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MOTILITY, RECOVERY RATE, AND PLASMA MEMBRANE INTEGRITY OF ONGOLE CROSSBREED BULL SPERMATOZOA AFTER CRYOPRESERVATION WITH THE ADDITION OF VITAMIN C, SODIUM SELENITE, AND THEIR COMBINATION IN SKIM MILK-EGG YOLK EXTENDER

Motiitas, *Recovery Rate*, dan Integritas Membran Plasma Spermatozoa Sapi Peranakan Ongole pasca Kiopreservasi dengan Penambahan Vitamin C, Natrium Selenite, dan Kombinasinya pada Pengencer Skim Milk-*Egg Yolk*

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Abstract

Ongole crossbred (OC) cattle are an important genetic resource that requires preservation, and one method to support this is gamete cryopreservation. However, the cryopreservation process often results in reduced sperm viability. This study aimed to evaluate the effect of adding sodium selenite, vitamin C, and its combination as antioxidants in the semen extender on post-thaw sperm quality. Semen was collected from five OC bulls aged 7–9 years. The study consisted of several stages: preparation of the extender, semen collection and evaluation, dilution and freezing, thawing, and post-thaw quality assessment. Sperm motility and membrane integrity were measured as quality parameters. Data were analyzed using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) with SPSS software. The results showed that supplementation with 1 µg/mL sodium selenite significantly maintained sperm motility (46.00 ± 2.23%), recovery rate (63.05 ± 2.94%), and membrane integrity (62.20 ± 1.92%) compared to the control. The addition of 5 mM vitamin C also preserved sperm quality, with motility (41.00 ± 2.23%), recovery rate (56.18 ± 2.85%), and membrane integrity (56.10 ± 1.81%). However, the combination of 5 mM vitamin C and 1 µg/mL sodium selenite resulted in lower values for motility (35.00 ± 3.53%), recovery rate (48.00 ± 5.36%), and membrane integrity (48.00 ± 2.23%). In conclusion, sodium selenite was more effective than vitamin C in preserving sperm membrane integrity, while the combination of both antioxidants showed a negative effect. Further studies are recommended to evaluate reactive oxygen species (ROS) levels in each treatment and to determine the optimal antioxidant dosage.

Keywords: Antioxidants, crossbreed bull, sodium selenite, spermatozoa, vitamin C

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Abstrak

Sapi peranakan Ongole (Ongole crossbred/OC) merupakan sumber daya genetik yang penting dan perlu dilestarikan, salah satunya melalui metode kriopreservasi gamet. Namun, proses kriopreservasi sering kali menyebabkan penurunan viabilitas spermatozoa. Penelitian ini bertujuan untuk mengevaluasi pengaruh penambahan natrium selenit, vitamin C, dan kombinasi keduanya sebagai antioksidan dalam pengencer semen terhadap kualitas spermatozoa pasca-pembekuan. Semen dikoleksi dari lima ekor sapi jantan OC berusia 7–9 tahun. Penelitian dilakukan melalui beberapa tahapan, yaitu persiapan bahan pengencer, pengambilan dan pemeriksaan semen segar, pengenceran dan pembekuan semen, proses thawing, serta evaluasi kualitas spermatozoa post-*thawing*. Parameter yang diamati meliputi motilitas dan integritas membran spermatozoa. Data dianalisis menggunakan uji one way ANOVA dan dilanjutkan dengan uji DMRT menggunakan perangkat lunak SPSS. Hasil menunjukkan bahwa penambahan natrium selenit 1 µg/mL secara signifikan mampu mempertahankan motilitas (46,00 ± 2,23%), recovery rate (63,05 ± 2,94%), dan integritas membran (62,20 ± 1,92%) dibandingkan dengan kontrol. Penambahan vitamin C 5 mM juga mampu mempertahankan motilitas (41,00 ± 2,23%), recovery rate (56,18 ± 2,85%), dan integritas membran (56,10 ± 1,81%). Namun, kombinasi vitamin C 5 mM dan natrium selenit 1 µg/mL justru menurunkan motilitas (35,00 ± 3,53%), recovery rate (48,00 ± 5,36%), dan integritas membran (48,00 ± 2,23%). Kesimpulannya, natrium selenit lebih efektif daripada vitamin C dalam mempertahankan integritas membran plasma spermatozoa, sementara kombinasi keduanya menunjukkan efek negatif. Penelitian lebih lanjut disarankan untuk mengevaluasi kadar *reactive oxygen species* (ROS) pada setiap perlakuan serta menentukan dosis antioksidan yang optimal.

