ผลของสารสกัดโปรตีนไหม (sericin) ต่อคุณภาพของน้ำเชื้อช้างเอเชีย (*Elephas maximus*) แช่แข็ง ภายหลังการอุ่นละลาย

Effect of Sericin Supplementation on Post-Thawed Asian Elephant (*Elephas maximus*) Semen Quality

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ABSTRACT

The development of optimal components for cryopreservation medium is critical for breeding management and genetic diversity preservation in endangered species like the Asian elephant (*Elephas maximus*). Sericin, a silk protein, has emerged as a novel cryoprotectant due to its antioxidative, membrane-stabilizing, and anti-apoptotic properties. This study evaluated the influence of sericin supplementation as a cryoprotectant on post-thaw Asian elephant sperm quality. Ten ejaculates were collected, diluted in a TEST with 20% egg yolk and 5% glycerol extender accompanied with varying sericin concentrations (0%, 0.5%, 1%, 1.5%), cryopreserved, and evaluated to determine post-thawed motility, viability, structural stability of the membrane and DNA, and motility parameters. The statistical analysis indicated notable enhancements in straightness and viability with 1.5% and 1% sericin, respectively, in comparison to the control group with

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p-value < 0.05. Based on previous findings in sericin studies involving different species, this investigation confirms that the inclusion of 1-1.5% sericin in freezing solutions improves specific parameters such as velocity, straightness, and viability of post-thaw Asian elephant sperm. Further research should clarify the exact mechanisms between semen-specific improvements and fertilizing ability according to the sericin supplementation in semen extender.

Keywords: Asian elephant, Spermatozoa, Cryopreservation, Sericin, Antioxidant

บทคัดย่อ

การพัฒนาองค์ประกอบสารละลายเพื่อการรักษาสภาพในระหว่างการแช่แข็งยังคงมีความจำเป็นต่อการจัดการความหลากหลายทางพันธุกรรมในสัตว์ที่อยู่ในภาวะใกล้สูญพันธุ์ อาทิเช่น ช้างเอเชีย (*Elephas maximus*) โดยจากการศึกษาในสปีชีส์อื่นก่อนหน้านี้เซริซิน (sericin) ให้ผลการทดลองในการคงคุณภาพน้ำเชื้อภายหลังผ่านกระบวนการแช่แข็งผ่านกลไกการต่อต้านอนุมูลอิสระ (antioxidative), คงสภาพเยื่อหุ้มเซลล์ของสเปิร์ม (membrane-stabilizing), และลดการถูกทำลายของเซลล์ (anti-apoptotic) ดังนั้น การศึกษาครั้งนี้จึงจัดทำขึ้นเพื่อวิเคราะห์ประสิทธิภาพของเซริซินที่ความเข้มข้นต่างๆ (0%, 0.5%, 1%, 1.5%) ในการคงสภาพตัวอย่างน้ำเชื้อช้างเอเชีย 10 ตัวอย่าง จากช้าง 7 เชือก ระหว่างการเก็บรักษาด้วยการแช่แข็งร่วมกับสารละลายมาตรฐานสูตร TEST 20% egg-yolk และ 5% glycerol โดยตรวจประเมินคุณภาพการเคลื่อนที่, สเปิร์มที่มีชีวิต (viability), ความสมบูรณ์ของอะโครโซม (acrosome), ความสมบูรณ์ของ DNA, และความสมบูรณ์ของเยื่อหุ้มเซลล์ (plasma membrane) หลังผ่านการวิเคราะห์ทางสถิติพบความแตกต่างอย่างมีนัยสำคัญที่ความตรงของการเคลื่อนที่ (straightness) และร้อยละของสเปิร์มที่มีชีวิต ที่ความเข้มข้นของเซริซิน 1.5% และ 1% ตามลำดับ เมื่อเปรียบเทียบกับการทดลองในสปีชีส์อื่นๆ จึงสนับสนุนได้ว่าเซริซินที่ความเข้มข้น 1% และ 1.5% สามารถช่วยคงคุณภาพน้ำเชื้อในกระบวนการแช่แข็งได้ อย่างไรก็ตามแม้มีการคาดการณ์กลไกการทำงานของเซริซินในทางชีวเคมี แต่ยังคงมีความจำเป็นในการศึกษาเพื่อระบุการทำงานที่ชัดเจนของเซริซินต่อไป

