Sperm morphology was expressed as the percentage of morphologically normal spermatozoa relative to the total number of spermatozoa observed.

2.2.4. Semen Processing and Cryopreservation: Semen processing and cryopreservation were performed following standard laboratory procedures with minor modifications (Sun et al., 2020). Fresh semen samples were diluted using a commercial soybean lecithin-based extender, AndroMed® (Minitube GmbH, Tiefenbach, Germany), according to the manufacturer's instructions. Diluted semen was loaded into 0.25 mL sterile straws, equilibrated at 4°C, frozen in liquid nitrogen vapor, and stored in liquid nitrogen at −196°C until further evaluation.

Before post-thaw assessment, frozen semen straws were thawed in a water bath at 37°C for 30 s. Frozen–thawed progressive motility and sperm morphology were evaluated using the same CASA-based and eosin–nigrosin staining procedures described for fresh semen assessment.

2.2.5. Cryopreservation Outcomes: Cryopreservation outcomes were assessed based on sperm motility recovery and sperm morphology preservation. Motility recovery was determined by comparing progressive motility after thawing with the corresponding fresh progressive motility value. Sperm morphology preservation was determined by comparing the percentage of morphologically normal spermatozoa after thawing with the corresponding fresh morphology value. These parameters were used to evaluate the ability of spermatozoa to maintain functional movement and structural integrity after cryopreservation. Both cryopreservation outcome variables were analyzed as post-classification endpoints and were not included in the initial BSE-based group classification (Tamburrino et al., 2025).

2.2.6. Proteomic Analysis and Data Processing: Proteomic analysis was performed using a Thermo Scientific™ Vanquish™ Horizon UHPLC system coupled to a Thermo Scientific™ Orbitrap™ Exploris 240 high-resolution mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) (Zheng et al., 2022). Peptide separation was carried out on a reversed-phase C18 analytical column using a binary mobile phase system consisting of MS-grade water with 0.1% formic acid as mobile phase A and MS-grade acetonitrile with 0.1% formic acid as mobile phase B. Peptide samples were injected into the LC system and separated using a gradient elution program optimized for peptide analysis. The gradient was initiated at a low proportion of mobile phase B, gradually increased to elute peptides according to their hydrophobicity, and then returned to the initial condition for column re-equilibration.

Mass spectrometric acquisition was performed using electrospray ionization in positive ion mode. Full-scan MS spectra were acquired across an appropriate peptide mass range, followed by data-dependent MS/MS acquisition of selected precursor ions. Peptide fragmentation was performed using higher-energy collisional dissociation with optimized collision energy settings, and nitrogen was used as the collision gas.

Raw LC–MS/MS data were processed using Proteome Discoverer software, version 3.0 (Thermo Fisher Scientific, Waltham, MA, USA). The acquired MS/MS spectra were searched against the Capra hircus protein database retrieved from UniProt, together with common contaminant sequences. Database searching was performed using trypsin as the digestion enzyme. Carbamidomethylation of cysteine was set as a fixed modification, while methionine oxidation was set as a variable modification. Protein identifications were filtered using a false discovery rate threshold of 1% at both peptide and protein levels (Diansyah et al., 2025c).

2.3. Data Analysis

2.3.1. Bioinformatic and Statistical Analysis: The resulting protein abundance matrix was used for downstream bioinformatic and statistical analyses. Protein identifiers were standardized using the UniProt database (https://www.uniprot.org/) to obtain accession numbers, gene names, protein names, and functional annotations. Protein distribution and overlap between SAT and UNS bucks were visualized using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) after protein identifier standardization.

Proteomic statistical analysis was performed using MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/). The protein abundance matrix was processed through missing value checking, normalization, transformation, and

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scaling before analysis. Differentially abundant proteins were identified using fold-change analysis and statistical significance testing. Proteins with fold change ≥2.0 and p-value <0.1 were considered exploratory differentially abundant candidate proteins. The comparison direction was set as SAT/UNS; therefore, positive log2 fold-change values indicated higher abundance in SAT bucks, while negative log2 fold-change values indicated higher abundance in UNS bucks.

We used principal component analysis, hierarchical clustering heatmap, volcano plot, and partial least squares–discriminant analysis to evaluate proteomic separation patterns between groups. Proteins with variable importance in projection scores >1.0 were considered important contributors to group discrimination. We performed functional enrichment analysis using g (https://biit.cs.ut.ee/gprofiler/) to identify enriched Gene Ontology terms related to biological process, molecular function, and cellular component. We prioritized candidate proteins by integrating differential abundance direction, VIP score, functional annotation, and biological relevance to sperm motility, morphology preservation, energy metabolism, membrane function, flagellar structure, oxidative stress response, and reproductive soundness status (Xu et al., 2022; Damayanti et al., 2026).

We performed correlation analysis to evaluate associations between selected candidate proteins and reproductive parameters, including progressive motility, normal sperm morphology, motility recovery, and morphology preservation. Pearson correlation analysis was used to determine the direction and strength of association, with statistical significance set at $P<0.05$.

Statistical analysis of BSE parameters, fresh semen quality, sperm motility recovery, and sperm morphology preservation was performed using IBM SPSS Statistics, version 27 (IBM Corp., Armonk, NY, USA). We assessed data normality using the Shapiro–Wilk test. Total BSE score, individual BSE components, fresh progressive motility, fresh sperm morphology, motility recovery, and sperm morphology preservation were compared between SAT and UNS bucks using an independent-samples t-test when data were normally distributed. Results were presented as mean ± standard deviation, with statistical significance set at $P<0.05$.

3. RESULTS

3.1. Breeding Soundness Examination and Classification of PE Bucks

Breeding Soundness Examination revealed differences in selected reproductive soundness parameters between SAT and UNS bucks (Table 2). Locomotion score and progressive motility were significantly higher in SAT bucks than in UNS bucks ($P<0.05$), whereas body condition score, scrotal circumference, and normal sperm morphology did not differ significantly between groups ($P>0.05$). Total BSE score was also significantly higher in SAT bucks compared with UNS bucks ($P<0.001$). Although the mean total BSE score of UNS bucks remained above the minimum numerical threshold, these bucks were classified as UNS because they failed to meet at least one major semen-based criterion, particularly progressive motility. These findings indicate that the BSE-based classification was primarily driven by the cumulative reproductive soundness score, locomotor capacity, and progressive sperm motility, whereas body condition, scrotal circumference, and sperm morphology showed relatively comparable values across groups.