Kata kunci: Antioksidan, sapi PO, natrium selenite, spermatozoa, vitamin C

INTRODUCTION

Cattle are integral to smallholder farming systems and are raised using diverse management practices. Beef cattle, in particular, are major contributors to meat production, fulfilling the demand for animal protein(Susanti et al., 2014) In 2021, Indonesia's beef cattle population reached 18 million head, a quantitative increase compared to 16.9 million head in 2019. However, this population growth has not been paralleled by an improvement in the genetic quality of local cattle breeds. The Ongole crossbred (OC) cattle, a prominent local beef breed, has reportedly experienced a decline in genetic quality (Dg Malewa et al., 2021). This situation is concerning given that OC cattle represent a vital genetic resource that requires preservation. Therefore, efforts to maintain and even enhance the genetic quality of Ongole crossbred cattle are essential to safeguard their existence as part of Indonesia's germplasm (Tety Hartatik, 2009).

One effective method for preserving the genetic quality of OC cattle is by storing the gametes of superior individuals. Semen cryopreservation is a crucial technique in animal reproduction, as it accelerates the dissemination of genetic diversity and facilitates the distribution of genetically superior livestock. Due to the critical role of cryobiology in reproductive technology, new protocols are continuously being developed, and cryoprotective agents are further tested to enhance sperm resilience during the cryopreservation process (Ugur et al., 2019). Recent discoveries regarding the effects of sperm cryopreservation have led to the development of novel cryopreservation techniques and methods. These advancements involve incorporating various proteins, antioxidants, and cryoprotective agents into the freezing media to improve spermatozoa cryosurvival. However, despite these improvements, the desired levels have not yet been fully achieved, as a significant number of spermatozoa still lose their viability

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after cryopreservation (Peris-Frau et al., 2020). Furthermore, cryopreservation has detrimental effects on spermatozoa, with studies showing a reduction in post-thaw sperm viability. Baust et al., (2009)noted that some effects of cell cryopreservation include hyperosmolarity, protein denaturation, membrane damage, ion imbalance, and biochemical changes within the cell, ultimately leading to cell death.

Uncontrolled production of Reactive Oxygen Species (ROS), exceeding the antioxidant capacity of seminal plasma, leads to oxidative stress which is detrimental to spermatozoa. All cellular components, including lipids, proteins, and nucleic acids, are potential targets of oxidative stress (Bansal & Bilaspuri, 2011). Antioxidants are key components that can be added during the cryopreservation process to mitigate the negative impacts of freezing. These agents interrupt oxidative reactions, thereby reducing oxidative stress. Antioxidants are supplemented to counteract the reduction in endogenous seminal plasma antioxidant levels caused by dilution and to minimize excessive ROS production during freezing (Marco-Jimenez & Hulya Akdemir, 2016). Numerous studies have demonstrated that antioxidant supplementation in cryopreservation extenders provides a protective effect on spermatozoa from various species, including bull, sheep, goats, wild boars, dogs, and even humans, thereby improving semen parameters such as sperm motility and post-thaw membrane integrity (Bucak et al., 2008).

