CarakaTani
Journal of Sustainable Agriculture
ISSN 2613-9456 (Print) 2599-2570 (Online)

Caraka Tani: Journal of Sustainable Agriculture, 41(1), 32-45, 2026
URL: https://jurnal.uns.ac.id/carakatani/article/view/108422
DOI: http://dx.doi.org/10.20961/carakatani.v41i1.108422

Regulation of Reactive Oxygen Species by Sodium Selenite in AndroMed® Extender Enhances Post-Thaw Saanen Buck Semen Quality

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Abstract

Semen cryopreservation is known to cause considerable damage to spermatozoa through the generation of free radicals. This damage can potentially be mitigated by the use of potent antioxidants, such as sodium selenite, which does not impair semen quality when administered in small doses. This study aimed to assess the impact of low-dose sodium selenite supplementation on post-thaw semen quality in Saanen bucks. Semen was collected from 2 superior Saanen bucks and evaluated according to standard criteria before dilution and freezing. Cryopreserved semen was stored in liquid nitrogen (LN_2) at a temperature of -196 °C for 90 days. The experimental treatments involved the addition of sodium selenite to the AndroMed® diluent medium at 3 different concentrations: without supplementation/control (C), 5 ppm (T1), and 10 ppm (T2). Frozen semen was thawed at 37 °C in a water bath for 30 seconds before post-thaw quality assessment. The parameters assessed included motility, viability, abnormalities, plasma membrane integrity (PMI), acrosome membrane integrity (AMI), and reactive oxygen species (ROS). The data were statistically analyzed using One-Way analysis of variance (ANOVA). The results showed that the addition of sodium selenite had a significant effect ($p < 0.05$) on motility, viability, PMI, AMI, and ROS production, but did not affect the abnormality ($p > 0.05$). Interestingly, T1 resulted in the most substantial improvements, yielding the highest percentages of post-thaw motility (71.81±1.05%), viability (73.36±1.08%), PMI (77.49±1.68%), and AMI (79.29±0.63%), as well as the lowest ROS production (13.67±0.50%). In conclusion, the addition of 5 ppm sodium selenite enhances post-thaw semen quality in Saanen bucks.

Keywords: frozen semen; ROS; Saanen buck; sodium selenite; sperm quality

Cite this as: Erlindra, C. P., Diatmono, D. F. F., Padmawati, F. G., Sastranegara, A. D. N., Sitaresmi, P. I., Bintara, S., & Widayati, D. T. (2026). Regulation of Reactive Oxygen Species by Sodium Selenite in AndroMed® Extender Enhances Post-Thaw Saanen Buck Semen Quality. *Caraka Tani: Journal of Sustainable Agriculture*, *41*(1), 32-45. doi: http://dx.doi.org/10.20961/carakatani.v41i1.108422

INTRODUCTION

The majority of livestock farming in Indonesia is predominantly managed by smallholder farmers employing traditional rearing systems. Among livestock, Saanen goats have gained popularity among local farmers. These goats, which originated from the Saanen Valley in Switzerland, exhibit high adaptability to tropical climates (Khandoker et al., 2018). To improve the genetic quality and performance of Saanen goat populations, strategic selection based on genetic, productive, and reproductive traits is necessary. Optimal reproductive efficiency can be attained

[*] *Received for publication August 25, 2025*
Accepted after corrections December 11, 2025

by implementing effective and well-structured reproductive management practices. Specifically, the utilization of sophisticated reproductive biotechnologies such as artificial insemination (AI) (Diskin and Kenny, 2016).

AI serves as a highly effective reproductive biotechnology tool for the widespread dissemination of superior male germplasm, especially for farmers in remote areas (Mathewos et al., 2023; Warman et al., 2025). The implementation of AI, when integrated with a comprehensive record management system, can effectively reduce the risk of inbreeding in livestock populations. Furthermore, the quality of frozen semen is one of the critical factors that significantly influence the success rate of AI programs (Yeste et al., 2017).

Frozen semen is produced by initially collecting fresh semen, followed by dilution with various types of extenders. The selection of specific extenders and cryopreservation protocols is contingent upon the kind of livestock species (Hollinshead and Hanlon, 2017). During semen freezing and thawing, oxidative stress is a key mechanism underlying sperm cryoinjury. The cryopreservation process disrupts the balance between reactive oxygen species (ROS) generation and antioxidant defenses, making spermatozoa more vulnerable to lipid peroxidation of their membranes (Wang et al., 2025). At the same time, physical factors, such as osmotic stress, ice crystallization, and the specific lipid composition of the sperm membrane, can modify membrane fluidity, thereby reducing motility and viability. This overall process reduces sperm quality and consequently impairs fertility (Jhamb et al., 2023). It is because spermatozoa experience oxidative stress, which can trigger the formation of ROS.

ROS are produced from an unstable and extremely reactive oxidized oxygen derivative because it possesses one or more unpaired electrons on its outermost orbital (Fitriana et al., 2025). While ROS are naturally formed and provide a functional effect for spermatozoa to balance the oxidation and reduction, excessive production leads to increased cell damage and decreased spermatozoa quality (Bui et al., 2018; Fitriana et al., 2025). It has also been established that ROS production in post-thaw frozen semen is higher than that in fresh semen (Baity et al., 2024; Bintara et al., 2025). The presence of ROS is a determining factor for damage to the spermatozoa membrane, so it needs to be minimized by adding antioxidants to the diluent (Bui et al., 2018).

