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Review article

Sericin in semen cryopreservation: A review of its potential and challenges

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Abstract

Semen cryopreservation plays a critical role in animal reproduction, but oxidative stress and reactive oxygen species (ROS) generation during freezing and thawing can significantly reduce sperm quality. Sericin, a silk-derived biopolymer, has gained attention as a promising cryoprotectant due to its potent antioxidant, radical-scavenging, and antibacterial properties. This review explores sericin's potential to improve sperm motility, viability, acrosome integrity, and DNA integrity across 12 species, including goats, bulls, buffalo, poultry, rabbits, boars, dogs, mice, horse, sheep, elephant, and humans by synthesizing findings from over 60 studies that utilized six different cryopreservation methodologies. Sericin has been shown to protect sperm during cryopreservation by reducing oxidative stress, preserving membrane integrity, and enhancing fertilizing capacity. Studies suggest that sericin supplementation may provide comparable or even superior protection compared to traditional cryoprotectants. Additionally, sericin can work synergistically with other additives, such as trehalose and glutathione, to further improve sperm motility and overall post-thaw quality. Despite these promising findings, several challenges hinder the widespread commercial application of sericin such as variability in composition, and scalability remain key limitations. Moreover, excessive sericin concentrations can lead to cytotoxicity, protein aggregation, osmotic imbalance, and increased viscosity, all of which may induce oxidative stress and ultimately impair sperm quality. Therefore, optimizing sericin concentrations is essential to maximize its cryoprotective benefits while minimizing potential adverse effects. Future research should focus on elucidating the molecular mechanisms underlying sericin's protective effects and conducting field trials to validate its efficacy in diverse reproductive contexts. With further development, sericin holds great potential for advancing semen preservation and enhancing reproductive technologies.

Keywords: Sericin, Semen cryopreservation, Antioxidant, Sperm quality, Cryoprotectant.

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INTRODUCTION

In animal reproduction, semen cryopreservation is essential for enabling long-term sperm storage, genetic preservation, and the widespread use of artificial insemination (AI) to enhance genetic improvement and breeding efficiency. However, maintaining sperm quality after thawing remains a significant challenge. Cryopreservation often leads to reduced motility, viability, acrosomal integrity, and fertilizing potential, ultimately affecting reproductive success (Küçük et al., 2021; Partyka and Niżański, 2021). During freezing and thawing, sperm cells undergo osmotic and thermal shocks, resulting in plasma membrane damage, lipid peroxidation, and apoptosis (Kadirvel et al., 2009). Additionally, the high polyunsaturated fatty acid content in sperm membranes makes them highly susceptible to oxidative stress, as reactive oxygen species (ROS) accumulate, impairing sperm motility, function, and DNA integrity (Aitken et al., 1993; Ugur et al., 2019).

To counteract these damaging effects, antioxidants such as vitamins C and E, glutathione peroxidase, and cysteine are commonly added to semen extenders. However, cryopreserved semen typically recovers only about 50% of the quality of fresh semen, highlighting the need for improved preservation strategies (Muiño et al., 2007; Ugur et al., 2019; Khalil et al., 2023). In response to these limitations, sericin, a silk-derived natural biopolymer, has emerged as a promising cryoprotective, antioxidant, and antimicrobial agent for semen preservation (Zhang, 2002). Recognized for its ability to mitigate oxidative stress, sericin has demonstrated potential in enhancing sperm viability, motility, and DNA integrity (Raza et al., 2019; Aghaz et al., 2020). These properties make it a valuable addition to semen extenders and a potential solution to the challenges associated with conventional cryopreservation methods.

Despite these promising findings, several barriers that must be addressed before sericin can be widely adopted in the semen preservation protocols. Key research gaps include a limited understanding of its precise molecular mechanisms, particularly how it protects sperm cells from oxidative stress and structural damage. Its efficacy across species with varying sperm membrane compositions remains unclear and requires more investigation. Additionally, optimal concentration, timing, and preservation protocols for maximizing sericin's benefits need to be established. Moreover, while the sericin shows promise, its potential synergy with emerging cryoprotectants such as nanoparticles has yet to be investigated. Large-scale trials in AI programs are essential to assess its practicality and cost-effectiveness for commercial use.

This review examines sericin's role in semen cryopreservation, focusing on its sources, properties, and protective mechanisms. It compares sericin with traditional cryoprotectants such as glycerol and egg yolk, analyzing their advantages, limitations, and species-specific applications. Additionally, the review critically evaluates the existing literature on sericin's efficacy as a cryoprotectant, explores the potential for sericin's commercialization, emphasizing the need for large-scale field trials and regulatory approval. Finally, it highlights the integration of sericin with emerging technologies, including nanoparticles, to enhance semen preservation strategies and improve reproductive outcomes.

SEMEN CRYOPRESERVATION: CURRENT LIMITATIONS AND NEEDS

Semen preservation is critical for AI and reproductive technologies, relying primarily on cryopreservation and chilling techniques to maintain sperm viability. However, cryopreservation faces several challenges that impact sperm quality, including physical, biochemical, and oxidative damage during freezing and

thawing. These damages often result in reduced sperm motility, viability, and fertilization potential (Watson, 2000).

Key issues in cryopreservation include damage to the sperm membrane, mitochondria, and acrosome, which are exacerbated by cryoprotectants, temperature fluctuations, and ROS (Parks and Graham, 1992). ROS, produced during freezing and thawing, lead to lipid peroxidation, DNA damage, and decreased motility (Aitken et al., 1993; Ugur et al., 2019). Spermatozoa, being highly susceptible to ROS, suffer from oxidative stress due to their polyunsaturated fatty acids, which are prone to lipid peroxidation. This oxidative stress is a primary cause of DNA fragmentation and reduced motility, especially in species such as buffalo, goat, and boar (Alvarez and Storey, 1989; Kadirvel et al., 2009; Ugur et al., 2019).

To mitigate cryoinjury, various cryoprotectants, antioxidants, and membrane stabilizers are employed. Common cryoprotectants include glycerol, egg yolk, and bovine serum albumin (BSA). However, high concentrations of cryoprotectants can be toxic, and traditional agents like egg yolk and BSA carry the risks of microbial contamination and batch variability (Johnson et al., 2000). Furthermore, chilling, although less harmful than cryopreservation, is unsuitable for long-term storage, as sperm viability deteriorates over time (Mazur, 2004). Many extenders also lack antibacterial properties, necessitating the use of antimicrobial agents to prevent bacterial contamination, which can further affect sperm quality (Hozbor et al., 2016).

