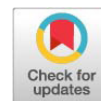


Research Article



Comparative Effects of Resveratrol and Melatonin Supplementation on Sperm Quality after Equilibration and Capacitation Like Incubation in Saanen Goats

YUDIT OKTANELLA^{1,2*}, IMAM MUSTOFA³, SUHERNI SUSILOWATI³, FITRI AULIA IZZATUL ISLAM⁴, CHRISTSANTY OMEGANINGTYAS⁴, RAGIL ANGGA PRASTIYA⁵

¹Doctorate Program of Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Kampus C Mulyorejo, Jl. Dr. Ir. H. Soekarno, Surabaya, East Java, 60115, Indonesia; ²Department of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Brauwijaya, St. Puncak Dieng Eksklusif, Dau, Malang, East Java, 65151, Indonesia; ³Division of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga, Kampus C Mulyorejo, Jl. Dr. Ir. H. Soekarno, Surabaya, East Java, 60115, Indonesia; ⁴Faculty of Veterinary Medicine, Universitas Airlangga, Kampus C Mulyorejo, Jl. Dr. Ir. H. Soekarno, Surabaya, East Java, 60115, Indonesia; ⁵Veterinary Professional Program, Department of Health and Life Sciences, Faculty of Health, Medicine and Life Sciences (FIKKIA), Universitas Airlangga, Surabaya, Indonesia.

Abstract | Semen processing and incubation under capacitation-like conditions may reduce sperm quality through membrane destabilization and oxidative damage. This pilot in vitro study evaluated the comparative effects of resveratrol and melatonin supplementation on Saanen goat sperm quality after equilibration and capacitation-like incubation. Semen was collected from one clinically healthy, sexually mature Saanen buck. Two ejaculates that met the minimum quality criteria were pooled and divided into three treatment groups: KN, negative control without antioxidant; KR, extender supplemented with 50 μ M resveratrol; and KM, extender supplemented with 0.2 mM melatonin. A total of 27.6 mL diluted semen was prepared and equally allocated into the three groups, resulting in 9.2 mL per group. Each group contained approximately 794×10^6 spermatozoa, equivalent to 86.30×10^6 spermatozoa/mL. Samples were equilibrated at 5 °C for 3 h and then incubated in phosphate-buffered saline supplemented with 5% bovine serum albumin at 38.5 °C for 60 min to create capacitation-like conditions. Progressive motility, viability, and plasma membrane integrity were evaluated. Ten technical replicate observations were used to describe laboratory repeatability; however, because the experimental unit was one pooled semen batch, statistical inference and definitive claims of biological superiority were not made. Resveratrol-treated samples showed numerically higher values than melatonin-treated and untreated control samples after equilibration and capacitation-like incubation. Although sperm quality decreased after capacitation-like incubation in all groups, antioxidant-treated samples maintained higher measured functional parameters than the untreated control. These preliminary findings suggest that resveratrol and melatonin may help preserve chilled Saanen goat sperm function, but validation using multiple bucks, independent semen collections, oxidative-stress biomarkers, extended CASA kinematics, and fertility endpoints are still required.

Keywords | Antioxidant, Spermatozoa, Capacitation-Like incubation, Melatonin, Resveratrol, Saanen goat

Received | April 26, 2026; **Accepted** | September 08, 2026; **Published** | September 19, 2026

***Correspondence** | Yudit Oktanella, Doctorate Program of Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Kampus C Mulyorejo, Jl. Dr. Ir. H. Soekarno, Surabaya, East Java, Indonesia; **Email:** yudit@ub.ac.id

Citation | Oktanella, Y., Mustofa I, Susilowati S, Islam FAI, Omeگانingtyas C, Prastiya RA (2026). Comparative effects of resveratrol and melatonin supplementation on sperm quality after equilibration and capacitation-like incubation in Saanen goats. J. Anim. Health Prod. 14(4): 1557-1564.

DOI | <https://dx.doi.org/10.17582/journal.jahp/2026/14.4.1557.1564>

ISSN (Online) | 2308-2801



Copyright: 2026 by the authors. Licensee ResearchersLinks Ltd, England, UK.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Saanen goats are among the most relevant milk goat breeds for intensive and semi-intensive production because of their high milk production rate and adaptability to managed breeding systems. The quality of semen used in artificial insemination and other assisted reproductive technologies is a key determinant of reproductive efficiency in dairy goats. Artificial insemination outcomes in goats are affected not only by female reproductive characteristics and insemination technique but also by semen quality during collection, dilution, cooling, equilibration, cryopreservation, thawing, and preparation for fertilization (Leboeuf *et al.*, 2000; Purdy, 2006; Nisfimawardah *et al.*, 2023).