Table 2: Breeding Soundness Examination parameters and group classification of PE bucks

Parameter	Mean ± SD		p-value
	SAT	UNS	
Body condition score	3.15 ± 0.34	2.95 ± 0.37	0.222
Locomotion score	4.40 ± 0.70	3.50 ± 1.08	0.040
Scrotal circumference(cm)	25.65 ± 0.61	25.21 ± 0.62	0.127
Progressive motility (%)	71.96 ± 2.05	68.77 ± 4.21	0.045
Sperm morphology normal (%)	81.60 ± 0.99	81.48 ± 2.02	0.868
Total BSE score	95.00 ± 5.77	74.50 ± 8.96	<0.001

Note: SAT=satisfactory; UNS=unsatisfactory; BSE=Breeding Soundness Examination.

3.2. Cryopreservation Resilience of SAT and UNS Bucks

Sperm motility recovery and morphology preservation after cryopreservation differed between SAT and UNS bucks (Fig. 1). SAT bucks showed significantly higher motility recovery than UNS bucks (59.8 ± 7.6% vs. 56.9 ± 7.9%; $p=0.047$; Fig. 1A). Morphology preservation was also significantly higher in SAT bucks compared with UNS bucks (92.4 ± 3.1% vs. 89.1 ± 3.6%; $p=0.041$; Fig. 1B). These findings indicate that BSE status was associated with post-thaw sperm resilience, particularly the maintenance of progressive motility and preservation of morphologically normal spermatozoa after cryopreservation.

3.3. Differentially Abundant Sperm Proteins between SAT and UNS Bucks

Differential abundance analysis identified 32 sperm proteins that differed between SAT and UNS bucks based

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on the exploratory screening criteria (Table 3; Fig. 2). Of these proteins, 22 showed higher abundance in SAT bucks, whereas 10 showed higher abundance in UNS bucks. The volcano plot showed a direction-specific distribution of differentially abundant proteins, with SAT-associated proteins located on the positive log2 (FC) side and UNS-associated proteins located on the negative log2 (FC) side.

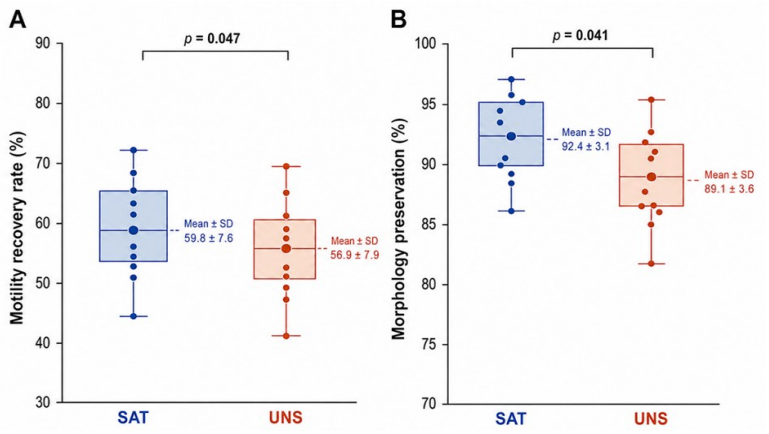

Fig. 1: Sperm motility recovery and morphology preservation after cryopreservation in SAT and UNS bucks. (A) Motility recovery rate (B) Morphology preservation.

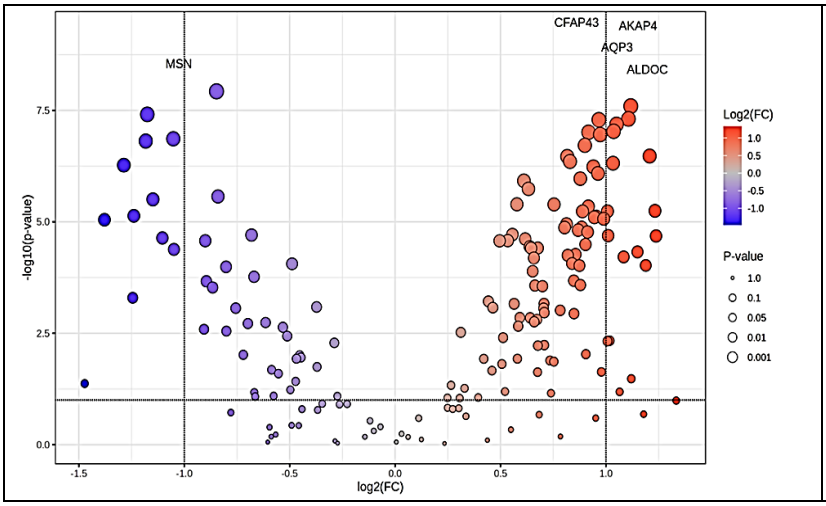

Fig. 2: Volcano plot of differentially abundant proteins between SAT and UNS bucks.

SAT-associated proteins included AKAP4, AQP3, ALDOC, CS, FLOT1, SPAG6, ENO1L, DNAI1, SEPT4, VDAC2, ATP5F1A, SPACA1, ACE, ENO1, SDHA, TUBA1B, MFGE8, ATP5F1B, IDH3A, FLOT2, and UPF157. In contrast, UNS-associated proteins included MMP9, EZR, PRDX1, HSPB1, FGB, CTSB, HEXB, MPO, H2BC1, UPF176, and UPF152. Several SAT-associated proteins were related to sperm structural organization, flagellar function, membrane regulation, and energy metabolism, whereas UNS-associated proteins were mainly linked to stress response, inflammatory activity, extracellular matrix remodeling, and altered cellular homeostasis. These findings indicate that BSE classification was accompanied by direction-specific changes in sperm protein abundance between SAT and UNS bucks.

3.4. Multivariate Proteomic Separation between SAT and UNS Bucks

Multivariate analysis showed a distinct separation pattern of sperm proteomic profiles between SAT and UNS bucks (Fig. 3). The PCA score plot demonstrated group clustering along PC1, which explained 43.6% of the total variation, whereas PC2 explained 5.5% of the variation (Fig. 3A). The biplot indicated that SAT-associated proteins, including CFAP43, AKAP4, AQP3, ALDOC, CS, FLOT1, and SPAG6, contributed to the SAT cluster, while MSN, ALB, and MMP9 contributed to the UNS cluster (Fig. 3B). Hierarchical clustering analysis further showed group-specific protein abundance patterns, with SAT bucks generally characterized by higher abundance of flagellar, membrane-associated, and metabolic proteins, whereas UNS bucks showed higher abundance of MSN, ALB, and MMP9 (Fig. 3C). The VIP score plot identified 15 proteins with VIP scores >1.0, indicating their major contribution to proteomic separation between SAT and UNS bucks (Fig. 3D). These findings indicate that BSE classification was accompanied by distinct sperm proteomic signatures in PE bucks.