Emerging techniques, such as lyophilization, monolayer centrifugation, and vitrification, offer potential solutions. Vitrification, which involves rapid freezing using high cryoprotectant concentrations, minimizes ice crystal formation and reduces freezing damage (Mazur, 2004). Additionally, nanoparticles like selenium are being explored to enhance antioxidant defenses, while omics techniques, such as proteomics, help identify molecular targets for more precise interventions. Plant-derived cryoprotectants are under development, focusing on reduced toxicity and improved sperm quality.

Despite these advances, challenges in cryopreservation remain, particularly in improving post-thaw sperm quality. Oxidative stress, limited protection from traditional cryoprotectants, and bacterial contamination persist. Both traditional cryoprotectants and nanoparticles have been explored to address these issues. Among traditional cryoprotectants, trehalose has proven particularly effective. Supplementing 100 mM trehalose reduced oxidative stress in frozen-thawed sheep semen (Bucak et al., 2007) and improved post-thaw semen characteristics in goats (Dutta et al., 2023). Adding 150 mM trehalose to a tris-citric acid-egg yolk-fructose diluent enhanced goat sperm motility, viability, plasma membrane integrity, and acrosome integrity (Kamal et al., 2023). Additionally, 30 mM trehalose significantly increased the motility, viability, and acrosome integrity of Hariana bull sperm ($P<0.05$), according to Kumar et al. (2023). However, some studies report conflicting results regarding trehalose's cryoprotective properties (Jia et al., 2021).

Nanoparticles such as lecithin, selenium, and zinc oxide have shown promise in reducing cryopreservation-induced damage by combating free radicals. For example, selenium nanoparticles (Se-NPs) improve post-thawed sperm motility, viability, and membrane integrity (Asadi et al., 2023), while thymoquinone nanoparticles (TQNPs) enhance buffalo bull sperm cryotolerance and fertility potential (Khalil et al., 2024). The supplementation of Beltsville Thawing Solution (BTS) with Se-NPs also improved semen quality and conception rates during short-term boar semen preservation (Paul et al., 2024).

Another significant factor in sperm preservation is the role of seminal vesicle proteins. Bull seminal vesicles produce acidic proteins (BSP-A1, BSP-A2, BSP-A3, and BSP-30-kDa), which bind to sperm membrane choline phospholipids during ejaculation. This binding leads to the loss of cholesterol and phospholipids, negatively affecting sperm preservation (Bergeron et al., 2004). Sperm from species with lower cholesterol content in their membranes are more susceptible to cold

shock than those with higher cholesterol levels (Darin-Bennett and White, 1977; Drobnis et al., 1993).

Incorporating milk caseins into semen extenders has been shown to inhibit BSP protein binding, reducing lipid depletion and improving sperm preservation in bovine samples (Bergeron et al., 2007). Similarly, caseinate, a casein derivative, effectively protects stallion sperm during cooling and freezing, performing similarly to whole milk (Lagares et al., 2012). In rooster semen cryopreservation, supplementing a casein-based extender with quercetin, a flavonoid with antioxidant properties, further improved post-thaw sperm quality (Appiah et al., 2020).

In conclusion, despite significant advancements in semen cryopreservation techniques, challenges such as oxidative stress, limited cryoprotectant efficacy, and bacterial contamination continue to compromise sperm quality. Future research aims to address these issues by optimizing cryopreservation protocols, incorporating nanoparticles, and exploring novel cryoprotectants, ultimately improving post-thaw sperm viability and enhancing outcomes in assisted reproduction and conservation efforts.

SERICIN: A PROMISING CRYOPROTECTANT? SOURCES AND PROPERTIES

Sericin is a glycoprotein primarily extracted from the silkworm *Bombyx mori* during the fifth larval instar (Kundu et al., 2008; Silva et al., 2022). It functions as an adhesive, binding fibroin filaments to form silk yarn (Mondal et al., 2007). Synthesized in the silk glands of *B. mori*, sericin belongs to a family of proteins produced by the alternative splicing of sericin genes (Michaille et al., 1989; Sehnal and Akai, 1990). The three genes responsible for its synthesis-Ser1, Ser2, and Ser3-give rise to variants (SER-1, SER-2, and SER-3) with distinct amino acid compositions and molecular weights (Das et al., 2021).

Sericin (Figure 1) is a globular protein with random coils and β-sheets. Its amino acid composition, including serine (31.64%) and threonine (7.20%), plays a key role in its antioxidative and radical-scavenging properties. The protein's hydrophilic nature, due to its high content of hydroxyl and carboxyl groups and polar amino acids, allows it to neutralize ROS, which contributes to oxidative damage during cryopreservation (Aad et al., 2024). Sericin is classified into three fractions based on solubility and molecular weight (Shaw and Smith, 1951).

Sericin A: Most water-soluble, found in the outer cocoon layer, containing 17.2% nitrogen.

Sericin B: Found in the intermediate cocoon layer, containing 16.8% nitrogen, like Sericin A.

Sericin C: Insoluble in hot water, found in the innermost cocoon layer, containing 16.6% nitrogen, and includes proline.

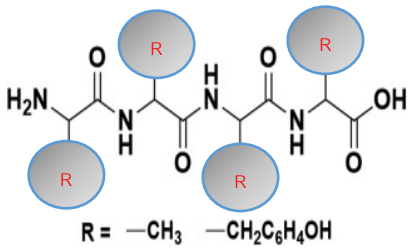

Figure 1 Structure of sericin.

The physicochemical properties of sericin can vary depending on extraction methods and silkworm lineage. It is soluble in water at temperatures above 50°C (Kweon et al., 2000) and forms a gel at lower temperatures (Kunz et al., 2016). The molecular weight of sericin ranges from 10 to 400 kDa, depending on extraction conditions such as temperature, pH, and processing time (Aramwit et al., 2012). Extraction methods—such as high temperature, urea, acids, alkalis, enzymes, and microwave-assisted processes-also contribute to variability (Aramwit and Sangcakul, 2007; Silva et al., 2012).

Sericin directly interacts with sperm plasma membranes by binding to phospholipids and cholesterol, stabilizing the membrane and preventing lipid loss (Miyamoto et al., 2012). Indirectly, it scavenges ROS and enhances antioxidant enzyme activity, reducing oxidative stress and protecting the membrane from lipid peroxidation. These combined actions help maintain sperm integrity during cryopreservation.