Saanen goat semen studies have mostly focused on diluents and cryopreservation systems, with sperm motility, viability, abnormality, and kinematics used as major indicators of semen quality (Nisfimawardah *et al.*, 2023). Spermatozoa are highly susceptible to temperature variation, osmotic pressure, dilution stress, cold shock, and biochemical changes during processing. Oxidative stress is a major source of sperm injury during semen storage because reactive oxygen species can damage sperm membranes, disturb mitochondrial function, and reduce fertilization competence (Dutta *et al.*, 2019; Mustofa *et al.*, 2021; Mustofa *et al.*, 2022; Oktanella *et al.*, 2024; Yi *et al.*, 2024). Polyunsaturated fatty acids in sperm plasma membranes are particularly vulnerable to lipid peroxidation, resulting in impaired membrane fluidity, motility, mitochondrial function, and acrosomal integrity (Chianese and Pierantoni, 2021; Wang *et al.*, 2025).

Reactive oxygen species also participate in normal sperm physiology, including capacitation, hyperactivation, acrosome reaction, and sperm-oocyte interaction at physiological levels. However, excessive reactive oxygen species (ROS) may cause premature capacitation-like changes, lipid peroxidation, mitochondrial dysfunction, DNA damage, and reduced sperm viability (de Lamirande *et al.*, 1997; Dutta *et al.*, 2019). Antioxidants in semen extenders may therefore provide protection during processing, although their final effect can vary according to antioxidant type, extender composition, dose, temperature, and processing stage (Silvestre *et al.*, 2021; Yi *et al.*, 2024).

Resveratrol is a naturally occurring polyphenol with antioxidant, anti-inflammatory, and mitochondrial-protective activities. In goat semen, resveratrol supplementation at moderate concentrations has been shown to enhance post-thaw motility, membrane integrity, acrosome integrity, mitochondrial activity, and viability (Lv *et al.*, 2019). Melatonin is an indoleamine that directly scavenges free radicals and indirectly supports antioxidant

enzyme systems. In buck semen extenders, 0.2 mM melatonin-maintained sperm quality during cold storage and enhanced antioxidant gene expression (Widyastuti *et al.*, 2024), although higher concentrations may produce inconsistent responses depending on extender composition and cryopreservation conditions (Monteiro *et al.*, 2022).

No previous study has directly compared resveratrol and melatonin supplementation under identical experimental conditions in Saanen goat semen, particularly after equilibration followed by capacitation-like incubation. Because capacitation is redox-sensitive, antioxidants that are useful during storage may also affect sperm survival and functional quality during subsequent incubation. However, post-incubation sperm quality should not be equated with confirmed capacitation unless capacitation-specific markers, such as protein tyrosine phosphorylation, cholesterol efflux, intracellular calcium changes, or chlortetracycline staining patterns, are directly assessed.

Therefore, this pilot laboratory study compared the effects of resveratrol and melatonin supplementation on Saanen goat sperm quality after equilibration and after capacitation-like incubation. Progressive motility, viability, and plasma membrane integrity were evaluated as post-treatment functional sperm quality endpoints. Abnormal morphology was used only as an initial fresh-semen screening criterion. The working hypothesis was that antioxidant-treated samples would maintain higher measured functional parameters than untreated control samples, while recognizing that the absence of biological replication limits inference to preliminary descriptive evidence.

MATERIALS AND METHODS

STUDY DESIGN, ANIMALS, REPLICATION, AND SAMPLE SIZE

A completely randomized pilot laboratory experimental design was used. Semen was collected from one clinically healthy, sexually mature Saanen buck aged four years and weighing 74-80 kg. The buck was maintained under standard feeding and management conditions at National Artificial Insemination Center (Balai Besar Inseminasi Buatan [BBIB]) Singosari, Indonesia. Two ejaculates were collected during one semen collection session on the same day. Only ejaculates that met the minimum quality criteria for fresh semen evaluation were included in the experiment.

The two qualifying ejaculates were pooled to obtain sufficient semen volume for laboratory treatment allocation and to reduce ejaculate-to-ejaculate variation within the same collection session. The pooled fresh semen was then diluted and allocated into three experimental treatment

groups: KN, negative control without antioxidant supplementation; KR, extender supplemented with 50 μ M resveratrol; and KM, extender supplemented with 0.2 mM melatonin.