Among the antioxidants that can be utilized are vitamin C and sodium selenite. Vitamin C is highly effective in neutralizing free radicals across numerous metabolic processes (Abdelazim et al., 2018). It can boost the percentage of live sperm and reduce the percentage of abnormal sperm during storage at 5ºC. Vitamin C protects spermatozoa by preventing the oxidation of their membrane lipids (Shahin et al., 2020). Sodium selenite is also an antioxidant known to improve post-thaw spermatozoa quality. Supplementation with sodium selenite can help mitigate the negative effects of semen cryopreservation on sperm quality characteristics. Specifically, sodium selenite can enhance sperm motility, viability, and plasma membrane integrity (Dorostkar et al., 2012).

Based on the foregoing, it is crucial for the authors to investigate the effects of adding vitamin C, sodium selenite, and their combination on the quality of OC bull spermatozoa post-thawing. This research aims to provide valuable reference material for the cryopreservation of superior OC bull in Indonesia.

RESEARCH METHODS

Ethical Clearance

The research conducted in this study has received ethical clearance for animal sample usage from the Research Ethics Committee, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, under approval number: 0113/EC-FKH/Int./2022.

Research Object

The research object used in this study was Ongole crossbred bull semen, collected from Ongole crossbred bull aged between 7 and 9 years.

Research Design

This study was conducted using a laboratory experimental method with a Completely Randomized Design. There were four treatment groups, This study was conducted using a laboratory experimental method with a Completely Randomized Design. There were four treatment groups, first group is control group which semen without any added antioxidants in the extender, 2nd group is vitamin C group where semen supplemented with 5 mM Vitamin C in the extender. The dosage for vitamin C was based on research by (Singh et al., 2020), the 3rd

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group is sodium selenite group where semen supplemented with 1 µg/ml sodium selenite in the extender. The dosage for sodium selenite was based on research by (Dorostkar et al., 2012) and the last group is combination group where semen supplemented with a combination of 5 mM Vitamin C and 1 µg/ml sodium selenite.

Research Variables

The independent variable in this study was the type of antioxidant used, vitamin C, sodium selenite, and their combination. The dependent variable observed in this research was the motility, recovery rate, and plasma membrane integrity of spermatozoa.

Data Collection Methods

The research procedure encompassed several key stages, preparation of extender, collection and examination of fresh semen, dilution and freezing of semen, thawing, and evaluation of spermatozoa quality 24 hours after freezing. The extender that used was a skim milk-egg yolk base, with glycerol serving as the cryoprotectant. The extender was prepared one day prior to semen collection. Semen collection was performed using the artificial vagina method. Immediately after collection, both macroscopic examination (including color, pH, odor, volume, and consistency) and microscopic examination (assessing mass movement, individual motility, and plasma membrane integrity) were conducted.

Plasma membrane integrity was assessed using the Hypoosmotic Swelling Test (HOST). For this test, 5 µL of semen was mixed with 50 µL of hypoosmotic solution (prepared from 7.35 g sodium citrate and 13.51 g fructose dissolved in 1000 ml distilled water, as described by Prochowska et al., (2022). The mixture was then incubated at 37ºC for 30 minutes. Following incubation, the sample was spread onto a glass slide, air-dried, and fixed. A total of 200 spermatozoa were counted under a microscope at 400x magnification. Spermatozoa with an intact plasma membrane displayed a swollen and coiled tail due to their presence in the hypotonic solution (Elsayed et al., 2019), whereas spermatozoa with damaged membranes exhibited straight tails (Bebas & Gorda, 2017).