คำสำคัญ: ช้างเอเชีย สเปิร์ม การเก็บรักษาสภาพด้วยการแช่แข็ง เซริซิน สารต้านอนุมูลอิสระ

Introduction

Importance of Asian Elephant

For centuries, elephants have shared a deep connection with humans. At present, only around 3,200 Asian elephants are documented in Thailand (Williams, 2020). Similar to their counterparts in the wild, captive groups face challenges concerning genetic diversity decline and the sustainability of their populations. A substantial factor contributing to the noticeable decrease in reproductive rates (Wiese, 2006). The declining captive populations have prompted an increased focus on elevating reproductive rates and fostering breeding within all remaining reproductively viable individuals.

Assisted Reproductive Technology & Extender

The importance of assisted reproductive technologies (ART) has gained prominence, notably in captive breeding management for species facing endangerment (Hermes, 2007). On a pragmatic level, the implementation of ART, including artificial insemination (AI), addresses the logistical challenges associated with physically transporting individuals for mating. It also proves valuable when dealing with behavioral incompatibility or inexperience among animals.

In contrast to the logistical challenges of chilled semen transport, cryopreservation offers several advantages for elephant AI programs.

It ensures the reliable presence of semen samples and facilitates the selection of genetically suitable donors globally. However, substantial within- and between- individual variations in an almost broad range of semen qualities have been documented among samples collected for both chilled and frozen specimens. (Thongtip *et al.*, 2008; Imrat *et al.*, 2013; Kiso *et al.*, 2013). Many samples exhibit morphological abnormalities, and low motility, even in bulls with natural breeding success. The underlying occasion remains unclear and persists as a widespread concern for captive facilities worldwide.

The variability in sperm quality across species, particularly in their susceptibility to freezing, is primarily linked to differences in the composition of the sperm plasma membrane (Bailey *et al.*, 2000). Discrepancies in the lipid ratios of spermatozoa, as observed in the case of Asian elephant semen in comparison to other species (Kiso, 2011; Kiso, 2012), potentially contribute to its comparatively lower freezability. Consequently, there is a pressing necessity to identify appropriate cryoprotectants to mitigate cryo-damage and enhance the freezability of Asian elephant spermatozoa.

Sericin

Sericin, a water-soluble globular protein (classified as a protein hydrolysate), is sourced from the silkworm Bombyx mori, representing a protein family with a molecular mass spanning 10 to 310 kDa (Wei *et al.*, 2005). Comprised of 18 varieties of amino acids, most notably characterized by robust polar side groups such as hydroxyl, carboxyl, and amino groups (Wei *et al.*, 2005), sericin is notably abundant in aspartic acid and serine (Kwang *et al.*, 2003), the latter exhibiting a high hydroxyl group content.

Amino acids have been shown to positively impact cryo-resistance (Khiabani *et al.*, 2017). Sericin exhibits cryoprotective activity by reducing lipid peroxidation from free radicals (Kato *et al.*, 1998). Previous research indicates sericin acts as a cryoprotectant for frozen bovine embryos (Isobe *et al.*, 2012; Takechi *et al.*, 2014). Various research has illustrated the potential of sericin to improve post-thaw semen quality in humans, rabbits, boars, bulls, and buffalo by safeguarding sperm against oxidative harm (Raza *et al.*, 2019; Aghaz *et al.*,2020; Ratchamak *et al.*, 2020; Yangngam *et al.*, 2021). However, sericin has not been evaluated in Asian elephants, which have distinct cryopreservation needs compared to other mammals. Moreover, since the optimal sericin dose varies between species such as 0.75% for boars (Ratchamak *et al.*, 2020) and 0.25% for bulls and buffalo (Kumar *et al.*, 2015; Yangngam *et al.*, 2021). Therefore, this study aims to assess the efficacy of different sericin concentrations added to freezing extender on post-thaw Asian elephant semen quality.

