



ANALYSIS OF Deoxyribonucleic Acid (DNA) FRAGMENTATION AND ACROSOME INTEGRITY AS BIOMARKERS OF FROZEN SEMEN QUALITY OF BULLS AT THE SPERM BANK OF SAMARINDA CITY

Analisis Biomarker Fragmentasi Deoxyribonucleic Acid (DNA) dan Status Akrosom pada Semen Beku Sapi yang Disimpan di Bank Sperma Kota Samarinda

Kirana Dara Dinanti Adiputra^{1*}, Novemia Fatmarischa¹, Dani Nur Arifin¹, Fikri Ardhani¹, Kumbawan Wibisono², Novi Ramadhani², Erni Damayanti³

¹Department of Animal Science, Faculty of Agriculture, Mulawarman University, Samarinda, Indonesia

²Food Security and Agriculture Office, Samarinda City Government, Indonesia

³Department of Animal Production, Faculty of Animal Science, Universitas Hasanuddin, Makassar, Indonesia

*Email: kiranadaradinanti28@gmail.com

ABSTRACT

This study aims to evaluate the quality of frozen bull semen at the Sperm Bank of Samarinda City based on parameters of motility, viability, morphological normality, plasma membrane integrity (PMI), acrosome integrity, and DNA fragmentation, as well as their effects on cattle breeds and storage duration. The samples consisted of frozen semen from five breeds (Limousin, Madura, Angus, Simmental, and Peranakan Ongole) with different storage periods (3, 4, and 5 years). Motility, viability, and morphological normality were tested according to the Indonesian National Standards (SNI). The PMI was assessed using the hypo-osmotic swelling test (HOST), acrosome integrity using peanut agglutinin (PNA) staining, and DNA fragmentation using acridine orange (AO) staining. Data were analyzed using two-way ANOVA, and if there was an effect, it was followed by a Tukey post hoc test. The findings indicated that motility and PMI remained relatively stable; however, the duration of storage significantly influenced PMI ($P < 0.05$). The breed of bull and storage duration had a significant effect on morphological normality, viability, acrosome integrity, and DNA fragmentation ($P < 0.05$). However, the two factors did not interact with each other. It can be concluded that the storage duration resulted in a decline in semen quality, specifically in terms of membrane and DNA integrity. Therefore, it is recommended to use semen straws ≤ 4 years old and conduct periodic quality re-qualification to maintain frozen semen fertility.

Keywords: *Acrosome Integrity, Breed, DNA Fragmentation, Frozen Semen, Storage Duration*

ABSTRAK

Penelitian ini bertujuan mengevaluasi kualitas semen beku sapi di Bank Sperma Kota Samarinda berdasarkan parameter motilitas, viabilitas, normalitas morfologi, membran plasma utuh (MPU), status akrosom dan fragmentasi DNA, serta pengaruhnya terhadap bangsa sapi dan lama masa simpan. Sampel terdiri dari semen beku lima bangsa sapi (Limousin, Madura, Angus, Simental, dan Peranakan Ongole) dengan lama masa simpan yang berbeda (3, 4 dan 5 tahun). Parameter motilitas, viabilitas dan abnormalitas morfologi diuji sesuai standar SNI; membran plasma utuh (MPU) dianalisis dengan metode *Hypo-osmotic Swelling Test* (HOST); status akrosom dengan pewarnaan *peanut agglutinin* (PNA); dan fragmentasi DNA dengan pewarnaan *acridine orange* (AO). Data dianalisis dengan ANOVA dua arah, dan jika terdapat pengaruh diikuti *post hoc test Tukey*. Hasil penelitian menunjukkan bahwa motilitas dan MPU

relatif stabil, namun lama masa simpan berpengaruh nyata terhadap MPU ($P < 0,05$). Sementara itu, normalitas, viabilitas, status akrosom, dan fragmentasi DNA dipengaruhi secara signifikan oleh bangsa sapi dan lama masa simpan ($P < 0,05$), meskipun tidak terdapat interaksi antara kedua faktor tersebut. Disimpulkan bahwa kualitas semen menurun seiring lama penyimpanan, terutama pada integritas membran dan DNA. Disarankan penggunaan straw ≤ 4 tahun dan re-kualifikasi mutu berkala untuk menjaga fertilitas semen beku.