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Table 3: Differentially abundant proteins identified between SAT and UNS bucks

Protein	FC	log2(FC)	p-value	-log10(p)	Direction
AKAP4	2.125	1.088	6.433×10^{-10}	9.192	SAT
AQP3	2.170	1.117	1.967×10^{-9}	8.706	SAT
ALDOC	2.190	1.131	2.309×10^{-9}	8.636	SAT
CS	2.217	1.149	5.182×10^{-9}	8.286	SAT
FLOT1	2.170	1.117	2.541×10^{-8}	7.595	SAT
MMP9	0.443	-1.175	3.879×10^{-8}	7.411	UNS
SPAG6	2.153	1.107	4.909×10^{-8}	7.309	SAT
ENO1L	2.071	1.050	6.530×10^{-8}	7.185	SAT
DNAI1	2.051	1.036	9.325×10^{-8}	7.030	SAT
EZR	0.482	-1.052	1.371×10^{-7}	6.863	UNS
PRDX1	0.441	-1.183	1.540×10^{-7}	6.812	UNS
SEPT4	2.309	1.207	3.348×10^{-7}	6.475	SAT
VDAC2	2.047	1.034	4.830×10^{-7}	6.316	SAT
HSPB1	0.410	-1.287	5.348×10^{-7}	6.272	UNS
FGB	0.451	-1.149	3.128×10^{-6}	5.505	UNS
ATP5F1A	2.350	1.232	5.665×10^{-6}	5.247	SAT
SPACA1	2.010	1.007	5.807×10^{-6}	5.236	SAT
CTSB	0.424	-1.239	7.337×10^{-6}	5.135	UNS
HEXB	0.385	-1.379	8.975×10^{-6}	5.047	UNS
ACE	2.014	1.010	2.042×10^{-5}	4.690	SAT
ENO1	2.358	1.238	2.080×10^{-5}	4.682	SAT
MPO	0.465	-1.104	2.304×10^{-5}	4.638	UNS
H2BC1	0.483	-1.049	4.155×10^{-5}	4.381	UNS
SDHA	2.219	1.150	4.737×10^{-5}	4.324	SAT
TUBA1B	2.120	1.084	6.120×10^{-5}	4.213	SAT
MFGE8	2.280	1.189	9.502×10^{-5}	4.022	SAT
UPF176	0.422	-1.245	5.059×10^{-4}	3.296	UNS
ATP5F1B	2.024	1.017	4.653×10^{-3}	2.332	SAT
IDH3A	2.010	1.007	4.757×10^{-3}	2.323	SAT
FLOT2	2.174	1.120	3.316×10^{-2}	1.479	SAT
UPF152	0.360	-1.472	4.258×10^{-2}	1.371	UNS
UPF157	2.092	1.065	6.514×10^{-2}	1.186	SAT

Note: SAT=satisfactory; UNS=unsatisfactory.

3.5. Functional Enrichment of BSE-associated Candidate Proteins

Functional enrichment analysis showed that BSE-associated candidate proteins were mainly related to sperm structural organization, flagellar function, and membrane-associated cellular components (Table 4). The enriched Gene Ontology terms consisted of one molecular function term, three biological process terms, and three cellular component terms. The most significant enriched term was 9+2 motile cilium (GO: 0097729; adjusted $P<0.01$), which involved CFAP43, AKAP4, SPAG6, DNAI1, and GSTM3.

Biological process enrichment was associated with epithelial cilium movement, sperm flagellum assembly, and axoneme assembly. These terms were represented primarily by flagellar and axonemal proteins, including CFAP43, SPAG6, AKAP4, and DNAI1. In addition, membrane-associated enrichment terms, including uropod and basolateral plasma membrane, were linked to MSN, FLOT1, and AQP3. These findings indicate that BSE-associated candidate proteins were functionally concentrated in processes related to sperm motility machinery, flagellar architecture, axonemal organization, and membrane regulation.

3.6. Candidate Protein Signatures of Breeding Soundness Examination Status

Candidate protein signatures associated with Breeding Soundness Examination status were prioritized by integrating abundance direction, VIP score, and Gene Ontology term assignment (Table 5). Of the 15 VIP-ranked proteins, 12 were retained as prioritized candidate proteins because they showed clear group direction, VIP score >1.0, and assignment to at least one enriched GO term.

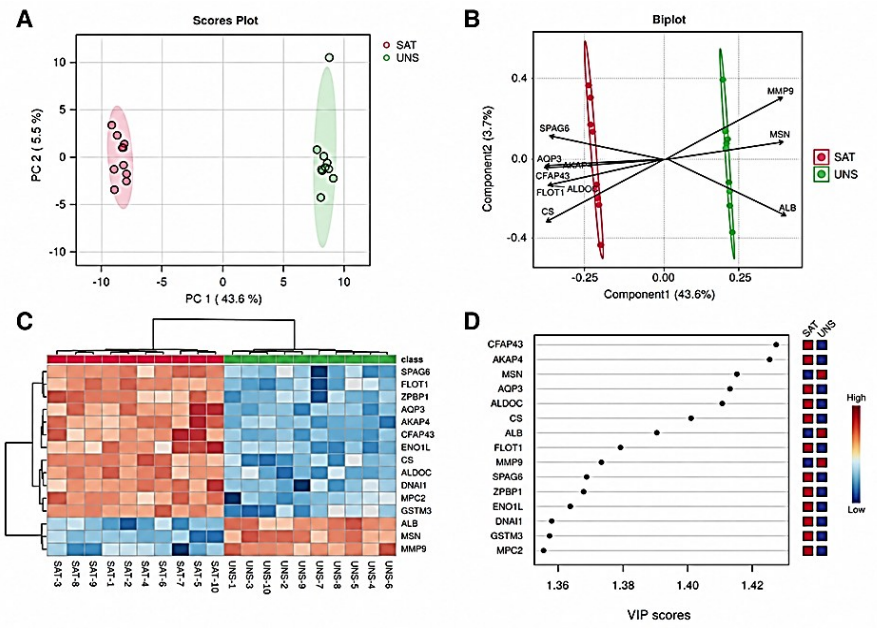

Fig. 3: Multivariate proteomic profiles and VIP-ranked proteins separating satisfactory (SAT) and unsatisfactory (UNS) PE bucks. (A) Principal component analysis score plot showing proteomic clustering between SAT and UNS bucks. (B) Biplot showing proteins contributing to group separation. (C) Hierarchical clustering heatmap showing group-specific protein abundance patterns. (D) Variable importance in projection (VIP) score plot showing the top proteins contributing to proteomic separation between groups.