This design generated one pooled semen batch; therefore, the biological experimental unit was the pooled semen batch, and biological replication was limited to $n = 1$. The 10 observations performed for each parameter were technical replicates intended to describe laboratory measurement repeatability and not independent biological replicates. Accordingly, the study should be interpreted as an exploratory pilot experiment, and the results should be validated in future studies using multiple bucks and independent semen collection days.

A total extender volume of 27.6 mL was prepared and divided equally among the three treatment groups, resulting in 9.2 mL of diluted semen per group. Based on the calculated sperm allocation, each treatment group contained approximately 794×10^6 spermatozoa in 9.2 mL of extender, equivalent to approximately 86.30×10^6 spermatozoa/mL. Therefore, each 50 μ L aliquot used for laboratory evaluation contained approximately 4.32×10^6 spermatozoa, whereas each 100 μ L aliquot contained approximately 8.63×10^6 spermatozoa.

The sample size was determined based on logistical constraints, the availability of high-quality Saanen buck semen, the total sperm concentration obtained from the pooled ejaculates, and the exploration objective of comparing antioxidant-treated samples under identical laboratory conditions. No formal power analysis was performed because the study did not include independent biological replicates and was not designed for definitive inferential testing.

The resveratrol dose was selected based on reports showing improved post-thaw goat sperm motility, membrane integrity, acrosome integrity, mitochondrial activity, and oxidative status at moderate concentrations (Lv *et al.*, 2019). The melatonin dose was selected because 0.2 mM melatonin improved buck sperm quality during cold storage by mitigating oxidative stress and supporting antioxidant gene expression (Widyastuti *et al.*, 2024). The use of melatonin also considered reports that its response may depend on concentration and extender composition (Monteiro *et al.*, 2022).

CHEMICALS AND REAGENTS

All chemicals used in this study were analytical or research grade. The basal extender was prepared using Trizma base/Tris base (Sigma-Aldrich, St. Louis, MO, USA), citric acid monohydrate (Merck KGaA, Darmstadt, Germany/Sigma-Aldrich, St. Louis, MO, USA), D-fructose (Sigma-

Aldrich, St. Louis, MO, USA), and fresh chicken egg yolk obtained from commercially available eggs in Indonesia. The antioxidant treatments used trans-resveratrol $\geq 99\%$ high-performance liquid chromatography (HPLC) (Sigma-Aldrich, St. Louis, MO, USA) and melatonin $\geq 98\%$ thin-layer chromatography (TLC) (Sigma-Aldrich, St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA) was used as the solvent for antioxidant stock solutions. For sperm washing and capacitation-like incubation, phosphate-buffered saline (PBS) (Sigma-Aldrich, St. Louis, MO, USA) and bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO, USA) were used. For sperm evaluation, eosin Y, nigrosine, sodium citrate tribasic dihydrate, and D-fructose were used.

SEMEN COLLECTION AND INITIAL EVALUATION

Semen was collected using a pre-warmed artificial vagina. Fresh semen was maintained at 37 °C and immediately analyzed macroscopically for volume, color, odor, pH, and consistency. Mass motility, individual motility, viability, concentration, and abnormal morphology were evaluated microscopically using standard semen evaluation procedures for small ruminants. Individual motility was assessed on a pre-warmed stage. Sperm concentration was determined by using a spectrophotometer. Ejaculates with individual motility of at least 70%, viability of at least 80%, and abnormality below 15% were processed further.

PREPARATION OF EXTENDER AND ANTIOXIDANT SOLUTIONS

The basal extender was a Tris-citric acid-fructose egg-yolk extender. Tris-based extenders are commonly used in goat semen preservation because they maintain osmotic balance, buffer pH, provide an energy source, and protect spermatozoa during cooling and cryopreservation (Purdy, 2006; Kamal *et al.*, 2023). Briefly, Tris base, citric acid monohydrate, and D-fructose were dissolved in sterile distilled water, and fresh egg yolk was added aseptically. The extender pH was adjusted to approximately 6.8-7.0 before use. Resveratrol and melatonin stock solutions were prepared in DMSO and stored in the dark. Resveratrol stock solution was prepared at 50 mM, while melatonin stock solution was prepared at 200 mM. The stock solutions were diluted into the extender to obtain final concentrations of 50 μ M resveratrol in KR and 0.2 mM melatonin in KM. The negative control received the same final DMSO concentration without antioxidant. The final DMSO concentration was maintained at 0.1% v/v in all groups to avoid solvent-related effects.