Sericin mitigates oxidative stress during semen storage through its antioxidative properties, primarily due to its high content of hydroxyl amino acids (Yasmin et al., 2015) and flavonoids (Zhao and Zhang, 2016). These components help sericin to scavenge ROS, reducing oxidative damage in sperm cells. This action prevents lipid peroxidation of the sperm plasma membrane, DNA damage, and reduced motility and fertilizing capacity (Aitken et al., 1993; Lucio et al., 2016). Furthermore, sericin exhibits a range of biological activities, such as ROS scavenging, anti-tyrosinase, anti-elastase properties, and immune regulation (Aramwit et al., 2010). By reducing ROS, sericin prevents cell death and promotes cellular growth, thus improving sperm viability during cryopreservation (Takahashi et al., 2003).

Sericin also protects against temperature-induced stress and the harmful effects of cryoprotectants on sperm (Sasaki et al., 2005). It enhances antioxidant enzyme activity, including superoxide dismutase, catalase, and glutathione peroxidase (Li et al., 2008), and its hydroxyl groups help chelate trace metals like copper and iron, further reducing oxidative stress (Miguel and Álvarez-López, 2020; Fatahian et al., 2021).

Beyond semen preservation, sericin supports reproductive health by mitigating photoperiod-induced disorders affecting testicular function, sex hormones, and sperm quality (Hassan et al., 2022). It also alleviates oxidative stress and restores spermatogenesis and steroidogenesis in mice (Habiba et al., 2023). Additionally, silk amino acids (SAA) enhance physical stamina, protect tissues, and boost male reproductive function by increasing testosterone levels and sperm count (Shin et al., 2010).

The unique structural properties of sericin, including its globular conformation and high content of serine and threonine, make it a valuable cryoprotectant. Its antioxidative and hydrophilic properties enable it to effectively scavenge ROS, reducing oxidative damage to sperm membranes, DNA, and motility. Additionally, sericin can chelate trace metals and enhance antioxidant enzyme activity that supports sperm viability during freezing and thawing. These structural and biochemical attributes highlight sericin's potential for improving sperm preservation and reproductive health across various species.

ROLE OF SERICIN IN CRYOPRESERVATION OF DIFFERENT CELLS

The potential of sericin in cryopreservation was first explored in the late 20th century, when its antioxidative properties were recognized as beneficial for preserving mammalian cells. Early studies by Kato et al. (1998) and Sasaki et al. (2005) demonstrated that sericin effectively neutralizes harmful effect of ROS, such as hydroxyl and superoxide radicals, thereby protecting cells from oxidative

damage during cryopreservation. Initially investigated as a serum replacement in cryopreservation media due to its radical-scavenging abilities, sericin's cryoprotective effects were later confirmed in a variety of applications.

For example, Isobe et al. (2013) showed successful post-thaw pregnancies in bovine embryos cryopreserved with 0.5% sericin. Furthermore, sericin has been shown to play a critical role in the cryopreservation of various cell types and embryos. In bovine and porcine embryos, sericin reduced oxidative stress and improved post-thaw development (Isobe et al., 2012; Do et al., 2014). It provides general cellular protection, preventing cell death and promoting growth under cryopreservation stressors such as freezing and exposure to ethanol (Takahashi et al., 2003; Sasaki et al., 2005).

The application of sericin extends beyond embryos; it has also been used as a replacement for bovine serum albumin and fetal bovine serum in oocyte maturation and embryo culture media, where it enhances the developmental competence of oocytes and embryos (Yasmin et al., 2015; Aghaz et al., 2016). However, sericin's antioxidative effects are dose dependent. Moderate concentrations (e.g., 0.5%) are beneficial, while excessive supplementation (e.g., 1.0%) can lead to lipid peroxidation and compromise cell quality (Wu et al., 2007; Aghaz et al., 2020).

In cryopreservation, sericin improves embryo viability and post-thaw outcomes across various cell types. For instance, 0.5% sericin enhanced ovine oocyte maturation and blastocyst development, making it a viable alternative protein supplement for in vitro maturation (IVM) and culture (Hajarian et al., 2017). Additionally, sericin's antioxidative and anti-apoptotic properties have led to its use as a serum substitute or additive for cell culture and cryopreservation media (Cao et al., 2017).

Low-molecular-weight sericin has also been shown to enhance mammalian cell culture in serum-free media (SFM), making sericin-based SFMs valuable for both cryopreservation and cell culture (Terada et al., 2005; Ohnishi et al., 2012). Additionally, an autoclavable cryopreservative solution containing sericin has been proven effective for mammalian cell preservation (Ikeda et al., 2010).

Sericin (1%) has been shown to mitigate oxidative stress and protect ovarian tissues during freezing and thawing via the PI3K/AKT/mTOR signaling pathway (Shua et al., 2023). It also serves as a substitute for fetal bovine serum in freezing media for human mesenchymal stromal cells (hMSCs), although it cannot replace DMSO (Verdanova et al., 2014). In reproductive biology, sericin enhances in vitro embryo production, as Cumulus–Oocyte Complexes (COCs) matured in sericin-supplemented media exhibited significantly higher meiotic maturation rates and accelerated blastocyst formation compared to controls (Khatun et al., 2024). This highlights sericin's potential in improving embryo viability and its application in assisted reproductive technologies (Khatun et al., 2018). The Table 1 summarizes the beneficial effects of sericin across different species and applications, demonstrating its versatility as a cryoprotectant and its potential for enhancing cell culture and cryopreservation outcomes in reproductive biology.