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Table 4: Functional enrichment terms and contributing candidate proteins

Source	Term ID	Enriched Term	Adjusted p-value	Protein
GO:MF	GO:0005515	Protein binding	3.691×10^{-3}	CFAP43, AKAP4, MSN, AQP3, ALDOC, CS, ALB, FLOT1, MMP9, SPAG6, DNAI1, GSTM3
GO:BP	GO:0003351	Epithelial cilium movement involved in extracellular fluid movement	1.577×10^{-3}	CFAP43, SPAG6, DNAI1
GO:BP	GO:0120316	Sperm flagellum assembly	5.974×10^{-3}	CFAP43, AKAP4, SPAG6
GO:BP	GO:0035082	Axoneme assembly	2.697×10^{-2}	CFAP43, SPAG6, DNAI1
GO:CC	GO:0097729	9+2 motile cilium	2.336×10^{-6}	CFAP43, AKAP4, SPAG6, DNAI1, GSTM3
GO:CC	GO:0001931	Uropod	7.179×10^{-4}	MSN, FLOT1
GO:CC	GO:0016323	Basolateral plasma membrane	1.388×10^{-2}	MSN, AQP3, FLOT1

Note: GO: MF=Gene Ontology Molecular Function; GO: BP=Gene Ontology Biological Process; GO: CC=Gene Ontology Cellular Component. Protein entries represent candidate proteins highlighted in the enrichment output for each Gene Ontology term.

Table 5: Candidate protein signatures of Breeding Soundness Examination status

Gene Name	Protein	Direction	VIP Score	Term ID
CFAP43	Cilia- and flagella-associated protein 43	SAT	1.43	GO:0005515; GO:0003351; GO:0120316; GO:0035082; GO:0097729
AKAP4	A-kinase anchoring protein 4	SAT	1.43	GO:0005515; GO:0120316; GO:0097729
MSN	Moesin	UNS	1.42	GO:0005515; GO:0001931; GO:0016323
AQP3	Aquaporin 3	SAT	1.41	GO:0005515; GO:0016323
ALDOC	Fructose-bisphosphate aldolase C	SAT	1.41	GO:0005515
CS	Citrate synthase	SAT	1.40	GO:0005515
ALB	Albumin	UNS	1.39	GO:0005515
FLOT1	Flotillin-1	SAT	1.38	GO:0005515; GO:0001931; GO:0016323
MMP9	Matrix metalloproteinase-9	UNS	1.37	GO:0005515
SPAG6	Sperm-associated antigen 6	SAT	1.37	GO:0005515; GO:0003351; GO:0120316; GO:0035082; GO:0097729
DNAI1	Dynein axonemal intermediate chain 1	SAT	1.36	GO:0005515; GO:0003351; GO:0035082; GO:0097729
GSTM3	Glutathione S-transferase Mu 3	SAT	1.36	GO:0005515; GO:0097729

Note: SAT=satisfactory; UNS=unsatisfactory.

The most prioritized candidate proteins were higher in SAT bucks, including CFAP43, AKAP4, AQP3, ALDOC, CS, FLOT1, SPAG6, DNAI1, and GSTM3. In contrast, MSN, ALB, and MMP9 were higher in UNS bucks. The highest VIP-ranked candidates included CFAP43, AKAP4, MSN, AQP3, and ALDOC, indicating their major contribution to the proteomic separation between BSE classifications.

SAT-associated candidate proteins were mainly linked to flagellar structure, axoneme organization, motile cilium components, membrane regulation, energy metabolism, and oxidative stress response. In contrast, UNS-associated candidate proteins were associated with membrane remodeling, extracellular matrix regulation, and altered sperm cellular status. We then used these prioritized proteins for correlation analysis with sperm quality and cryopreservation-related outcomes.

3.7. Association between Candidate Protein Signatures and Sperm Quality and Cryopreservation Outcomes

Correlation analysis showed that selected candidate protein signatures were associated with sperm quality and cryopreservation-related outcomes (Fig. 4). Among SAT-associated proteins, ALDOC was positively correlated with progressive motility ($P<0.05$), while AKAP4, AQP3, DNAI1, and GSTM3 were positively correlated with morphology preservation after cryopreservation ($P<0.05$). In contrast, UNS-associated proteins showed negative associations with reproductive outcomes. ALB and MMP9 were negatively correlated with progressive motility ($P<0.05$), whereas MSN and MMP9 were negatively correlated with morphology preservation, with the strongest negative association observed for MMP9 ($P<0.01$).

We observed no significant correlations between the selected candidate proteins and normal sperm morphology or motility recovery rate. These findings indicate that the prioritized candidate protein signatures were more closely associated with progressive sperm movement and preservation of sperm morphology after cryopreservation than with baseline sperm morphology alone.

4. DISCUSSION

This study provides an integrated interpretation of reproductive soundness in Peranakan Etawah bucks by combining BSE-based classification, cryopreservation outcome assessment, and sperm proteomic profiling. The main findings showed that SAT bucks had higher total BSE score, locomotion score, progressive motility, motility recovery, and morphology preservation than UNS bucks. These phenotypic differences were accompanied by distinct sperm proteomic signatures, particularly involving candidate proteins related to flagellar organization, axonemal structure, membrane regulation, energy metabolism, oxidative stress response, and altered cellular status.

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The prioritized SAT-associated proteins included CFAP43, AKAP4, AQP3, ALDOC, CS, FLOT1, SPAG6, DNAI1, and GSTM3, whereas the prioritized UNS-associated proteins included MSN, ALB, and MMP9. These findings indicate that BSE status in PE bucks was associated not only with conventional reproductive traits but also with molecular differences that may be relevant to sperm function and post-thaw resilience.

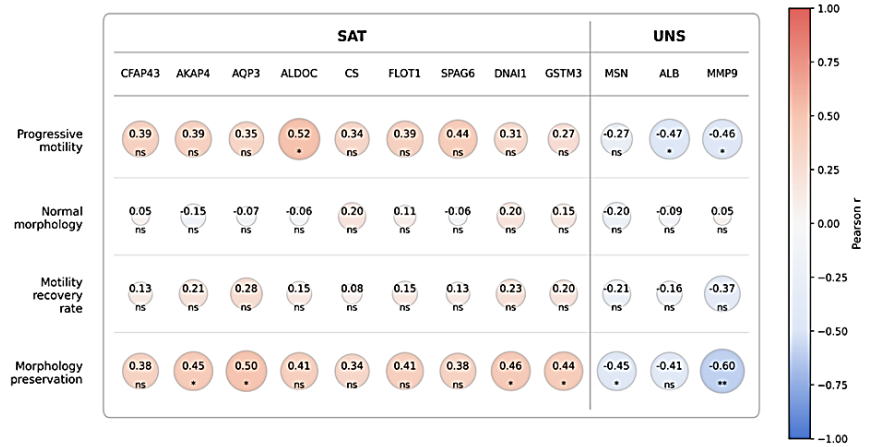

Fig. 4: Correlation of candidate protein biomarkers with sperm quality and cryopreservation outcomes. *P<0.05; **P<0.01; ns=not significant.

