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Regulatory functions of lecithin nanoparticles on post-thaw quality of buffalo bull semen: a morphological analysis of spermatozoa

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ABSTRACT. This study was designed to compare the addition of lecithin (LC) and lecithin nanoparticles (LC-NPs) to a semen extender (control group – egg yolk, EY) during a storing period in relation to its effectiveness on buffalo sperm cryopreservation. Ejaculates were collected from six mature, healthy Egyptian buffalo bulls using an artificial vagina, and diluted with nine extenders. LC-NPs and LC were tested at concentrations of 0.25, 0.5, 1.00, and 1.50 µg/ml compared to the egg yolk extender. The results indicated an overall enhancement in sperm quality: increased progressive motility and improved hypo-osmotic swelling test (HOST), reduced instances of dead sperm, abnormal morphology and chromatin damage in the LC-NPs group compared to both LC and the control groups ($P < 0.05$), apart from group 0.25 µg/ml. Furthermore, LC-NPs demonstrated superior viability at a concentration of 1 µg/ml. This concentration exhibited lower rates of early and late apoptotic events, as well as reduced necrosis compared to lower concentrations. The results showed significantly increased ($P < 0.05$) antioxidant indices, including superoxide dismutase (SOD), glutathione peroxidase, and total antioxidant capacity, with the inclusion LC-NPs, particularly notable at concentrations of 1.00 and 1.50 µg/ml, surpassing the effects observed in other groups. In contrast, the levels of malondialdehyde decreased significantly ($P < 0.05$) with LC-NPs, particularly at 1.00 µg/ml in comparison to the LC and control groups. Moreover, the fortification of cryopreserved spermatozoa with 1 or 1.5 µg/ml LC-NPs resulted in the preservation of acrosomal and plasma membrane integrity, as well as normal ultrastructure. So, the addition of LC-NPs, particularly of 1 or 1.5 µg/ml, significantly enhanced sperm quality by improving antioxidant indices, reducing apoptosis, and preserving ultrastructure integrity of buffalo sperm after the post-thawing process.

Introduction

Sperm freezing is vital for the preservation of animal biodiversity, offering a practical approach to mitigate the risk of breed and population extinctions. The utilisation of frozen sperm not only contributes to a reduction in the likelihood of such extinctions but also proves cost-effectiveness in terms of transportation expenses (Khalil et al., 2023). In the context of buffalo sperm, cryopreservation can help lower the costs of feeding large numbers of buffaloes and facilitate the dissemination and monitoring of genetic progress. However, the process of cryopreservation and subsequent thawing can have

detrimental effects on sperm viability and motility, leading to a decline in fertility (Ganesan and Govindarajan, 2023). In recent years, concerns have also been raised regarding conventional sperm extenders, primarily due to the unpredictability in their composition and risk associated with both microbiological and exotic disease agent contaminants in egg yolk (EY) extenders (Sun et al., 2021). Despite their undeniable importance, freezing, cooling, and thawing processes can compromise sperm quality and fertility. These adverse effects manifest through the breakage of sperm membrane, damage to nuclear DNA, and the generation of reactive oxygen species (Ugur et al., 2019). Notably, plasma membranes are particularly vulnerable during cryopreservation compared to other cellular components, with over half of the sperm population experiencing cell membrane damage (Abdelnour et al., 2022). During cryopreservation, male gametes are stored in various aquatic media as semen extenders, each formulated with a specific composition. Intensive efforts have been devoted in recent years to modify the chemical composition of diluents in order to improve protection against cold or freezing shock, enhance nutrient uptake, reduce microbial attack, and lower the negative impacts on sperm function and structure (Hashem and Gonzalez-Bulnes, 2021). Many attempts have been made to mitigate the detrimental effects of cryopreservation by incorporating antioxidants, antifreeze proteins, fatty acids, nanoparticles, plant oils, soybean lecithin (LC), and low-density lipoproteins into extenders (Pytlik et al., 2022).