Spermatozoa have limited antioxidant capacity, making them vulnerable to excessive ROS production, which leads to oxidative stress, particularly during cryopreservation. This stress causes lipid peroxidation in the sperm membrane and DNA damage, impairing fertilization potential, and is regulated by enzymatic antioxidants like glutathione (Cardenas-Padilla et al., 2024). Antioxidants are chemical compounds that are used to protect the biological system against the potentially harmful effects of oxidation reactions (Su et al., 2019; Authaida et al., 2025). They prevent cellular damage by interrupting the chain reactions of free radicals and mitigating the detrimental effects of oxidants within the organism (Authaida et al., 2025).

Selenium, specifically sodium selenite, is one form of antioxidant that has the potential to counteract the formation of ROS. This is because sodium selenite serves as a cofactor in glutathione peroxidase (GPx), which is an antioxidant enzyme (Andrade et al., 2021). The enzymatic activity of GPx mitigates lipid peroxidation in cellular membranes and preserves mitochondrial integrity by forming protective cross-links with associated proteins (Khalil et al., 2019). A study conducted by Dolník and Mudroňová (2021) shows that selenium is an important compound in selenoprotein components that influence biological processes, spermatogenesis, and semen quality. Sodium selenite is required as an oral additive to protect and maintain spermatozoa quality during cryopreservation, as selenium deficiency can result in 20 to 40% of infertility in human spermatozoa (Alahmar, 2023). However, the incorporation of selenium in semen extenders, particularly in small ruminants, necessitates further exploration, especially when administered at low dosages.

Several studies have reported the effect of selenium addition, showing improvements in sperm quality across various species, particularly in terms of motility, morphology, viability, and deoxyribonucleic acid (DNA) integrity. The study of Rezaeian et al. (2016) observed the most significant improvement in human sperm motility and viability in the group treated with selenium at a dose of 5 ppm after freezing and thawing procedures. The study by Zubair et al. (2015) added that the level of 2 ppm selenium can improve the quality of fresh and frozen semen in male buffaloes, rats, humans, sheep, cattle,

and other species. In addition, long-term exposure to selenium in animals showed improvement in semen quality (El-Sharawy et al., 2017).

To date, no in-depth study has investigated the addition of sodium selenite as an antioxidant to AndroMed® diluent for post-thaw semen production in Saanen bucks. Therefore, based on this prior knowledge, this study is crucial for evaluating the ability of antioxidants to maintain post-thawing semen quality in Saanen bucks. Also, to determine whether the addition of sodium selenite at concentrations of 5 or 10 ppm has a positive impact, including on ROS production as an indicator of oxidative status during cryopreservation.

MATERIALS AND METHOD

Ethical approval

The management of the bucks and sample collection were conducted in accordance with the standards of the Artificial Insemination Center (BIB) Lembang. The BIB Lembang testing laboratory maintains national recognition and international accreditation (SNI ISO/IEC 17025:2017). Furthermore, all procedures within this study were approved by the Research Ethics Committee (3/EC-FKH/int./2024), Faculty of Veterinary Medicine, Universitas Gadjah Mada (UGM), which was valid for the duration of the study.

Animals used, research period, and study locations

This study included 2 superior Saanen bucks. Saanen bucks were provided a daily feeding regimen consisting of 0.45 kg of concentrate, administered twice daily (6:15 to 7:00 AM and 10:00 to 11:00 AM). Forage was offered 3 times daily, 2 kg in the morning (7:30 to 8:30 AM) and afternoon (3:30 to 5:00 PM), followed by 1 kg at midday (11:00 AM to 12:00 PM). The study was conducted from December 2024 to March 2025. The processing of frozen semen, involving semen collection, dilution, filling, sealing, and freezing steps, was performed at the BIB Lembang, West Java. Fresh semen samples were collected and processed for a total of 9 replicates, and a total of 66 straws were utilized in this study, with 18 repetitions for the cellular quality variable and 5 repetitions for the analysis of ROS production. Meanwhile, semen storage for 90 days and post-thawing quality analysis were performed at the Laboratory of Animal Physiology and Reproduction, Faculty of Animal Science, UGM.

Preparation of sodium selenite solution

The sodium selenite stock solution was prepared by dissolving 50 mg of sodium selenite in 10 ml of water for injection. This solution was then diluted according to the required dosage to obtain final concentrations of 5 and 10 ppm. The dilution was determined using Equation 1.

Semen collection

The semen collection process was carried out twice a week using a sterile artificial vagina, with 9 replications. False mounting was done 3 times before collection to obtain semen free from urine and contaminants. This was indicated by the semen color, which ranged from yellowish-beige to milky and was not excessively watery in consistency. Fresh semen samples were immediately transported to the laboratory for initial assessment. The initial assessment included both macroscopic and microscopic examinations to confirm the quality of the fresh semen samples as per the required standards for further processing. The fresh semen used in this study was required to have progressive motility ≥ 70% with ≤ 15% morphologically abnormal spermatozoa (Indonesian National Standard, 2023). The results of the initial quality assessment of the fresh semen samples used in this study are presented in Table 1.