BSE remains a practical and structured approach for evaluating reproductive suitability in male livestock because it integrates physical condition, locomotor ability, scrotal development, and semen quality parameters (Diaz-Miranda et al., 2024). In the present study, SAT bucks had significantly higher total BSE scores than UNS bucks, supporting the ability of the modified BSE framework to separate bucks according to reproductive soundness status. However, not all BSE components differed between groups. Body condition score, scrotal circumference, and normal sperm morphology were relatively comparable, whereas locomotion score and progressive motility were significantly higher in SAT bucks. This pattern suggests that the classification difference was mainly driven by cumulative reproductive soundness, locomotor capacity, and sperm movement rather than general body condition, scrotal size, and baseline sperm morphology.

The classification of UNS bucks despite a mean total BSE score above the minimum numerical threshold highlights the importance of major semen-based criteria in the modified BSE system. Bucks with acceptable total scores may still be classified as unsatisfactory when they fail to meet key semen quality criteria, particularly progressive motility. Progressive motility is one of the most direct indicators of sperm functional competence because it reflects the ability of spermatozoa to move effectively and participate in fertilization-related processes (Li et al., 2016; Harighi et al., 2022). Therefore, reduced progressive motility in UNS bucks may represent a functional limitation that is not fully captured by physical traits alone.

Cryopreservation further differentiated SAT and UNS bucks. SAT bucks showed higher motility recovery and morphology preservation after thawing, indicating better post-thaw sperm resilience. Although the numerical differences were relatively small, the significant group differences suggest that BSE status may be associated with the ability of spermatozoa to withstand freezing and thawing stress. Cryopreservation can expose spermatozoa to cold shock, osmotic imbalance, membrane destabilization, oxidative stress, and structural disruption (Milewska et al., 2024; Ananda et al., 2025). Therefore, the better post-thaw outcomes observed in SAT bucks are consistent with greater sperm functional and structural stability during cryopreservation.

The prioritized SAT-associated protein signatures provide a molecular explanation for the better reproductive and post-thaw outcomes observed in SAT bucks. CFAP43, AKAP4, SPAG6, and DNAI1 are closely related to sperm flagellar structure, axonemal organization, and motile cilium function. These proteins are relevant because sperm progressive motility depends on the structural integrity and coordinated activity of the flagellum and axoneme (Liang et al., 2020; Zhou et al., 2023). The higher contribution of these proteins to the SAT proteomic profile supports the interpretation that SAT bucks had a more favorable molecular background for maintaining sperm movement.

AKAP4 is particularly important because it is a major component of the sperm flagellar fibrous sheath and helps organize signaling complexes involved in motility regulation (Blommaert et al., 2019). Together with CFAP43, SPAG6, and DNAI1, the higher abundance of AKAP4 in SAT bucks is consistent with the enrichment of sperm flagellum assembly, axoneme assembly, and 9+2 motile cilium terms. This finding strengthens the biological link between BSE status, progressive motility, and the molecular architecture of sperm motility machinery.

Energy metabolism also appeared to be an important feature of the SAT-associated protein profile. ALDOC

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and CS were prioritized as SAT-associated candidate proteins and may reflect the metabolic support required for sperm movement. ALDOC is involved in glycolytic metabolism, while CS is associated with mitochondrial energy metabolism (Kang et al., 2020; Jia et al., 2021). Spermatozoa require continuous ATP supply to maintain flagellar beating and progressive movement. Therefore, the positive association between ALDOC and progressive motility suggests that metabolic protein support may contribute to better sperm functional competence in SAT bucks.

Membrane regulation was another important component of the SAT-associated signature. AQP3 and FLOT1 were higher in SAT bucks and were linked to membrane-associated enrichment terms. AQP3 is involved in water and small solute transport, whereas FLOT1 is associated with membrane microdomain organization (Delgado-Bermúdez et al., 2021; Lu et al., 2026). During cryopreservation, sperm membranes are exposed to osmotic and temperature-related stress. Therefore, the SAT-associated increase in AQP3 and FLOT1 may indicate better membrane adaptation and structural stability during freezing and thawing.

The presence of GSTM3 among SAT-associated candidate proteins suggests that antioxidant-related mechanisms may also be involved in post-thaw resilience. Cryopreservation can increase reactive oxygen species production and damage sperm membranes, proteins, and cellular structures (Llavanera et al., 2019). GSTM3, as a glutathione S-transferase family protein, may contribute to cellular defense against oxidative stress. Although oxidative stress markers were not directly measured in this study, the positive association between GSTM3 and morphology preservation supports the interpretation that antioxidant-related protein signatures may contribute to better structural preservation after cryopreservation.

In contrast, the prioritized UNS-associated proteins, including MSN, ALB, and MMP9, reflected a different molecular profile. MSN is a membrane–cytoskeleton linker protein involved in membrane organization and cytoskeletal remodeling (Cohen et al., 2022). Its higher abundance in UNS bucks may indicate altered membrane–cytoskeletal dynamics, cellular remodeling, and reduced structural stability. The negative association between MSN and morphology preservation further supports the interpretation that UNS-associated membrane remodeling signatures may be linked to lower post-thaw structural resilience.

MMP9 was another important UNS-associated candidate protein. MMP9 is involved in extracellular matrix remodeling and has been associated with inflammatory and tissue remodeling processes (Wolosowicz et al., 2025). In this study, MMP9 was negatively associated with both progressive motility and morphology preservation, indicating that higher MMP9 abundance may accompany reduced sperm function and lower post-thaw structural stability. This does not imply a direct causal role, but it suggests that MMP9 may represent part of an unfavorable sperm protein signature in UNS bucks.

ALB was also higher in UNS bucks and showed a negative association with progressive motility. Albumin may originate from seminal plasma carryover, extracellular fluid, and sample-associated protein components during sperm preparation (Almadaly et al., 2023). Therefore, ALB should be interpreted cautiously and not as a direct causal factor. In this study, higher ALB abundance may indicate an altered extracellular or sample-associated protein environment accompanying reduced sperm functional status in UNS bucks.

Functional enrichment analysis strengthened the biological interpretation of these candidate protein signatures. The enriched terms were mainly associated with protein binding, sperm flagellum assembly, axoneme assembly, 9+2 motile cilium, and membrane-associated cellular components. The enrichment of 9+2 motile cilium involving CFAP43, AKAP4, SPAG6, DNAI1, and GSTM3 is particularly relevant because mammalian sperm motility depends on the highly organized axonemal structure of the flagellum (Liang et al., 2020). This enrichment pattern provides a mechanistic link between BSE status, progressive motility, and the molecular organization of sperm motility machinery.