Material & Method

Ethic

Semen sample collection was conducted in compliance with the Animal Care and Use Protocol and received approval under Project NRIIS 269284, granted by the Zoological Park Organization of Thailand under the Royal Patronage of H.M. the King.

Chemical

Unless specified otherwise, all compounds employed in this study were obtained from the Sigma Chemical Company (Sigma, St. Louis, MO, USA).

Animal

Between March 2019 and September 2020, this investigation took place at two sites: the Khao-Kheaw Open Zoo (KKOZ) in Chonburi and the Thailand Elephant Conservation Center (TECC) in Lampang. A total of 10 ejaculates were obtained from 7 elephants, aged between 28 to 47 years, with weights ranging from 1,900 to 3,500 kilograms at the time of sampling. During the period preceding sample collection, these individuals had not undergone manual rectal massage or engaged in natural mating for approximately 3 months. The animals were provided a diet consisting mainly of pangola grass, sugarcane, bananas, and corn, supplemented with concentrated feed to meet a nutritional plan comprising 8% protein, 2% fat, and 20% fiber. They were allowed freedom of movement during the day and appropriately confined or tethered at night.

Semen Collection & Cryopreservation

Prior to semen collection, assessments of the accessory sex glands (prostate, seminal vesicle, and ampulla) were conducted using transrectal ultrasonography (LogicQ, GE Healthcare, United States). Specimens were obtained employing the manual rectal massage technique, adhering to Schmitt and Hildebrandt's guidelines (Schmitt,1998). To prevent contamination from urine, collection tubes were regularly replaced, and samples were collected in several portions. The ejaculate fractions demonstrating the maximal sperm motility evaluated through phase-contrast microscopy were retained. Further immediate measurements were performed including semen volume, pH (pH indicator strips; Universal Indicator, Merck, Germany), sperm concentration using a Neubauer hemocytometer following Macfarlane's approach (Macfarlane, 1991), and viability (using eosin-nigrosin staining). The manipulation, dilution, and evaluation of samples were executed at room temperature. Only fresh ejaculates exhibiting total motility equal to or exceeding 50% were kept. Thongtip and colleagues (Thongtip, 2004) delineated the freezing protocol. Essentially, freshly collected sperm was promptly diluted to a concentration of 100 x 106 cells/mL using TEST extender, supplemented with 20% chicken egg yolk. Different concentrations of commercial sericin (Sigma-Aldrich: Cat. No. S5201), specifically 0% (control), 0.5%, 1%, and 1.5%, were incorporated into the extender.

The sericin additives were prepared following the manufacturer's guidelines. For instance, the process of incorporating sericin powder into 100 mL of the prepared TEST extender is detailed below.

$$0.5\ \%w/v = \frac{\text{weight of sericin (gram)} \times 100}{100\ \text{mL}}$$

$$\text{weight of sericin} = \frac{0.5 \times 100}{100}$$

weight of sericin = 0.50 grams

Subsequently, 0.50 grams of sericin powder will be incorporated into the 100 mL of the TEST extender.

Following equilibration, the specimen was meticulously transferred into 0.5 mL semen straws (Kruuse, Ltd., Leeds, UK), and positioned on a stainless-steel rack, suspended 2.5 cm on top of the liquid nitrogen surface for 10 minutes, then plunging through submersion until thawing.

The frozen semen samples were preserved in a liquid nitrogen tank at a controlled temperature of -196°C for a period of one month. Thawing was conducted by submerging

the collective straws in water at 37°C for 30 seconds. Subsequently, the specimen was transferred to snap tubes for immediate assessment post-thaw, ensuring the samples remained at a consistent temperature of 37°C throughout the evaluation process.

