Buletin Peternakan 48 (4): 233-241, November 2024

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternakv%vi%i.96459

Sperm Kinematics of Pesisir Bull Thawed at Different Temperatures and Times

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Article history
Submitted: 26 May 2024
Accepted: 3 September 2024

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ABSTRACT

Pesisir bulls from West Sumatra, Indonesia, have the potential to be valuable meat producers due to their adaptability and unique characteristics, but reproductive challenges necessitate the use of artificial insemination (AI) technology. The success of AI relies on the quality of frozen semen, which is influenced by thawing conditions, making the evaluation of sperm kinematics through Computer-Assisted Sperm Analysis (CASA) crucial for a comprehensive assessment of sperm function. This research evaluated the kinematics of frozen semen spermatozoa from Pesisir bulls. Frozen semen samples (n = 20) were thawed at 37°C for 10, 20, and 30 s and at 25°C for 30, 45, and 60 s. Kinematic parameters were observed using a Computer-Assisted Sperm Analyzer (CASA), including total motility (M), progressive motility (PM), the velocity of the average path (VAP) (μm/sec), the velocity of curvilinear (VCL) (μm/sec), the velocity of straight line (VSL) (μm/sec), straightness (STR) (%), linearity (LIN) (%), wobble (WOB) (%), amplitude of lateral head displacement (ALH) (μm), and beat/cross-frequency (BCF) (Hz). Thawed frozen semen at a temperature of 37°C for 20 s significantly ($p<0.05$) increased M, PM, and ALH. Furthermore, thawed frozen semen at 37°C for all durations and at 25°C for 60 s showed better quality for VAP, VCL, and VSL ($p<0.05$). Meanwhile, thawed frozen semen at 25°C increased STR, LIN, and WOB. However, thawing temperature and duration had no significant effect on BCF. Correlation analysis conducted on semen thawed at a temperature of 37°C for 20 s found that VAP was correlated with VCL, VSL, and ALH, while semen thawed at a temperature of 25°C for 60 s found that VAP was correlated with VCL, VSL, STR, LIN, and ALH. Most kinematic parameters were significantly better in the thawing treatment at 37°C compared to 25°C, suggesting careful consideration of frozen semen treatment before conducting artificial insemination in Pesisir bulls.

Keywords: Pesisir bulls, Sperm, Kinematics, Temperatures, Thawing

Introduction

Pesisir bulls are native to West Sumatra, Indonesia, and can potentially be valuable meat producers (Hendri, 2013). Their unique and specific characteristics, such as low body weight and small body size, contribute to their efficiency in space utilization. These traits, coupled with good adaptation to coastal environmental conditions and the ability to thrive on limited forage, present opportunities for developing these bulls in coastal areas across Indonesia.

Challenges in improving the physical and genetic performance of Pesisir bulls in West Sumatra are associated with the reproductive system, which is closely linked to rearing management. Breeders must address two crucial aspects regarding bull regulation: inbreeding and using unsuitable bulls. As per Wirdahayati and Bamualim (2007), minimal breeder intervention in regulating livestock mating results in a relatively high frequency of inbreeding between offspring and mothers or among siblings. Using unsuitable bulls is indicated when the livestock is too young and exhibits below-standard performance. Adrial (2016) also reported a similar issue, noting that bulls in pastures generally consist of cattle aged <2 years. Additionally, breeders sometimes use Pesisir bulls that the market rejects due to poor genetic quality. Wirdahayati and Bamualim (2007) emphasized that the scarcity of superior males meeting the requirements for seed purposes poses a challenge to the reproduction of Pesisir bulls.

