VOLUME 12 NOMOR 1 JUNE 2025 ISSN 2548 – 611X

JURNAL
BIOTEKNOLOGI & BIOSAINS INDONESIA

Homepage Jurnal: http://ejournal.brin.go.id/JBBI/index

KINEMATICS OF SPERMATOZOA IN FRESH AND FROZEN SEMEN OF PESISIR CATTLE

Kinematika Spermatozoa Semen Segar dan Beku Sapi Pesisir

Rio Ramadhan[1], Ananda[2], Hendri[2]*
[1]Undergraduate Program, Department of Animal Production Technology, Faculty of Animal Science, Universitas Andalas, Padang, Indonesia
[2]Department of Animal Production Technology, Faculty of Animal Science, Universitas Andalas, Padang, Indonesia
*Email: hendri@ansci.unand.ac.id

ABSTRACT
This study aimed to evaluate the quality and kinematics of spermatozoa in fresh and frozen semen of coastal cattle. Semen of coastal cattle was collected using an artificial vagina from 2 males, fresh semen was tested and frozen using tris egg yolk diluent. Fresh and frozen semen of coastal cattle were evaluated using a Computer Assisted Sperm Analyzer (CASA). This study used a t-test analysis by comparing the quality and kinematics of spermatozoa of fresh semen and frozen semen of coastal cattle. The parameters observed were macroscopic, microscopic and CASA quality observations. The results showed the kinematics of fresh and frozen semen motility (83.83%; 71.68%) and the kinematics of progressive motility of fresh and frozen semen (80.81%; 59.56%). Based on the results of the t-test analysis, it was found that t count > t table, the kinematics of fresh semen spermatozoa had a significant effect on the frozen semen of Pesisir cattle on the motility of fresh semen and frozen semen, on the progressive motility of fresh semen and frozen semen. The characteristic values of kinematics in fresh semen and frozen semen of Pesisir cattle were respectively velocity curvilinear (VCL) 178.94 µm/s and 108.52 µm/s; velocity straight line (VSL) 96.61 µm/s and 49.34 µm/s; velocity average path (VAP) 114.99 µm/s and 64.04 µm/s; linearity (LIN) 0.53% and 0.44%; straightness (STR) 0.83% and 0.75%; wobble (WOB) 0.64% and 0.58%; lateral head displacement (ALH) amplitude 5.83 µm and 5.15 µm; beat cross frequency (BCF) 35.28 Hz and 24.65 Hz. The kinematics of fresh and frozen semen spermatozoa significantly affect the value of motility characteristics. The conclusion of the results of this study is that there is a decrease in the quality of fresh semen kinematics and frozen semen kinematics spermatozoa on the value of motility characteristics, namely on the speed of spermatozoa at the velocity curvilinear (VCL), velocity straight line (VSL), and velocity average path (VAP), linearity (LIN), straightness (STR), and wobble (WOB) and on the distance value of spermatozoa amplitude lateral head displacement (ALH) and beat cross frequency (BCF).

Keywords: *CASA, Evaluation, Fresh semen, Frozen semen, Pesisir cattle*

ABSTRAK
Penelitian ini dilakukan untuk mengetahui kualitas dan kinematika spermatozoa pada semen segar dan semen beku sapi Pesisir. Semen sapi Pesisir dikoleksi dengan alat yang digunakan yaitu vagina buatan dari 2 ekor pejantan, semen segar di uji dan dibekukan dengan mengunakan pengencer tris kuning telur. Semen segar dan semen beku sapi Pesisir di evaluasi menggunakan *Computer Assisted Sperm Analyzer* (CASA). Penelitian ini menggunakan analisis uji t yaitu dengan membandingkan kualitas dan kinematika spermatozoa semen segar semen beku sapi Pesisir. Parameter yang diamati adalah pengamatan kualitas *makroskopis,*