While EY has long been employed as a sperm cryoprotectant, it presents certain drawbacks and adverse effects on sperm processing. The most commonly reported problems include heterogeneity in EY composition, potential microbial contamination or pathogen transmission, endotoxin presence, and difficulties in quality assessment. These disadvantages have increased the demand for non-animal-derived, chemical-free alternatives to conventional extenders (Miguel-Jimenez et al., 2020). Replacing non-permeable, animal-based protectants with plant-based alternatives offers a potential solution to mitigate the risks associated with microbial contamination or disease transmission. This transition can contribute to improved standardization, biosecurity, and product traceability in sperm processing (Hassan et al., 2022). However, ongoing debates persist regarding the effect of LC on sperm quality both *in vitro* and *in vivo*, when it is included in sperm diluents (Leite et al. 2010). In this context, the size of lecithin nanoparticles (LC-NPs) in extenders is widely recognized as a crucial factor influencing the quality of frozen-thawed semen. Nano-lecithin particles, characterised by smaller size and higher solubilising capacity resulting from nano-treatment, play a significant role in enhancing sperm interaction. This, in turn, reduces the potential for cold shock injury during the freezing-thawing processes (Nadri et al., 2019). Moreover, findings by Mousavi et al. (2019) suggest that a reduction in the size of LC-NPs is associated with an increase in sperm antioxidant capacity following freezing and thawing.

Therefore, the present study investigates the differences between the addition of LC and its nanoparticles at different concentrations to a semen extender. The objective is to assess their protective impact on the quality of spermatozoa after post-thawing in buffalo bull spermatozoa. This evaluation encompasses considerations of sperm quality, cell apoptosis, and antioxidant performance, with special attention to changes in post-thawing morphological characteristics.

Material and methods

Animal care

This study adhered to all ethical requirements established by the Institutional Animal Care and Use Committee (ARC-IACUC) in Egypt, with the assigned protocol number: ARC-APRI-60-23.

Animal selection, semen collection, evaluation, and processing

The study utilised six mature, healthy Egyptian buffalo bulls, aged between 3.5 to 5 years, used as semen donors. All bulls were housed at the experimental station of the Animal Production Research Institute (APRI) in Mahallet Mousa, Kafrelsheikh governorate, Egypt. Bulls were fed and managed following industry standards. Sperm was collected once a week for 20 weeks (100 ejaculates), and an artificial vagina (Neustadt/Aisch, Müller, Nürnberg, Germany), prewarmed to 42 °C, was employed for the collection. In this experiment, only ejaculates with a mass motility of 75% and a sperm concentration of 0.7×10^9 sperm/ml were pooled for freezing (Ijaz et al., 2009).

Following semen collection, the pool of fresh sperm was divided into nine aliquots. Each aliquot was individually diluted with Tris-extender at varying concentrations of LC or LC-NPS: 0.0, 0.25, 0.5, 1.0 and 1.50 µg/ml LC, and 0.25, 0.5, 1.0 and 1.50 µg/ml LC-NPs.

Within 15 min, the diluted semen was gradually cooled to 5 °C and equilibrated for 4 h at 4–5 °C in a refrigerator (equilibration period) at 37 °C with each extender at a ratio of 1:10. During the equilibration period, the semen was delivered to the laboratory at APRI International Livestock Management Training Centre. The equilibrated semen was automatically packaged into French straws (0.25 ml, IVM technologies, L′Aigle, France), positioned 5 cm above liquid nitrogen (LN) for 10 min, and subsequently frozen in LN at −196 °C. Straws were thawed for 30 s in a 37 °C water bath and maintained at that temperature. Measurements of progressive motility, death, abnormality, plasma membrane integrity, and chromatin damage were conducted, with results expressed in percentages (Singh et al., 2018).

Preparation and characteristics of lecithin nanoparticles

Lecithin nanoparticles were prepared through reverse-phase evaporation, following the methodology outlined by Tar et al. (2021). The particle size and zeta potential of the produced nanoparticles were analysed using a Zetasizer Nano ZS analyser (Malvern Instruments, Malvern, UK). After appropriate dilution with distilled water (1:10), measurements were made in triplicate and morphology was determined using transmission electron microscopy (JEOL JEM-2100, JEOL Ltd., Tokyo, Japan) (Soliman et al., 2021). Lecithin nanoparticles were used and stored at 4 °C for future use in semen extender.