Table 1 Summary of sericin in cryopreservation of different cells

Species	Application	Concentration	Effect
Bovine Embryos	Cryopreservation	0.5%	Improved post-thaw development and successful pregnancies (Isobe et al., 2013)
Porcine Embryos	Cryopreservation	0.5%	Reduced oxidative stress, improved post-thaw development (Isobe et al., 2012)
Ovine Oocytes	In vitro maturation (IVM) and embryo culture	0.5%	Enhanced maturation and blastocyst development (Hajarian et al., 2017)
Human Mesenchymal Stromal Cells (hMSCs)	Cryopreservation	1%	Effective substitute for fetal bovine serum (Verdanova et al., 2014)
Ovarian Tissues	Cryopreservation	1%	Protection during freezing/thawing via PI3K/AKT/mTOR signaling pathway (Shua et al., 2023)
Cumulus-Oocyte Complexes (COCs)	In vitro embryo production (IVP)	Sericin-supplemented media	Higher meiotic maturation rates and accelerated blastocyst formation (Khatun et al., 2024)

SERICIN'S EFFECTS ON SPERM PARAMETERS

Sperm motility is crucial for fertilization, as sperm must swim through the female reproductive tract to reach and fertilize the egg. It is a key parameter of sperm quality that influences fertility and reproductive outcomes. Poor motility often results in decreased fertility, making the preservation of motility during semen storage and cryopreservation essential for artificial insemination and breeding. Sericin supplementation has been shown to improve sperm motility, especially in cryopreserved semen. For example, frozen-thawed mouse sperm supplemented with 0.25% and 0.5% sericin exhibited improved motility (Ghasemi et al., 2018). Similarly, in Kankrej bull semen, supplementation with sericin in TFYG extender resulted in higher motility and lower lipid peroxidation (Shaikh et al., 2016). Patel et al. (2019) and other studies (Dorji et al., 2015; Kumar et al., 2015) also observed improved motility and reduced oxidative stress in bovine semen cryopreserved with sericin. In rooster semen, sericin supplementation improved total and progressive motility and reduced MDA levels after cryopreservation (Ratchamak et al., 2023). However, concentrations above 1% may negatively impact sperm motility (Terada et al., 2002). For instance, in buffalo semen, sericin concentrations between 0.1% and 0.5% improved post-thaw motility, while higher concentrations were detrimental (Kumar et al., 2015; Dorji et al., 2015).

Sericin also helps maintain sperm motility during storage at low temperatures. Aad et al. (2024) found that 0.1% sericin helped maintain motility in sperm stored at 15°C for up to 72 hours. However, sperm quality declined on days 2 and 3 of storage due to oxidative damage, particularly in species like rabbits (Roca et al., 2000; Rosato and Iaffaldano, 2011). Studies by Johinke et al. (2014) and Raza et al. (2024) suggest that sericin improves sperm kinematics, which may enhance fertility. In chickens, 0.025%-0.2% sericin in sperm diluent improved motility and vigor, especially after 5 days of storage (Sonseeda et al., 2015). In dairy bulls, 0.25% and 0.5% sericin improved motility and curvilinear velocity after thawing (Yangngam et al., 2021), while 2.5%-5% sericin supplementation improved human sperm motility and viability (Aghaz et al., 2020). In stallion semen, sericin, combined with glutathione, significantly enhanced motility and kinematic parameters, particularly class A sperm (Nasirabadi et al., 2019). In Hariana bull semen, 0.25% sericin showed the highest progressive motility (65.42±0.67%), followed by 0.50% sericin (60.42±0.67%), while the control group had the lowest motility (56.25±0.69%) (Yadava et al., 2018).

Moreover, other cryoprotectants like trehalose and glutathione can also increase sperm motility. Trehalose enhances human sperm cryopreservation by improving motility, viability, cellular integrity (membrane, DNA, acrosome), and mitochondrial function (Gholami et al., 2023). It also benefits sperm

cryopreservation across species: in ram sperm, it improves post-thaw motility, viability, and mitochondrial activity (Öztürk et al., 2020); in buck sperm, it enhances membrane integrity and motion parameters (Xu et al., 2022); and in canine sperm, it reduces damage to motility and acrosomes (Park et al., 2018). Additionally, glutathione supplementation improves sperm motility parameters (Lenzi et al., 1992).

In conclusion, sericin supplementation improves sperm motility by reducing oxidative stress, preserving membrane integrity, and enhancing metabolic activity. Lower concentrations (0.25%-0.5%) are typically most effective, while higher concentrations may have negative effects. The observed inconsistencies in sericin's effects on sperm motility may stem from variations in semen extenders, differences in cryopreservation protocols, and species-specific sperm physiology. Further research is needed to better understand the mechanisms by which sericin helps maintain sperm motility over a prolonged period (3-5 days), and to identify the specific pathways involved in energy production. Therefore, the hypothesis that sericin acts as an energy substrate needs to be verified.

Sperm viability is crucial for fertilization, as it ensures that sperm can maintain motility and integrity to reach and fertilize the egg. It is a key indicator of semen quality and reflects the sperm's ability to survive and retain fertilizing potential. Maintaining high sperm viability during cryopreservation is essential for the future successful use of stored sperm. Sericin, through its antioxidant and antibiotic properties, protects sperm from oxidative stress, preserving viability during storage and cryopreservation. For example, rabbit sperm pretreated with 0.5% sericin showed higher viability after thawing (Raza et al., 2019). Similarly, Ghasemi et al. (2018) found that frozen-thawed sperm supplemented with 0.25% and 0.5% sericin had higher viability. In chicken semen, 0.025% sericin improved viability at day 1 post-storage, while 0.25% sericin enhanced viability in chilled semen (Sonseeda et al., 2015). Adding sericin to freezing and thawing media also benefited human sperm viability, with 5% sericin increasing total viability (Aghaz et al., 2020). Yangngam et al. (2021) reported higher sperm viability and mitochondrial function with 0.25% sericin compared to controls and 1.0% sericin. Studies in rooster semen showed that 0.5% of the sericin improved viability, while higher concentrations (0.75%) decreased it (Ratchamak et al., 2023). According to Yadava et al. (2018), the 0.25% sericin group had the highest percentage of live spermatozoa in Hariana bull semen (82.13±0.68%), followed by the 0.50% sericin group (76.54±0.63%), while the control group had the lowest viability (71.33±0.68%). Sericin supplementation in extenders also maintained sperm viability better than extenders without sericin (Khye et al., 2021).

Sericin at low to mid concentrations improves sperm viability, while higher doses reduce viability and mitochondrial function, indicating a dose-dependent effect. The observed inconsistencies in sericin's effects may stem from differences in extender additives, cooling rates, equilibration times, and species-specific sperm physiology, all of which can significantly alter sperm responses. Additionally, factors such as membrane lipid composition and intrinsic antioxidant capacity influence sperm sensitivity to oxidative stress. Overall, sericin's ability to enhance sperm viability is crucial for preserving sperm quality during storage and cryopreservation. The Table 2 summarizes the effective sericin concentrations across different species, demonstrating its role in enhancing sperm viability and its potential for optimizing cryopreservation protocols in reproductive biology.