EQUILIBRATION PROCEDURE

Fresh semen samples meeting the quality criteria were pooled and divided equally among the three treatment

groups. Each aliquot was diluted with the selected extender and adjusted to the same sperm concentration across groups. Diluted semen samples were equilibrated at 5 °C for 3 h. After equilibration, aliquots from each group were collected for functional sperm quality evaluation.

CAPACITATION-LIKE INCUBATION PROCEDURE

Following equilibration, sperm samples from each group were washed with PBS by centrifugation at $500 \times g$ for 10 min. The sperm pellet was resuspended in PBS supplemented with 5% BSA and incubated at 38.5 °C for 60 min. BSA was used because albumin promotes cholesterol efflux from the sperm plasma membrane, a key event associated with capacitation-related membrane remodeling (Visconti *et al.*, 1999). Because this study did not directly evaluate capacitation-specific biomarkers, this step is described as capacitation-like incubation rather than definitive capacitation induction. After incubation, sperm quality was assessed using the same functional parameters measured after equilibration.

SPERM QUALITY ASSESSMENT

Individual sperm motility was evaluated using a portable computer-assisted sperm analysis system, AndroScope® (Minitüb GmbH, Tiefenbach, Germany). The system was used to obtain objective motility data and reduce observer subjectivity compared with conventional microscopic estimation (Minitüb GmbH, 2023). The semen sample was gently homogenized, placed on a pre-warmed analysis slide or chamber, and examined according to the manufacturer's instructions (Becherer, 2023). At least five microscopic fields were recorded for each technical replicate. Motility was expressed as progressive motility according to the system output. Additional computer-assisted sperm analysis (CASA) kinetic variables, including curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), linearity (LIN), straightness (STR), amplitude of lateral head displacement (ALH), and beat-cross frequency (BCF) were not exported in the present dataset and should be included in future studies.

Sperm viability and abnormal morphology were assessed using eosin-nigrosin staining. A thin smear was prepared by mixing semen with eosin-nigrosin stain, air-dried, and examined under a light microscope. Live spermatozoa remained unstained, whereas dead spermatozoa absorbed eosin and appeared pink or red. At least 200 spermatozoa were counted per sample, and results were expressed as percentages (Björndahl *et al.*, 2003; Nisfima Wardah *et al.*, 2023).

Plasma membrane integrity was evaluated using the hypo-osmotic swelling test. The hypo-osmotic solution was prepared using sodium citrate tribasic dihydrate and D-fructose dissolved in distilled water. Semen samples were incubated in the hypo-osmotic solution at 37 °C for

30 min. Spermatozoa with swollen or curled tails were classified as having intact plasma membranes, whereas spermatozoa without tail swelling were classified as membrane-damaged (Jeyendran *et al.*, 1984).

Acrosome integrity, mitochondrial activity, oxidative stress biomarkers, and capacitation-specific endpoints, including protein tyrosine phosphorylation, chlortetracycline staining patterns, intracellular calcium, cholesterol efflux, or induced acrosome reaction, were not measured in the present study.

STATISTICAL ANALYSIS

Data was presented as mean $\pm$ standard deviation of technical replicate observations. Because all aliquots originated from one pooled semen batch prepared from two ejaculates collected from one buck during one collection session, the treatment groups did not represent independent biological experimental units. Therefore, one-way ANOVA, post hoc testing, and statistical significance claims were not applied. No exact inferential p-values are reported because such p-values would be based on technical pseudo replication and would not support biological inference. The numerical differences among groups are presented descriptively to support interpretation of this pilot laboratory experiment. Future confirmatory studies should include multiple bucks, independent collection days, formal sample-size calculation, and statistical models that treat the biological donor or collection batch as the experimental unit.

RESULTS

FRESH SEMEN QUALITY

Fresh semen used for processing showed acceptable quality based on macroscopic and microscopic evaluation. Semen was creamy white, had characteristic odor, moderate-to-thick consistency, and pH within the physiological range. Initial motility, viability, concentration, and abnormality values met the minimum criteria for further equilibration and capacitation-like incubation (Table 1). Formal statistical comparisons were intentionally omitted because the experimental unit consisted of a single pooled semen batch; accordingly, all findings are presented descriptively.