Kata kunci: *Bangsa Sapi, Fragmentasi DNA, Lama Masa Simpan, Status Akrosom, Semen Beku*

INTRODUCTION

Enhancing livestock productivity has emerged as a principal objective in the advancement of Indonesia's livestock sector. Artificial insemination (AI) is one of the reproductive technologies long relied upon to support this goal (Adiputra et al., 2022). The success of AI programs is largely determined by the quality of the frozen semen used (Wanma et al., 2022). However, the field generally still focuses on conventional indicators like the motility and morphology of frozen semen for quality assessment. However, more fundamental markers related to genetic material stability and functional structure integrity of sperm are often overlooked (Adiputra et al., 2023; Brillianti et al., 2021).

DNA damage in sperm is known to reduce fertilization and inhibit embryo development (Gustina et al., 2024). Conversely, the acrosome is essential for penetrating the zona pellucida of the oocyte; damage or loss of acrosome function can inhibit fertilization, even if the sperm appears morphologically normal (Adiputra et al., 2022; Syafi'i & Rosadi, 2022; Baharun et al., 2023; Sophian et al., 2025). Therefore, these two biomarkers can serve as more accurate molecular indicators to assess frozen semen quality.

The AI program in East Kalimantan Province, specifically in Samarinda City, is rapidly expanding to accommodate the growing cattle population. Based on iSIKHNAS (Integrated National Animal Health System) data validated by the East Kalimantan Provincial Livestock and Animal Health Service (2024), the dynamics of AI success rates show a decline from 25.86% in 2022 to 10.10% in 2023, followed by an increase to 45.36% in 2024 (Adiputra et al., 2025). Although improving, this achievement remains

below the national standard considered adequate at around 50% and far from the ideal range in several developed countries, which is around 65–75% (Masrurroh et al., 2019).

Previous studies have shown that high DNA fragmentation correlates with decreased fertilization rates and poorer early embryo development, whereas good acrosome integrity positively correlates with higher fertilization rates (Ardhani et al., 2020; Rangkuti et al., 2021). Reports indicate that the cryopreservation process, breed, and storage duration in liquid nitrogen, in addition to biological factors in the bulls, influence both biomarkers. However, local evidence that comprehensively examines DNA fragmentation and acrosome integrity in frozen semen stored at the Sperm Bank of Samarinda City, while also evaluating the effects of breed and storage duration, remains limited.

Therefore, the novelty of this study lies in providing contextual data that can be directly adopted to strengthen the quality assurance of the semen bank as a basis for operational AI decision-making in the region, thereby supporting improved reproductive efficiency and livestock productivity in Samarinda, East Kalimantan.

MATERIALS AND METHODS

Study Location

The study used frozen semen samples obtained from the Sperm Bank of the Food Security and Agriculture Agency (DIS-TAPANGTANI) in Samarinda City, East Kalimantan (geographical coordinates: $-0.502106, 117.153709$). The sperm bank functions as a storage and distribution center for frozen semen used in artificial insemination (AI) programs. The frozen semen came from the Singosari National Artificial

Insemination Center (Singosari-NAIC) in Malang, East Java. Laboratory analysis was conducted at the *In Vitro* Embryo Laboratory of Hasanuddin University, Makassar, South Sulawesi (geographical coordinates: -5.132618, 119.488578).

Experimental Materials

The study material consisted of frozen semen samples from five different breeds of bulls, namely Simmental, Limousin, Madura, Peranakan Ongole, and Angus, produced in 2020, 2021, and 2022 (storage periods of 3, 4, and 5 years). All samples were stored in the Sperm Bank in containers containing liquid nitrogen (LN₂) at -196°C until further examination.

The reagents used in this study included eosin–nigrosine solution (0.067 g eosin and 1.0 g nigrosin dissolved in 10 mL of 0.9% NaCl solution); hypo-osmotic solution (HOS) (0.147 g sodium citrate dihydrate and 0.270 g fructose in distilled water up to 20 mL); acridine orange (AO) (Sigma-Aldrich, St. Louis, MO, USA); peanut agglutinin (PNA) (Sigma-Aldrich, St. Louis, MO, USA); and Carnoy’s fixative (methanol : acetic acid, 3:1).