Table 2 Effective sericin concentrations per species for sperm viability

Species	Concentration	Effect on Sperm Viability	Reference
Rabbit	0.5%	Higher viability after thawing	Raza et al. (2019)
Hariana Bull	0.25% - 0.50%	Highest viability at 0.25% (82.13%), followed by 0.50% (76.54%)	Yadava et al. (2018)
Chicken	0.025% - 0.25%	0.025% improved viability on day 1; 0.25% improved viability in chilled semen	Sonseeda et al. (2015)
Rooster	0.5%	Increased viability; 0.75% reduced viability	Ratchamak et al. (2023)
Human	5%	Increased total viability	Aghaz et al. (2020)
Frozen-Thawed Sperm	0.25% - 0.50%	Improved viability post-thawing	Ghasemi et al. (2018)
Multiple Species	0.25%	Higher viability and mitochondrial function	Yangngam et al. (2021)
Extender Studies	Various concentrations	Sericin extenders preserved sperm viability better than controls	Khye et al. (2021)

Acrosome integrity is vital for successful fertilization, as it contains enzymes necessary for sperm to penetrate the egg's outer layers. During fertilization, the acrosome undergoes a reaction, releasing these enzymes to help the sperm fuse with the egg. Damage to the acrosome can reduce sperm function, motility, and the ability to fertilize, leading to infertility or suboptimal fertility outcomes. Maintaining acrosome integrity is vital during sperm processing, such as cryopreservation and thawing, which can cause damage. Supplementing with 0.25% sericin significantly improved acrosome integrity in bull sperm compared to the control and 1.0% sericin (Yangngam et al., 2021). Similarly, in buffalo semen, 0.25% sericin supplementation significantly enhanced acrosome integrity (87.2±1.6 vs. 79.8±5.5) compared to the control group (Demra et al., 2017). Rabbit spermatozoa pretreated with 0.5% sericin showed significantly higher acrosome integrity after thawing (Raza et al., 2019). In a study on post-thawed bull spermatozoa, acrosome integrity was significantly higher in the sericin-treated group (58.0±3.6%) compared to the control (50.8±3.6%) reported by Saphungthong et al. (2023). Yadava et al. (2018) observed that the 0.25% sericin group had the highest percentage of undamaged acrosomes in Hariana bull semen (74.92±0.86%), followed by the 0.50% sericin group (70.25±0.75%), while the control group had the lowest percentage (66.58±0.92%). However, in canine semen, no significant differences were observed in acrosome and plasma membrane integrity among sericin-treated groups (Khye et al., 2021).

Research has shown that higher sericin concentrations may impair sperm quality. For example, increasing sericin concentration to 1.0% negatively affected total motility in dairy bull semen compared to lower concentrations (Yangngam et al., 2021). Similarly, Kumar et al. (2015) reported harmful effects on sperm cells at sericin concentrations between 1% and 2%. Conversely, a study on human spermatozoa revealed that supplementation with 2.5% and 5% sericin improved sperm viability and motility, emphasizing the need for concentration optimization to achieve protective benefits while avoiding toxicity (Aghaz et al., 2020). The adverse effects observed at higher sericin concentrations may be linked to osmotic imbalance, which can cause cellular dehydration or swelling and compromise sperm viability; protein aggregation, which may impair membrane stability and function; increased viscosity in the cryopreservation medium, which may reduce sperm motility and cryoprotectant distribution; and cytotoxicity, which can trigger oxidative stress or disrupt cellular signaling pathways, leading to increased lipid peroxidation. Excess antioxidant concentrations can cause hypertonic conditions, leading to dehydration of sperm cells (Bucak et al., 2007). Elevated antioxidant levels may disturb the balance between free radicals and antioxidants in mammalian cells (Rahal et al., 2014). For instance, antioxidant overdoses have been shown to reduce the functional integrity of the acrosome and plasma

membrane (Atessahin et al., 2008). Similarly, higher DHA concentrations have been reported to impair sperm motility (Losano et al., 2018).

Sericin's effectiveness in semen preservation varies across species due to differences in sperm membrane composition, antioxidant capacity, and metabolism. Species with higher unsaturated fatty acid content in their sperm membranes, like boars and rams, are more vulnerable to lipid peroxidation, particularly during cryopreservation due to increased ROS production (Hezavehei et al., 2018). Species with lower cholesterol-to-phospholipid ratios, such as bulls and roosters, are more susceptible to cold shock and membrane damage (Holt, 2000). In these species, sericin helps by stabilizing membranes in those with lower cholesterol content and providing antioxidant protection in those with higher unsaturated fatty acids. Species with strong antioxidant defenses, like stallions, may see limited benefits, while those with weaker defenses, like roosters, experience more significant improvements. Additionally, species with higher metabolic rates generate more ROS and are more responsive to sericin's antioxidant effects. Overall, sericin is the most effective in species with vulnerable sperm membranes or weaker antioxidant systems.

Membrane cholesterol-to-phospholipid (C:P) ratio influences sperm sensitivity to cold shock damage (Holt, 2000). Sperm with elevated C:P ratios (e.g., rabbit and human sperm, 0.88 and 0.99, respectively) are more resistant to cold shock damage compared to sperm with lower C:P ratios (e.g., boar, ram, and bull sperm, 0.35, 0.37, and 0.45, respectively) (Davis, 1981; White, 1993). Therefore, these findings highlight the critical need to determine appropriate sericin concentrations in semen extenders to ensure optimal sperm quality post-thaw. Figure 2 illustrates the protective role of sericin against oxidative stress in sperm cells, highlighting its antioxidant properties and its potential to improve sperm function and preservation in reproductive biology.