POST-EQUILIBRATION SPERM QUALITY

After equilibration, antioxidant-treated samples maintained numerically higher measured sperm quality than untreated control. Resveratrol-treated samples showed the highest measured progressive motility, viability, and plasma membrane integrity, followed by melatonin-treated and control samples. These values are descriptive technical observations from one pooled semen batch and should not be interpreted as independent biological evidence of statistical significance (Table 2).

Table 1: Fresh semen quality of Saanen goats before treatment.

Parameter	Result (mean $\pm$ SD)	Acceptance criterion
Volume (mL)	1.6 (2 ejaculates)	Laboratory standard
Color/consistency	White/very thick	Creamy white; moderate-thick
pH	7.0	Physiological range
Mass motility	+++	Acceptable wave motion
Individual/progressive motility (%)	85%	$\geq 70\%$
Viability (%)	90%	$\geq 80\%$
Sperm concentration ($\times 10^9$ sperm/mL)	2293	Laboratory standard
Abnormality (%)	<5%	<15%

Table 2: Descriptive post-equilibration sperm quality in control, resveratrol, and melatonin groups.

Parameter	KN control	KR Resveratrol 50 μ M	KM Melatonin 0.2 mM
Motility (%)	68.3 $\pm$ 2.5	76.0 $\pm$ 2.0	73.7 $\pm$ 2.1
Viability (%)	73.4 $\pm$ 2.1	82.1 $\pm$ 1.9	79.4 $\pm$ 2.2
Plasma membrane integrity (%)	58.2 $\pm$ 2.4	68.6 $\pm$ 2.1	65.8 $\pm$ 1.8

Values represent mean $\pm$ SD of technical replicate observations from one pooled semen batch.

POST-CAPACITATION-LIKE SPERM QUALITY

Sperm quality decreased in all groups after capacitation-like incubation compared with post-equilibration values. However, antioxidant-treated samples maintained numerically higher functional parameters than untreated control samples. Resveratrol-treated samples showed the highest post-incubation progressive motility, viability, and plasma membrane integrity, followed by melatonin-treated and control samples (Table 3).

Table 3: Descriptive post-capacitation-like sperm quality in control, resveratrol, and melatonin groups

Parameter	KN control	KR Resveratrol 50 μ M	KM Melatonin 0.2 mM
Motility (%)	28.4 $\pm$ 1.8	36.2 $\pm$ 2.1	33.5 $\pm$ 1.9
Viability (%)	46.3 $\pm$ 2.7	55.8 $\pm$ 2.5	52.1 $\pm$ 2.4
Plasma membrane integrity (%)	35.2 $\pm$ 2.1	45.1 $\pm$ 2.3	41.8 $\pm$ 2.0

Values represent mean $\pm$ SD of technical replicate observations from one pooled semen batch.

RELATIVE DECLINE FROM EQUILIBRATION TO CAPACITATION-LIKE INCUBATION

The relative decline in progressive motility from post-equilibration to post-incubation was greatest in the control group and lowest in the resveratrol-treated group. This pattern suggests that antioxidant supplementation may reduce functional deterioration during capacitation-like incubation; however, this interpretation remains descriptive and indirect because capacitation-specific biomarkers were not measured (Table 4).

Table 4: Relative decline in sperm functional parameters from equilibration to capacitation-like incubation.

Group	Post-equilibration motility (%)	Post-capacitation motility (%)	Relative decline (%)
KN Control	68.3 $\pm$ 2.5	28.4 $\pm$ 1.8	58.4
KR Resveratrol 50 μ M	76.0 $\pm$ 2.0	36.2 $\pm$ 2.1	52.4
KM Melatonin 0.2 mM	73.7 $\pm$ 2.1	33.5 $\pm$ 1.9	54.5

Relative decline was calculated descriptively from group means and was not subjected to inferential testing.

DISCUSSION

Fresh semen generated from the Saanen buck met the inclusion criteria for quality, as demonstrated by motility, viability, abnormality, concentration, pH, and macroscopic features, which were acceptable for laboratory processing. This quality control step was critical because semen with poor initial quality could compromise the interpretation of treatment responses during equilibration and subsequent post-incubation evaluation. The revised methodological guidelines help specify the inclusion criteria, the experimental unit, the pooling method, and the method of replication to enhance transparency and reproducibility.