Data Analysis

Spermatozoa motility, recovery rate, and membrane integrity data were then tabulated and analyzed using a one-way ANOVA, followed by Duncan's multiple range test to determine significant differences among treatments ($P<0.05$), utilizing SPSS version 25.0 (SPSS Inc., Chicago, IL, USA)

RESULTS AND DISCUSSION

Results

Macroscopic and Microscopic Evaluation of Fresh Semen

This study investigated the macroscopic and microscopic characteristics of fresh semen from 5 OC bulls. All measured parameters, namely volume, color, odor, consistency, pH, mass motility, individual motility, sperm concentration, abnormality, viability, and sperm membrane integrity, are presented in detail in Table 1. The average semen volume collected was 5.40±2.96 mL, with an average pH value of 6.57±0.98. Meanwhile, sperm concentration analysis using a spectrophotometer showed an average of 1166.20±89.11×10^6 cells/mL. For motility parameters, the average observed mass motility was (++) and the average individual motility reached 73.00±2.73%. The average sperm abnormality was 4.60±3.91%, with an average viability of 80.80±1.92% and an average membrane integrity of 80.00±2.00%. Qualitatively, all successfully collected semen samples consistently exhibited a characteristic semen odor, had a medium consistency, and were cream-colored.

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Post Thawing Evaluation

Detailed characteristics of post-thawing spermatozoa, including motility, recovery rate, and membrane integrity, for the control group as well as the sodium selenite, vitamin C, and combination treatment groups, are presented in Table 2. The highest post-thawing motility was observed in the sodium selenite group (46.00±2.23%), exceeding the control group (40.00±3.53%) and the vitamin C group (41.00±2.23%). Meanwhile, the combination group exhibited the lowest motility, at 35.00±3.53%. Similar to motility, the best recovery rate was recorded for the sodium selenite group (63.05±2.94%). This value was higher compared to the control group (54.76±3.86%), the vitamin C group (56.18±2.85%), and the combination group (48.00±5.36%). Regarding membrane integrity, the sodium selenite group again showed the highest average value (62.20±1.92%). The control group had a membrane integrity of 55.00±1.22%, the vitamin C group showed 56.10±1.81%, and the combination group recorded the lowest integrity at 48.00±2.23%.

Discussion

Fresh Semen Evaluation

The data in Table 1 indicate that all measured parameters, including volume, color, odor, consistency, pH, mass motility, individual motility, viability, abnormality, membrane integrity, and sperm concentration, are within the generally normal physiological range for fertile bull semen. This robust initial semen quality is crucial as it provides a solid baseline for accurately assessing the effects of cryopreservation and antioxidant supplementation on post-thawing parameters.

The semen volume obtained in this study ranged from 3.5 to 10 mL, with an average of 5.40±2.96 mL. This average was lower than the findings reported by Prihantoko et al. (2020), which were 6.7±1.5 mL. However, our results were higher than those reported by Muthiapriani et al. (2019), who found an average volume of 2.1±0.7 mL. Several factors can influence differences in ejaculate volume, including age, body weight (Saputra et al., 2017), testis size (Saurabh et al., 2021) and weather conditions (Aisah et al., 2017). Saputra et al. (2017) stated that younger bulls typically exhibit lower semen volume and concentration compared to adult bulls. Variations in testis size among cattle breeds can also lead to differences in fresh semen volume, as larger testes contain more seminiferous tubules, thereby increasing spermatozoa production supported by a greater volume of seminal plasma. Furthermore, weather, particularly variations in temperature and sunlight exposure, can inhibit follicle-stimulating hormone (FSH) production, subsequently hindering spermatogenesis within the testes (Aisah et al., 2017).

The average pH of the semen used in this study was 6.57±0.98, which falls within the range reported by Isnaini et al. (2019) and Anggraeny et al. (2025), specifically 6.3±0.01 to 6.92±0.13. The semen consistency obtained in this study was moderate, with a creamy color. These findings align with Suretno et al. (2018), who reported that the consistency of OC semen, regardless of the rainy or dry season, is predominantly thin to moderate. Normal bovine semen color is milky white or creamy and turbid. The creamy color in semen is attributed to riboflavin pigment and does not adversely affect sperm fertility (Suretno et al., 2018).