The development of Pesisir bulls can be improved through artificial insemination (AI) technology. However, the pregnancy rate from AI varies significantly and tends to be low (Ansori *et al*., 2021), a variability closely linked to the quality of the frozen semen used (Hastuti, 2008). Thawing prior to insemination is a critical step that must be carefully managed, as the correct temperature and thawing duration are crucial factors affecting

spermatozoa motility. The quality of frozen semen after thawing can be compromised by plasma membrane instability caused by heat stress or prolonged exposure to air (Novita, 2020). There is a lack of consensus in the scientific literature regarding thawing procedures, as evidenced by disparate data on the quality of frozen semen for different bull breeds after the thawing process. A standardized procedure is necessary to achieve optimal results for successful fertilization with frozen semen. Maintaining semen quality post-thawing depends critically on the thawing method, temperature, and duration. Therefore, it is essential to investigate how thawing methods affect the quality of frozen semen, specifically from Pesisir bulls. The influence of thawing time and temperature on bull sperm quality is crucial for optimizing AI outcomes. Research indicates that both the duration and temperature of thawing significantly affect sperm motility, viability, and overall quality. For instance, thawing bull sperm in a water bath at 33-35°C for 30-40 s is considered optimal, as this combination maximizes motility and membrane integrity post-thaw (Yılmaz *et al*., 2020). This aligns with findings from Zhang *et al*. (2015), who report that the motility and membrane integrity of bull sperm decreases progressively during the freezing-thawing process, emphasizing the importance of controlled thawing conditions.

Conventionally, spermatozoa quality is assessed using a phase-contrast microscope to measure sperm concentration, motility, and morphology (Iguer-ouada and Verstegen, 2001). However, assessing the kinematics of spermatozoa cells, including velocity, swimming patterns, and sperm head behavior, is also essential for a more comprehensive understanding of sperm function. Compared to traditional methods, measuring spermatozoa kinematics using Computer-Assisted Semen Analysis (CASA) provides a more objective assessment of spermatozoa quality. The application of this method relies on the advancement of digital image technology, enabling rapid and accurate analysis of spermatozoa, thus improving and standardizing the testing of spermatozoa motility parameters relevant for fertility assessment (Simmet, 2004). Greater predictive power regarding the fertility potential of ejaculated semen is provided by CASA, which measures not only the proportion of moving spermatozoa but also various individual sperm movement parameters (Hirano *et al*. 2001). In bulls, spermatozoa kinematic parameters positively correlate with fertility (Tanga *et al.,* 2021).

CASA is also helpful for researching the outcomes of different in vitro tests, such as examining the phenomenon of sperm hyperactivation and the impact of particular diluent ingredients on sperm motility. For fertilization, advancements in spermatozoa motility and other kinematic parameters are essential. Bull fertility and spermatozoa kinematic parameters like PM, VSL, VCL, ALH, and LIN are positively correlated (Perumal *et al.,* 2011). The use of CASA to measure spermatozoa kinematics has been reported in several livestock species, including bulls (Fernandez-Novo *et al*., 2021; Maulana *et al*., 2022; Syarifuddin *et al*., 2018), buffalo (Kumar *et al*., 2011; Prete *et al*., 2022), sheep (Van de Hoek *et al*., 2022), goats (Del Gallego *et al*., 2017), pigs (Boe-Hansen and Satake, 2019), and chickens (Svoradova *et al*., 2021). The latest report on the kinematics of Pesisir bull spermatozoa using CASA by Wahyudi *et al*., (2023) found that the treatment before freezing, conducted at a distance of 16 cm above liquid nitrogen for 9 min. However, there is no information regarding the effect of different temperatures and variations in thawing time on the kinematics of Pesisir bull spermatozoa. Our study aimed to ascertain the kinematic properties of Pesisir bull spermatozoa that had been thawed at different times and temperatures. The findings of this study can be used to forecast spermatozoa's capacity for fertilization prior to insemination in Pesisir bulls, which are native bulls of Indonesia.

Materials and Methods

Frozen Semen and Thawing Treatment

Frozen semen from Pesisir bulls was sourced from the Department of Animal Husbandry and Animal Health of West Sumatra, Tuah Sakato Technology and Resource Development Center, Indonesia. A total of 120 straws of frozen semen were utilized in this study, involving a combination of six treatments (Table 1).