After equilibration and capacitation-like incubation, sperm quality decreased, particularly in the control group. Such a pattern is biologically plausible, as goat spermatozoa are prone to cooling, dilution, osmotic, and membrane destabilization stress. Spermatozoa have a high content of polyunsaturated fatty acids, while their cytoplasmic antioxidant reserves are limited, making them susceptible to lipid peroxidation and functional deterioration (Aitken *et al.*, 2016; Suprayogi *et al.*, 2022). However, ROS, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, or total antioxidant capacity were not measured in the current study. Thus, oxidative stress is described as a potential explanatory mechanism supported by previous literature, rather than as a specific mechanism explicitly demonstrated in the present dataset.

After equilibration and capacitation-like incubation, resveratrol-treated samples exhibited the highest measured functional parameters. Higher descriptive values for progressive motility, viability, and plasma membrane integrity suggest better preservation of membrane-dependent and movement-related sperm functions compared with untreated control samples. This pattern aligns with previous reports showing that resveratrol at moderate concentrations improved the quality and oxidative status of goat sperm post-thaw (Lv *et al.*, 2019). Related antioxidant-promoting effects have similarly been observed in small-ruminant semen supplementation studies, such as in ram semen supplemented with resveratrol (Zhu *et al.*, 2023). However, because a single pooled semen batch was used in this study, the findings should be considered an indication of a numerical trend rather than a conclusion about biological superiority.

Melatonin-treated samples also showed numerically higher measured functional sperm quality than the untreated control, but the numerical response was generally lower than that observed in the resveratrol-treated samples. Melatonin can act as a direct free-radical scavenger and promote enzymatic antioxidant systems. The current descriptive pattern aligns with Widyastuti *et al.* (2024), who reported that 0.2 mM melatonin maintained buck sperm quality during cold storage and favored antioxidant gene expression. Melatonin efficacy, however, depends on concentration, species, extender composition, storage temperature, and processing stage. Reports of inconsistent responses at higher concentrations in frozen goat semen indicate that melatonin may have a relatively narrow effective concentration range (Monteiro *et al.*, 2022).

We referred to the post-incubation period throughout the manuscript as capacitation-like incubation (not confirmed capacitation). BSA-containing medium can promote cholesterol efflux and capacitation-associated membrane remodeling (Visconti *et al.*, 1999), yet sperm function subsequent to this incubation does not demonstrate capacitation unless tyrosine phosphorylation, intracellular calcium, cholesterol efflux, chlortetracycline staining, or induced acrosome reaction are measured. Therefore, the conclusion is limited to functional sperm quality after incubation under capacitation-like conditions.

The novelty of this study lies in comparing resveratrol and melatonin in Saanen goat semen using the same experimental approach, in which the functional quality of sperm was evaluated not only after equilibration but also after a capacitation-like incubation. But the novelty must be interpreted with caution, since antioxidant supplementation for goat semen preservation has been widely studied. The revised discussion avoids overstating originality and highlights that the current work offers

preliminary comparative functional evidence that should be validated with mechanistic and fertility-related outcomes.

There are several limitations that must be recognized. First, one buck, one semen collection session, and one semen batch were used; thus, the study lacks biological replication and should be considered as an exploration pilot experiment. Second, technical replicate observations were not considered independent biological units, and inferential p-values were not reported because these would be biologically misleading. Third, all the biomarkers of oxidative stress, such as ROS, MDA, total antioxidant capacity, SOD, GPx, and catalase, were not measured, and antioxidant mechanisms are still inferential. Fourth, biomarkers specifically on acrosome integrity, mitochondrial activity, and capacitation were not evaluated. Fifth, other fertility-related endpoints (*in vitro* fertilization, embryo development, conception rate, pregnancy rate following artificial insemination) were not assessed. Sixth, one dose of resveratrol and one dose of melatonin were compared, preventing dose-response interpretation. Seventh, additional CASA kinematic variables, including VCL, VSL, VAP, LIN, STR, ALH, and BCF, were not present in this dataset. These studies need to combine multiple biological donors, separate days of sample collection, statistical power analysis, oxidative biomarkers, capacitation variables, dose-response analysis, CASA kinematics, and fertility measures.

CONCLUSION

In the present pilot *in vitro* experiment, resveratrol (50 μ M) and melatonin (0.2 mM) were associated with higher descriptive functional sperm quality following equilibration and a capacitation-like incubation, compared with untreated control samples in Saanen goat semen. Samples treated with resveratrol showed the highest measured progressive motility, viability, and plasma membrane integrity. As it was a pooled semen batch of one buck, and because oxidative stress biomarkers, capacitation-specific markers, acrosome integrity, mitochondrial activity, and fertility outcomes were not directly measured, the findings align with initial trends in function but offer no further definitive statistical, mechanistic, or fertility evidence. Additional research should assess biological replication, oxidative stress indices, capacitation biomarkers, full CASA kinematics, dose-response assessment, and fertility-related endpoints to verify these results.