Correlation analysis provided additional support for the relationship between prioritized candidate proteins and sperm functional outcomes. ALDOC was positively associated with progressive motility, while AKAP4, AQP3, DNAI1, and GSTM3 were positively associated with morphology preservation after cryopreservation. Conversely, ALB and MMP9 were negatively associated with progressive motility, while MSN and MMP9 were negatively associated with morphology preservation. These associations indicate that SAT-associated proteins were linked to favorable sperm outcomes, whereas UNS-associated proteins were linked to reduced sperm functional and structural resilience.

Interestingly, no significant correlations were observed between the selected candidate proteins and baseline normal sperm morphology or motility recovery rate. This finding may indicate that the prioritized protein signatures were more closely related to progressive sperm movement and preservation of sperm morphology after cryopreservation than to baseline morphology alone. Baseline sperm morphology is a useful phenotypic indicator, but it may not fully reflect the molecular capacity of spermatozoa to withstand freezing and thawing stress. In contrast, morphology preservation after thawing represents a dynamic outcome that reflects structural stability under cryopreservation conditions.

From an applied perspective, these findings suggest that BSE classification may be strengthened by molecular

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information related to sperm function and cryopreservation resilience. Conventional BSE remains essential because it is practical, accessible, and applicable under field conditions. However, sperm proteomic profiling can provide additional biological insight into why bucks with different BSE status differ in post-thaw semen quality. In semen production programs, prioritized protein signatures such as CFAP43, AKAP4, AQP3, ALDOC, DNAI1, GSTM3, MSN, ALB, and MMP9 may help guide future studies on molecular indicators of sperm quality and freezing tolerance. Nevertheless, these proteins should be considered exploratory candidate signatures rather than validated biomarkers at this stage.

This study has several limitations. First, the sample size was relatively limited, with 10 bucks per group, which may affect the generalizability of the findings. Second, the study used an exploratory proteomic approach, and the prioritized candidate proteins were not validated using targeted proteomics, Western blotting, ELISA, and independent biological cohorts. Third, fertility outcomes after artificial insemination were not included, so the relationship between the identified protein signatures and actual fertility remains to be confirmed. Fourth, oxidative stress, mitochondrial activity, membrane integrity, and DNA integrity were not directly measured, although several identified proteins were biologically related to these processes. Future studies should include larger populations, targeted protein validation, functional sperm assays, and fertility outcome data to confirm the biological and practical relevance of the candidate protein signatures identified in this study.

Overall, these findings support the interpretation that BSE status in PE bucks is accompanied by differences in post-thaw sperm resilience and sperm proteomic signatures, particularly proteins related to flagellar architecture, energy metabolism, membrane regulation, oxidative stress defense, and altered cellular status. The integration of BSE, cryopreservation outcomes, and sperm proteomics provides a more comprehensive framework for understanding reproductive soundness in PE bucks and supports the use of sperm protein signatures as complementary indicators for evaluating semen quality and cryopreservation resilience.

5. CONCLUSION

This study demonstrates that BSE status in Peranakan Etawah bucks is associated with sperm cryopreservation resilience and distinct sperm proteomic profiles. SAT bucks showed higher total BSE score, locomotion score, progressive motility, motility recovery, and morphology preservation than UNS bucks. The SAT-associated protein signatures, including CFAP43, AKAP4, AQP3, ALDOC, CS, FLOT1, SPAG6, DNAI1, and GSTM3, were mainly related to flagellar organization, axonemal structure, membrane regulation, energy metabolism, and oxidative stress response, whereas UNS-associated proteins, including MSN, ALB, and MMP9, were linked to altered membrane remodeling, extracellular protein environment, and reduced sperm functional or structural resilience. These findings indicate that integrating BSE classification, post-thaw semen assessment, and sperm proteomic profiling provides a more comprehensive framework for evaluating reproductive soundness in PE bucks, although the identified protein signatures remain exploratory candidates that require further validation using larger populations, targeted protein analysis, functional sperm assays, and fertility outcome data.

Declarations

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Data Availability: All data supporting the findings of the study are available within the manuscript.

Ethics Statement: All experimental procedures involving animals were conducted in accordance with institutional guidelines for animal care and use. Ethical approval for this study was obtained from the Animal Research Ethics Committee of the Department of Animal Science, Faculty of Agriculture, Lambung Mangkurat University, South Kalimantan, Indonesia (Approval No. 381/UN8.1.23.5/PG/2026).

The use of animal biological materials was reviewed and approved by the Ethics Committee of the Department of Animal Science, Faculty of Agriculture, Lambung Mangkurat University, South Kalimantan, Indonesia

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(Approval No. 381/UN8.1.23.5/PG/2026). All procedures were conducted in accordance with institutional and national ethical standards for research involving animal biological samples.

Author's Contribution: RR, ANC, DTF, AMD, MR and MFA: Conceived, designed, and coordinated the study. RR, ANC, AMD and DTF: Principal investigators. MR, AMD, MY, MFA, and AMA: Designed data collection tools. RR, ANC, DTF, NAS, AMD, MY, MFA, and AMA: Supervised field sampling, data collection, laboratory work, and data entry. RR, AMD, MY, MFA, MM and AMA: Performed statistical analysis, interpreted the data, and drafted the manuscript. All authors contributed to the study design, critically reviewed the manuscript, and approved the final version of the manuscript.

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REFERENCES

Alam, K., Srivastava, S., Kumar, R., Saurabh, Verma, A. K., Verma, S. K., Jaiswal, A., & Verma, H. C. (2024). Testicular biometry and its relationship with body weight and semen parameters of Sirohi and Jamnapari bucks. The Indian Journal of Animal Reproduction, 45(1), 29–34. https://doi.org/10.48165/ijar.2024.45.01.7

Almadaly, E. A., Abdel-Salam, A. S., Sahwan, F. M., Kahilo, K. A., Abouzed, T. K., & El-Domany, W. B. (2023). Fertility-associated biochemical components in seminal plasma and serum of buffalo (Bubalus bubalis) bulls. Frontiers in Veterinary Science, 9, 1043379. https://doi.org/10.3389/fvets.2022.1043379

Ananda, A., Gusdinal, H., Ramadhan, R., Abimanyu, A. A., Ningsih, W. H., & Jaswandi. (2025). Impacts of cryopreservation on semen quality and sperm protein profiles of Pesisir bulls. Tropical Animal Science Journal, 48(3), 189–198. https://doi.org/10.5398/tasj.2025.48.3.189