Table 1. Thawing treatment of frozen semen of Pesisir bull (n = 20)

Temperature (°C)	Times (s)
37	10
	20
	30
25	30
	45
	60

Kinematics Evaluation

The kinematics of frozen semen from Pesisir bulls were evaluated using a Computer-Assisted Semen Analyzer (CASA) equipped with Sperm Vision™ Version 3.7.5 software. The CASA device was mounted on an Olympus BX 51 microscope (Olympus, Tokyo, Japan) and configured with SpermVision software designed explicitly for bovine sperm. The camera operated at 60 frames per second, and the microscope had a 20× objective lens and a 10× eyepiece. Sperm samples (5 µL) from 20 straws in each treatment

were extracted and placed under a glass cover on a slide. CASA settings are detailed in Table 2. Post-thawing kinematic parameters include total motility, progressive motility, VAP (velocity of average path), VCL (velocity of curve linear), VSL (velocity of straight line), STR (straightness) = VSL/VAP, LIN (linearity) = VSL/VCL, WOB (wobble) = VAP/VCL, ALH (amplitude of lateral head movement), and BCF (beat cross frequency).

Table 2. Parameter settings for computer-assisted sperm analysis (CASA)

Variable	Units
Particle size range	20 – 100 µm
Immotile sperm	20 – 100 µm
Locally motile sperm	AOC < 5; BCF < 0.2 ; VSL < 0.2
Hypermotile	DSL < 4.5
Progressively motile sperm	VCL > 80; LIN < 0.65; ALH > 6.5

Description: AOC (average orientation change), BCF (beat cross frequancy), VSL (straight line velocity), DSL (distance straight line), VCL (curvilinear velocity), LIN (linearity), and ALH (amplitude of lateral head).

Statistical analysis

The data obtained from the sperm kinematics of Pesisir bulls were analyzed using Analysis of Variance (ANOVA) within a Completely Randomized Design (CRD). A total of 120 frozen semen straws, produced by Tuah Sakato Artificial Insemination Center, Payakumbuh, West Sumatra, Indonesia, were thawed at different temperatures (37°C and 25°C) for varying times (10, 20, 30 s at 37°C and 30, 40, 60 s at 25°C). Each treatment group consisted of 20 replicates.

To compare kinematic parameters across different thawing temperatures and times, ANOVA was employed to determine the statistical significance of differences between groups. Following this, the treatment that exhibited the optimal kinematic parameters at each thawing temperature was further analyzed using Pearson correlation coefficients to assess the relationships between kinematic parameters, with significance set at $p<0.05$. Specifically, we compared two groups of thawing temperatures with the highest progressive motility (PM) values: semen thawed at 37°C for 20 s and at 25°C for 30 s. The t-test was not utilized, as the primary objective was to compare multiple groups across different thawing conditions, which is more appropriately addressed by ANOVA.

Results and Discussion

Post-thawing Motility

Sperm individual motility is a test for assessing the quality of frozen semen, involving observing the progressive movement of spermatozoa. The individual movement of spermatozoa is a crucial factor influencing the success of artificial insemination (AI). Typically, spermatozoa exhibit progressive movement, advancing to facilitate fertilization. This progressive movement is essential for their transport within the female reproductive tract, allowing for successful fertilization (Yumte *et al*., 2013).

The analysis of variance results revealed that variations in temperature and thawing time significantly influenced progressive motility in frozen semen from Pesisir bulls ($p<0.05$) (Table 1). The optimal motility, at 64.26 ± 6.50%, was observed when using a temperature of 37°C with a thawing time of 20 s. In contrast, the lowest progressive motility of spermatozoa, at 42.93 ± 4.14%, occurred when using a temperature of 25°C with a thawing time of 60 s. These findings slightly outperformed previous research on Simmental bulls, which reported a motility of 50.83% when thawed at 38°C for 15 s (Darmasasmita *et al*., 2016). For Bali bull semen, a temperature of 37°C with a thawing time of 30 s resulted in progressive motility ranging from 27.89% to 55.73%, depending on the diluent used (Amal *et al*., 2019).