Research Method

The study used a completely randomized design (CRD) factorial with treatment combinations based on the bull's breed (Simmental, Limousin, Madura, Peranakan Ongole, and Angus) and storage duration (3, 4, and 5 years), with replicates for each combination.

Semen was evaluated at the *In Vitro* Embryo Laboratory, Hasanuddin University. The samples were SNI-certified frozen semen straws of beef cattle from the Sperm Bank of Samarinda. Before analysis, semen samples were thawed for 15–20 seconds in

accordance with Indonesian National Standard (SNI, 2024).

The observed parameters included post-thaw motility, viability and morphological normality assessed using *eosin–nigrosine* staining; plasma membrane integrity (PMI) analysed using the *hypo-osmotic swelling test* (HOST); DNA fragmentation assessed by *acridine orange* (AO) staining; and acrosome integrity assessed using *peanut agglutinin* (PNA) staining.

Statistical Analysis

Observation data were analysed using a two-way analysis of variance (ANOVA) based on a factorial completely randomized design (CRD) to determine the effect of breed and storage duration, as well as the interaction between the two, on the evaluated parameters. When significant differences among treatments were detected (P < 0.05), Tukey’s post hoc test was applied at a 95% confidence level.

The results are presented as mean ± standard deviation (mean ± SD). Statistical analysis was performed using IBM SPSS Statistics Version 29 (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

Frozen Semen Quality of Bulls from Different Breeds at Various Storage Durations

As a basis for assessing the fertility potential of bulls through artificial insemination, a multi-parameter evaluation was conducted to examine post-thawing motility, normality, viability, plasma membrane integrity (PMI), acrosome integrity, and DNA fragmentation in five bull breeds stored for 3–5 years. Table 1 presents the analysis results.

Table 1. Frozen Semen Quality of Bulls from Different Breeds at Various Storage Durations

Parameters	Bulls	Time Storage Duration			Average
		3 years	4 years	5 years	
Motility (%)	Limousin	55.06±3.15	54.27±5.01	56.00±1.68	55.11±3.30
	Madura	55.06±3.15	54.27±5.01	56.84±4.61	55.39±4.08
	Angus	57.27±4.05	57.00±2.16	56.89±0.80	57.05±2.44
	Simmental	57.36±1.94	56.33±0.48	57.82±2.60	57.17±1.83
	Peranakan Ongole	58.41±2.60	55.84±1.01	58.88±5.53	57.70±3.52
	Average	56.63±3.05	55.54±3.19	57.28±3.29	56.49±3.20