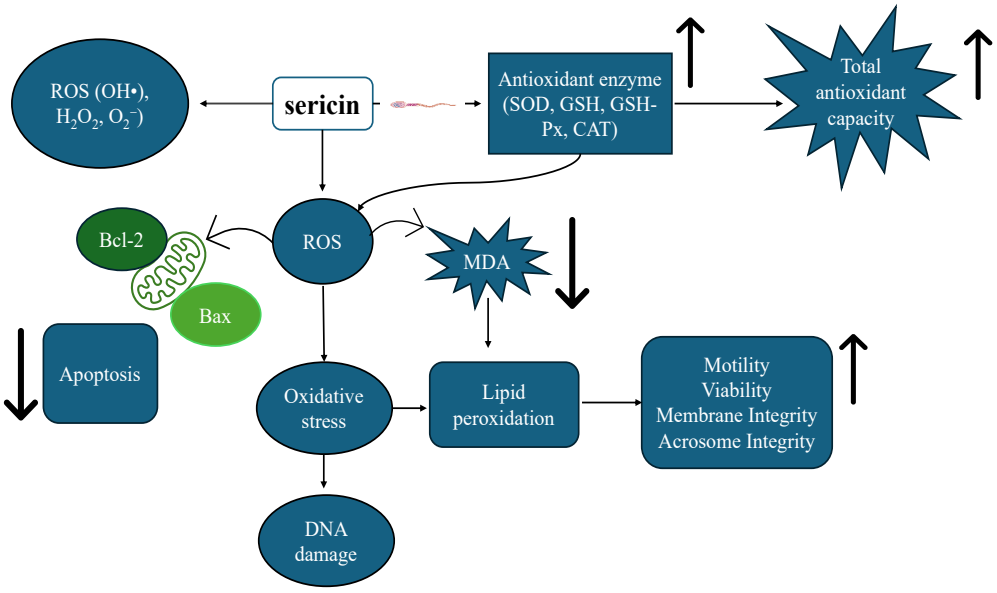

Figure 2 Protective role of sericin against oxidative stress in sperm cells

SERICIN'S IMPACT ON FERTILITY OUTCOMES

Sericin, a silk-derived protein, has demonstrated potential in improving sperm cryopreservation outcomes and fertility rates. In native chickens, fertility was highest in the 0.50% sericin group (54.92±0.50%), significantly surpassing the control group (36.41±0.68%) and other sericin concentrations. Lower fertility rates were observed in the 0.75% (37.39±0.36%) and 0.25% (33.45±0.15%) sericin groups, emphasizing the optimal effect of 0.50% sericin on fertility enhancement (Ratchamak et al., 2023). Similarly, in rabbits, the use of 0.1% sericin at storage temperatures of 4°C and 15°C resulted in higher conception rates of 66.6% and 71.4%, respectively (Reza et al., 2019). These findings suggest that sericin can improve sperm quality and fertility outcomes across different species.

Despite its promising effects, the application of sericin in commercial artificial insemination (AI) programs remains limited. Most of the research has been done in lab settings, and there aren't many extensive field tests evaluating sericin's effectiveness in actual AI applications. Therefore, further research is necessary to validate these findings in commercial settings and optimize sericin concentrations for different species and storage conditions.

SERICIN VS. TRADITIONAL CRYOPROTECTANTS

Sericin, derived from silkworm cocoons, has strong antioxidative and radical-scavenging properties, making it highly effective in protecting sperm from oxidative damage and improving post-thaw quality, including motility and acrosome integrity. Additionally, sericin possesses antibacterial activity, reducing the risk of contamination. These characteristics position it as a safer and more consistent alternative to traditional cryoprotectants, particularly in advanced reproductive technologies such as oocyte maturation and embryo culture. However, its concentration must be carefully optimized, as high levels (>0.5%) can induce lipid peroxidation and negatively impact sperm quality.

Traditional cryoprotectants like BSA and egg yolk have long been used for sperm preservation, each with distinct properties. BSA stabilizes sperm membranes and protects against oxidative stress through its antioxidative and osmoregulatory functions. However, its antioxidant capacity may be weaker than that of sericin, and it carries risks such as pathogen contamination, batch variability, and incompatibility with certain biotechnological applications. Egg yolk, while effective in preserving sperm membranes during cooling and freezing, lacks inherent antioxidants or antibacterial properties. Additionally, it raises biosecurity concerns, requiring stringent quality control measures.

In contrast, sericin presents a cost-effective alternative to traditional cryoprotectants like BSA, glycerol, dimethyl sulfoxide (DMSO), and egg yolk. Because sericin is derived from textile industry byproducts, its raw material costs are significantly lower than those of chemically synthesized cryoprotectants. The extraction process is simple and inexpensive, involving boiling in water, making it economically viable for large-scale use. Furthermore, sericin’s stability in dry or lyophilized form reduces logistical costs, unlike DMSO and glycerol, which require strict storage conditions. Additionally, sericin’s potent antioxidant and protective properties may allow its use at lower concentrations, further enhancing its cost-effectiveness.

Beyond its individual benefits, sericin may work synergistically with other additives to further optimize sperm cryopreservation. For example, Kankrej bull semen treated with 100 mM trehalose and sericin showed improved motility and reduced oxidative stress (Shaikh et al., 2016). Similarly, combining sericin with

glutathione significantly enhanced sperm quality, particularly in class A sperm, sperm velocity, and trajectory (Nasirabadi et al., 2019).

While the sericin shows promising protective effects, recent advancements in nanoparticle-based cryoprotectants provide new avenues for enhanced cryopreservation. Nanoparticles loaded with antioxidants such as quercetin, selenium, or zinc oxide offer targeted delivery, controlled release of protective agents, and improved cellular uptake. Combining sericin with nanoparticle-based cryoprotectants could enhance overall effectiveness by leveraging sericin's antioxidant and membrane-stabilizing properties while utilizing nanoparticles for precise cryoprotectant delivery. This synergistic approach could improve sperm quality and survival rates by addressing both oxidative stress and membrane integrity more effectively.

However, sericin's natural origin, affordability, and potent cryoprotective properties make it a promising candidate for sperm cryopreservation and reproductive biotechnology. As research progresses, its integration into commercial cryopreservation protocols could offer a practical and economical alternative to traditional cryoprotectants. Table 3 summarizes the complications in optimizing sericin concentrations and the use of alternative cryoprotectants across different species and studies, highlighting key factors such as species, breed, semen type, extender, tested concentrations, optimum dose, and observed effects on sperm quality.