Semen dilution process

The diluent was made using AndroMed® (Minitube, Germany), which was diluted with water injection (Generic, Indonesia) in a ratio of 1:4. The diluent was freshly prepared on the morning before semen collection. The AndroMed® and water injection solution was then homogenized with sodium selenite (Merck, Germany) antioxidant. The ratio between

Table 1. Macroscopic and microscopic parameters of fresh Saanen buck semen

Parameter	Mean±SEM
Volume (ml)	1.40±0.62
Color	Yellowish-beige
pH value	7.34±0.19
Smell	Distinctive
Consistency	Thick
Concentration (10^6 ml^{-1})	2,300.00±470.00
Motility (%)	87.59±0.51
Viability (%)	92.09±0.52
Abnormalities (%)	5.83±0.34
Plasma membrane integrity (%)	96.03±0.24
Acrosome membrane integrity (%)	91.04±1.93

the antioxidant and diluent used was 1:150. The volume of dilution was calculated using the concentration formula in each collection result and homogenized with 0.5 ml of fresh semen. Three equal semen ejaculates were separated and diluted with a base diluent (0 ppm) as a control (C) and a diluent containing sodium selenite at 5 (T1) and 10 ppm (T2) levels, resulting in each concentration containing 50 million cells per straw. The dilution formula applied to frozen was calculated using Equations 2, 3, and 4.

Freezing process

Before freezing, the semen underwent 2 stages of evaluation, including fresh semen assessment and liquid semen assessment after equilibration. The semen was then processed through printing, filling, and sealing, followed by a pre-freezing stage where the temperature was lowered from 5 to -140 °C over 9 minutes. The next stage was freezing, which was done in a container (Taylor Warten, USA). The straws containing semen were cryopreserved by direct immersion in liquid nitrogen (LN_2) at a temperature of -196 °C. Following storage in LN_2 for 90 days, the straws were retrieved for post-thaw quality analysis.

Evaluation of semen cellular quality

Final stage examination of the semen samples was done using several microscopic assessments under a light microscope (Yazumi 107 BN, Japan), equipped with an advanced observer (Optilab, United States of America). The data recorded included assessments of motility, viability, abnormalities, plasma membrane integrity (PMI), and acrosome membrane integrity (AMI) percentages. Frozen semen containing sodium selenite was then individually thawed at 37 °C in a water bath for 30 seconds. Motility was assessed by dropping 10 µl of semen-sodium selenite onto an object glass and covering it with a cover glass (Baity et al., 2024), which was then immediately examined under a light microscope.

Sperm viability and abnormalities were assessed using the eosin method, in which 10 µl of semen was mixed with 5 µl of stain on a slide, smeared, and heat-fixed (Fitriana et al., 2025). Non-viable sperm absorbed the eosin stain (stained), whereas viable sperm retained a white head (unstained). In this study, sperm abnormalities were evaluated using viability preparations. Both primary and secondary abnormalities were assessed, including broken heads, small or large heads, coiled or folded tails, and broken tails (Novita et al., 2025).

The observation of PMI was conducted using the hypo-osmotic swelling (HOS) test method. The procedure involved exposing 10 µl of semen-sodium selenite to 30 µl hypo-osmotic solvent, followed by incubation in a water bath at 37 °C for 60 minutes. The solution was then disseminated on the object glass covered with cover glass (Prihantoko et al., 2021). AMI examination was performed in accordance with the study from Prihantoko et al. (2020), using Giemsa staining (Merck, Germany) with methanol fixation for 10 minutes. The solution was incubated in a water bath at 37 °C for 2 hours.

$$V_1 \times M_1 = V_2 \times M_2 \quad (1)$$

Where, V_1 = Initial volume, M_1 = Initial concentration, V_2 = Final volume, and M_2 = Final concentration.

$$\text{Number of straws} = \frac{\text{Semen volume} \times \text{Concentration} \times 10^6}{50 \times 10^6} \quad (2)$$

$$\text{Final volume (ml)} = \text{Number of straws} \times 0.25 \text{ ml} \quad (3)$$

$$\text{Extender volume (ml)} = \text{Final volume} - \text{Semen volume} \quad (4)$$

$$\text{Recovery rate (\%)} = \frac{\text{Post-thawing quality}}{\text{Fresh semen quality}} \times 100 \quad (5)$$

$$Yik = \mu + \alpha i + oak \quad (6)$$

Where, Yik = The value of the observation of the quality of frozen semen, µ = The average value, αi = The factor or treatment of selenium supplementation, and oak = The random error.

All observations for each variable (viability, abnormalities, PMI, and AMI) were performed on 200 spermatozoa cells at 400× magnification. Sperm recovery rate (Equation 5) was obtained by subtracting the post-thawing assessment results from the fresh semen described by Missio et al. (2018), whereby a greater recovery rate value indicated better spermatozoa quality.

Evaluation of ROS production

Intracellular ROS production in spermatozoa was measured by flow cytometry using the cell-permeable probe 2', 7'-dichlorofluorescein diacetate (DCFH-DA) staining (Sigma, Germany) as described by Ruijter et al. (2024). A 20 µM DCFH-DA working solution was prepared in 20 µM dimethyl sulfoxide (DMSO), and thawed semen was diluted in phosphate-buffered saline (PBS) to a concentration of approximately 5×10^6 sperm ml^{-1}. The suspension was centrifuged at 1,000× g for 10 minutes. The supernatant was discarded, and the pellet was resuspended in DCFH-DA solution. An additional aliquot was incubated with hydrogen peroxide (H_2O_2) as a positive control. The samples were incubated at 37 °C for 60 minutes in the dark. Following the incubation process, the suspension was centrifuged to remove residual extracellular probes, 20 µl of PBS was added, and the samples were analyzed.