Parameters	Bulls	Time Storage Duration			
		3 years	4 years	5 years	Average
Viability (%)	Limousin	81.41±0.51 ^{ab}	81.41±0.51 ^{ab}	81.21±1.31 ^a	81.34±0.79 ^p
	Madura	81.41±0.51 ^{ab}	81.41±0.51 ^{ab}	82.41±1.34 ^{ab}	81.74±0.93 ^{pq}
	Angus	84.21±1.16 ^{abc}	84.56±1.22 ^{abc}	81.55±0.38 ^{ab}	83.44±1.67 ^{qr}
	Simmental	87.05±2.06 ^c	83.88±2.48 ^{abc}	82.15±1.90 ^{ab}	84.36±2.88 ^r
	Peranakan Ongole	84.52±1.87 ^{abc}	85.28±3.31 ^{bc}	82.31±0.73 ^{ab}	84.04±2.41 ^r
	Average	83.72±2.50 ^y	83.31±2.40 ^y	81.93±1.21 ^x	82.98±2.22 [*]
Normality (%)	Limousin	83.19±1.75 ^{ab}	81.02±0.48 ^a	80.68±1.40 ^a	81.63±1.67 ^p
	Madura	83.19±1.75 ^{ab}	81.02±0.49 ^a	82.19±1.27 ^a	82.13±1.48 ^{pq}
	Angus	81.33±0.48 ^a	81.43±0.53 ^a	81.18±0.64 ^a	81.31±0.51 ^p
	Simmental	81.93±0.18 ^a	81.89±0.10 ^a	81.60±1.95 ^a	81.81±1.04 ^p
	Peranakan Ongole	85.91±2.91 ^b	83.15±0.48 ^{ab}	80.98±1.18 ^a	83.35±2.68 ^q
	Average	83.11±2.23 ^y	81.70±0.90 ^x	81.33±1.32 ^x	82.05±1.74 [*]
Plasma Membran Integrity (%)	Limousin	85.68±1.66	86.22±0.81	80.57±1.62	84.16±2.95
	Madura	85.68±1.66	86.22±0.81	81.27±1.21	84.39±2.59
	Angus	87.15±4.62	83.25±2.16	80.63±0.42	83.67±3.87
	Simmental	82.25±0.60	83.54±1.72	81.37±1.68	82.39±1.60
	Peranakan Ongole	85.16±4.09	86.20±2.05	81.50±1.34	84.29±3.26
	Average	85.18±3.11 ^y	85.09±2.02 ^y	81.07±1.24 ^x	83.78±2.94
Acrosome Integrity (%)	Limousin	90.03±3.41	80.69±2.98	79.82±1.46	83.51±5.43 ^p
	Madura	90.03±3.41	80.69±2.98	83.10±6.69	84.60±5.91 ^p
	Angus	87.69±0.75	80.77±2.95	80.35±2.35	82.94±4.05 ^p
	Simmental	89.24±2.04	89.52±1.53	87.45±5.20	88.74±3.17 ^q
	Peranakan Ongole	88.66±2.38	79.64±2.00	81.87±1.38	83.39±4.39 ^p
	Average	89.13±2.48 ^y	82.26±4.39 ^x	82.52±4.55 ^x	84.64±5.01
DNA Fragmentation (%)	Limousin	11.76±1.46	11.26±7.41	16.53±3.12	13.18±4.94 ^{pq}
	Madura	11.76±1.46	11.26±7.41	17.80±9.49	13.60±7.05 ^{pq}
	Angus	14.32±2.83	13.30±0.67	20.07±0.98	15.90±3.50 ^r
	Simmental	11.28±4.50	6.62±2.02	10.63±4.06	9.51±3.97 ^p
	Peranakan Ongole	14.72±3.02	20.39±2.82	16.80±2.99	17.30±3.61 ^r
	Average	12.77±2.96 ^y	12.56±6.36 ^y	16.37±5.50 ^x	13.90±5.36

Notes:

Different lowercase superscripts in the same column (p, q, r) indicate significant differences among bull breeds ($p < 0.05$). Different lowercase superscripts in the same row (x, y) indicate significant differences among storage durations of frozen semen ($p < 0.05$). Different lowercase superscripts (a, b, c) within the same row or column indicate significant interactions between breed and storage duration ($p < 0.05$). The values are presented as the mean \pm standard deviation (mean \pm SD).

Sperm Motility

The results indicated that the average post-thaw sperm motility ranged from 55.11±3.30% to 57.70±3.52%, which remains within the acceptable range for use in artificial insemination. The highest value was obtained from the Peranakan Ongole bulls (57.70±3.52%), while the lowest was found in the Limousin bulls (55.11±3.30%). Based on the storage duration, motility after 3, 4, and 5 preservations was 56.63±3.05%, 55.54±3.19%, and 57.28±3.29%, respectively. The results of the analysis of variance indicated that the breed of bull,

storage duration, and their interaction had no significant effect ($P > 0.05$) on sperm motility.

The stability of motility values across treatments indicated that frozen semen from various breeds, stored for up to 5 years, did not significantly reduce sperm motility, thus satisfying the minimum eligibility criterion (40%) set by the Indonesian National Standard (2024).

The present findings show higher or better motility when compared to Goshme et al. (2021), which reported 31.5–41% in frozen semen of bovine. Low frozen semen

motility can be influenced by several factors, including the distribution and filling process of straws, the thawing technique used, the frequency of liquid nitrogen refilling, and the management of storage containers (Witri et al., 2025).

Sperm Viability

Analysis of variance shows that the bull breed, storage duration, and their interaction significantly affected ($P < 0.05$) sperm viability. The average viability decreased from $83.72 \pm 2.50\%$ at 3 years of storage to $81.93 \pm 1.21\%$ at 5 years of storage. Simmental and Peranakan Ongole bulls showed the highest viability ($84.36 \pm 2.88\%$ and $84.04 \pm 2.41\%$, respectively), indicating better resistance of the sperm plasma membrane to cold shock during freezing. The highest value ($87.05 \pm 2.06\%$) was found in Simmental bulls that had been stored for 3 years, and the lowest value ($81.21 \pm 1.31\%$) was found in Limousin bulls that had been stored for 5 years.