Preparation of extenders and sperm motility

The 20% Tris-EY extender contained the following components: 20 ml of chicken yolk, 3.025 g of Tris (hydroxyl methyl amino methane), 1 675 g of citric acid, 0.75 g of glucose, 7 ml of glycerol, 0.25 g of lincomycin, 0.005 g of streptomycin, and pure water to a final volume of 100 ml.

Assay of sperm quality after thawing

Individual progressive motility

Sperm progressive motility was assessed using a phase-contrast microscope (Leica DM 500, Leica, Germany) with a heated plate, magnification x100. Briefly, a thawed drop of the sample was placed on a pre-warmed (37 °C) slide and covered with a coverslip, and each sperm sample was examined in 10 microscopic fields (Gamal et al., 2016).

Determination of semen parameters

The functional and structural integrity of buffalo sperm plasma membrane was analysed using the supravital hypo-osmotic swelling test (HOST), as described by Ansari et al. (2016). Clear heads and tails, along with swollen tails, indicated intact, biochemically active sperm membranes, whereas pink heads and tails, and unswollen tails, were indicative of damaged, inactive sperm membranes. Moreover, in a previous study of Menon et al. (2011), sperm viability and abnormalities were calculated based on observations in five fields. Chromatin damage was evaluated using an assay described by Agarwal and Said (2003). Flow cytometry was employed to identify apoptotic cells in post-thawed spermatozoa, as described by Khalil et al. (2023).

Antioxidant assessment

Assessments of total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) levels in buffalo spermatozoa during the post-thawing stages of cryopreservation were conducted following the methodologies outlined by Garg et al. (2009) and Khalil et al. (2023). All analyses were carried out using a spectrophotometer (Spectro UV-Vis Auto, UV-2602; Culver City, CA, USA), and commercial kits were purchased from Biodiagnostic (Giza, Egypt) and used according to the manufacturers' instructions.

Ultrastructural evaluation of spermatozoa by transmission electron microscopy

With minor modifications, sperm samples were prepared for transmission electron microscopy (TEM) as described by Khalil et al. (2018). Briefly, the semen sample (500 µl) was centrifuged and resuspended in a fixative solution containing 2.5% glutaraldehyde for 24 h at 4 °C. Samples were dehydrated in an ethanol gradient, embedded in Epon resin, and ultrathin-sectioned (60–70 nm) for TEM after washing and post-fixing in 1% osmium tetroxide for 1.30 h. A JEOL 2100 TEM operating at 80 kV was utilised to examine ultrathin sections at 160 kV of accelerating voltage. The ultrastructure of sperm at the head (n = 300 for each sample) was evaluated, specifically focusing on the plasma membrane, acrosome, and nucleus morphology. Additionally, the morphology of the mitochondria in the mid-piece region was also examined (n = 300 per sample).

Statistical analysis

Levene's test was used to assess normality of distribution and variance homogeneity. Statistical analysis was conducted using IBM SPSS ver. 22 statistical software (SPSS Inc., IL, USA). In all cases, one-way ANOVA was used, and upon

detecting significant differences, multivariate post-hoc Tukey's assessments were employed to quantify statistical differences between lecithin levels in different treatments. The T-test was utilised to compare LC and LC-NPs in both groups, with statistical significance set at $P < 0.05$.

Results and discussion

Effect of lecithin in extenders on sperm characteristics after thawing

In comparison to the impact of extenders utilising different forms of lecithin, our observations indicated that progressive motility and membrane integrity were significantly higher in the extender containing LC, especially the nanoparticle form in most treatments. In contrast, lower values of dead spermatozoa, abnormalities, and chromatin damage were recorded in the LC-containing extender (Table 1). Moreover, the result showed that the LC-NPs extender at a concentration of 1 µg/ml was more effective in preserving spermatozoa compared to other concentrations. It is noteworthy that the control group (LC-0) exhibited a comparatively reduced ability to preserve spermatozoa than the groups subjected to LC-containing extenders.