The average mass motility of spermatozoa in the semen samples used in this study was ++. Rachmawati et al. (2021) reported that the normal range for mass motility of fresh bovine semen is between ++ and +++. Mass motility serves as an indicator of individual sperm motility; a higher proportion of actively motile or progressively motile spermatozoa reflects better semen quality (Junaedi et al., 2016). Sperm motility is a commonly utilized parameter for predicting

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fertility (Magdanz et al., 2019). The individual sperm motility of the semen samples in this study ranged from 70-75%, with an average value of 73.00±2.73%. These results indicate that the individual sperm motility of the samples falls within the normal category, defined as a minimum of 70% (Said et al., 2016) Similarly, Ina & Kaka, (2020) stated that sperm motility in fresh bovine semen typically ranges from 70-90%. Differences in motility values can be influenced by various factors, including temperature, contaminants, and the proportion of dead spermatozoa.

Sperm concentration is defined as the number of sperm cells per mL of semen. It is important to note that a bull may have a low semen volume but a high sperm concentration, and conversely, some bulls may exhibit high semen volume but low concentration (Santoso et al., 2021). In this study, the sperm concentration of the examined samples ranged from 1031×10^6 cells/mL to 1241×10^6 cells/mL, with an average value of $1166.20\pm89.11\times10^6$ cells/mL. These results indicate that the semen samples utilized in this research still possess a normal level of sperm concentration. Several factors influence the concentration of spermatozoa in bovine semen, including testis size and semen collection frequency. Scrotal circumference, for instance, has been shown to positively correlate with sperm concentration (Saputra et al., 2017)

The sperm viability in this study ranged from 78-83%, with an average value of 80.80±1.92%. This result was lower than the findings reported by Prihantoko et al. (2020) for Ongole Crossbreed (OC) bull, which were 86.56±1.24% and 84.73±0.49%. However, our viability results were higher compared to those reported by (Ina & Kaka, 2020), who found an average of 78.75±3.93%, and also higher than the study by (Sulistyowati et al., 2018), which showed 73.70±9.39%. Despite these variations, the viability observed in this study remains within the normal range, as values above 70% are considered normal. Differences in age are one of the factors that can influence variations in viability results (Lodu et al., 2021)

The sperm abnormality in fresh OC bull semen observed in this study ranged from 1-11%, with an average value of 4.60±3.91%. This finding is lower than the results reported by (Lodu et al., 2021), which was 6.15±1.81%, and (Sulistyowati et al., 2018), who found 7.40±2.12%. However, the abnormality percentage in this study was higher compared to Nurcholis et al. (2021) at 3.2±0.30%. Despite these variations, our results remain within the normal range, as Bustamante-Filho et al. (2022) state that a good sperm abnormality percentage should be below 20%. Saputra et al. (2017) indicated that improvements in body condition positively impact semen production, leading to a decrease in the percentage of abnormal spermatozoa as the animal's physical condition improves.

The sperm membrane integrity in fresh OC bull semen in this study ranged from 78-82%, with an average value of 80.00±2.00%. This result was higher than the findings reported by Prihantoko et al. (2020) , which showed values of 71.09±0.68% and 72.55±0.57%. However, our results were lower compared to those found by Rachmawati et al. (2021) which was 92.59±4.15%. Despite these variations, the sperm membrane integrity observed in this study falls within the normal category, defined as values ≥80% (Rachmawati et al., 2021). Several factors can influence the percentage of sperm membrane integrity, such as the percentage of semen with progressive motility, as motility relies on the transport of compounds across the sperm membrane, and the total abnormality of spermatozoa in the utilized semen samples (Kumar et al., 2015).