Differences in viability among cattle breeds indicate diversity in the ability of sperm plasma membranes to maintain their function and integrity during the

cryopreservation process. Conversely, decreased sperm viability in frozen semen stored for longer periods is thought to be associated with a decrease in membrane phospholipid levels and an increase in free radical accumulation, reduced tolerance to osmotic stress, and ice crystal formation during freezing and thawing. According to Prihantoko et al. (2022) and Gallo et al. (2021), elevated levels of reactive oxygen species (ROS) during the freezing process, particularly during the equilibration stage, can cause damage to the plasma membrane, which leads to a decrease in viability.

Sperm with more stable membrane structures, such as those of the Simmental and Peranakan Ongole bulls, exhibit greater resilience to oxidative stress and cold shock, thus maintaining a higher proportion of live cells after thawing. In addition to genetic factors, these differences may also be due to the initial quality of fresh semen, the effectiveness of cryoprotectants in the diluent, and the consistency of the freezing and liquid nitrogen storage procedures used. Figure 1 illustrates the comparison between live and dead sperm.



Figure 1. Red arrows indicate dead sperm; black arrows indicate live sperm

The results of this study showed higher sperm viability compared to those reported by Ardhani et al. (2020), with frozen semen stored for 3 and 5 years maintaining viability above 50%. According to Adiputra et al. (2025), sperm viability after thawing must be in the range of 60%–75% live cells to still meet the requirements as insemination material suitable for use in production.

Sperm Morphological Normality

Analysis of variance shows that the sperm morphological normality of frozen semen is influenced by the bull breed, storage

duration, and the interaction between breed and storage duration ($P < 0.05$). This suggests that genetic variations among cattle breeds influence the ratio of normal sperm following the freezing and storage procedure. This finding is consistent with Khoirunisa et al. (2024), who found that differences in cattle breeds can affect semen quality, including sperm morphology normality.

The decline in morphological normality with increasing storage duration indicates a higher proportion of abnormal sperm, which is mainly caused by osmotic stress and extreme temperature exposure during

cryopreservation. Primary abnormalities in sperm generally include head shapes such as oversized heads (macrocephaly), undersized heads (microcephaly), short or broad heads, the presence of two tails on a single sperm cell, and short tails. Meanwhile, secondary abnormalities usually arise due to the storage process or during the

cryopreservation stage. These types of abnormalities include folded tails, the appearance of cytoplasmic granules in the proximal or distal parts, the release of the acrosome from the head without the tail, and the separation of the tail from the sperm body (Patmawan et al., 2020). Figure 2 displays the sperm morphological abnormalities.

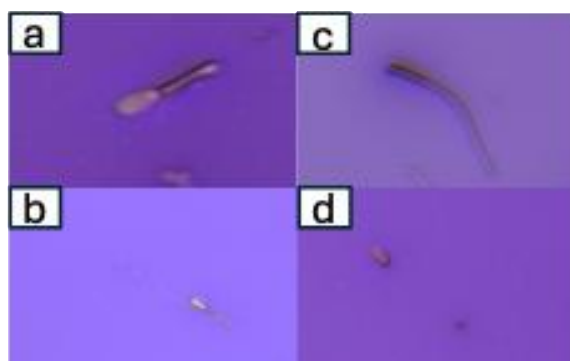


Figure 2. (a–b) Primary abnormalities; (c–d) Secondary abnormalities.

The average normal morphology in this study ranged from 80.68% to 85.91%. The highest value was obtained in Peranakan Ongole ($85.91 \pm 2.91\%$) stored for 3 years, while the lowest value was found in Limousin ($80.68 \pm 1.40\%$) with a storage duration of 5 years. Thus, the range of abnormality values was 14%–19%. The decrease in normal morphology could be caused by subcellular damage due to exposure to free radicals and changes in membrane protein stability. The values obtained in this study show higher abnormalities compared to Dianyah et al. (2022), where Bali, Brahman, Limousin, and Simmental cattle ranged from 13 to 15%. However, the results of this study are still considered acceptable for artificial insemination, as the abnormality rate remained below the 20% threshold (Pasyah et al., 2021).