Moreover, our hypothesis posits that the incorporation of LC-NPs in semen extenders may enhance sperm biological effectiveness by improving its stability and solubility properties. Lecithin nanoparticles, owing to its elevated surface-area ratio, serve as a protective barrier for spermatozoa, mitigating cryoinjuries incurred during the freezing-thawing process (Ganesan and Govindarajan, 2023). In addition, it is important to highlight the potential adverse effects of soy LC, which at elevated levels may exhibit toxicity and adversely affect sperm motility and viability, as highlighted by Forouzanfar et al. (2010).

Effect of lecithin in extenders on sperm apoptotic-like changes in post-thawed buffalo bull sperm

Data concerning the impact of LC-NPs in individual extenders on various features of buffalo spermatozoa reveal the effectiveness of LC, particularly in its nanoparticle forms (Table 2). Sperm viability was significantly increased in all samples analysed ($P < 0.05$), particularly those containing LC, compared to the control sample, except for the 0.25 µg/ml concentration (LC an LC-NPs), which did not differ significantly from the control. Additionally, at a concentration of

Table 1. Effect of lecithin (LC) or lecithin nanoparticles (LC-NPs) in extender on progressive motility, death, membrane integrity, abnormalities, and chromatin damage of buffalo bull spermatozoa

Spermatozoa		Control	T1	T2	T3	T4	*P*-value
Progressive motility	LC	31.41 ± 1.81^{Ac}	33.14 ± 2.47^{Bc}	41.25 ± 1.83^{Ab}	48.22 ± 1.11^{Ba}	44.94 ± 1.54^{Aa}	<0.05
	LC-NPs	31.41 ± 1.81^{Ad}	34.22 ± 1.47^{Ac}	39.85 ± 2.11^{Bc}	58.52 ± 1.04^{Aa}	42.91 ± 1.44^{Bb}	<0.03
Dead	LC	55.28 ± 1.52^{Aa}	53.61 ± 1.07^{Aa}	51.42 ± 0.84^{Aa}	40.12 ± 2.10^{Ab}	42.39 ± 1.14^{Bb}	<0.03
	LC-NPs	55.28 ± 1.52^{Ab}	51.14 ± 2.03^{Bc}	39.22 ± 1.82^{Bd}	36.51 ± 1.64^{Be}	57.39 ± 1.33^{Aa}	<0.04
Membrane integrity	LC	38.15 ± 2.04^{Ab}	35.72 ± 1.23^{Bc}	36.11 ± 2.07^{Bc}	48.69 ± 1.54^{Ba}	46.48 ± 1.47^{Aa}	<0.02
	LC-NPs	38.15 ± 2.04^{Ac}	36.21 ± 2.15^{Ac}	37.91 ± 1.22^{Ac}	57.61 ± 1.06^{Aa}	45.47 ± 1.76^{Ab}	<0.05
Abnormality	LC	26.44 ± 0.53^{Aa}	24.18 ± 1.46^{Ab}	23.22 ± 0.52^{Ab}	17.10 ± 0.61^{Ad}	19.92 ± 0.33^{Ac}	<0.02
	LC-NPs	26.44 ± 0.53^{Aa}	23.51 ± 0.46^{Bb}	18.23 ± 0.91^{Bc}	14.21 ± 0.39^{Bd}	16.55 ± 0.77^{Be}	<0.05
Chromatin damage	LC	13.59 ± 0.73^{Aa}	11.42 ± 0.42^{Ab}	10.13 ± 0.39^{Ab}	8.36 ± 0.28^{Ac}	9.17 ± 0.47^{Ac}	<0.02
	LC-NPs	13.59 ± 0.73^{Aa}	10.21 ± 0.61^{Bb}	7.40 ± 0.18^{Bc}	6.60 ± 0.14^{Bd}	7.0 ± 1.02^{Bc}	<0.03

freezing extender fortified with various levels of LC or LC-NPs: µg/ml: control 0, T1 0.25, T2 0.50, T3 1.00 and T4 1.50, respectively. Data are presented as mean value ± standard error of the mean. $^{a-e}$ – means in the same row with different superscripts are significantly different at $P < 0.05$ (ANOVA); ^{AB} – means in the same column with different superscripts are significantly different

Previously, researchers have found that inclusion of an extender with LC positively affects sperm properties such as motility, life, and chromatin integrity (Falchi et al., 2018; Tar et al., 2021). Pillet et al. (2012), based on studies involving goats, noted reduced motion parameters with progressive motility following freezing when using a liposome-based semen extender as opposed to an EY extender.