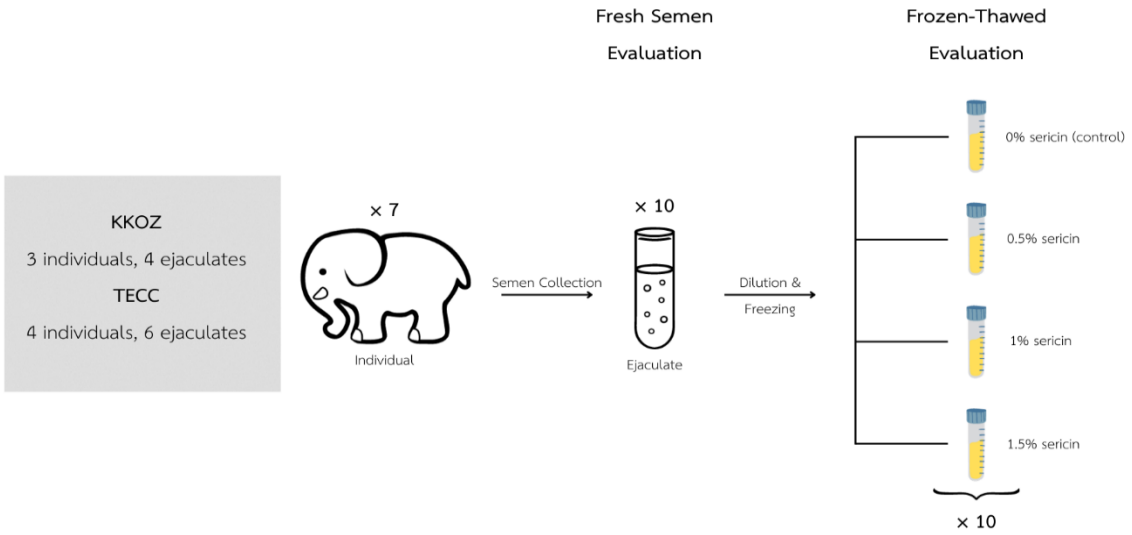

Figure 1 Concise Summary of the Process

Computer Assisted Sperm Analyzer

The Computer-Assisted Sperm Analyzer (CASA; IVOS Animal Breeder, Hamilton–Thorne Biosciences, Beverly, MA, USA) parameters followed standard procedures for analyzing Asian elephant spermatozoa (Thongtip, 2008). Immediately after thawing, a 7 µL portion of semen was placed in a pre-warmed 20-mm 2X-CEL chamber (Hamilton–Thorne Biosciences) maintained at a temperature of 37°C on a heating plate. The analysis encompassed various kinematic characteristics, including (1) percentage of motility, (2) percentage of progressive motility, (3) average path velocity (VAP), (4) straight-line velocity (VSL), (5) curvilinear velocity (VCL), (6) amplitude of lateral head displacement (ALH), (7) beat-cross frequency (BCF), (8) straightness (STR), and (9) linearity (LIN). At least 500 spermatozoa, randomly selected from multiple fields of view, were recorded for analysis.

Eosin-Nigrosin Staining

Spermatozoa were mixed in equal proportions with eosin-nigrosin stain (Björndahl, 2003). A smear was created and allowed to air-dry, then examined under bright-field microscopy (×1000 magnification) using oil immersion. A minimum of 200 spermatozoa per sample were classified as viable (exhibiting no stain uptake) or non-viable (demonstrating partial or complete pinkish stain uptake). The assessment was conducted by a single technician.

Coomassie-Blue Staining

The Coomassie blue staining method (Larson, 1999) involved initial fixation of 10-20 µL of the specimen in 250 µL of 4% paraformaldehyde, stored at 4°C prior to additional procedures. The spermatozoa underwent centrifugation (2000 g, 8 min) and then were washed twice with 500 µL of 0.1 M ammonium acetate (pH 9.0). The pellet was

mixed with 30-50 µL of ammonium acetate, and 20 µL of this mixture was spread on a glass slide and air-dried. Smears were submerged in a Coomassie blue staining solution for 90 seconds. Then they were softly rinsed using tap water and air-dried once more. Bright-field microscopy (×1000) under oil immersion examined at least 200 stained spermatozoa per sample to assess acrosomal integrity. Spermatozoa with uniform staining over the acrosomal region were classified as having intact acrosomes, while those showing irregular staining and unusual morphology were categorized as having damaged acrosomes. The assessment was conducted by a single technician.