Sperm Plasma Membrane Integrity (PMI)

The findings demonstrated that the percentage of plasma membrane integrity (PMI) in frozen-thawed bull semen diminished as the duration of storage increased. The highest PMI value was found in 3-year storage at $85.18 \pm 3.11\%$, followed by 4-year

storage at $85.09 \pm 2.02\%$, and the lowest in 5-year storage at $81.07 \pm 1.24\%$. Analysis of variance indicated that storage duration had a significant effect ($P < 0.05$), but the breed and the interaction between storage duration and breed had no significant effect ($P > 0.05$) on PMI. This finding indicates that PMI is influenced more by storage duration than by breed variations among bulls. Prolonged storage may cause structural damage due to the cryopreservation process and prolonged exposure to liquid nitrogen temperatures (Min et al., 2025).

The plasma membrane integrity (PMI) in this study was higher than that reported by Bahmid et al. (2023), who found a 37% IPM percentage in frozen semen from Bali cattle. The PMI percentage in the sperm of each cattle breed was still above 85%. Genetic factors inherited from parents likely influence the variation in values among individuals, but they are still considered advantageous. This finding aligns with Abbaspour et al. (2022), who stated that semen can be categorized as high quality if the PMI is at least 60%. Figure 3 displays the integrity of the plasma membrane.

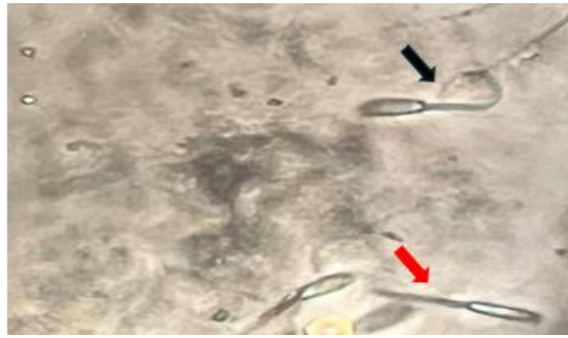


Figure 3. The red arrow shows that the plasma membrane is not intact, and the black arrow shows the plasma membrane integrity

The plasma membrane integrity is closely associated with the progressive motility of active sperm. A plasma membrane integrity is essential for sperm function, as any structural damage may disrupt metabolic processes and negatively affect both motility and viability (Arvioges et al., 2021).

Sperm Acrosome Integrity

The findings demonstrated that acrosome integrity varied significantly among bull breeds ($P < 0.05$) and diminished with prolonged frozen semen storage duration ($P < 0.05$). However, the interaction between breed and storage duration had no significant effect ($P > 0.05$). Simmental cattle had the best acrosome integrity ($88.74 \pm 3.17\%$), while Angus cattle had the worst ($82.94 \pm 4.05\%$). Based on storage duration, there was a decrease in acrosome integrity

from $89.13 \pm 2.48\%$ in 3-year storage to $82.52 \pm 4.55\%$ in 5-year storage. These results indicate that the genetic factors of the cattle breed and the length of storage affect the quality and stability of the acrosome structure, which reflects the overall integrity of the sperm membrane in frozen semen.

Acrosome integrity is closely associated with the condition of the cell membrane. Damage to the plasma membrane can reduce acrosome integrity and may lead to deficiencies in essential proteins, such as protamine (Handarini et al., 2024). In this study, acrosome integrity was denoted by a yellow or yellowish hue at the apex of the sperm head, whereas damaged acrosomes were signified by a red hue on the sperm head. Visualization of acrosome status is presented in Figure 4.

