Review Article

Silymarin as a promising antioxidant for sperm cryopreservation: a narrative review

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Background and Objective: Sperm cryopreservation is a key technology in preserving male fertility, particularly for patients undergoing chemotherapy, radiotherapy, or those with compromised semen quality. However, the cryopreservation and thawing processes frequently lead to oxidative stress, resulting in significant deterioration of sperm motility, viability, morphology, and DNA integrity. This review explores the potential role of plant-derived antioxidants in mitigating cryodamage, focusing on silymarin—a polyphenolic flavonoid extracted from Silybum marianum. The objective is to evaluate the effect of silymarin on post-thaw sperm parameters in comparison to traditional antioxidants such as vitamin C and vitamin E.

Methods: This narrative review was conducted by systematically searching PubMed, Scopus, Web of Science, and ScienceDirect for publications between 2015 and 2025. Relevant studies examining the influence of silymarin and other antioxidants on sperm cryopreservation outcomes were identified and analyzed. Emphasis was placed on experimental models that assessed motility, viability, morphology, DNA fragmentation, and reactive oxygen species (ROS) levels.

Key Content and Findings: Findings suggest that all three antioxidants demonstrate potential in enhancing post-thaw sperm quality. However, silymarin consistently exhibited superior effects on sperm motility, membrane integrity, and antioxidant defense, particularly when used at optimized concentrations. Despite these promising results, clinical protocols for silymarin remain undeveloped.

Conclusions: Silymarin shows promise as a multifunctional antioxidant for improving post-cryopreservation sperm quality. Its incorporation into assisted reproductive technology (ART) protocols may enhance reproductive outcomes. Further clinical research is needed to validate its efficacy and determine optimal dosing for human applications.

Keywords: Sperm cryopreservation; plant-based antioxidants; silymarin; vitamin C; vitamin E

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Introduction

Sperm cryopreservation has been recognized by Ali *et al.* [2017] as a crucial technology for preserving male fertility and enhancing the effectiveness of assisted reproductive technology (ART) (1). This method is widely used for patients with various diseases associated with male reproductive dysfunction. Cryopreservation is particularly essential for individuals undergoing chemotherapy or radiation therapy, though these processes significantly compromise sperm and testicular cell integrity.

The process of cryopreservation affects not only spermatozoa but also critical support cells, such Sertoli cells (involved in sperm development) and Leydig cells (responsible for hormone production), as recognized by Delessard *et al.* [2020] (2). Damage to these cells further exacerbates the negative impact on male fertility. Sertoli cells, in particular, form a crucial protective barrier between the blood and the lumen of the seminiferous tubules, preventing harmful substances and antibodies from reaching developing spermatozoa.

Leydig cells produce testosterone in response to luteinizing hormone from the pituitary gland, as described by Elder *et al.* [2020] (3). Damage to these cells reduces hormonal support for spermatogenesis, ultimately affecting male fertility.

In addition, genetic damage to germ cells is caused by cytotoxic treatment, as stated by Hezavehei *et al.* [2018], thus leading to a high risk of disease inheritance in offspring (4). The clinical relevance of sperm cryopreservation is highlighted by its inclusion in the management of nonmalignant diseases, such as diabetes and autoimmune disorders, which lead to impaired sperm production, as noted by He *et al.* [2021] (5).

Despite the relevance of cryopreservation, the freezing and thawing of spermatozoa is a complex process accompanied by significant cell damage. Parameters such as motility, viability, and morphology are often affected. An increase in the number of defects in the sperm head region, midpiece, and tail has been reported by Li *et al.* [2019] due to freezing (6). Those defects include abnormal head shape, absence of the acrosome, tail curvature, and enlargement of the sperm neck. Tail deformation often occurs due to changes in osmolarity, which causes membrane damage. The reduction in sperm motility may be associated with damage to mitochondrial membranes. The energy supply for spermatozoa is provided by mitochondria through the production of ATP via oxidative phosphorylation, which is essential for their motility, as stated by Rotimi *et al.* [2024] (7). This process is disrupted by damage to mitochondrial membranes, as described by Sharma *et al.* [2015], reducing energy production and consequently decreasing the level of available ATP in spermatozoa (8).