Acridine-Orange Staining

The assessment of sperm chromatin integrity was performed using acridine orange (AO) as described by Tejada (1984). Shortly, 20 µL of the specimen was spread onto a glass slide, air-dried, and fixed with Carnoy's solution for at least 8 hours or overnight. After fixation, the slides were air-dried, and immersion in a freshly prepared AO for 10 minutes under conditions devoid of light. Subsequently, the slides were rinsed, air-dried, and subjected to examination under a fluorescent microscope (Olympus BX50).

The same examiner evaluated at least 200 sperm cells on each slide, with the precaution that the assessment duration per field of view did not surpass 40 seconds to mitigate potential photo-bleaching effects. Spermatozoa exhibiting green fluorescence were classified as having intact DNA content. While those exhibiting a range of yellow to red fluorescence were classified as fragmented DNA.

Hypo-Osmotic Swelling Test

Evaluation of the function of the sperm plasma membrane was conducted using the Hypo-Osmotic Swelling Test (HOST), following a modified protocol based on Jeyendran (1992) for Asian elephant specimens. In this test, a 20 µL sample was combined with 250 µL of a hypo-osmotic solution (100 mOsm/kg). The mixture was then incubated at 37 ^{o}C for 30 minutes. Following incubation, formalin (20 µL) was added to fix the spermatozoa, and the samples were stored at 4 ^{o}C. An amount of 5-10 µL was positioned on slides with covers and observed using phase-contrast microscopy (400 × magnification). At least 200 spermatozoa were analyzed by a single technician, and an affirmative reaction to hypo-osmotic pressure was indicated by spermatozoa displaying tail bending or enlargement to different extents, as characterized by Jeyendran. (Jeyendran, 1984) which indicates the integrity and functionality of the plasma membrane in a normal state.

Statistical Analysis

The data underwent analysis utilizing the R language with RStudio version 4.2.2 (R Core Team, 2022) For the statistical analysis of both fresh and post-thawed semen evaluations, essential assumptions were verified, encompassing normality and homogeneity of variance. Comparative analysis was conducted through Kruskal-Wallis's test, followed by post hoc using Dunn's test with Bonferroni correction. All values denote the mean and standard error of the mean (mean ± SEM). Statistical significance was considered at $p < 0.05$. Various R packages, such as tidyverse, rcompanion, PMCMRplus, and

agricolae, were employed, chosen for their reliability in scientific research.

Results

The analysis of fresh semen is presented in (Table1) categorized based on the collection sites. Despite undergoing rigorous statistical analysis, the multiple comparisons revealed no significant differences across any of the parameters

Table 1 Semen characteristics of fresh semen sample, separated by location of collection including (1) KKOZ = Khao Kheaw Open Zoo, Chonburi, Thailand and (2) TECC = Thailand Elephant Conservation Center, Lumpang, Thailand. Summarized data were stated in mean ± SEM.

Parameters	Locations	
	KKOZ (4 ejaculates)	**TECC (6 ejaculates)**
Age (year)	36.75 ± 4.23	43.67 ± 1.52
Volume (mL)	23.32 ± 11.21	61.87 ± 17.80
Spermic Volume (mL)	15.45 ± 11.52	20.28 ± 8.84
Motility (%)	72.50 ± 4.79	58.33 ± 4.01
Concentration ($\times 10^6$/mL)	1,067.50 ± 188.03	1,493.33 ± 275.41
pH	7.75 ± 0.25	7.17 ± 0.48
Viability (%)	85.12 ± 4.55	80.50 ± 4.19
Acrosome Integrity (%)	86.75 ± 4.01	59.92 ± 10.87
Normal Head (%)	80.50 ± 7.12	84.92 ± 5.12
Normal Tail (%)	66.00 ± 6.67	67.42 ± 3.58
Membrane Integrity (%)	62.75 ± 7.28	44.58 ± 6.60

For the effects of varying sericin concentrations on cryopreserved and post-thawed semen quality, Table 2 presents all parameters across the 0% (control), 0.5%, 1%, and 1.5% groups. Statistical analyses revealed two parameters that were significantly different from the control: Straightness (STR) and viable sperm count by Eosin-Nigrosin staining (Viability), which were higher in the 1.5% and 1% sericin groups respectively (p-value < 0.05).