A temperature of 37°C with a duration of 15–20 s aligns with the physiological condition of spermatozoa, providing sufficient time to melt ice crystals in the diluent. Conversely, frozen semen thawed at 37°C for 10 s exhibited progressive motility of 69.56 ± 7.30. The lower motility observed during this treatment suggests that thawing too quickly immobilizes many spermatozoa. This finding is consistent with previous research indicating that rapid thawing leads to spermatozoa remaining still or moving backward (Kusumawati *et al*., 2016). Notably, spermatozoa motility significantly decreased ($p<0.05$) when thawed at 37°C for 30 s compared to thawing for 20 s. This decrease is attributed to a potential heat shock effect experienced by spermatozoa thawed at a higher temperature for a more extended period. The heat shock effect may result from a substantial temperature difference between thawing and the environmental temperature, reducing spermatozoa motility (Aprilina *et al*., 2014). Meanwhile, the treatment with a temperature of 37°C for 10 s exhibited the lowest spermatozoa motility, consistent with previous research on the adverse effects of rapid thawing (Kusumawati *et al*., 2016; Ramadhani *et al*., 2022).

At a temperature of 25°C for 60 s, spermatozoa motility appeared to be the lowest among the other treatments. Meanwhile, spermatozoa thawed at 25°C for 30 s showed quite good motility. Thawing for an extended duration can significantly increase metabolic activity, resulting in elevated lactic acid production. This increase in toxic lactic acid concentration can diminish spermatozoan movement power and accelerate death (Salim *et al*., 2012). This occurrence demonstrates that low temperatures in the thawing process and prolonged thawing duration will reduce the motility percentage of frozen semen. Darmasasmita *et al*. (2016) stated that a longer thawing duration increases spermatozoa metabolism and energy expenditure,

depleting available energy more quickly. Depletion of energy content can lead to the cessation of spermatozoa fibril movement, halting the overall spermatozoa movement. Increased metabolism can also increase lactic acid content and decrease pH, slowing spermatozoa movement. Prolonged thawing time may cause a low percentage of frozen semen motility, rendering it unsuitable for Artificial Insemination if it does not reach 40% (Pratama *et al*., 2018).

Spermatozoa motility rates are also associated with metabolic activities within the spermatozoa. The energy required by spermatozoa in the movement process is sourced from nucleotides through the breakdown of adenosine triphosphate (ATP) in cells, leading to chemical reactions and the formation of adenosine diphosphate (ADP) and adenosine monophosphate (AMP). Essentially, spermatozoa activity originates from organelles in the mitochondria. Disturbances and unstable mitochondrial function are linked to temperature changes, thereby reducing the movement power of spermatozoa (Sukmawati *et al*., 2015).

Sperm Kienematics

Computer-Assisted Sperm Analysis (CASA) was used to assess the kinematics and motility parameters of sperm, and the results are shown in Table 3. Thawed spermatozoa at 37°C for 20 s had a significantly ($p<0.05$) higher percentage of total motility (TM) and progressive motility (PM). Similarly, spermatozoa thawed at 37°C for 20 s had a significantly ($p<0.05$) higher velocity parameter. Therefore, as part of the artificial insemination process, this method can be used as a guide to improve semen quality.