Cryopreservation-induced oxidative stress primarily results from excessive production of reactive oxygen species (ROS). ROS cause significant structural and functional damage to spermatozoa, including DNA fragmentation, lipid peroxidation of sperm membranes, and mitochondrial dysfunction. Collectively, these damages severely impair sperm viability and reduce fertilization capacity after thawing.

Furthermore, excessive ROS may also trigger intrinsic apoptotic pathway in sperm cells. The activation of caspase 3, a key executor in apoptosis, has been observed after the freezing and thawing process, as reported by Asadi *et al.* [2021] (9). In addition, ROS-induced lipid peroxidation impairs membrane fluidity and permeability, as noted by Van der Paal *et al.* [2016] (10). High levels of oxidative stress also contribute to DNA fragmentation and may lead to double-strand breaks, which are further aggravated by defects in DNA repair enzymes and disruptions in genomic regions, as noted by Ugur *et al.* [2019] (11). Moreover, cryodamage has been associated with alterations in chromatin integrity, potentially affecting the faithful transmission of paternal genetic and epigenetic information to the offspring, as reported by Bao *et al.* [2016] (12).

In the process of cryopreservation, freezing media contain cryoprotectants, which are used to achieve optimal cooling rates and increase cell survival. Cryoprotectants are low-molecular-weight substances added to the medium for the freezing process, as stated by Grötter *et al.* [2019] (13). The formation of intracellular ice is prevented, the freezing point is lowered, a protective layer forms around the sperm membrane, and both intracellular and extracellular electrolytes undergo changes during the freezing process. Cryoprotectants are divided into penetrating and non-penetrating. Penetrating cryoprotectants include glycerol, dimethyl sulfoxide (DMSO), dimethyl acetal, propylene glycol, and ethylene glycol. This type of cryoprotectant prevents the formation of intracellular ice and stabilizes the lipid bilayer by penetrating the cell membrane, as noted by Tamburrino *et al.* [2023] (14). Non-penetrating cryoprotectants include raffinose, sucrose, egg yolk citrate, albumin, polyethylene glycol, and polyvinylpyrrolidone. In this case, the reduction of unfrozen water in the cell and the influence on the concentration of saline solutions are

achieved by Bartolac *et al.* [2018], rather than by penetrating the cell through the plasma membrane (15).

This narrative review primarily focuses on the effect of the antioxidant silymarin in freezing media, while offering limited comparison with traditional antioxidants such as vitamin C and vitamin E for context. The inclusion of silymarin, a flavonoid belonging to polyphenolic antioxidants, has been explored by Surai *et al.* [2015] (16). This antioxidant is extracted from the seeds and fruits of the milk thistle plant (Silybum marianum) and is obtained in the form of structural isomers—silybin, silydianin, and silychristin—by Taleb *et al.* [2018] (17). The strong antitumor and protective properties of Silymarin, along with its antibacterial and antimutagenic activity, have been demonstrated by Akbari *et al.* [2022] (18). The strong antioxidant properties of Silymarin in combating oxidative stress in the liver have previously been reported by Aghemo *et al.* [2022] (19). An earlier study by Yue *et al.* [2023] demonstrated improvements in motility, plasma membrane integrity, and acrosomal membrane integrity of boar spermatozoa post-cryopreservation after the addition of silymarin (20). Another study mentioned the effect of silymarin in increasing the viability of thawed boar sperm, as demonstrated by Ali *et al.* [2022] (21). In a recent investigation, the effect of silymarin on bull sperm after cryopreservation was evaluated. The results presented by Ali *et al.* [2022] showed an increase in sperm motility and a decrease in the percentage of dead, abnormal, and defective cells compared to the control group (21). This suggests that plant-derived antioxidants have significant potential to reduce oxidative stress and improve semen quality under cryopreservation conditions.

Compared to silymarin, vitamins C and E have previously shown improvements in semen parameters by removing ROS. However, it was found that in some cases, these antioxidants do not have a positive effect. For example, in a previous study, buffalo sperm motility was reduced in the group of sperm with added vitamin C compared to the control group, as shown by Ivanova *et al.* [2020] (22). In the case of vitamin E, improvements in some sperm parameters such as motility were found. However, the effect on sperm viability and DNA integrity was not proven (Ebrahimi *et al.*, 2022) (23). It is worth taking into account that the results of studies may vary depending on many factors, including freezing methods, ways of measuring parameter improvements, sperm preparation methods for cryopreservation, and supplement dosages.