***Received**: 25 May 2025* ***Accepted**: 27 May 2025* ***Published**: 10 June 2025*

mikroskpis dan CASA. Hasil penelitian menunjukan kinematika motilitas semen segar dan semen beku (83,83%; 71,68%) dan nilai kinematika motilitas progresif semen segar dan semen beku (80,81%; 59,56%). Berdasarkan hasil analisis uji t didapatkan bahwa $t_{hitung} > t_{tabel}$, kinematika spermatozoa semen segar berpengaruh nyata terhadap semen beku sapi Pesisir terhadap motilitas semen segar dan semen beku, terhadap motilitas progresif pada semen segar dan semen beku. Nilai karakteristik kinematika pada semen segar dan semen beku sapi Pesisir masing-masing adalah *velocity curvilinear* (VCL) 178,94 µm/s dan 108,52 µm/s; *velocity straight line* (VSL) 96,61 µm/s dan 49,34 µm/s; *velocity average path* (VAP) 114,99 µm/s dan 64,04 µm/s; *linearity* (LIN) 0,53 % dan 0,44 %; *straightness* (STR) 0,83 % dan 0,75 %; wobble (WOB) 0,64 % dan 0,58 %; *amplitude lateral head displacement* (ALH) 5,83 µm dan 5,15 µm; *beat cross frequency* (BCF) 35,28 Hz dan 24,65 Hz. Kinematika spermatozoa semen segar dan semen beku berpengaruh nyata terhadap nilai karakteristik motilitas. Kesimpulan dari hasil penelitian ini yaitu terdapat penurunan kualitas spermatozoa kinematika semen segar dan kinematika semen beku pada nilai karakteristik motilitas yaitu pada kecepatan spermatozoa pada kecepatan *velocity curvilinear* (VCL), *velocity straight line* (VSL), dan *velocity average path* (VAP), *linearity* (LIN), *straightness* (STR), dan *wobble* (WOB) dan pada nilai jarak spermatozoa *amplitude lateral head displacement* (ALH) dan *beat cross frequency* (BCF).

Kata Kunci: *CASA, Evaluasi, Kinematika, Sapi Pesisir, Semen beku, Semen segar*

INTRODUCTION

Local cattle contribute significantly to the national economy by supporting livelihoods and fulfilling the demand for animal protein through meat and related products. Compared to imported breeds, indigenous cattle exhibit superior adaptability to low-quality feed, compatibility with traditional farming systems, and resistance to diseases and parasites, although they generally show lower productivity.

Pesisir cattle, one of Indonesia's five indigenous breeds alongside Bali, Aceh, Sumbawa, and Madura cattle, possess valuable traits such as adaptability to poor-quality forage, resilience under extensive farming conditions, and strong disease resistance (Farid, 2018). Despite their small body size, Pesisir cattle efficiently utilize limited space and thrive in tropical coastal regions, making them a promising genetic resource for national beef production. Phenotypically, they are characterized by a brick-red coat, distinct dorsal stripes, and small body conformation, as defined by Indonesian agricultural regulations (Ministry of Agriculture Decree No. 2908, 2011).

However, Pesisir cattle populations are declining due to extensive farming practices, high slaughter rates of productive animals, limited feed availability, and a shortage of breeding males (Udin et al., 2017). Conservation efforts are essential to preserve their genetic diversity and improve productivity, with reproductive management and fertility evaluation as key strategies.

In 2014, conservation initiatives led by BPTU-HPT Padang Mengatas began focusing on maintaining Pesisir cattle as breeding stock. Nonetheless, reproductive performance remains suboptimal, mainly due to uncontrolled natural mating and lack of structured sire selection, which increases inbreeding risks and associated negative effects such as deformities and mortality (Hendrik & Kalinowski, 2000; Meagher et al., 2000).

Artificial insemination (AI) offers a viable approach to reduce inbreeding and enhance reproductive efficiency by optimizing sire utilization (Arifiantini, 2012). However, AI application in Pesisir cattle faces challenges, including the approximately 30% sperm loss during semen cryopreservation that reduces fertility (Susilawati et al., 2016). Moreover, Pesisir semen availability remains limited and has yet to meet national quality standards. Due to their small size, Pesisir cows require breed-specific semen to avoid dystocia, emphasizing the need for high-quality frozen semen from Pesisir bulls (Afriani et al., 2019).

Sperm kinematic analysis has been conducted on various cattle breeds, including Holstein, Brahman, Bali, Madura,

Limousin, Ongole, and Simmental. The most recent study on Pesisir cattle semen using Computer-Assisted Sperm Analysis (CASA) was reported by Wahyudi et al. (2023), which examined sperm kinematics following Bovine Serum Albumin (BSA) treatment. However, baseline data on the kinematics of fresh and frozen semen in Pesisir cattle have not yet been reported. Therefore, this study aims to evaluate the sperm kinematics of fresh and frozen semen of Pesisir cattle as part of efforts to conserve Indonesia's local cattle genetic resources, particularly those originating from West Sumatra.

MATERIALS AND METHOD

Study Location and Period

This study was conducted at the Artificial Insemination Laboratory, UPTD BPTSD Tuah Sakato, Payakumbuh, West Sumatra, from February 1 to August 8, 2024.