1 µg/ml (irrespective of LC presence), sperm maintained a higher viability than the control group ($P < 0.05$). The result also showed that the addition of LC to the extender had an observable effect at 0.5 µg/ ml, and this effect increased to 1 µg/ml and tended to decrease again at a concentration of 1.5 µg/ ml. However, the optimum concentration appears to be greater than 0.5 µg/ml.

Table 2. Effect of lecithin (LC) in extender on apoptotic-like changes in buffalo bull spermatozoa

Items		Control	T1	T2	T3	T4	*P*-value
Viable	LC	10.28 ± 0.71^{Ab}	11.23 ± 0.92^{Bb}	28.17 ± 1.23^{Ba}	35.28 ± 3.11^{Ba}	32.79 ± 2.57^{Ba}	<0.04
	LC-NPS	10.28 ± 0.71^{Ac}	12.37 ± 1.08^{Ac}	30.61 ± 2.08^{Ab}	51.12 ± 1.56^{Aa}	37.34 ± 2.44^{Ab}	<0.05
Early apoptotic	LC	4.83 ± 0.48^{Aa}	4.11 ± 0.52^{Aa}	3.14 ± 0.46^{Ab}	2.75 ± 0.15^{Ac}	3.07 ± 0.71^{Ab}	<0.02
	LC-NPS	4.83 ± 0.48^{Aa}	3.81 ± 0.39^{Bb}	2.94 ± 0.45^{Bc}	1.63 ± 0.22^{Bd}	2.62 ± 0.70^{Bc}	<0.05
Late apoptotic	LC	75.61 ± 1.78^{Aa}	71.22 ± 2.62^{Aa}	59.43 ± 2.14^{Ab}	53.27 ± 1.05^{Ac}	56.88 ± 1.14^{Ab}	<0.05
	LC-NPS	75.61 ± 1.78^{Aa}	69.87 ± 1.91^{Ba}	51.47 ± 2.35^{Bb}	35.40 ± 1.18^{Bd}	42.11 ± 2.11^{Bc}	<0.04
Necrosis	LC	24.16 ± 1.33^{Aa}	22.46 ± 1.04^{Ab}	21.37 ± 0.92^{Ab}	16.58 ± 1.08^{Ac}	18.78 ± 1.11^{Ac}	<0.03
	LC-NPS	24.16 ± 1.33^{Aa}	19.07 ± 2.06^{Bb}	17.63 ± 1.71^{Bc}	10.97 ± 1.63^{Bd}	14.53 ± 2.02^{Bc}	<0.04

LC-NPS – lecithin nanoparticle; freezing extender fortified with various levels of LC or LC-NPs: µg/ml: control 0, T1 0.25, T2 0.50, T3 1.00 and T4 1.50, respectively. Data are presented as mean value ± standard error of the mean. $^{a-e}$ – means in the same row with different superscripts are significantly different at $P < 0.05$ (ANOVA); ^{AB} – means in the same column with different superscripts are significantly different

Notably, the events of early apoptotic, late apoptotic, and necrotic spermatozoa were significantly less frequent in cells preserved in the extender containing 1 µg/ml LC-NPs compared to other treatments. Consequently, LC-NPs demonstrated a decreasing effect on sperm apoptosis and necrosis, but not when added at a concentration of 0.25 µg/ml. A more significant effect of LC-NPs and LC, compared to all other groups (Table 2), was observed for the 1 µg/ml concentration. The present findings are consistent with the study of Tar et al. (2021), who reported a significant influence of LC on the viability of thawed ram spermatozoa. Similarly, congruent results were documented by Naz et al. (2018) regarding the impact of LC on sperm acrosome integrity.