This article is based on the study of the addition of the antioxidant silymarin to thawed spermatozoa since the effect and protective properties of a new plant antioxidant on spermatozoa after the thawing process have not been studied. Thus, this article aims to study the effect of silymarin on the condition of sperm after cryopreservation and the reduction of oxidative stress. Moreover, comparing the new plant antioxidant with traditional antioxidants such as vitamin C and vitamin E will help better understand the impact of antioxidants on sperm parameters. This, in turn, opens up opportunities for researching new antioxidant supplements based on plants, fruits, and vegetables. We present this article in accordance with the Narrative Review reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-2025-141/rc).

Methods

This study is a literature review aimed at systematizing and analyzing existing scientific data on the use of antioxidants for improving sperm quality after cryopreservation. No original experimental research was conducted.

A comprehensive literature search was performed using four electronic databases: PubMed, Scopus, Web of Science, and ScienceDirect, covering the period from 2015 to 2025. The search included a combination of MeSH and free-text terms such as ‘silymarin’, ‘sperm cryopreservation’, ‘plant-derived antioxidants’, ‘vitamin C’, ‘vitamin E’, ‘motility’, ‘viability’, ‘morphology’, and ‘reactive oxygen species’. Additional sources were identified through reference lists of selected studies. The search strategy, inclusion criteria, and selection methodology are summarized in *Table 1*.

Only peer-reviewed articles published in English were included. Included studies examined the effects of silymarin and/or other antioxidants on sperm parameters post-cryopreservation and provided sufficient methodological detail (e.g., antioxidant concentration, treatment duration, and outcomes measures such as motility, viability, and morphology). Publications lacking experimental clarity or relevance were excluded.

The selected studies were reviewed and systematized to extract key information regarding the effectiveness, dosages, and mechanisms of action of each antioxidant. A comparative analysis was conducted to assess the relative efficacy of silymarin versus traditional antioxidants (vitamin C and vitamin E). This included evaluation of similarities and differences in sperm quality outcomes, antioxidant types, and cryopreservation conditions.

Additionally, experimental and theoretical data from the

Table 1 The search strategy summary

Items	Specification
Date of search	15 February 2025
Databases and other sources searched	PubMed, Scopus, Web of Science, and ScienceDirect
Search terms used	Silymarin, sperm cryopreservation, plant-derived antioxidants, vitamin C, vitamin E, motility, viability, morphology, reactive oxygen species
Timeframe	2015–2025
Inclusion and exclusion criteria	Inclusion criteria: studies published in English from 2015 to 2025, evaluating the effect of antioxidants (vitamin C, vitamin E, silymarin) on sperm cryopreservation parameters
	Exclusion criteria: studies not involving antioxidants, abstracts without full texts, duplicate studies, and articles not related to sperm cryopreservation
Selection process	The selection process was conducted by G.Z. In the cases of discrepancies, a consensus was reached through joint discussion

reviewed literature were analyzed to elucidate the biological mechanisms through which antioxidants exert their effects, particularly in relation to oxidative stress reduction, membrane stabilization, and DNA integrity preservation under cryogenic conditions.

Methodological limitations across the studies were taken into account, including variability in protocols, sample characteristics (e.g., species or patient fertility status), and antioxidant administration. Based on the reviewed findings, general trends were identified and potential recommendations for future studies and clinical applications were proposed.

Discussion

Sperm analysis

Several studies included in this review utilized standard techniques for the assessment of sperm parameters after cryopreservation and antioxidant treatment. Sperm motility was typically evaluated using the computer-assisted semen analysis (CASA) system, with Leja slides and a 20-µm chamber depth being commonly employed. Parameters such as curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), linearity (LIN), and beat cross frequency (BCF) were among the most frequently reported metrics (Schubert *et al.*, 2019) (24).

Sperm viability was most often assessed using the eosin-nigrosin staining method. This approach allows differentiation between live and dead sperm cells based on membrane integrity and dye exclusion, and was consistently applied in a study (Agarwal *et al.*, 2022) (25). Morphological assessment was commonly performed using the Diff-Quik staining, which involves sequential exposure to xanthene, triarylmethane, and thiazine dyes. Studies by Xu *et al.* [2022] and Chenoweth *et al.* [2022] applied this method to evaluate head, midpiece, and tail abnormalities in at least 200 spermatozoa under oil immersion microscope at 1,000× magnification (26,27).