Assessing sperm motility through conventional microscopic methods poses challenges due to its subjective nature, with reported high variations even within the same ejaculate (Mortimer *et al*., 1986). CASA is a precise technique for evaluating Pesisir bulls spermatozoa motility and velocity parameters. CASA allows for individualized analysis within a short time, even under conditions of high spermatozoa concentration (Verstegen *et al*., 2002). It is important to remember that many variables can affect motility and velocity parameters. These include age, the length of time spent collecting samples, the intervals between ejaculations, sperm energy stores, the presence of surface-active substances in cell membranes like detergents and agglutinins, viscosity, osmolarity, pH, temperature, ionic concentration in seminal plasma, and the presence of minerals like Cu, Zn, and Mn, in addition to hormones and prostaglandins (Blasco, 1984).

Spermatozoa mobility is closely related to several kinematic parameters detected by CASA. Parameters such as VSL, LIN, and BCF contribute to the overall motility characteristics of Pesisir bull spermatozoa because they all correlate significantly ($p<0.05$) with sperm mobility. In this study, various kinematic parameters showed varied results in each treatment group (Table 3), such as VCL, VSL, and VAP, which were significantly higher in spermatozoa thawed at 25°C for 60 s. Meanwhile, STR was significantly higher in spermatozoa thawed at 25°C for 45 s. LIN showed that spermatozoa thawed at 25°C for 30 and 45 s were significantly higher than other treatment groups. WOB parameters were significantly higher in spermatozoa thawed at 25°C for 30 s. The ALH and BCF parameters significantly increased in spermatozoa that were thawed at 37°C for 20 and 30 s. The LIN parameter measures linearity, and the BCF motion parameter indicates the number of times the sperm path crosses a smooth path, indicating linear development. So, it can be assumed that spermatozoa thawed at 25°C or 37°C can swim straight and fast. This finding can provide good information regarding the mobility of frozen sperm as a parameter for detecting fertility (Froman *et al*., 1999; Froman & Feltmann, 1998).

Table 3. Kinematics of Pesisir bull spermatozoa with various thawing treatments

Kinematics	37°C for 10s	37°C for 20s	37°C for 30s	25°C for 30s	25°C for 45s	25°C for 60s
TM (%)	69.56±7.30b	76.42±6.44a	69.92±4,68b	71.3±6.55b	60.01±6.40c	55.15±5.37d
PM (%)	57.74±7.25b	64.26±6.50a	57.49±5.89b	55.3±5.72b	44.66±5.17c	42.93±4.14c
VAP (m/sec)	61.60±3.82a	61.79±4.39a	61.31±4.74a	53.23±3.48c	56,51±5.39b	62.71±8.10a
VCL (m/sec)	111.49±9.07a	111.82±8.77a	110.37±9.69a	93.31±7.46b	100.13±12.18b	113.66±20.81a
VSL (m/sec)	40.93±2.42a	40.37±2.96a	40.95±2.99a	35.62±1.92c	38.36±3.27b	41.18±4.27a
STR (%)	0.66±0.02ab	0.65±0.02b	0.66±0.02ab	0.67±0.02ab	0.68±0.05a	0.66±0.04ab
LIN (%)	0.36±0.02ab	0.36±0.01b	0.37±0.02ab	0.38±0.02a	0.38±0.04a	0.36±0.04ab
WOB (%)	0.55±0.02b	0.55±0.01b	0.55±0.02b	0.57±0.02a	0.56±0.03ab	0.55±0.04ab
ALH (m)	6.17±0.30bc	6.45 ± 0.33a	6.25±0.30ab	5.95±0.36b	5.91±0.45b	6.11±0.62bc
BCF (Hz)	19.68±0.88a	19.36 ± 1.07a	19.83±1.27a	19.20±0.36a	19.56±1.40a	19.34±1.33a

Description: TM (total motility), PM (progressive motility), VAP (velocity of average path), VCL (velocity of curve linear), VSL (straight line velocity), STR (straightness), LIN (linearity), WOB (wobble), ALH (amplitude of lateral head), and BCF (beat cross frequency). Different letters a,b, and c on the same line indicate statistical difference ($p<0.05$).