Table 2 Comparison of semen qualities from previous 10 ejaculates of KKOZ and TECC among different sericin concentrations, compact-letter-display indicates significant differences among concentrations (p-value < 0.05). Summarized data were stated in mean ± SEM.

parameters	sericin concentrations			
	0%	**0.5%**	**1%**	**1.5%**
Motility (%)	3.75 ± 1.81	4.50 ± 2.66	3.75 ± 1.26	11.05 ± 4.99
Progressive (%)	0.45 ± 0.26	0.30 ± 0.21	0.35 ± 0.15	3.20 ± 2.68
VAP (μm/s)	32.83 ± 6.13 **ab**	33.02 ± 5.15 **a**	51.49 ± 2.61 **b**	47.89 ± 4.95 **ab**
VSL (μm/s)	28.25 ± 4.81 **ab**	24.51 ± 4.01 **a**	38.31 ± 2.68 **ab**	40.35 ± 4.14 **b**
VCL (μm/s)	54.33 ± 10.03 **ab**	55.47 ± 8.82 **a**	83.17 ± 4.51 **b**	73.26 ± 6.62 **ab**
ALH (μm)	2.89 ± 0.79 **ab**	2.00 ± 0.69 **a**	3.20 ± 0.82 **ab**	5.28 ± 0.87 **b**
BCF (Hz)	10.04 ± 2.01	10.78 ± 2.30	15.07 ± 2.50	17.29 ± 2.60
STR (%)	51.96 ± 6.72 **b**	52.64 ± 6.94 **ab**	73.04 ± 3.42 **ab**	75.71 ± 3.93 **a**
LIN (%)	37.14 ± 5.10	34.96 ± 4.96	49.14 ± 2.96	51.93 ± 3.57
Viability (%)	15.71 ± 2.07 **b**	18.91 ± 2.75 **ab**	29.8 ± 4.19 **a**	16.93 ± 1.73 **ab**
Acrosome Integrity (%)	19.91 ± 4.00	25.54 ± 4.71	21.55 ± 4.03	23.75 ± 4.76
DNA Integrity (%)	87.02 ± 2.71	84.70 ± 2.30	89.89 ± 1.86	90.14 ± 1.77
Membrane Integrity (%)	36.17 ± 4.74 **ab**	47.12 ± 3.86 **ab**	56.92 ± 5.20 **a**	34.42 ± 5.16 **b**

Despite noteworthy distinctions among experimental groups in VAP, VSL, VCL, ALH, and intact plasma membrane by Hypo-Osmotic Swelling Test based on multiple comparisons, these parameters did not exhibit significant differences from the control group.

To identify appropriate sericin doses for Asian elephant semen cryopreservation, a detailed examination of each experimental group's differentiation remains valuable. Notably, in velocity profiles, VAP and VCL demonstrated the highest results at 1% sericin, while VSL exhibited the highest result at 1.5%. Additionally, ALH showed the highest result at 1.5% sericin among the experimental groups. Furthermore, intact plasma membrane by Hypo-Osmotic Swelling Test demonstrated the highest result at 1% sericin among the experimental groups.

Finally, this study underscores those parameters including motility, progressive motility, beat cross frequency (BCF), linearity (LIN), acrosome integrity, and DNA integrity did not manifest significant differences following multiple comparisons between the control and experimental groups.

In summary, this study suggests 1-1.5% sericin may improve select sperm quality indicators like Straightness and Viability in frozen-thawed Asian elephant semen.

Discussion

The similarity between fresh semen evaluations from KKOZ and TECC, as well as

the congruence with the range of semen qualities in previous studies of Asian elephants (Pinyopummin *et al.*, 2018; Thongphakdee *et al.*, 2022), suggests that our study was not affected by the inclusion of low-quality samples prior the collection and accurately reflect the baseline of fresh semen quality in Thailand.

Our investigation indicated that supplementation with 1% and 1.5% sericin notably improved sperm straightness (STR) and viability, as observed through Eosin-Nigrosin staining, compared to other groups. However, parameters such as motility, progressive motility, VAP, VSL, VCL, ALH, BCF, LIN, acrosome integrity, DNA integrity, and plasma membrane integrity exhibited no significant differences between the control and sericin groups, despite observable enhancements in certain measurements.