Statistical analysis

The observed data included assessments of motility, viability, abnormalities, PMI, AMI, and ROS production. The data were analyzed using a One-Way analysis of variance (ANOVA) with a completely randomized design (CRD). To identify significant differences between groups, the ANOVA was followed by Duncan's multiple range test (DMRT). A significance level of $p < 0.05$ was used for all statistical analyses, which were performed with IBM SPSS 30.0 software (IBM Corp., United States of America). The mathematical model for the ANOVA is represented as Equation 6.

RESULTS AND DISCUSSION

The results revealed that varying sodium selenite concentrations in the AndroMed® diluent medium had a significant effect ($p < 0.05$), compared with the control. In particular, T1 yielded the most substantial improvements in post-thawing, resulting in the highest motility (71.81±1.05%), viability (73.36±1.08%), and AMI (79.29±0.63%). While sodium selenite supplementation generally enhanced sperm PMI compared to the control group, no significant difference ($p > 0.05$) was observed between the T1 and T2. Importantly, sodium selenite supplementation did not significantly influence sperm abnormalities rates ($p > 0.05$). Overall, all cellular quality parameters in semen treated with sodium selenite-supplemented AndroMed® diluent surpassed those of the control group (C) across all tested concentrations (T1 and T2). Detailed post-thawing cellular quality of frozen semen at each sodium selenite concentration is presented in Tables 2 and 3.

The process of freezing and thawing (cryopreservation) exerts an oxidative effect on the physiological characteristics of sperm membranes. The results (Table 2) showed that the motility and viability values of post-thawed semen from Saanen bucks with the addition of sodium selenite antioxidant experienced a significant growth. This indicates an increase in progressive sperm motility, particularly in the recovery rate of motile spermatozoa with the addition of the antioxidant sodium selenite in post-thawing semen, compared to the control group (Figure 1).

Motility is important in spermatozoa transportation and ovum fertilization process, as well as serving as a determining factor in

Table 2. Pre-freezing and post-thawing cellular quality of Saanen buck semen supplemented with various concentrations of sodium selenite (Mean±SEM)

Sodium selenite level	Motility (%)		Viability (%)		Abnormalities (%)	
	Pre-freezing	Post-thawing	Pre-freezing	Post-thawing	Pre-freezing	Post-thawing
C	65.69±1.30[a]	48.38±1.87[a]	69.59±2.23[a]	55.67±1.12[a]	12.28±1.29	18.22±1.07
T1	74.33±1.33[b]	71.81±1.05[c]	77.48±2.17[b]	73.36±1.08[c]	9.77±0.88	15.36±0.69
T2	72.33±2.52[b]	65.47±0.61[b]	76.31±1.59[b]	68.39±1.25[b]	10.44±0.93	16.61±1.29
F-value	6.267	88.473	4.556	62.822	1.530	1.877
p-value	0.006	0.000	0.021	0.000	0.237	0.175

Note: Different superscripts within the same column denote a statistically significant difference ($p < 0.05$). AndroMed® diluent medium: C = Without sodium selenite, T1 = 5 ppm sodium selenite, T2 = 10 ppm sodium selenite supplementation

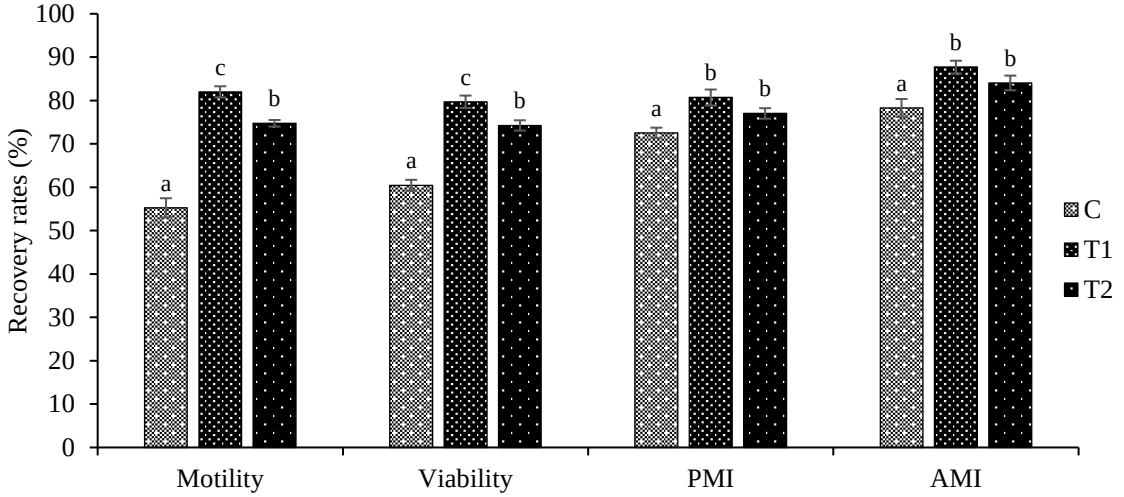

Figure 1. Post-thawing recovery rates of cellular quality parameters of Saanen buck semen for each sodium selenite supplementation level