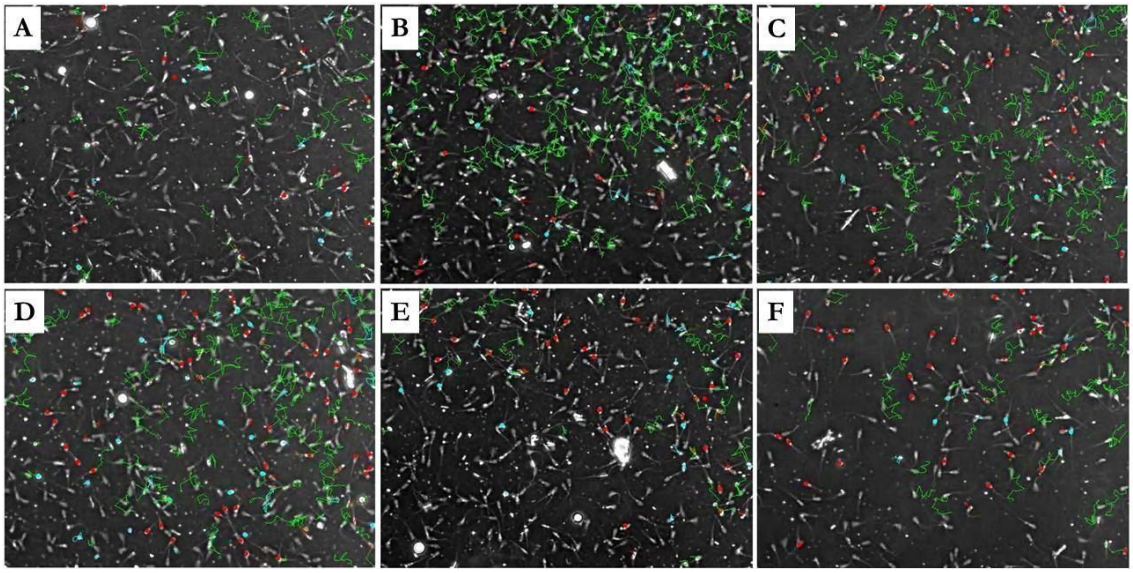

Figure 1. Motility of Pesisir bull spermatozoa in various treatment variations was analyzed using CASA. A: 37°C for 10 s; B: 37°C for 20 s; C: 37°C for 30 s; D: 25°C for 30 s; E: 25°C for 45 s; F: 25°C for 60 s.

Our findings show that semen samples with high PM and TM do not always have a positive correlation with frozen semen kinematic parameters. In the semen treatment thawed at 37°C for 20 s, TM and PM only correlated with an increase in ALH (Table 4). Meanwhile, in the semen treatment that was thawed at a temperature of 25°C for 30 s, PM was correlated with VAP, VCL, LIN, and ALH (Table 5). This is different from what Kumar *et al*. (2011) and Perumal *et al*. (2011) reported for line velocity. Progressive velocity, track velocity, and ALH all exhibited a substantial and positive correlation with average track velocity. The velocity parameters demonstrated correlations and interconnections with ALH, evident in the strong positive correlations observed between VAP, VSL, VCL, and ALH, as well as between VSL and VCL and between ALH and VAP, VSL, and VCL. The average values of parameters describing the behavior of sperm heads, ALH, and BCF encompass a wide range. Additionally, a pronounced negative correlation was identified between STR and WOB. (Kumar *et al*., 2011; Perumal *et al*., 2011).

Table 4. Correlation between kinematic parameters of Pesisir bull spermatozoa thawed at 37°C for 20s.