Sperm cryopreservation

Cryopreservation protocols typically involve the gradual mixing of sperm suspensions with cryoprotectant media to minimize osmotic shock and maximize cell survival. Zohrabi *et al.* [2024] described a method where spermatozoa are mixed dropwise with equal volume of cryoprotectant at a 1:1 ratio to ensure uniform distribution (28). A Tris-egg yolk-based medium has been commonly used as a cryoprotective agent, providing membrane stabilization and protection against freezing damage, as demonstrated by Buranaamnuay *et al.* [2017] (29). Following the addition of cryoprotectant, samples are aliquoted into 0.5 mL straws, sealed, and subjected to controlled cooling—first by exposure to liquid nitrogen vapor at –80 ℃, and subsequently by immersion in liquid nitrogen at –196 ℃ for long-term storage.

These approaches, illustrated schematically in *Figure 1A*, are widely adopted to preserve sperm viability and structural integrity during cryopreservation.

Sperm thawing

The thawing of cryopreserved sperm samples typically

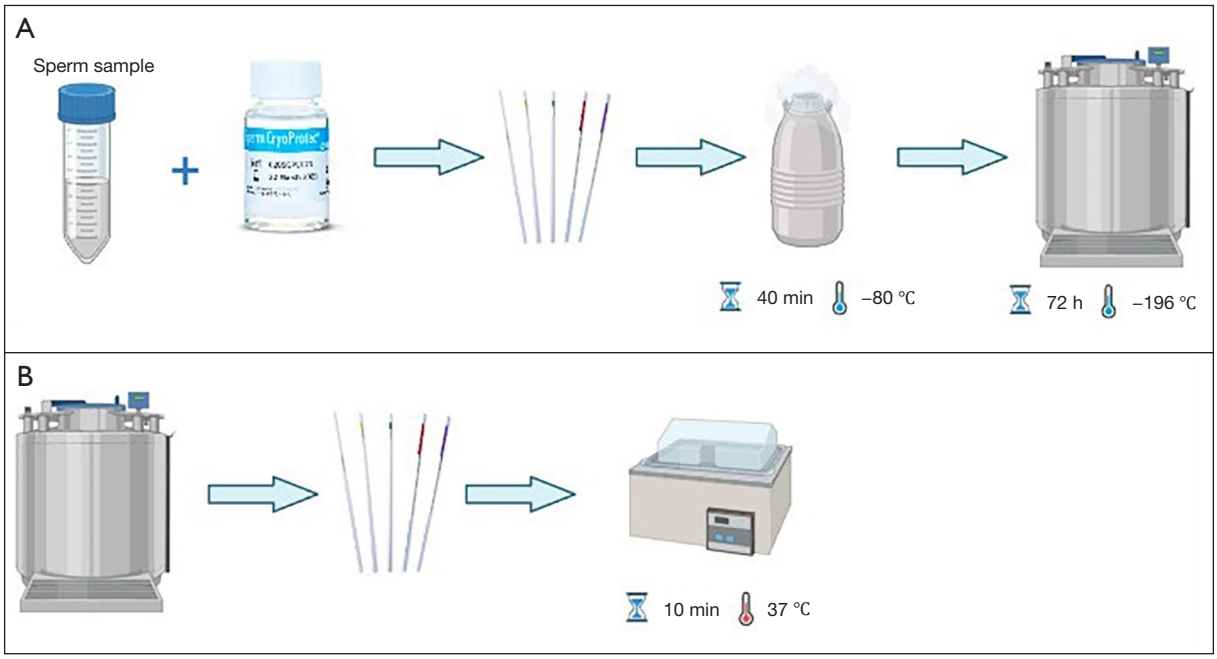

Figure 1 Cryopreservation and thawing of sperm. (A) The cryopreservation process includes the addition of a cryoprotectant, loading the sperm samples into straws, exposing them to nitrogen vapors, and ultimately storing them in a liquid nitrogen tank at −196 ℃ for 72 hours. (B) The thawing process involves placing the sperm samples in a water bath at 37 ℃ for 10 minutes. The figure was created using BioRender.

involves the rapid warming of straws in a water bath at physiological temperatures to minimize the formation of ice crystals and reduce cellular damage. Muratori *et al.* [2019] demonstrated that thawing straws at 37 ℃ for approximately 10 minutes provides optimal recovery of sperm motility and viability (30). The procedure is schematically illustrated in *Figure 1B*.