Note: Different notation in each parameter group indicates a significant difference ($p < 0.05$). AndroMed® diluent medium: C = Without sodium selenite, T1 = 5 ppm sodium selenite, T2 = 10 ppm sodium selenite supplementation; PMI = Plasma membrane integrity; AMI = Acrosome membrane integrity

penetration to cumulus cells and zona pellucida (Baity et al., 2024). The observed variations in sodium selenite concentration within the diluent medium exert a discernible influence on both the motility and viability (Tethool et al., 2022). This effect is likely attributed to the unique conditions inherent in cryopreservation, wherein semen is subjected to extremely low temperatures (-196 °C), which is defined as the temperature of LN_2. At these temperatures, the metabolic processes of spermatozoa cells are effectively halted, resulting in a significant deceleration or complete cessation of enzymatic reactions and cellular functions (Baity et al., 2024; Fitriana et al., 2025). However, while metabolic activity is suppressed, the inherent vulnerabilities of sperm cells to oxidative stress remain.

Selenium, a critical component of GPx, which is a potent antioxidant enzyme, plays a pivotal role in mitigating this stress (Leszto et al., 2024). Cryopreservation, despite its benefits, inevitably generates ROS, unstable molecules that can inflict substantial damage on sperm cell membranes, DNA, and other vital cellular components (Ofosu et al., 2023). Sodium selenite, through its incorporation into GPx, effectively neutralizes these ROS, thereby safeguarding sperm cells from oxidative stress and preserving their structural and functional integrity (Yuan et al., 2024).

By reducing oxidative damage, sodium selenite contributes to the preservation of sperm viability (the ability of sperm to remain alive) and motility (the ability of sperm to swim effectively). These attributes are indispensable for successful fertilization, as sperm must be both viable and motile to navigate the female reproductive tract and penetrate the ovum (Espinoza et al., 2008; Ofosu et al., 2023). The ability of sodium selenite to maintain sperm viability and motility in the presence of cryopreservation-induced oxidative stress is crucial for ensuring the fertility potential of frozen semen (Zubair et al., 2015; Moya et al., 2021). Therefore, different concentrations of sodium selenite allow for varying levels of ROS neutralization, leading to the observed differences in sperm motility and viability (Zubair et al., 2015).

The capacity of spermatozoa cells to endure within the reproductive tract of the female goat until fertilization is achieved, known as sperm viability (Figure 2), is a fundamental determinant of reproductive success (Sati and Huszar, 2015; Fitriana et al., 2025). In this study, T1 resulted in the greatest improvement in viability compared to the control and T2 groups (Table 2). Furthermore, the T1 group showed the highest recovery rate for the viability parameter (Figure 1), differing significantly from the other groups (C and T2). Notably, the sperm viability values of frozen semen, as documented in previous studies by Handayani et al. (2021) and Susilowati et al. (2021), demonstrated a comparative level of quality, with percentages of viable sperm ranging from 40 to 50%. This consistency across studies underscores the importance of maintaining adequate sperm viability during cryopreservation.

As established in several previous studies, the reduction in post-thawing semen viability is attributed to excessive ROS production (Shi et al., 2024; Fitriana et al., 2025). Based on this premise, the findings of this study demonstrated a significant effect of sodium selenite concentration on the sperm viability of Saanen bucks. These results consequently demonstrate that the antioxidant activity of sodium selenite inhibits free radicals that can damage the sperm plasma membrane, which otherwise would decrease sperm motility and viability (Novita et al., 2025). This occurs through the GPx mechanism, which catalyzes the reaction to neutralize various types of free radicals, such as H_2O_2 and organic peroxides (Bouhadana et al., 2025). This clearly indicates that the addition of sodium selenite in AndroMed® medium dilution plays a crucial role in enhancing the survival of sperm during the freezing until thawing process, thereby improving the overall quality of frozen semen for AI.

The difference in the concentration of sodium selenite supplementation in the diluent does not have a significant effect ($p > 0.05$) on the percentage of the morphological abnormalities of spermatozoa cells (Table 2). The highest observed abnormalities were in the control group post-thawing, while the abnormalities in the T1 and T2 groups were lower. All values for sperm abnormalities remained well below the 20% threshold, which is a critical standard for semen appropriateness for AI, as outlined by Indonesian National Standard (2023). Adhering to these standards is essential, as a high abnormal sperm count may reduce the success of AI. This adherence to established standards is paramount, as an elevated count of abnormal sperm in cryopreserved semen significantly increases the likelihood of fertilization failure (Baity et al., 2024). Specifically, structural defects in the midpiece and endpiece of the sperm cell disrupt the intricate metabolic processes necessary for energy production. As previously described, this energy is essential for sperm survival and the vigorous motility required to navigate the female reproductive tract and reach the egg (Widayati and Pangestu, 2020; Baity et al., 2024).