Semen Collection

The semen extender used was Tris-egg yolk (TKT). Semen was collected using an artificial vagina (AV) after preparing the bull, teaser animal, restraining chute, and AV set. The AV was assembled, filled with warm water (40–52°C), and lubricated externally up to one-third of its length. Bulls were trained prior to collection to reduce stress and minimize injury. Collected semen was immediately transported to the laboratory for evaluation.

Semen Quality Evaluation

Fresh Semen Evaluation

Fresh semen evaluation included both macroscopic and microscopic assessments. Macroscopically, the volume was measured directly from the collection tube, while color, consistency, and odor were visually and physically assessed.

Microscopic evaluation included mass motility, sperm concentration, progressive motility, viability, morphological abnormalities, and plasma membrane integrity. Mass motility was observed under a light microscope at 100× magnification with reduced light. It was graded based on the strength of wave motion, ranging from no movement (–) to strong wave motion (+++).

Fresh Semen Quality Evaluation of Pesisir Bulls

Fresh semen kinematics of Pesisir cattle were evaluated using a Computer-Assisted Semen Analysis (CASA). A diluted semen sample was placed on a pre-warmed slide and analyzed under a microscope connected to the CASA system. Parameters observed included total motility, progressive motility, VCL (curvilinear velocity), VSL (straight-line velocity), VAP (average path velocity), ALH (amplitude of lateral head displacement), and BCF (beat cross frequency).

Data Analysis

This study involved two treatment groups: fresh and frozen semen from Pesisir cattle. Semen was collected from two bulls, with 16 replicates per treatment. Data were analyzed using independent t-tests to compare the groups. Results are presented as mean ± standard deviation (SD) in tables.

RESULTS AND DISCUSSION

Semen evaluation is a method used to determine the quality of collected spermatozoa.

Table 1. Evaluation of fresh and frozen semen of Pesisir cattle

Parameter	Fresh Semen ± SD	Frozen Semen ± SD
Viability (%)	87.31±1.52	74.10±2.64
MPU (%)	85.51±1.33	72.54±3.40
Abnormalities (%)	3.07±0.83	10.44±1.74

The viability of fresh and frozen semen of Pesisir cattle is higher compared to sperm motility. This is because some spermatozoa are alive but not motile, which lowers the motility value. Muzakkir et al. (2017) reported that the percentage of live spermatozoa is generally higher than the percentage of motile spermatozoa, as some

live sperm are not progressively motile but remain alive and thus are not stained during the viability assessment.

Intact plasma membrane (IPM) refers to spermatozoa with preserved membrane integrity. The average percentage of spermatozoa with intact membranes in fresh and frozen semen of Pesisir cattle, based on 16 collections, was 85.51% for fresh semen and 72.54% for frozen semen. The decline in IPM quality is caused by the increasing duration of storage, which leads to a decrease in the percentage of intact plasma membranes. Susilawati (2011) reported that lipoproteins and lecithin play a role in maintaining and protecting the integrity of the spermatozoa plasma membrane, thereby reducing damage to the membrane.

Spermatozoa abnormalities are physical defects of the sperm cells, which can be classified into two categories: primary and secondary abnormalities. The results of the study on Pesisir cattle semen, obtained from 16 collections, showed an average abnormality rate of 3.07% in fresh semen and 10.44% in frozen semen. In this study, the increase in abnormalities is suspected to be influenced by a decrease in semen pH, osmotic pressure, and the effects of cold shock occurring from the fresh semen stage up to just before the freezing process.

Table 2. Kinematic fresh and frozen semen of Pesisir cattle

Parameter	Fresh Semen ± SD	Frozen Semen ± SD	Difference
VCL (μm/s)	178.94±16.31	108.52±28.83	70.42
VSL (μm/s)	96.61±12.19	49.34±21.22	47.27
VAP (μm/s)	114.99±11.98	64.04±19.72	50.95
LIN (%)	0.53±0.04	0.44±0.07	0.094
STR (%)	0.83±0.02	0.75±0.07	0.084
WOB (%)	0.64±0.03	0.58±0.04	0.053
ALH (μm)	5.83±0.49	5.15±0.73	0.684
BCF (Hz)	35.28±3.00	24.65±6.29	10.625

Velocity or movement speed pattern of spermatozoa in fresh and frozen semen of Pesisir cattle exhibits distinct kinematic characteristics. The average VCL value (curvilinear velocity) for fresh semen from 16 collections was 178.94 μm/s, while for frozen semen it was 108.52 μm/s. The VCL kinematics obtained in this study were lower compared to the results of a previous study by Hendri et al. (2024), which reported a curvilinear velocity of frozen-thawed Pesisir cattle semen of 111.82 ± 8.77 μm/s at 37ºC after 30 seconds. These results are higher than those reported by Sarastina et al. (2006), VCL values of 121.04 μm/s and 117.34 μm/s for Bali and Madura cattle, respectively. The VCL value only indicates the strength of spermatozoa movement but does not provide information about spermatozoa progressiveness or the direction of movement (Perreault, 2002).