	TM	PM	VAP	VCL	VSL	STR	LIN	WOB	ALH	BCF
TM	1.00	0.920** 0.000	0.228 0.335	0.133 0.575	– 0.004 0.985	– 0.479 0.032	– 0.336 0.147	0.184 0.436	0.588** 0.006	0.068 0.774
PM		1.00	0.088 0.711	0.015 0.951	– 0.137 0.564	– 0.480 0.032	– 0.342 0.141	0.108 0.650	0.445* 0.049	– 0.140 0.557
VAP			1.00	0.956** 0.000	0.877** 0.000	– 0.188 0.427	– 0.270 0.249	– 0.128 0.591	0.554* 0.011	0.141 0.552
VCL				1.00	0.853** 0.000	– 0.147 0.535	– 0.396 0.084	– 0.404 0.077	0.497* 0.026	0.201 0.396
VSL					1.00	0.302 0.195	0.131 0.581	– 0.110 0.644	0.407 0.075	0.242 0.303
STR						1.00	0.792** 0.000	0.027 0.910	– 0.253 0.281	0.201 0.394
LIN							1.00	0.579** 0.007	– 0.314 0.177	0.087 0.715
WOB								1.00	0.043 0.856	– 0.126 0.596
ALH									1.00	0.007 0.977
BCF										1.00

Description: TM (total motility), PM (progressive motility), VAP (velocity of average path), VCL (velocity of curve linear), VSL (straight line velocity), STR (straightness), LIN (linearity), WOB (wobble), ALH (amplitude of lateral head), and BCF (beat cross frequency). **=Correlation is significant at the 0.01 level, *=Correlation is significant at the 0.05 level.

Table 5. Correlation between kinematic parameters of Pesisir bull spermatozoa thawed at 25°C for 30s.

	TM	PM	VAP	VCL	VSL	STR	LIN	WOB	ALH	BCF
TM	1.00	0.752** 0.000	0.309 0.185	0.238 0.312	0.247 0.294	– 0.224 0.342	– 0.110 0.643	0.019 0.935	0.506* 0.023	0.153 0.521
PM		1.00	0.507* 0.023	0.547* 0.013	0.378 0.1	– 0.392 0.87	– 0.496* 0.026	– 0.378 0.1	0.606** 0.005	0.260 0.269
VAP			1.00	0.935** 0.000	0.853** 0.000	– 0.545* 0.013	– 0.580** 0.007	– 0.349 0.131	0.789** 0.000	0.130 0.585
VCL				1.00	0.779** 0.000	– 0.557* 0.011	– 0.779** 0.000	– 0.653** 0.002	0.795** 0.000	0.305 0.191
VSL					1.00	– 0.035 0.882	– 0.225 0.341	– 0.286 0.222	0.672** 0.001	– 0.25 0.918
STR						1.00	0.775** 0.000	0.268 0.253	– 0.477* 0.034	– 0.357 0.122
LIN							1.00	0.790** 0.000	– 0.554* 0.011	– 0.542* 0.014
WOB								1.00	– 0.471* 0.036	– 0.592** 0.006
ALH									1.00	0.264 0.261
BCF										1.00

Description: TM (total motility), PM (progressive motility), VAP (velocity of average path), VCL (velocity of curve linear), VSL (straight line velocity), STR (straightness), LIN (linearity), WOB (wobble), ALH (amplitude of lateral head), and BCF (beat cross frequency). **=Correlation is significant at the 0.01 level, *=Correlation is significant at the 0.05 level.

Spermatozoa must possess specific velocity parameters and progressive motility (PM) to reach the oocyte and facilitate fertilization. Bull fertility is correlated with kinematic variables of spermatozoa, such as PM, VSL, VCL, ALH, and LIN (Farrell *et al*., 1996; Perumal *et al*., 2011). Significant bending in the middle and a large amplitude of lateral head displacement are indicated by high VCL and ALH of spermatozoa. This implies that spermatozoa undergo hyperactivation, a higher energy state in spermatozoa, which is necessary for sperm to pass through cervical mucus and fuse with the oocyte (Aitken *et al*., 1985). Sperm velocity and motility are indirect indicators of mitochondrial activity. Certain movement characteristics in bulls have been linked to fertility. (Budworth et al., 1987; P. B. Farrell et al., 1996). However, threshold levels for these movement characteristics still need to be determined to meet the standard criteria for male spermatozoa quality in kinematics.