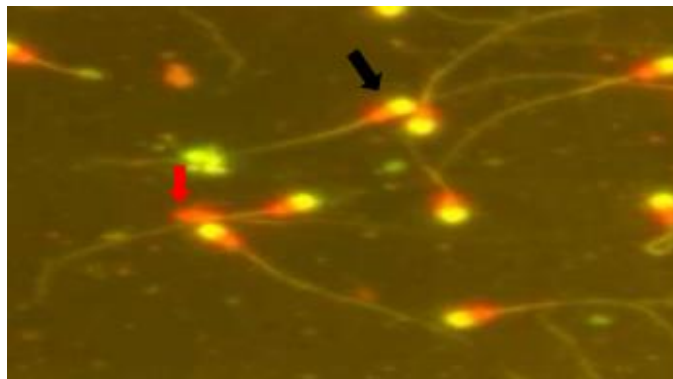


Figure 4. Red arrow indicates a damaged acrosome; the black arrow indicates an intact acrosome

Damage or alterations to the acrosomal cap represent a form of abnormality in the sperm head. Damage to the acrosome cap reduces the ability of sperm to fertilize, because it contains important hydrolytic enzymes that are necessary for the sperm to penetrate the zona pellucida of the oocyte,

thus directly implying a decrease in the fertility of frozen semen (Aldini et al., 2022).

Sperm DNA Fragmentation

The results indicated that sperm DNA fragmentation in frozen-thawed semen was significantly affected by cattle breed and

storage duration ($P < 0.05$), although the interaction between these two factors was not significant ($P > 0.05$). The lowest DNA fragmentation was found in Simmental ($9.51 \pm 3.97\%$), and the highest was found in Peranakan Ongole ($17.30 \pm 3.61\%$). The average sperm DNA fragmentation increased with storage duration, from $12.77 \pm 2.96\%$ at 3 years of storage to $16.37 \pm 5.50\%$ at 5 years of storage. These findings indicate that prolonged storage tends to increase sperm DNA damage due to oxidative stress during cryopreservation.

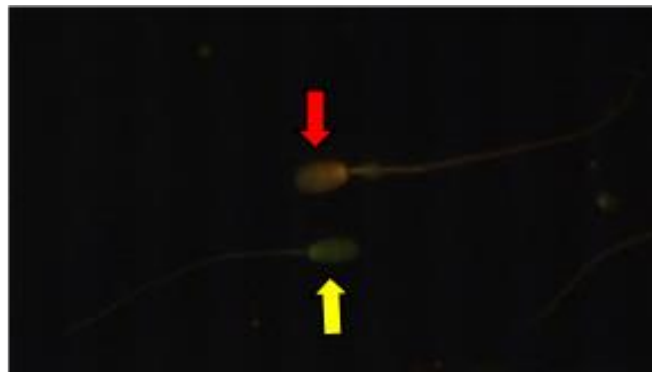


Figure 5. Red arrow indicates DNA fragmentation; the yellow arrow indicates DNA integrity

The DNA fragmentation observed in this study exceeded the approximately 96% reported by Baharun et al. (2025) in Donggala cattle. The main factors causing DNA damage in sperm cells are reactive oxygen species (ROS), generally produced by sperm undergoing apoptosis. Baity et al. (2024) added that sperm oxidation causes a high frequency of single and double DNA strand breaks, and oxidative stress conditions can alter the structure of nucleoproteins, disulfide bonds, and protamine-DNA complexes.

Sperm DNA integrity is an important indicator of male fertility in livestock. DNA damage not only affects fertilization capacity but is also associated with overall semen quality, making the maintenance of sperm DNA stability a key focus in semen reproduction and cryopreservation management (Kumaresan et al. 2017).

CONCLUSION

The breed of cattle and the duration of storage affect the quality of frozen bull semen at the Sperm Bank of Samarinda City.

Increased DNA fragmentation in semen stored for longer durations may be caused by the accumulation of oxidative stress and cellular damage due to free radicals during storage (Min et al., 2025). In addition, Safa et al. (2025) and Le et al. (2019) explain that freezing and thawing semen can trigger the formation of ice crystals that damage the sperm plasma membrane and disrupt organelle function, thereby increasing DNA fragments. Figure 5 displays DNA fragmentation.

Parameters such as sperm morphological normality, viability, acrosome integrity, and DNA fragmentation show significant differences between breeds and storage durations, while motility and intact plasma membrane remain relatively stable despite a slight decline during longer storage.

The quality of the semen tends to go down the longer it is stored. The best performance is seen after 3–4 years of storage, especially in terms of membrane integrity and DNA. Therefore, for operational reasons, it is best to use straws that have been stored for less than four years and to verify their quality every year.

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