Table 3 Complications in the optimization of sericin concentration and alternative cryoprotectants across species and studies

Species	Breed	Semen Type	Extender	Concentration	Optimum Dose	Effect	References
Goat	Barbari	Frozen	Not mention	0, 0.25 and 0.50%	0.25%	Sericin upregulates HSP70	Reddy et al. (2018)
Goat	Chengde hornless black goats	Frozen	Optixcell	0.2%, 0.4%, 0.6%, 0.8%, and 1%	0.6%	Sericin enhances frozen semen quality	Zhang et al. (2024)
Stallion	Not mention	Frozen	INRA 82 medium: 2% egg yolk, 20 mm HEPES, 2.5% glycerol	0, 0.25% Sericin, 1.5 mm Glutathione, 0.25% Sericin + 1.5 mm Glutathione	0.25% Sericin + 1.5 mm Glutathione	Sericin preserves DNA and resists oxidative damage	Nasirabadi et al. (2019)
Bull	Holstein-Friesian crossbred	Frozen	Tris-extender (without egg yolk and glycerol)	(0, 0.25, 0.5, and 1.0% (wt/vol)	0.25%	Sericin improves post-thaw quality, reduces peroxidation	Yangngam et al. (2021)
Chicken	Thai native	Cold	Iggkph diluent	0.025, 0.05, 0.1 and 0.2%	0.03%	Sericin enhances chicken semen preservation	Sonseeda et al. (2015)
Buffalo	Not mention	Frozen	Tris egg yolk extender	0.25, 0.5, 0.75 and 1.0%	0.25%	Sericin enhances buffalo semen cryopreservation	Demra et al. (2017)
Rabbit	New Zealand white	Short term preserved	Tris–citric acid–glucose (TCG)	0, 0.1%, and 0.5%	0.5%	Sericin boosts rabbit sperm fertility	Raza et al. (2024)
Human	-	Frozen	Spermfreeze	0, 0.5, 1, 2.5, and 5%	5%	Sericin improves motility, viability, reduces DNA fragmentation	Aghaz et al. (2020)

Table 3 Cont.

Species	Breed	Semen Type	Extender	Concentration	Optimum Dose	Effect	References
Rabbit	Not mention	Frozen	Me2SO (TCG-sucrose-Me2SO)	0, 0.1% or 0.5% (w/v)	0.10%	Sericin improves sperm quality, and osmotic tolerance	Raza et al. (2019)
Boar	Not mention	Frozen	Modena extender (1:1) (v/v)	0, 0.25, 0.50, 0.75, 1%	0.75%	Sericin is recommended as an alternative component in freezing	Ratchamak et al. (2020)
Mice	Not mention	Frozen	Modified 18% raffinose pentahydrate and 3% skimmed milk	0, 0.25, 0.5, and 0.75%	0.50%	Sericin improves frozen-thawed sperm quality, embryo development	Ghasemi et al. (2018)
Goat	Pantja bucks	Frozen	Tris Extender	EYT was used, with 4.0% Rosemary and 0.25% Sericin	Both demonstrated better cryoprotective effects	Sericin enhances cryoprotection, post-thaw semen quality	Kumar et al. (2024)
Canine/dog	Not mention	Chilled	Tris-egg yolk	0%, 0.1%, 0.25%, and 0.5%	0.25% and 0.5%	Sericin preserves motility, viability for 72 hours	Khye et al. (2021)
Bull/buffalo	Gir and Murrah bulls	Frozen	Tris-fructose egg yolk glycerol	0, 0.1, 0.25, 0.50 and 1.0%, (w/v)	0.5% and/or 0.25%	Sericin optimizes cryopreservation, enhances post-thaw quality	Patel et al. (2019)
Bull/buffalo	Gir and Murrah	Frozen	TFYG extender	Mifepristone (10 µg/ml), Sericin (5 mg/ml), Taurine (4 mg/ml)	Mifepriston (10 |jg/ml)	Sericin enhances motile sperm percentage	Chaturvedi et al. (2022)
Bull	Not mention	Frozen	Not mention	0, 0.25, 0.5, 1.5 and 2%	0.25–0.5%	Sericin improves frozen-thawed semen quality	Kumar et al. (2015)
Buffalo	Jaffarabadi	Frozen	Andromed® extender	0, 0.25, 0.50, 0.75, 1.0%	0.25 or 0.50% (w/v)	Sericin improves post-thaw quality	Vijyeta et al. (2024)
Chicken	Not mention	Frozen	Schramm extender	0%, 0.25%, 0.50%, and 0.75%	0.50%	Sericin improves cryopreserved rooster semen quality	Ratchamak et al. (2023)
Bull	Hariana	Frozen	Egg yolk tris citrate (EYTG)	0, 0.25, 0.50%	0.25%	Sericin enhances cryo-protection in Hariana semen	Yadava et al. (2018)
Bull	Not mention	Frozen	Egg yolk-tris-citric extender with and without glycerol	0.50%	0.50%	Sericin preserves motility without affecting fertilization	Saphungthong et al. (2023)
Bull	Gir and Murrah	Frozen	Tris-citrate-fructose-yolk-glycerol extender	0.1, 0.25, 0.5 and 1.0% (w/v)	0.50%	Sericin improves post-thaw sperm quality, reduces stress	Patel et al. (2020)
Elephant	Asian	Frozen	TEST (20% egg yolk and 5% glycerol) extender	0, 0.5%, 1%, and 1.5%	1.0-1.5%	Increase motility and plasma membrane integrity	Keatisak et al. (2023)

Table 3 Cont.

Species	Breed	Semen Type	Extender	Concentration	Optimum Dose	Effect	References
Sheep	Akkaraman	Frozen	Tris-based	50, and 100mM (Trehalose)	100mM	Significantly elevated vitamin E (vit E) levels	Bucak et al. (2007)
Goat	Assam Hill	Frozen	Tris-citric acid fructose egg yolk glycerol	50 and 100 mM (Trehalose)	100 mM	Enhance sperm membrane protein retention (29 kDa and 42 kDa bands)	Dutta et al. (2023)
Goat	Not mention	Frozen	tris-citric acid-egg yolk-fructose	75, 150, 450, and 900 mm (Trehalose)	150 mM	Progressive Motility, Total Motility, and plasma membrane integrity increase	Kamal et al. (2023)
Cattle	Hariana	Frozen	Tri-Fructose Yolk Glycerol (TFYG)	10, 30 and 50 mM (Trehalose)	30 mM	Significantly($P<0.05$) higher percentage of individual sperm motility, sperm viability, and intact acrosome	Kumar et al. (2023)
Pig	Hampshire crossbred	Frozen	Beltsville Thawing Solution	0.5, 1, and 2μgml−1 of Se-NPs	1μgml−1	Improved semen quality and achieved the highest conception rate	Paul et al. (2024)
Chicken	Not mention	Frozen	Not mention	Casein extender with quercetin concentrations: CS-Q 0.040, CS-Q 0.020, CS-Q 0.010, and CS-Q 0.005 (mg/mL).	0.010 mg/mL quercetin	Enhanced post-thaw sperm quality and antioxidant activity.	Appiah et al. (2020)
Chicken	Ross 308	Frozen	Lake extenders	Control: No ZnONP; ZnO100: 100-μg ZnO; ZnONP50: 50-μg ZnONP; ZnONP100: 100-μg ZnONP; ZnONP200: 200-μg ZnONP.	50- to 100-μg ZnONP	Improved sperm motility, membrane integrity, mitochondrial activity, acrosome integrity, viability, and fertility, while reducing DNA fragmentation, lipid peroxidation, and ROS levels.	Karimi-Sabet et al. (2024)
Bull	Not mention	Frozen	TRIS-egg yolk	0.01, 0.1, and 1 μM PQQ; 10, 100, and 1000 μM Ergothioneine; 10, 100, and 1000 μM Vitamin C	1 μM PQQ, 100 μM ergothioneine or1000 μM vitamin C	PQQ and VC reduced mitochondrial ROS; Ergo and VC reduced cytosolic ROS.	Younus et al. (2024)