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

Bodu, M., Hitit, M., Greenwood, O. C., Murray, R. D., & Memili, E. (2025). Extender development for optimal cryopreservation of buck sperm to increase reproductive efficiency of goats. Frontiers in Veterinary Science, 12, 1554771. https://doi.org/10.3389/fvets.2025.1554771

Cohen, J., Wang, L., Marques, S., Ialy-Radio, C., Barbaux, S., Lefèvre, B., Gourier, C., & Ziyyat, A. (2022). Oocyte ERM and EWI proteins are involved in mouse fertilization. Frontiers in Cell and Developmental Biology, 10, 863729. https://doi.org/10.3389/fcell.2022.863729

Damayanti, E., Diansyah, A. M., Azis, I. U., Irawan, F., Maulana, T., Muhajir, M., Dagong, M. I. A., & Kartika, N. A. (2026). Proteomic profile of seminal plasma in tropical Saanen bucks under heat stress using 1D-SDS PAGE. Open Veterinary Journal, 16(1), 354–362. https://doi.org/10.5455/OVJ.2026.v16.i1.32

Delgado-Bermúdez, A., Recuero, S., Llavanera, M., Mateo-Otero, Y., Sandu, A., Barranco, I., Ribas-Maynou, J., & Yeste, M. (2021). Aquaporins are essential to maintain motility and membrane lipid architecture during mammalian sperm capacitation. Frontiers in Cell and Developmental Biology, 9, 656438. https://doi.org/10.3389/fcell.2021.656438

Diansyah, A. M., Nurlatifah, A., Damaris, C., Rahmat, R., Saili, T., Amrullah, M. F., Alfian, A. M., Adam, A. A. S., Kaiin, E. M., & Damayanti, E. (2025a). Evaluation of sperm sexing technique using BSA column medium in Garut rams: Enhancing sperm quality, kinematics, and fertility performance for improved breeding efficiency. Open Veterinary Journal, 15(8), 3727–3736. https://doi.org/10.5455/OVJ.2025.v15.i8.37

Diansyah, A. M., Santoso, S., Herdis, H., Hasbi, H., Saili, T., Suyatno, S., Said, S., Kostaman, T., Surachman, M., Pamungkas, F. A., Adiati, U., Anwar, R. I., Iskandar, H., Rahmat, R., & Hasrin, H. (2025b). Proteomic analysis of seminal plasma in Bali-polled bulls: Identifying biomarkers for fertility assessment. Journal of Advanced Veterinary and Animal Research, 12, 698–708. https://doi.org/10.5455/javar.2025.l933

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. (2025c). Identification of reproductive performance in Bali-polled bulls using computer-assisted semen analysis and plasma seminal proteomics. Veterinary World, 18, 102–109. https://doi.org/10.14202/vetworld.2025.102-109

Diansyah, A. M., Yusuf, M., Toleng, A. L., & Dagong, M. I. A. (2023). The effect of thawing duration on the sperms quality of Bali polled bull. AIP Conference Proceedings, 2628(1). https://doi.org/10.1063/5.0146414

Diaz-Miranda, E. A., Penitente-Filho, J. M., Gomez-Leon, V. E., Neto, T. M., Guimarães, S. F., Siqueira, J. B., & Guimarães, J. D. (2024). Selection based on the breeding soundness evaluation is associated with the improvement of the reproductive quality of young Nellore bulls. Theriogenology, 226, 369–377. https://doi.org/10.1016/j.theriogenology.2024.06.032

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Open Access Journal

Harighi, M. F., Wahid, H., Khumran, A. M., & Baiee, F. (2022). Breeding soundness examination (BSE): A decision-making tool that requires a particular guideline for male goats. Tropical Animal Health and Production, 54, 174. https://doi.org/10.1007/s11250-022-03178-8

Harrison, T. D., Chaney, E. M., Brandt, K. J., Ault-Seay, T. B., Schneider, L. G., Strickland, L. G., Schrick, F. N., & McLean, K. J. (2022). The effects of differing nutritional levels and body condition score on scrotal circumference, motility, and morphology of bovine sperm. Translational Animal Science, 6, txac001. https://doi.org/10.1093/tas/txac001

Husen, A. F., Suyadi, S., Parwoto, Y., Sairi, M., Crooijmans, R., & Nurgiartiningsih, V. M. A. (2025). Estimating genetic parameters and principal component analysis of breeding values for morphometric traits in Indonesian Etawah goats. Cogent Food & Agriculture, 11, 2520964. https://doi.org/10.1080/23311932.2025.2520964

Jia, B., Larbi, A., Lv, C., Liang, J., Xiang, D., Zhang, B., Fang, Y., Shen, W., Wu, G., & Quan, G. (2022). Identification and validation of ram sperm proteins associated with cryoinjuries caused by the cryopreservation process. Theriogenology, 184, 191–203. https://doi.org/10.1016/j.theriogenology.2022.03.015

Jia, B., Liang, J., Lv, C., Memon, S., Fang, Y., Wu, G., & Quan, G. (2021). The characteristics of proteome and metabolome associated with contrasting sperm motility in goat seminal plasma. Scientific Reports, 11, 15562. https://doi.org/10.1038/s41598-021-95138-9

Kang, W., Harada, Y., Yamatoya, K., Kawano, N., Kanai, S., Miyamoto, Y., Nakamura, A., Miyado, M., Hayashi, Y., Kuroki, Y., Saito, H., Iwao, Y., Umezawa, A., & Miyado, K. (2020). Extra-mitochondrial citrate synthase initiates calcium oscillation and suppresses age-dependent sperm dysfunction. Laboratory Investigation, 100, 583–595. https://doi.org/10.1038/s41374-019-0353-3

Keller, A., Keller, E., Vorwald, M., Selsby, J. T., Perry, G., & Kerns, K. (2026). Comparative proteomic analysis of bovine spermatozoa before and after cryopreservation. Frontiers in Animal Science, 7, 1781535. https://doi.org/10.3389/fanim.2026.1781535

Lukusa, K., & Kabuba, J. (2020). Semen collection methods and cooling rates affect post-thaw sperm motility and kinematic parameters of Saanen goat. Asian Pacific Journal of Reproduction, 9(5), 239–246. https://doi.org/10.4103/2305-0500.294666

Li, Y., Kalo, D., Zeron, Y., & Roth, Z. (2016). Progressive motility: A potential predictive parameter for semen fertilization capacity in bovines. Zygote, 24(1), 70–82. https://doi.org/10.1017/S0967199414000720