Furthermore, Singh et al. (2018) found in a study on Boer goats that an EY extender improved sperm plasma membrane integrity to a higher extent than a soybean LC-based extender. The potential vital physiological role of soy LC during freezing lies in its ability to improve the protective characteristics of the sperm plasma membrane. This enhancement is attributed to the physical protection mechanism of exogenous lipids, preventing cryoinjuries, which increases the strength and stability of the sperm membrane and reduces mechanical damage (Singh et al., 2012). In addition, LC-NPs may improve the ability to protect spermatozoa against osmotic stress and cold shock by regulating membrane fluidity and permeability. This is particularly pertinent during the freezing process, where the formation of ice crystals in spermatozoa can intensify damage to the acrosomal region (Mousavi et al., 2019).

Impact of lecithin in extenders on antioxidant properties of buffalo bull spermatozoa

Table 3 demonstrates a significant increase in antioxidant enzyme (total antioxidant capacity (TAC), gluthationeperoxidase(GPx)andsuperoxidedismutase (SOD)) activities, and decreased malondialdehyde (MDA) levels across all groups, particularly in the LC-NPs and LC groups. The levels of TAC, GPx, SOD, and, to some extent, MDA at concentrations of

Table 3. Effect of lecithin (LC) in extender on antioxidant parameters of buffalo bull spermatozoa

Items		Control	T1	T2	T3	T4	*P*-value
TAC, µm	LC	0.11 ± 0.01^{Ad}	0.14 ± 0.03^{Bc}	0.21 ± 0.02^{Bb}	0.31 ± 0.01^{Ba}	0.27 ± 0.02^{Ba}	<0.03
	LC-NPS	0.11 ± 0.01^{Ae}	0.19 ± 0.01^{Ad}	0.33 ± 0.02^{Ac}	0.51 ± 0.01^{Aa}	0.44 ± 0.03^{Ab}	<0.02
GPx, U/ml	LC	44.12 ± 2.77^{Ab}	48.19 ± 1.52^{Bb}	52.41 ± 2.04^{Ba}	58.88 ± 1.38^{Ba}	54.24 ± 1.66^{Ba}	<0.05
	LC-NPS	44.12 ± 2.77^{Ab}	51.52 ± 1.67^{Ab}	56.82 ± 2.35^{Aa}	64.11 ± 1.38^{Aa}	60.81 ± 2.53^{Aa}	<0.03
SOD, U/ml	LC	27.32 ± 1.13^{Ad}	31.12 ± 1.55^{Bc}	34.24 ± 0.93^{Bb}	39.28 ± 1.26^{Ba}	37.55 ± 1.48^{Ba}	<0.04
	LC-NPS	27.32 ± 1.13^{Ad}	33.92 ± 1.16^{Ac}	38.44 ± 1.47^{Ab}	44.28 ± 1.82^{Aa}	41.57 ± 1.66^{Aa}	<0.05
MDA, nmol/ml	LC	10.22 ± 1.11^{Aa}	9.14 ± 0.76^{Aa}	8.12 ± 0.71^{Ab}	7.18 ± 0.48^{Ab}	7.81 ± 0.58^{Ab}	<0.05
	LC-NPS	10.22 ± 1.11^{Aa}	8.62 ± 0.17^{Bb}	7.43 ± 0.23^{Bc}	6.22 ± 0.11^{Bd}	6.94 ± 0.26^{Bc}	<0.05

LC-NPS – lecithin nanoparticle, TAC – total antioxidant capacity, GPx – glutathione peroxidase, SOD – superoxide dismutase, MDA – malondialdehyde; freezing extender fortified with various levels of LC or LC-NPs: µg/ml: control 0, T1 0.25, T2 0.50, T3 1.00 and T4 1.50, respectively. Data are presented as mean value ± standard error of the mean. $^{a-e}$ – means in the same row with different superscripts are significantly different at $P < 0.05$ (ANOVA); ^{AB} – means in the same column with different superscripts are significantly different