Effect of Sodium Selenite on Post-thawing Sperm Quality

The findings of this study clearly demonstrate that sodium selenite supplementation significantly improved post-thaw sperm quality parameters. The sodium selenite group exhibited the highest post-thaw motility (46.00±2.23%), recovery rate (63.05±2.94%), and

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plasma membrane integrity (62.20±1.92%) compared to the control and other treatment groups. These results are highly consistent with the existing literature supporting the beneficial effects of selenium supplementation in cryopreservation. Selenium (Se) is an essential trace element and a potent antioxidant, serving as an essential cofactor for the enzyme glutathione peroxidase (GPx). GPx is a key enzyme in the cellular antioxidant defense system, actively catalyzing the reduction of hydrogen peroxide and organic hydroperoxides. This process effectively scavenges harmful reactive oxygen species (ROS), thereby protecting vital cellular components, particularly spermatozoa and cell membranes, from lipid peroxidation (Salimi et al., 2024). In addition to its direct antioxidant enzymatic role, selenium also plays a crucial role in regulating the broader mammalian antioxidant system and is essential for normal spermatogenesis as well as optimal sperm function (Li et al., 2023). The robust improvement observed in plasma membrane integrity within the sodium selenite group is a direct manifestation of these protective mechanisms, as GPx activity consistently correlates with the maintenance of healthy sperm membranes (Salimi et al., 2024).

Numerous contemporary studies corroborate the positive impact of selenium supplementation on the quality of frozen-thawed spermatozoa in various species, including roosters (Safa et al., 2016), camels (Shahin et al., 2020), sheep (Nateq et al., 2020), bull (Khalil, 2023), and deer (Hadary et al., 2023). Specifically, the addition of selenium nanoparticles has been shown to significantly enhance motility, viability, mitochondrial activity, plasma membrane integrity, and acrosome integrity in bovine spermatozoa (Berean et al., 2024). The findings of the present study are in strong agreement with these established benefits, further reinforcing the role of selenium in mitigating cryopreservation-induced damage in bovine semen.

The superiority of selenium over Vitamin C is likely attributed to its direct involvement in enzymatic antioxidant pathways, which offer a more robust and sustainable defense mechanism. The data from this study clearly indicate that sodium selenite individually outperforms Vitamin C. Numerous literature sources consistently highlight selenium's role as a cofactor for GPx, which is an enzymatic antioxidant. Enzymatic systems generally provide a more efficient and regenerative defense against oxidative stress compared to non-enzymatic antioxidants like Vitamin C, which are depleted in the process. This fundamental mechanistic difference provides a strong biological rationale for the observed superior performance of sodium selenite (Salimi et al., 2024)

Effect of Vitamin C on Post-thawing Sperm Quality

In this study, Vitamin C supplementation resulted in moderate improvements in post-thaw sperm motility (41.00±2.23%), recovery rate (56.18±2.85%), and membrane integrity (56.10±1.81%) compared to the control group (motility: 40.00±3.53%; recovery rate: 54.76±3.86%; membrane integrity: 55.00±1.22%). Although these benefits were evident, its overall efficacy was notably lower than that achieved with sodium selenite. Ascorbic acid (Vitamin C) is a well-documented water-soluble non-enzymatic antioxidant, naturally present in high concentrations in seminal plasma (Qamar et al., 2023). Its primary mode of action involves the direct scavenging of reactive oxygen species (ROS) by reducing oxygen radicals and interrupting free-radical chain reactions (Hadary et al., 2023). This effectively protects critical sperm components, including DNA (Singh et al., 2020) and lipid membranes, from oxidative damage (Hungerford et al., 2024). Furthermore, Vitamin C contributes to the regeneration of other endogenous antioxidants, thereby strengthening the overall antioxidant defense system (Qamar et al., 2023).

The effectiveness of Vitamin C is highly concentration-dependent. The effects of Vitamin C often exhibit a bell-shaped dose-response curve (Singh et al., 2020). While optimal

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concentrations can significantly enhance sperm viability (Hungerford et al., 2024), supraphysiological levels can paradoxically act as a pro-oxidant. This occurs when Vitamin C reduces trace transition metals, leading to the formation of additional harmful ROS and exacerbating oxidative damage (Kaźmierczak-Barańska et al., 2020). This dual nature of Vitamin C could explain the intermediate efficacy observed in the present study, suggesting that the concentration used, while beneficial, may not have been entirely optimal or could have been approaching a threshold at which pro-oxidant effects begin to emerge.