ACKNOWLEDGMENTS

The authors gratefully acknowledge DPPSPP FKH UB 2025 for providing research funding support under Contract Number: 01559/LN10.F1401/B/PT/2025. The

authors also express their sincere appreciation to National Artificial Insemination Center (Balai Besar Inseminasi Buatan [BBIB]) Singosari, Indonesia, for technical support and laboratory facilities during semen collection and analysis.

NOVELTY STATEMENT

This pilot study provides a direct comparison of resveratrol and melatonin supplementation in Saanen goat semen extender under the same experimental conditions, with sperm functional quality evaluated sequentially after equilibration and capacitation-like incubation. This approach provides preliminary comparative evidence on the ability of these two antioxidants to preserve progressive motility, viability, and plasma membrane integrity across two distinct semen-processing stages.

AUTHOR'S CONTRIBUTION

YO conceived the study design, supervised the research, and drafted the manuscript. IM, SS, and WW contributed to methodology, data interpretation, and manuscript revision. FAII and CO contributed to sample processing, laboratory observation, and data tabulation. All authors read and approved the final manuscript.

ETHICAL APPROVAL

All procedures involving animals were approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, Indonesia, under ethical clearance number 34-KEP-FKHUB-2025. All procedures were performed in accordance with institutional guidelines for the care and use of experimental animals.

GENERATIVE AI AND AI ASSISTED TECHNOLOGY STATEMENT

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

- Aitken RJ, Gibb Z, Mitchell LA, Lambourne SR, Connaughton HS, De Iuliis GN (2016). Causes and consequences of oxidative stress in spermatozoa. *Reprod. Fertil. Dev.*, 28(1-2): 1-10.
- Becherer D (2023). How to interpret motility analysis in AndroVision® and AndroScope: Technical report 04/2023. Minitüb GmbH.
- Björndahl L, Söderlund I, Kvist U (2003). Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. *Hum. Reprod.*, 18(4): 813-816. <https://doi.org/10.1093/humrep/deg199>
- Chianese R, Pierantoni R (2021). Mitochondrial reactive oxygen species (ROS) production alters sperm quality. *Antioxidants*, 10(1): 92. <https://doi.org/10.3390/antiox10010092>
- de Lamirande E, Jiang H, Zini A, Kodama H, Gagnon C (1997). Reactive oxygen species and sperm physiology. *Rev. Reprod.*, 2(1): 48-54. <https://doi.org/10.1530/ror.0.0020048>
- Dutta S, Majzoub A, Agarwal A (2019). Oxidative stress and sperm function: A systematic review on evaluation and management. *Arab J. Urol.*, 17(2): 87-97. <https://doi.org/10.1080/2090598X.2019.1599624>
- Hernawati T, Susilowati S, Mulyati S, Oktanella Y (2024). Optimizing equilibration protocols through ubiquinone supplementation in goat frozen semen diluent. *Interciencia*, 40(5): 65-83. <https://doi.org/10.59671/gvis9>
- Jeyendran RS, Van der Ven HH, Perez-Pelaez M, Crabo BG, Zaneveld LJD (1984). Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J. Reprod. Fertil.*, 70(1): 219-228. <https://doi.org/10.1530/jrf.0.0700219>
- Kamal MM, Alam ME, Das SK, Yeasmin MS, Ahmed S, Rahman MA, Das DK, Gofur MR, Masum MA (2023). Effects of glucose and trehalose on tris-citric acid-egg yolk-fructose diluents for semen cryopreservation in goat. *J. Adv. Vet. Anim. Res.*, 10(2): 169-177. <https://doi.org/10.5455/javar.2023.j666>
- Leboeuf B, Restall B, Salamon S (2000). Production and storage of goat semen for artificial insemination. *Anim. Reprod. Sci.*, 62(1-3): 113-141. [https://doi.org/10.1016/S0378-4320\(00\)00156-1](https://doi.org/10.1016/S0378-4320(00)00156-1)
- Lv C, Larbi A, Wu G, Hong Q, Quan G (2019). Improving the quality of cryopreserved goat semen with a commercial bull extender supplemented with resveratrol. *Anim. Reprod. Sci.*, 208: 106127. <https://doi.org/10.1016/j.anireprosci.2019.106127>
- Minitüb GmbH (2023). AndroScope: Mobile CASA system for computer-assisted semen analysis. Minitüb GmbH, 2023.
- Monteiro MM, Silva RAJA, Arruda LCP, Oliveira AS, Mergulhão FCC, Monteiro Júnior PLJ, Seal DCM, Trevisan M, Batista AM, Guerra MMP (2022). Effect of melatonin in different extenders on the quality of frozen semen of goats. *eAnim. Sci.*, 1: 100015. <https://doi.org/10.1016/j.eas.2022.100015>
- Mustofa I, Susilowati S, Suprayogi TW, Oktanella Y, Purwanto DA, Akintunde AO (2022). Combination of nanoparticle green tea extract in Tris-egg yolk extender and 39 °C thawing temperatures improve the sperm quality of post-thawed Kacang goat semen. *Anim. Reprod.*, 19(4): e20220025. <https://doi.org/10.1590/1984-3143-ar2022-0025>
- Mustofa I, Susilowati S, Wurlina W, Hernawati T, Oktanella Y (2021). Green tea extract increases the quality and reduced DNA mutation of post-thawed Kacang buck sperm. *Heliyon*, 7(3): e06372. <https://doi.org/10.1016/j.heliyon.2021.e06372>
- Nisfimawardah L, Firmawati A, Ihsan MN, Susilawati T, Wahjuningsih S (2023). Semen cryopreservation quality and sperm kinematics of Saanen goats using different diluents. *World Vet. J.*, 13(2): 300-309. <https://doi.org/10.54203/scil.2023.wvj32>
- Oktanella Y, Mustofa I, An-Haru FAR, Putri DDM, Hendrawan VF, Susilowati S, Degu NY, Hernawati T (2024). Conserving goat sperm post-thawed gene expression and cellular characteristics using the antioxidant coenzyme Q10