Moreover, abnormalities affecting the sperm head (Figure 3) can impede the crucial fusion event between the sperm and egg, a fundamental step in fertilization. As corroborated by Prinosilova et al. (2012), a high prevalence of sperm abnormalities compromises the ability of spermatozoa to reach the site of fertilization, successfully fertilize the ovum, or support the early stages of embryonic development. Building upon this, Ardhani et al. (2020) and Baity et al. (2024) further emphasize the severity of sperm abnormalities, revealing that percentages exceeding 30 to 35% are indicative of infertility. Consequently, while the sperm abnormality rates in this context remained within acceptable limit. However, the underlying principle remains: maintaining low levels of sperm abnormalities is absolutely critical for ensuring successful fertilization and the subsequent development of a viable embryo (Widayati and Pangestu, 2020).

The addition of sodium selenite to the AndroMed® diluent medium demonstrably influences the integrity quality of both the PMI (Figure 4) and the AMI (Figure 5) in post-thaw Saanen buck semen (Table 3). The plasma membrane, serving as a critical physiological

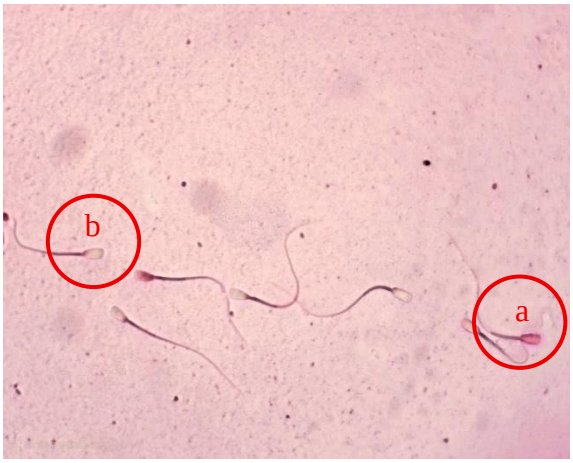

Figure 2. Sperm viability assessed with eosin-nigrosin staining at 400× magnification: a) Dead spermatozoa, identified by their pink- or red-stained heads, and b) Live spermatozoa, identified by their unstained (transparent) heads

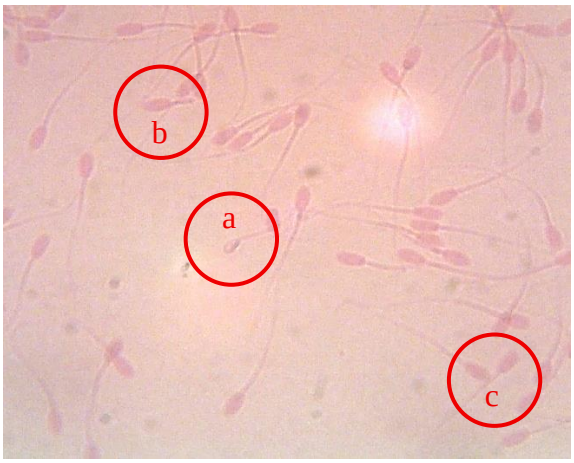

Figure 3. Sperm morphology analysis at 400× magnification, indicating primary and secondary abnormalities: a) Sperm with a small head, representing a primary abnormality, b) Sperm with a folded tail, representing a secondary abnormality, and c) Normal sperm for comparison

barrier, regulates ion transport and prevents the influx of external fluids, necessitating the maintenance of its structural integrity (Prihantoko et al., 2020). Due to their high content of polyunsaturated fatty acids (PUFAs), which feature multiple carbon double bonds, mammalian sperm membranes are extremely susceptible to oxidative damage (Gautier and Aurich, 2022; Kowalczyk, 2022). The weakened carbon-hydrogen bonds associated with the unconjugated double bonds in the PUFAs readily lose hydrogen to free radicals, initiating lipid peroxidation. This process, driven by ROS, can result in the loss of up to 60% of membrane fatty acids, leading to detrimental effects such as reduced membrane fluidity, increased ion permeability, and impaired enzyme and receptor function, thus disrupting overall sperm membrane integrity (Dutta et al., 2019).

Varying concentrations of sodium selenite demonstrated a statistically significant effect ($p < 0.05$) on sperm PMI post-thawing. Specifically, the T1 (77.49±1.68%) and T2 (73.95±1.07%) groups yielded the highest observed post-thawing PMI, compared to the control group (69.67±1.11%). Furthermore, the T1 group exhibited the highest AMI percentage (79.29±0.63%), which was significantly higher than both the T2 group (76.29±0.63%) and the control group (71.04±1.41%). This substantial preservation of membrane integrity in Saanen buck post-thawing semen can be attributed to

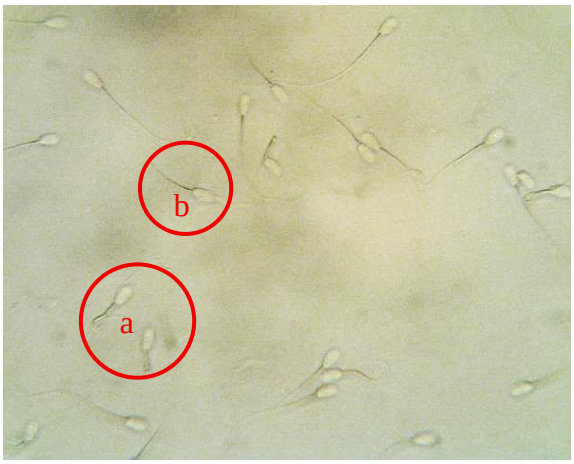

Figure 4. Sperm PMI as assessed by the HOS-test at 400× magnification: a) A coiled tail sperm, indicating a positive reaction to the HOS solution and an undamaged plasma membrane, and b) Sperm with an uncoiled tail, indicating a negative reaction to the HOS solution and a damaged plasma membrane