Following thawing, many protocols recommend the gradual addition of warming medium supplemented with human serum albumin (HSA) to minimize osmotic stress. Subsequent centrifugation steps are applied to remove residual cryoprotectant, with careful handling of the sperm pellet to avoid mechanical damage. These approaches aim to restore sperm functionality and integrity for further evaluation.

Incubation and analysis of semen parameters

Determining the optimal concentration of antioxidants is critical for enhancing the quality of thawed spermatozoa post-cryopreservation. Treulen *et al.* [2019] demonstrated that varying antioxidant concentrations can have significant effects on sperm motility, viability, and morphology (31). In their experimental design, thawed sperm samples were divided into multiple groups, each incubated at 37 ℃ for several hours with different concentrations of antioxidants. Typical concentrations explored ranged from 0.2 to 1 mM for vitamin C, vitamin E, and silymarin.

The spermatozoa were subsequently evaluated to determine how antioxidant dosage influenced the key sperm parameters. These approaches have underscored the importance of dose optimization, as inappropriate concentrations may lead not only to reduce oxidative stress but, paradoxically, to detrimental effects on sperm function. A schematic overview of the incubation and evaluation workflow is presented in *Figure 2*. Comparison across different antioxidants at their optimal concentrations provides valuable insights into their relative efficacy in mitigating cryopreservation-induced damage.

Comparison with other antioxidants: zinc, selenium, magnesium

Although silymarin has demonstrated positive effects on sperm motility, morphology, membrane integrity, and DNA fragmentation in animal models, direct comparative studies with other antioxidants such as zinc, selenium, and magnesium are still lacking. Zinc plays a critical role in sperm

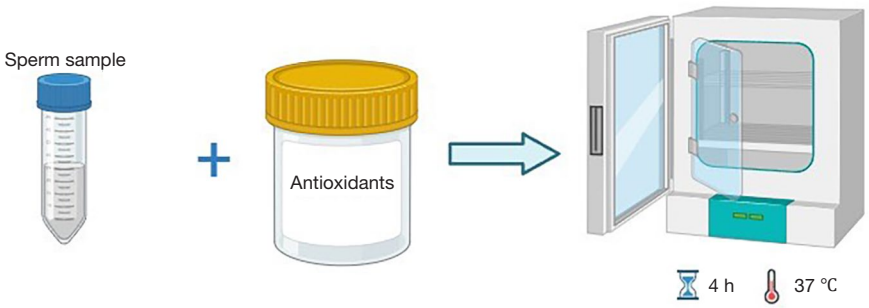

Figure 2 The procedure of incubating a sperm sample with antioxidants at 37 ℃ for a duration of 4 hours.

maturation and structural stability; supplementation at a dose of 68 mg for 26 weeks was shown by Ahmadi *et al.* [2016] to improve sperm concentration, although other parameters remained unaffected (32). Selenium, a key component of glutathione peroxidase, has been associated with improved motility and morphology in a dose-dependent manner, with higher concentrations yielding more favorable outcomes, as described by Dolník *et al.* [2021] (33). The role of magnesium in male fertility is less clear, but recent evidence by Mirgalooye Bayat *et al.* [2024] suggests that magnesium oxide nanoparticles may have cytotoxic effects on both fresh and frozen spermatozoa (34). These comparisons highlight the need for further direct studies evaluating the relative efficacy and safety of these antioxidants versus silymarin in sperm cryopreservation.

Optimization of antioxidant concentration

The optimization of antioxidant concentrations is critical for enhancing the quality of thawed spermatozoa post-cryopreservation. Hungerford *et al.* [2024] reported that vitamin C showed optimal antioxidant activity at a concentration of 0.4 mM, whereas higher concentrations were associated with reduced efficacy and potential pro-oxidant effects (35). Similarly, Zhu *et al.* [2015] found that vitamin E provided the best protection against oxidative damage at a concentration of 0.2 mM (36).