- supplementation. *Vet. World*, 17(7): 1637-1647. <https://doi.org/10.14202/vetworld.2024.1637-1647>
- Purdy PH (2006). A review on goat sperm cryopreservation. *Small Rumin. Res.*, 63(3): 215-225. <https://doi.org/10.1016/j.smallrumres.2005.02.015>
- Silvestre MA, Yániz JL, Peña FJ, Santolaria P, Castelló-Ruiz M (2021). Role of antioxidants in cooled liquid storage of mammal spermatozoa. *Antioxidants*, 10(7): 1096. <https://doi.org/10.3390/antiox10071096>
- Suprayogi TW, Susilowati S, Hernawati T, Hafidha FG, Wening CA, Purnawan AB (2022). Improved quality of Kambing Kacang sexing frozen semen with the addition of green tea extract. *J. Adv. Vet. Anim. Res.*, 9(3): 412-418. <https://doi.org/10.5455/javar.2022.i609>
- Visconti PE, Ning X, Fornés MW, Alvarez JG, Stein P, Connors SA, Kopf GS (1999). Cholesterol efflux-mediated signal transduction in mammalian sperm. *J. Biol. Chem.*, 274(5): 3235-3242. <https://doi.org/10.1074/jbc.274.5.3235>
- Wang Y, Fu X, Li H (2025). Mechanisms of oxidative stress-induced sperm dysfunction. *Front. Endocrinol.*, 16: 1520835. <https://doi.org/10.3389/fendo.2025.1520835>
- Widyastuti R, Prastowo S, Jaswandi J, Lubis A, Setiawan R, Ridlo MR, Boediono A (2024). Effect of melatonin supplementation on sperm quality parameters and expression of antioxidant genes during cold storage of buck semen extenders. *Vet. World*, 17(4): 863-870. <https://doi.org/10.14202/vetworld.2024.863-870>
- Yi X, Qiu Y, Tang X, Lei Y, Pan Y, Raza SHA, Althobaiti NA, Albalawi AE, Al Abdulmonem W, Makhlof RTM, Alsaad MA, Zhang Y, Sun X (2024). Effect of five different antioxidants on the effectiveness of goat semen cryopreservation. *Reprod. Sci.*, 31: 1472-1484. <https://doi.org/10.1007/s43032-024-01452-8>
- Zhu Z, Zhao H, Cui H, Adetunji AO, Min L (2023). Resveratrol improves the frozen-thawed ram sperm quality. *Animals*, 13(24): 3887. <https://doi.org/10.3390/ani13243887>