1 and 1.5 µg/ml LC-NPs were significantly greater than those observed in the other groups ($P < 0.05$). However, no statistically significant differences were seen between the concentrations of 1 and 1.5 µg/ml ($P < 0.05$). The LC-NPs group produced the best results in terms of TAC, GPx, SOD, and MDA values, followed by the LC group. Notably, the accumulation of reactivr oxygen species (ROS) induces cell DNA damage or the degradation of cell components, including lipids and proteins. Hence, spermatozoa, being living cells, employ various enzymes and antioxidants such as CAT, GPX, and SOD to neutralise ROS and maintain cellular integrity.

Furthermore, SOD is considered an enzyme capable of cellular repair, preserving the integrity of cells by removing superoxide (O_2), the most common free radical in the body known to induce cell damage. Malondialdehyde is a by-product of lipid peroxidation formed by ROS, ultimately leading to changes in cell membrane structure. In this regard, numerous studies have emphasised the significant role of nanoparticles in ameliorating the adverse effects of oxidative stress and enhancing the antioxidant capacity, thereby improving sperm quality (Mehdipour et al., 2017 and Pinto et al., 2020). Considering these findings, it can be deduced that the breakdown of the plasma membrane, resulting from lipid disruption, may escalate cellular damage, ultimately leading to sperm death (Abdelnour et al., 2022).

Characteristics of lecithin nanoparticles

The average particle size, zpotential, and polydispersity index (PDI) of LC-NPs were 81.29 ± 0.76 nm, −23 ± 0.60 mV, and 0.36 ± 0.03, respectively, and Figure 1 depicts the morphology of LC-NPs. The z-potential amplitude indicated the stability of the colloidal system potential. Similarly, low PDI values implied that nanoparticles exhibited a restricted distribution, were homogeneously manufactured, and showed improved colloidal stability without aggregate formation. These properties were consistent with those described by Nadri et al. (2019) and Ezekiel et al. (2021).

Alterations in spermatozoa ultra-structure after thawing

The ultrastructure of damages in buffalo spermatozoa was assessed using transmission electron microscopy (TEM) (Figure 2A–I) to gain further insight and understanding of changes in the subcellular structure of spermatozoa after freezing. The examination of buffalo sperm revealed notable alterations, including enlarged plasma membranes, damage to the tail and neck, acrosome disruption, as well as altered acrosome membranes (Figure 2A–D). In contrast, the addition of 0.5 g/ml LC-NPs (Figure 2E, F) reduced nuclear damage, and satisfactory acrosome and plasma membrane integrity was recorded. Notably, concentrations of 1 and 1.5 µg/ml LC-NPs proved to be more effective than other treatments in mitigating issues related to acrosomal integrity, plasma membrane, and sperm tail rupture rates post-thawing (Figure 2G–I). The present study is in line with prior investigations of Khalil et al. (2018) and Abdelnour et al. (2023), who have demonstrated that the incorporation of nanoparticles into a freezing extender effectively preserved the ultrastructural integrity of sperm and enhanced its functional attributes across different species such as bulls, rabbits, and buffaloes.