The LIN value represents the linearity or straightness of spermatozoa swimming movement (El-Bahrawy et al., 2017). The LIN kinematics obtained in this study were higher compared to the results of a previous study by Hendri et al. (2024), which reported a linearity value of 0.36 ± 0.01 for frozen-thawed Pesisir cattle semen at 37ºC after 30 seconds.

STR (straightness of the linear curve path), was 0.833 for fresh semen and 0.75 for frozen semen. The STR kinematics obtained in this study were higher compared to the results of a previous study by Hendri et al. (2024), which reported a straightness value of 0.65 ± 0.2 for frozen-thawed Pesisir cattle semen at 37ºC after 30 seconds. Frozen semen spermatozoa on average move in a linear manner, as indicated by LIN values greater than 0.35 and STR values greater than 0.5.

The WOB (wobble) value of fresh semen was 0.64 ± %, while that of frozen semen was 0.58 ± %. The WOB kinematics obtained in this study were higher compared to the results of a previous study by Hendri et al. (2024), which reported a wobble value of 0.55 ± 0.01 for frozen-thawed Pesisir cattle semen at 37ºC after 30 seconds. The WOB

value of fresh Pesisir cattle semen in this study was approximately 0.64%, while that of frozen semen was 0.58%. These results are comparable to those of other local cattle breeds, such as Bali and Madura cattle, which had WOB values of 0.60% and 0.62%, respectively (Sarastina et al., 2006).

The ALH (Amplitude of Lateral Head displacement) value is obtained through a mathematical calculation of the maximum excursion distance from the sperm's trajectory and the average path distance. In this study, the ALH value for fresh semen was 5.38 ± μm, while for frozen semen it was 5.15 ± μm. The ALH kinematics obtained in this study were lower compared to the results of a previous study on frozen Pesisir cattle semen conducted by Hendri et al. (2024), which reported an ALH value of 6.25 ± 0.30 μm at 37ºC within 30 seconds. Sperm loses energy due to oxidative stress and decreased seminal plasma quality, resulting in inefficient movement. This energy loss can reduce ALH movement, as the sperm is unable to produce optimal motion. According to Chatiza et al. (2012), changes in movement patterns are caused by flagellar activity, which occurs when spermatozoa undergo capacitation. In this study, the Pesisir bull sperm had not yet undergone the capacitation process. However, the spermatozoa exhibited a shift toward hyperactive motility, indicated by ALH values ≥ 7 μm (Verstegen et al., 2002), and they are capable of penetrating cervical mucus when the ALH reaches 4.5 μm (Mortimer, 1997).

BCF (Beat Cross Frequency) is calculated based on the number of times a spermatozoon crosses its average path per second. In this study, the BCF value of fresh semen was 35.28 ± 3.00 Hz, while that of frozen semen was 24.65 ± 6.29 Hz. The BCF kinematics obtained in this study were higher compared to the findings of a previous study on frozen Pesisir cattle semen conducted by Hendri et al. (2024), which reported a BCF value of 19.36 ± 1.70 Hz at 37ºC within 30 seconds.

The frequency of spermatozoa with progressive motility is 60 hertz; the lower frequency is due to decreased temperature and the cessation of progressive movement (Sarastina et al., 2006). The study by Sarastina et al. (2006) reported that the BCF value for Bali cattle was 30.24 Hz and for Madura cattle was 34.76 Hz. Sarastina et al. (2006) stated that the values of VCL, VAP, VSL, ALH, and BCF are spermatozoa motion variables that serve as indicators of fertility and are highly correlated with the probability of successful pregnancy.

CONCLUSION

Based on the results of this study, it can be concluded that the kinematic evaluation of spermatozoa revealed a decrease in the motility characteristics of fresh semen after the freezing process, as observed using a Computer Assisted Sperm Analyzer (CASA). The velocity of spermatozoa in fresh semen falls into the hyperactive category, while the velocity in frozen semen is classified as low.