The Combination Effect of Sodium Selenite and Vitamin C on Post-thawing Sperm Quality

The most significant and unexpected finding of this study was the clear detrimental effect observed in the combination group (Vitamin C and Sodium Selenite). This group consistently exhibited the lowest post-thaw motility (35.00±3.53%), recovery rate (48.00±5.36%), and membrane integrity (48.00±2.23%) among all treatment groups, even performing worse than the non-supplemented control. Potential reasons for these antagonistic or detrimental effects may be that spermatozoa function optimally within a very delicate balance between ROS production and antioxidant defense mechanisms (Qamar et al., 2023). While excessive ROS leads to oxidative stress, "an over-response to oxidation or excessive use of antioxidants can result in reductive stress" (Panner Selvam et al., 2020), a state that is also detrimental to cellular function and can disrupt various physiological processes (Sadeghi et al., 2023). The combination of two potent antioxidants may have created an overly reductive intracellular environment, disrupting essential physiological redox signaling pathways crucial for normal sperm functions such as capacitation, which paradoxically require low levels of ROS.

The detrimental combination results underscore the critical need for precise dose optimization and a thorough understanding of interaction mechanisms when designing multi-antioxidant therapies for cryopreservation. The unexpected and significant negative outcome of the combination treatment serves as an important cautionary tale. This implies that merely administering multiple compounds known for their individually beneficial antioxidant properties is insufficient. Instead, a careful understanding of their specific biochemical interaction pathways, potential synergistic or antagonistic effects, and the precise optimal combined concentrations is paramount.

CONCLUSION AND SUGGESTIONS

Conclusion

The supplementation of antioxidants in extender can yield both positive and negative effects on spermatozoa quality. Administration of sodium selenite at a dose of 1 µg/ml had a significant positive effect on the post-thaw quality of PO bull spermatozoa. Administration of Vitamin C at a dose of 1 µg/ml maintained the post-thaw quality of PO bull spermatozoa, whereas the combined administration of 5 mM Vitamin C and 1 µg/ml sodium selenite had a considerably detrimental effect on the post-thaw quality of PO bull spermatozoa.

Suggestions

Further research should be conducted on the free radical levels for each treatment group. Additionally, various doses of both Vitamin C and sodium selenite could be utilized to identify a potential optimal dosage for the combination of Vitamin C and sodium selenite.

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Table

Table 1. Fresh Semen Examination Results

Variable	Mean ± SD
Macroscopic Examination	
Volume (ml)	5,40 ± 2,96
pH	6,57 ± 0,98
Consistency	medium
Color	cream
Odor	typical semen
Microscopic Examination	
Mass Motility	++
Konsentrasi ($\times 10^6$sel/ml)	1166,20 ± 89,11
Motility (%)	73,00 ± 2,73
Viability (%)	80,80 ± 1,92
Abnormality (%)	4,60 ± 3,91
Membrane Integrity (%)	80,00 ± 2,00

SD: standard deviation

Table 2. Post-thawing Sperm Examination Results

Variable	Motility ± SD (%)	*Recovery Rate* ± SD (%)	Membrane Integrity ± SD (%)
Control	40,00 ± 3,53^{b}	54,76 ± 3,86^{b}	55,00 ± 1,22^{b}
Sodium Selenite	46,00 ± 2,23^{c}	63,05 ± 2,94^{c}	62,20 ± 1,92^{c}
Vitamin C	41,00 ± 2,23^{b}	56,18 ± 2,85^{b}	56,10 ± 1,81^{b}
Combination	35,00 ± 3,53^{a}	48,00 ± 5,36^{a}	48,00 ± 2,23^{a}

^{abc} Different letters following numbers in the same column indicate significant differences ($P<0.05$). SD: standard deviation

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