Compared to the percentage of total motility, CASA variables such as linearity or higher linear motility indicate that spermatozoa have a higher fertilization potential (Cremades *et al*., 2005). Frozen semen samples containing these spermatozoa show a higher fertility and pregnancy rate following artificial insemination (Farrell *et al*., 1998). The minimum required motility percentage in the bovine artificial insemination industry is 50% (Hallap *et al*., 2004). For bovine semen motility standards in Indonesia, the minimum motility that must be achieved is 40%. In this study, the thawing treatment of frozen semen at 37°C for 20 s demonstrated the highest motility, indicating that applying temperature and thawing time is highly suitable for frozen semen from Pesisir bulls.

According to earlier studies, spermatozoa motility tests may not be the most accurate way to determine the quality of frozen semen. Alternatively, more accurate predictions of semen fertility potential have been found using objective and quantitative measurements of different sperm movement characteristics obtained from observing individual sperm cells using CASA (Mortimer, 1994). Kinematic parameters are crucial in spermatozoa development within the female reproductive tract, including navigating through dense molecular cervical mucus and penetrating the oocyte zona pellucida (Verstegen *et al*., 2002).

A positive correlation has been established in vitro between sperm velocity and oocyte fertilization rates in human studies (Donnelly *et al*., 1998). Spermatozoa that succeeded in penetrating showed noticeably higher values in Beat Cross Frequency (BCF) and Velocity Curvilinear (VCL) compared to those that failed (Fetterolf & Rogers, 1990). In addition to helping estimate semen fertility, CASA can be used to examine the effects of different in vitro treatments on sperm motility and the phenomenon of sperm hyperactivation (Farrell *et al*., 1993).

This study's findings differ from those published by other authors (Farrell *et al*., 1998; Kumar *et al*., 2011; Perumal *et al*., 2011). Various factors, such as semen collection method, initial semen quality, thawing procedure, semen processing for CASA, the time elapsed between collection and analysis, instrument settings for sample analysis, the accuracy of the sample chamber, available space, quantity of samples, and the comprehensiveness of field and sperm examination, could contribute to this variation. Each of these elements ensures sufficient statistical sample data is available for analysis (Farrell *et al*., 1995).

Conclusion

Most of the CASA sperm motility and velocity parameters were significantly higher in frozen semen thawed at 37°C for 20 s compared to other thawing treatments. This indicates that the frozen semen of Pesisir bulls, when thawed at a temperature of 37°C for 20 s, maintains structural stability during the thawing process compared to

other thawing treatments. Consequently, the frozen semen of Pesisir bulls thawed at this specific temperature and duration exhibits higher functionality in terms of sperm structure, enabling faster and forward movement. Moreover, the CASA system has demonstrated its utility in routinely evaluating Pesisir bull semen, providing a more accurate measurement of spermatozoa quality.

Conflict of interest

The authors have no conflict of interest to declare. All authors have seen and agree with the contents of the manuscript.

Funding statement

This research was supported by Faculty of Animal Science, Universitas Andalas for the year 2023, with contract number 001.h/UN.16.06.D/PT.01/SPP.RD/FATERNA/2023.

Acknowledgments

The authors are grateful for support by Rio Ramadhan, Harif Gusdinal, and UPTD BPTSD Tuah Sakato, Payakumbuh, Indonesia.

Author's contribution

A analyzed the data and wrote the manuscript. A, H, and J designed the concept, searched for funding, and compiled and reviewed the paper. A and RI oversaw field and laboratory work, conducted field and laboratory work, and performed data tabulation.

Ethics approval

There are no human subjects in this article and informed consent is not applicable. This study was approved by the Administration Committee of Experimental Animals, Universitas Andalas.

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