ACKNOWLEDGMENTS

The author would like to express sincere gratitude to the Institute for Research and Community Service (LPPM), Universitas Andalas, for funding this research through the Undergraduate Thesis Research Grant (Penelitian Skripsi Sarjana), Contract Number 167/UN16.19/PT.01.03/PSS/2024, dated July 17, 2024. The author also extends appreciation for the support provided by UPTD BPTSD Tuah Sakato, Payakumbuh, Indonesia.

REFERENCES

Afriani, T., P. M. Agusta, Yurnalis, F. Arlina dan P. D. E. 2019. Estimasi dinamika populasi dan pembibitan sapi potong di Kecamatan Bayang, Kabupaten Pesisir Selatan. Jurnal Fakultas Peternakan Universitas Andalas, Padang, Peternakan Indonesia. 21(2): 130-142.

Arifiantini, I. R. 2012. Teknik Koleksi dan Evaluasi Semen Pada Ternak. PT Penerbit IPB Press. Bogor.

Chatiza, F. P., P. Bartels, T. L. Nedambale and G. M. Wagenaar. 2012. Computer assisted sperm analysis of motility patterns of postthawed epididymal sperms of springbok (antidorcas

marsupialis), impala (aepyceros melampus), and blesbok (Damaliscus dorcus philipsi) incubated under conditions supporting domestic cattle *in vitro* fertilization. Theriogenology. 46: 402-414.

El-Bahrawy, K. A. 2017. The influence of caffeine supplementation and concerted utilization of enzymatic and mechanical semen liquefaction on freezability of dromedary camel sperms. International Journal of Veterinary Medicine. 5: 121-127.

Farid. 2018. Pengaruh Waktu Ekuilibrasi terhadap Kualitas Semen Sapi Pesisir Sebelum Pembekuan. Skripsi Fakultas Peternakan. Universitas Andalas, Padang.

Hendri, Jaswandi, R. Indriastuti and Ananda. 2024. Sperm kinematics of pesisir bull thawed at different temperatures and times. Bulletin of Animal Science. doi:10.21059/buletinpeternakv%vi%i.96459.

Hendrik, P. W. and S. T. Kalinowski 2000. Inbreeding depression in conservation biology. Annual Review of Ecology and Systematics. 31: 130-162.

Meagher, S., D. J. Penn and W. K. Potts. 2000. Male-male competition magnifies inbreeding depression in wild house mice. Proceedings of the National Academy of Sciences. 97: 3324-3329.

Menteri Pertanian. 2011. Penetapan Rumpun Sapi Pesisir. Keputusan Menteri Pertanian nomor 2908/Kpts/OT.140/6/2011. Menteri Pertanian. Jakarta.

Mortimer, S. 1997. A critical review of the physiological importance and analysis of sperm movement in mammals. Journal Reprodution Animals. 3: 403-439.

Muzakkir, Dasrul, S. Wahyuni, M. Akmal dan M. Sabri. 2017. Pengaruh lama ekuilibrasi terhadap kualitas spermatozoa sapi Aceh setelah pembekuan menggunakan pengencer andromed. Jurnal Ilmiah Peternakan. 5(2): 115-128.

Perreault, S. D. 2002. Smart use of computer-aided sperm analysis (CASA) to characterize toxicology division. U.S. Environmental Protection Agency National Health and Environmental Effects Research Laboratory.

Sarastina, T. Susilawati dan G. Ciptadi. 2006. Analisa beberapa parameter motilitas spermatozoa pada berbagai bangsa sapi menggunakan computer assisted sperm analyzer (CASA). Jurnal Ternak Tropika. 6(2): 1-12.

Susilawati, T. 2011. Spermatologi. Universitas Brawijaya (UB) Press. Malang. ISBN 978-602-8960-045.

Susilawati. T., N. Isnaini, A. P. A. Yekti, I. Nurjanah, Errico dan D. Costa. 2016. Keberhasilan inseminasi buatan menggunakan semen beku dan semen cair pada sapi peranakan ongole (PO). Jurnal Ilmu-Ilmu Peternakan. 26(3): 14-19.

Udin, Z dan A. Agustar. 2022. Mengenal Sapi Pesisir Rumpun Sapi Lokal. Andalas University Press. Padang.

Wahyudi, D., Z. Udin and Afriani. 2023. Analysis of motility characteristics of Pesisir bulls sexed semen with different pre-freezing methods based on Computer Assisted Sperm Analyzer (CASA). Universitas Andalas, Padang.