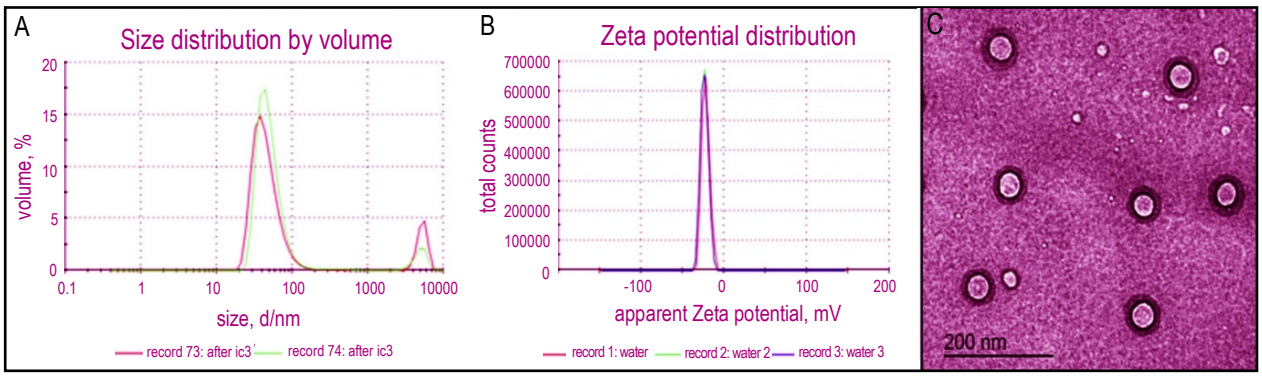

Figure 1. Histogram of lecithin particle size and zeta potential distribution (A, B), and lecithin nanoparticles morphology by transmission electron microscopy (C)

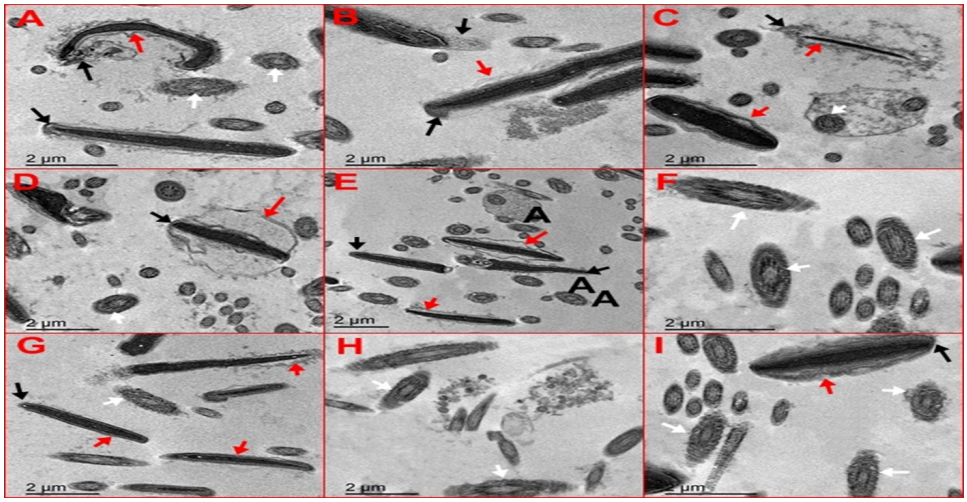

Figure 2. Transmission electron microscopic photomicrographs of sections of the buffalo spermatozoa head and mid pice in control 1% without nano (A,B), showing the plasma membrane was slightly swollen (red arrow), swelling of acrosomal ground substance with vesicles of fused plasma and outer acrosomal membranes (black arrow) and showing mild degree of mitochondrial sheath damage (white arrow), in lecithin nanoparticles (LC-NPs) 0.25% (C,D) showing complete loss of plasma membranes (PM), abnormal nucleus with necrosis chromatin and vacuolated mitochondria C, in D showing extenuation and undulating PM. LC-NPs 0.5% (E,F) showing slightly swollen PM and normal mitochondria (white arrow). LC-NPs 1% (G,H) showing numerous normal spermatozoa with intact plasma membrane, acrosomal, and normal nucleus with condensed chromatin and mitochondria was regularly placed in longitudinal sections (white arrow). LC-NPs 1.5% (I) showing abnormal plasma membrane, acrosomal, and normal mitochondria

Conclusions

The addition of lecithin nanoparticles (LC-NPs) at a concentration of 1.00 µg/ml to semen extender may serve as a suitable complementary mediator for the cryopreservation of buffalo sperm due to its potential to enhance several aspects of post-thawed sperm quality. These include improvements in motility parameters, antioxidant indices and apoptotic status. However, further investigations are warranted to comprehensively evaluate the cryoprotective attributes of LC-NPs in buffalo sperm cryopreservation. Specifically, exploring transcriptomics, proteomics, and lipidomics would provide a more in-depth understanding of the molecular and biochemical mechanisms underlying the observed improvements.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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