Liang, C., He, Y., Liu, Y., Gao, Y., Han, Y., Li, X., Zhao, Y., Wang, J., & Zhang, J. (2020). Fluoride exposure alters the ultra-structure of sperm flagellum via reducing key protein expressions in testis. Chemosphere, 246, 125772. https://doi.org/10.1016/j.chemosphere.2019.125772

Llavanera, M., Delgado-Bermúdez, A., Fernandez-Fuertes, B., Recuero, S., Mateo, Y., Bonet, S., Barranco, I., & Yeste, M. (2019). GSTM3, but not IZUMO1, is a cryotolerance marker of boar sperm. Journal of Animal Science and Biotechnology, 10, 61. https://doi.org/10.1186/s40104-019-0370-5

Lu, M. A., Qian, Y., Ma, L., Hong, J., Li, X., Yu, L., Guo, Q., & Gao, N. (2026). Molecular mechanisms of flotillin complexes in organizing membrane microdomains. Nature Communications, 17, 2541. https://doi.org/10.1038/s41467-026-69197-3

Milewska, A. J., Kuczyńska, A., Pawłowski, M., Martynowicz, I., Deluga-Białowarczuk, S., Sieczyński, P., Kuczyński, W., & Milewski, R. (2024). The influence of cryopreservation on sperm morphology and its implications in terms of fractions of higher-quality sperm. Journal of Clinical Medicine, 13, 7562. https://doi.org/10.3390/jcm13247562

Ouchene-Khelifi, N. A., & Ouchene, N. (2021). Relationships between age, body measurements, and testicular measurements in Arabia bucks in Algeria. Tropical Animal Health and Production, 53, 91. https://doi.org/10.1007/s11250-020-02500-6

Perrett, J., Harris, I. T., Maddock, C., Farnworth, M., Pyatt, A. Z., & Sumner, R. N. (2021). Systematic analysis of breed, methodological, and geographical impact on equine sperm progressive motility. Animals, 11(11), 3088. https://doi.org/10.3390/ani11113088

Rahmat, R., Rizal, M., Syarifuddin, N. A., Riyadhi, M., Diansyah, A. M., Amrullah, M. F., Churriyah, A. N., Wahdi, A., Adam, A. A. S., Alfian, A. M., & Rizal, M. (2026). Effect of Moringa oleifera leaf extract supplementation on motility and viability of Boer crossbreed buck spermatozoa during cold storage. Open Veterinary Journal, 16(5), 2823–2830. https://doi.org/10.5455/OVJ.2026.v16.i5.24

Sahiruddin, S., Yusuf, M., Diansyah, A. M., Masturi, M., Herdis, H., Maulana, T., Sihombing, V. R., Rahmat, R., Amrullah, M. F., Adam, A. A. S., Said, S., & Alfian, A. M. (2026). Proteomic profiling of bull spermatozoa and seminal plasma to inform the rational development of functionally targeted semen extenders for tropical cattle. Veterinary World, 19, 1163–1177. https://doi.org/10.14202/vetworld.2026.1163-1177

Said, S., Diansyah, A. M., Rahayu, J. D., Maulana, T., Gunawan, M., & Kaiin, E. M. (2025). A comparative analysis of semen quality traits and sperm kinematic parameters in relation to fertility prediction in Murrah buffaloes. Journal of advanced veterinary and animal research, 12(3), 751–759. https://doi.org/10.5455/javar.2025.l938

Sun, L., Fan, W., Wu, C., Zhang, S., Dai, J., & Zhang, D. (2020). Effect of substituting different concentrations of soybean lecithin and egg yolk in tris-based extender on goat semen cryopreservation. Cryobiology, 92, 146–150. https://doi.org/10.1016/j.cryobiol.2019.12.004

Suo, J., Wang, J., Zheng, Y., Xiao, F., Li, R., Huang, F., Niu, P., Zhu, W., Du, X.X., He, J., Gao, Q., & and Khan, A. (2024). Recent advances in cryotolerance biomarkers for semen preservation in frozen form–A systematic review. PLoS ONE, 19(5), e0303567. https://doi.org/10.1371/journal.pone.0303567

Tamburrino, L., Traini, G., Ragosta, M. E., Dabizzi, S., Vezzani, S., Scarpa, F., Vignozzi, L., Baldi, E., & Marchiani, S. (2025). Semen cryopreservation and storage in liquid nitrogen: Impact on chromatin compaction. Andrology, 13, 1780–1796. https://doi.org/10.1111/andr.13806

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

Tanga, B. M., Qamar, A. Y., Raza, S., Bang, S., Fang, X., Yoon, K., & Cho, J. (2021). Semen evaluation: Methodological advancements in sperm quality-specific fertility assessment—A review. Animal Bioscience, 34, 1253–1270. https://doi.org/10.5713/ab.21.0072

Warmadewi, D. A., Ardika, I. N., Melati, N. P. Y., Bidura, I. G. N. G., Aryawan, I. N. A., & Murtika, I. L. (2026). Identifying potential breeding candidates in Etawah crossbred (EC) goats in Jembrana, Indonesia. Advances in Animal and Veterinary Sciences, 14(2), 356–363. https://doi.org/10.17582/journal.aavs/2026/14.2.356.363

Whitesell, K., Stefanovski, D., McDonnell, S., & Turner, R. (2020). Evaluation of the effect of laboratory methods on semen analysis and breeding soundness examination (BSE) classification in stallions. Theriogenology, 142, 67–76. https://doi.org/10.1016/j.theriogenology.2019.09.035

Wolosowicz, M., Prokopiuk, S., & Kaminski, T. W. (2025). Matrix metalloproteinase-9 (MMP-9) as a therapeutic target: Insights into molecular pathways and clinical applications. Pharmaceutics, 17, 1425. https://doi.org/10.3390/pharmaceutics17111425

Xu, B., Wang, Z., Wang, R., Song, G., Zhang, Y., Su, R., Liu, Y., Li, J., & Zhang, J. (2022). Metabolomics analysis of buck semen cryopreserved with trehalose. Frontiers in Genetics, 13, 938622. https://doi.org/10.3389/fgene.2022.938622

Zheng, R., Stejskal, K., Pynn, C., Mechtler, K., & Boychenko, A. (2022). Deep single-shot NanoLC-MS proteome profiling with a 1500 bar UHPLC system, long fully porous columns, and HRAM MS. Journal of Proteome Research, 21(10), 2545–2551. https://doi.org/10.1021/acs.jproteome.2c00270

Zhou, Y., Wang, Y., Chen, J., Wu, B., Tang, S., Zhang, F., Liu, C., & Wang, L. (2023). Dnali1 is required for sperm motility and male fertility in mice. Basic and Clinical Andrology, 33, 32. https://doi.org/10.1186/s12610-023-00205-y

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