These findings highlight the delicate balance required when applying antioxidants: suboptimal doses may be insufficient to counteract oxidative stress, while excessive doses may exacerbate cellular damage through pro-oxidant mechanisms. For example, an excessively high concentration of vitamin C may increase oxidative stress rather than mitigate it, and an excess of vitamin E may induce cytotoxicity by disrupting membrane integrity.

The optimal concentration of silymarin, however, remains to be clearly established. Although preliminary experimental data suggest that silymarin improves sperm motility, viability, and morphology, the exact dose that maximizes these effects without inducing cytotoxicity has not been standardized. A study on bovine spermatozoa showed that silymarin supplementation reduces ROS levels, increases mobility, and decreases the percentage of dead and abnormal spermatozoa (22). Future studies are warranted to define the dose-response relationship for silymarin more precisely, ensuring its safe and effective application in sperm cryopreservation protocols.

Potential cytotoxicity of silymarin in high concentrations

Although silymarin demonstrated promising antioxidant properties, its potential cytotoxic effect at high concentrations remains an important consideration. Current experimental data on human spermatozoa are limited. Animal studies by Ali *et al.* [2022] and Yue *et al.* [2023] have reported no adverse effects even at elevated doses (20,21). Furthermore, clinical trials in the context of liver disease, such as the investigation by Gillessen and Schmidt [2020], found that daily administration of up to 2,100 mg of silymarin over several months resulted in only minor side effects (37).

Despite these reassuring findings, the lack of targeted reproductive studies necessitates further investigation. Specifically, future research should address whether prolonged or high-dose exposure to silymarin could induce subtle cytotoxic effects on human spermatozoa or testicular tissues. Clarifying the safety profile of silymarin in the context of male fertility is essential before recommending

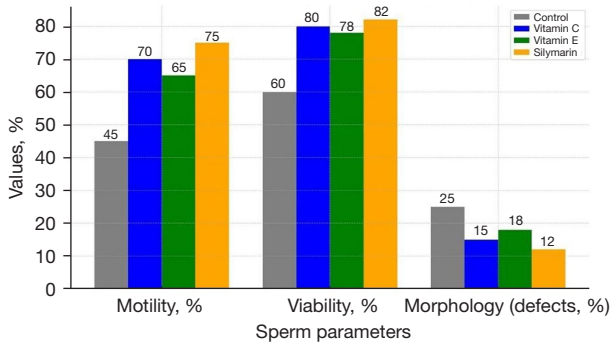

Figure 3 Comparison of the impact of antioxidants on sperm parameters.

its routine clinical use in cryopreservation protocols.

Comparative efficacy of silymarin, vitamin C and vitamin E

Comparative evaluation of antioxidant efficacy in cryopreservation models suggests that silymarin may offer superior protective effects compared to traditional antioxidants such as vitamin C and vitamin E. As illustrated in *Figure 3*, silymarin demonstrated the most significant improvements in sperm motility, viability, and morphology following thawing.

While vitamin C and vitamin E have well-documented antioxidant properties and have shown benefits in improving post-thaw sperm parameters, their effectiveness appears to be dose-sensitive, with a narrower therapeutic range. In contrast, silymarin, derived from plant polyphenols, exhibited consistent antioxidant benefits across various parameters in experimental models.

These findings highlight silymarin's potential as a promising agent for enhancing sperm cryopreservation outcomes. However, further clinical studies are necessary to validate these comparative advantages in human fertility preservation settings.

Conclusions

The review highlights the critical impact of cryopreservation on sperm quality, with anticipated declines in motility, morphology, and viability relative to World Health Organization (WHO) standards. The addition of antioxidants, particularly vitamin C, vitamin E, and silymarin, shows potential for mitigating cryodamage and improving post-thaw sperm parameters.

Among the antioxidants evaluated, silymarin demonstrated the most consistent benefits across motility, viability, morphology, and DNA integrity in experimental models. While these findings are promising, clinical studies are needed to establish the optimal concentrations and confirm the safety and efficacy of silymarin in human fertility preservation protocols.

Overall, silymarin emerges as a promising multifunctional antioxidant for improving outcomes in sperm cryopreservation, supporting its further investigation in reproductive medicine.

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Footnote

Reporting Checklist: The authors have completed the Narrative Review reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-2025-141/rc

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