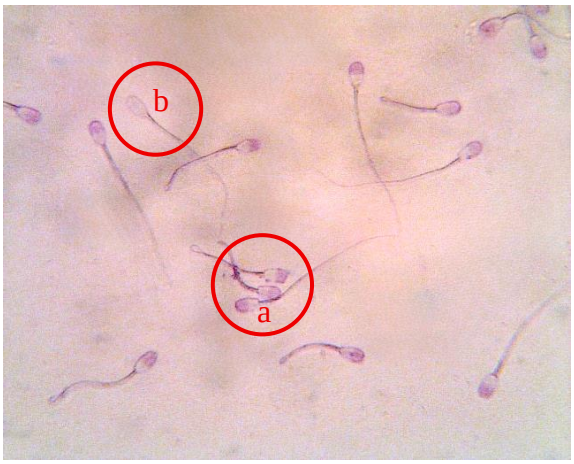

Figure 5. Sperm AMI, as assessed by a Giemsa staining procedure at 400× magnification: a) Sperm with a partially stained head, indicating an intact acrosome, and b) Sperm with an unstained or fully stained head, indicating a damaged acrosome

the mitigation of oxidative stress during cryopreservation (Bintara et al., 2025; Bouhadana et al., 2025).

At extremely low temperatures, cellular respiration, a process involving oxygen (O_2) consumption and carbon dioxide (CO_2) production, is effectively halted. Consequently, sperm cells, which do not actively engage in these metabolic processes at such temperatures, experience a reduced risk of oxidative stress and cellular damage (Asni et al., 2022; Baity et al., 2024). The profound temperature reduction in LN_2 significantly diminishes and effectively ceases metabolic activity, thereby inhibiting the generation of ROS, the primary instigators of oxidative damage (Kumar et al., 2019; Su et al., 2019).

Sperm cell membranes, characterized by their high content of PUFAs, are particularly vulnerable to lipid peroxidation, a process initiated by ROS (Fitriana et al., 2025). Sodium selenite, through its incorporation into GPx, effectively reduces ROS levels (Figure 6), thereby preserving the integrity of these membranes, which are indispensable for sperm viability and motility (Zubair et al., 2015; Alahmar, 2023). Furthermore, ROS can induce DNA damage, leading to mutations and compromised sperm function (Martins et al., 2021). The DNA destruction can cause various problems, such as embryonic development failure and hereditary genetic problems that will interfere with embryo formation and development (Baity et al., 2024). Consequently, antioxidant capabilities of selenium contribute to the protection of sperm DNA from oxidative lesions, thus safeguarding the genetic material (Moya et al., 2021). Selenium, a vital coenzyme in antioxidants, aids in scavenging and neutralizing ROS as well as maintaining the delicate redox balance within cells, which is necessary for normal cell function but is susceptible to disruption during cryopreservation, thereby effectively preventing damage to plasma membrane and acrosome integrity (Moya et al., 2021; Leszto et al., 2024). This protective mechanism is pivotal for preserving the quality and functionality of Saanen buck frozen semen.

Table 3. Sperm membrane integrity of Saanen buck semen pre-freezing and post-thawing supplemented with various concentrations of sodium selenite (Mean±SEM)

Sodium selenite level	PMI (%)		AMI (%)	
	Pre-freezing	Post-thawing	Pre-freezing	Post-thawing
C	78.11±1.26^{a}	69.67±1.11^{a}	77.61±1.86^{a}	71.04±1.41^{a}
T1	81.98±1.67^{b}	77.49±1.68^{b}	81.98±1.67^{a}	79.29±0.63^{c}
T2	89.53±2.08^{b}	73.95±1.07^{b}	89.53±2.08^{b}	76.29±0.63^{b}
F-value	8.567	8.829	10.337	20.262
p-value	0.002	0.001	0.001	0.001

Note: Different superscripts within the same column denote a statistically significant difference ($p < 0.05$). AndroMed® diluent medium: C = Without sodium selenite, T1 = 5 ppm sodium selenite, T2 = 10 ppm sodium selenite supplementation; PMI = Plasma membrane integrity; AMI = Acrosome membrane integrity