The optimal sericin concentration varies across species. Most animals benefit from concentrations of 0.25–0.50%, though higher concentrations are more effective in boars (0.75%) and humans (5%). Goats showed the best results at 0.25–0.6% (Kumar et al., 2024; Zhang et al., 2024), while in buffalo and bulls, 0.25–0.50% was optimal for post-thaw sperm quality (Demra et al., 2017; Patel et al., 2020; Vijyeta et al., 2024). In chickens, concentrations of 0.03–0.50% enhanced semen preservation (Sonseeda et al., 2015; Ratchamak et al., 2023). Rabbits benefited from 0.10–0.50% (Raza et al., 2019, 2024), while mice showed the best results at 0.50% (Ghasemi et al., 2018).

Despite its varying degrees of effectiveness across different species, sericin generally enhances sperm quality. However, its impact on fertilization rates remains uncertain. Saphungthong et al. (2023) reported that while sericin preserved sperm motility, it did not significantly improve fertilization rates, suggesting that its effectiveness may depend on specific conditions or extender compositions.

SERICIN IN COMMERCIAL APPLICATIONS: CHALLENGES AND FUTURE DIRECTIONS

Sericin, a silk-derived protein, offers antioxidant and cryoprotective benefits for commercial semen preservation. It scavenges free radicals, stabilizes membranes, enhances sperm motility and viability, and reduces DNA fragmentation. Its inclusion in semen extenders has shown promise across species like cattle, sheep, and poultry.

Despite these advantages, several barriers hinder the widespread commercial adoption of sericin in semen cryopreservation. Key challenges are variations in purity and composition due to differences in extraction methods, and the lack of standardized processing techniques. Limited standardization makes it difficult to optimize the sericin for commercial applications, as the ideal concentration varies across species and cryopreservation protocols.

Scalability is another major concern. Industrial-scale extraction and processing methods must be optimized to maintain sericin's bioactivity while ensuring cost-effectiveness. Additionally, regulatory hurdles, such as the need for comprehensive safety and efficacy testing-can slow its approval for clinical and agricultural use. Moreover, further research is required to assess potential interactions with existing cryopreservation media and to determine the long-term effects of sericin in sperm quality.

Although laboratory studies highlight sericin's cryoprotective potential, its integration into commercial semen preservation remains in its early stages. There is currently little evidence of its widespread adoption in AI programs. Its effectiveness also varies across species, emphasizing the need for species-specific research. Additionally, the molecular mechanisms underlying sericin's interaction with sperm membranes remain poorly understood.

Future research should focus on determining the optimal concentrations of sericin, evaluating its long-term effects on sperm DNA integrity, and exploring its synergy with advanced cryoprotectants, such as nanoparticles. Nanoparticle-based cryoprotectants offer targeted delivery of antioxidants and controlled release of protective agents, potentially enhancing sericin's efficacy. Large-scale trials are essential to validate laboratory findings and establish sericin's viability in commercial applications.

The promise of sericin as a substitute sperm energy source has not been confirmed, even though it has been shown to sustain sperm motility for up to 72 to 120 hours. Comparative studies with other antioxidants and cryoprotectants are also lacking. To fully optimize the sericin for commercial use, more comprehensive, large-scale, and comparative studies are needed to evaluate its benefits across different species and reproductive contexts.

CONCLUSIONS AND PERSPECTIVES

In conclusion, sericin has demonstrated significant promise as a cryoprotectant in semen preservation across a wide range of species, including ruminants, poultry, rabbits, and humans. Optimal sericin concentrations for improving semen quality and post-thaw outcomes typically range between 0.25% and 0.6% in ruminants, and 0.03%–0.5% in poultry and rabbits, with concentrations up to 5% benefiting human sperm. Its ability to stabilize sperm

membranes, preserve DNA integrity, reduce oxidative stress, and serve as an important component of semen extenders, especially those used in assisted reproductive technologies (ARTs).

The observed discrepancies in sericin's effects could be caused by differences in cryopreservation techniques, species-specific sperm physiology, and semen extenders. Variations in cooling rates, equilibration times, and additives can change the sperm response, whereas antioxidant capacity and membrane lipid composition affect the sensitivity to oxidative stress. Small sample sizes, erratic experimental designs, and insufficient controls can also lead to contradictory findings. To determine the best way to employ sericin in sperm cryopreservation, these considerations highlight the necessity of consistent procedures and cross-species examinations.

However, careful species-specific optimization is crucial, as higher concentrations above 1% may induce negative effects such as cellular dehydration, membrane disruption, and oxidative stress, limiting its broader application. The main barriers to using sericin include the need to find the right dosage and the difficulty of improving semen extender formulas so they work well without any negative effect. Future research should focus on understanding how sericin protects sperm, especially how it helps reduce oxidative stress. It's also important to find out whether sericin can reduce oxidative stress in the sperm's cytoplasm or mitochondria. In addition, studying how sericin might work together with other protective substances could help improve semen quality. By solving these challenges and using sericin more effectively, it could help improve artificial insemination programs, boost fertility rates, and be used in other species.

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AUTHOR CONTRIBUTIONS

MYA, AK: conceptualization, paper collection, reading, writing-original draft, and review & editing. Both authors contributed to the final version of the manuscript and approved it.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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