In multiple species, an influential factor in the degradation of sperm qualities during cryopreservation involves the increased production of reactive oxygen species (ROS) (Kadirvel, 2009). While ROS naturally occur during sperm metabolism, an excess leads to oxidative stress, resulting in detrimental impacts such as lipid membrane peroxidation and DNA fragmentation (Aitken, 2006; Koppers, 2008). Spermatozoa with a high content of polyunsaturated fatty acids in the membrane, are particularly susceptible to oxidative stress (Jones, 1979). Earlier studies in Asian elephants revealed a link between elevated levels of sperm membrane lipid and protein peroxidation and suboptimal semen quality in freshly collected samples (Satitmanwiwat, 2017; Thongtipsiridech, 2013). Furthermore, prolonged processing and cold storage induce considerable DNA damage in Asian elephant spermatozoa (O'Brien, 2013; Wattananit, 2012).

While sperm from various species naturally contains antioxidants counteracting lipid peroxidation (Kelso, 1997; Marti, 2007), this inherent antioxidant capacity may be insufficient over prolonged storage. Augmenting the semen extender with exogenous antioxidants is a commonly employed strategy to mitigate lipid peroxidation. This approach has shown efficacy in protecting spermatozoa against ROS impact and enhancing both sperm straightness and viability during liquid storage across various mammalian species, including rams (Maxwell & Watson, 1996), dogs (Michael *et al.*, 2009), and boars (Funahashi & Sano, 2005).

Since an observed enhancement in sperm straightness (STR) with 1.5% sericin mirrors findings in buffalo semen by Kumar (2015), this improvement in sperm movement likely stems from sericin's ability to enhance energy production in sperm cells, aiding motility and enabling straighter swimming trajectories (Kumar *et al.*, 2015). Sericin probably ameliorates STR by safeguarding the sperm membrane from cryopreservation-induced damage. The susceptibility of the lipid bilayer to alterations during freezing and thawing, due to osmotic, cold shock, and ice crystal stresses, can compromise mechanisms regulating flagellar beating, impacting motility. Sericin's antioxidant properties likely prevent lipid peroxidation, while its hydrophilic nature may stabilize the membrane structure (Miyamoto *et al.*, 2012). Preserving membrane and flagellar structures crucial for movement may underlie sericin's maintenance of higher sperm straightness.

Furthermore, the notable enhancement in viability with 1.0% sericin aligns with results from (Ratchamak, 2023) demonstrating increased viability in rooster sperm with sericin supplementation. Sericin's combination of antioxidant, membrane-

stabilizing, energy-enhancing, and anti-inflammatory properties underlies its ability to safeguard viability. Although the precise antioxidative mechanisms of sericin protein remain under study, Kato (1998) proposed that hydroxy amino acids, serine, and threonine abundantly present in sericin, might contribute to its scavenging function. Our study accentuates that sericin functions as a highly effective neutralizing agent, guarding sperm from oxidative damage, as evidenced by improved spermatozoa viability.

Sericin's benefits in cryopreserving diverse mammalian cells, such as islet cells for transplantation (Ohnishi, 2012) adipose stem/progenitor cells for regenerative medicine (Miyamoto, 2012) and embryos for IVF (Isobe, 2013; Isobe, 2012) are well-documented. Beyond its antioxidative effects, sericin acts as a shield promoting cell growth (Takahashi, 2003) and resilience against cryoprotectant, thermal, and other stresses (Sasaki, 2005) This suggests that sericin safeguards sperm during cooling and exposure to cryoprotectants by preserving critical membrane structures and functions vital for mobility and fertility.

Conclusion

In the current study, sericin supplementation at 1-1.5% enhances post-thaw Straightness and Viable Sperm Count in Asian elephant cryopreserved semen. Further research should clarify links between specific improvements and fertilization capacity. Moreover, the specific causes behind the suboptimal quality of Asian semen samples remain elusive. Probable factors include the possibility of incomplete ejaculation during the collection process, inconsistencies among individuals, and dietary restrictions during musth periods. As the definitive reasons are yet to be identified, further research should focus on delineating the varying constituents between high and low semen quality to identify crucial elements and their proportional significance.

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Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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