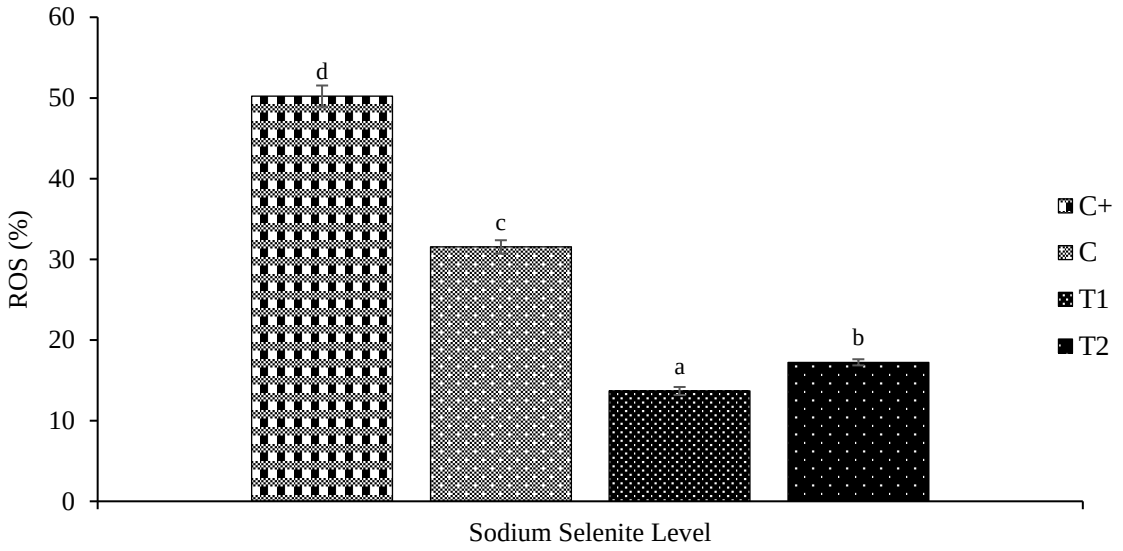

Figure 6. ROS production (%)

Note: Different notation in each treatment group indicates a significant difference ($p < 0.05$). C+ = Positive control; AndroMed® diluent medium: C = Without sodium selenite, T1 = 5 ppm sodium selenite, T2 = 10 ppm sodium selenite supplementation

The pattern in the graph (Figure 6) indicates that the positive control (50.23±1.31%) exhibited the highest generation of ROS, followed by the untreated control group (31.53±0.83%). In contrast, both T2 (17.23±0.38%) and T1 (13.67±0.50%) sodium selenite treatment groups further reduced ROS production. The lowest ROS production was observed in semen extended with 5 ppm sodium selenite (T1), while 10 ppm (T2) still reduced ROS relative to both controls, affirming notable distinctions across all treatments ($p < 0.05$). During cryopreservation, exposure to very low temperatures can impair sperm structure and function, leading to mitochondrial and membrane damage, reduced motility and viability, alterations in plasma membrane lipid composition, and the onset of premature capacitation (Benko et al., 2022).

Premature capacitation, often referred to as cryocapacitation, describes a condition in which spermatozoa undergo capacitation-like changes during freezing-thawing. Cryocapacitation arises from increased generation of ROS and reactive nitrogen species (RNS) during the freezing-thawing process (Upadhyay et al., 2021). During the freeze-thaw cycle, membrane lipid phase transitions disrupt phospholipid packing and calcium homeostasis, promoting premature membrane maturation and acrosome reaction (Talukdar et al., 2015). This condition alters the sperm membrane, increasing environmental sensitivity and shortening sperm survival in the female reproductive tract (Upadhyay et al., 2021). One strategy to reduce cryocapacitation- and cryopreservation-related damage is the inclusion of antioxidants in the cryopreservation protocol (Rajoriya et al., 2020).

Incorporation of sodium selenite antioxidant into the cryopreservation extender at physiologically relevant doses appears to modulate redox balance during the freezing-thawing process in Saanen buck spermatozoa. This antioxidant serves as an accessible source of sodium selenite, facilitating the production and function of GPx (Lee et al., 2025). This selenoenzyme catalyzes the reduction of ROS, particularly H_2O_2, thereby mitigating oxidative stress (Zhang et al., 2020). This effect is critical because cryogenic procedures markedly increase ROS generation, which can trigger lipid peroxidation in the PUFAs-rich sperm membrane and induce DNA damage, ultimately compromising motility and survival (Hai et al., 2024). By enhancing GPx-mediated detoxification, sodium selenite helps preserve membrane integrity and supports mitochondrial function. This ensures more efficient adenosine triphosphate (ATP) production required for progressive motility after thawing (Yuan et al., 2024). Nonetheless, the beneficial window of sodium selenite supplementation is narrow, and when present at excessive concentrations, sodium selenite may exert pro-oxidant or toxic effects on sperm cells.

CONCLUSIONS

The addition of the antioxidant sodium selenite at a concentration of 5 ppm was effective in mitigating the negative effects of the cryopreservation process, significantly improving several post-thawing cellular qualities, including motility, viability, PMI, AMI, and ROS production. A future comprehensive study is suggested to explore post-thawing DNA damage, malondialdehyde (MDA) level measurement, and *in vitro* fertilization (IVF) success rates to further elucidate the protective effects of this antioxidant on cryopreserved Saanen buck semen.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the research funding provided by Universitas Gadjah Mada through *Rekognisi Tugas Akhir (RTA)*/ Final Project Recognition Program (Grant No. 4193/UN1.P1/Dit-Lit/PT.01.03/2025), as well as partial support from the Ministry of Higher Education, Science, and Technology through *Pendidikan Magister menuju Doktor untuk Sarjana Unggul*/Master's-to-Doctoral Program for Outstanding Bachelor's Graduates Program (PMDSU) (Grant No. 067/C3/DT.05.00/PL/ 2025). The authors also thank the Artificial Insemination Center (BIB) Lembang, West Java, and the Faculty of Animal Science, Universitas Gadjah Mada, for providing facilities, technical assistance, and support throughout the study.

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