

Effect of palmitic acid nanoparticles on the quality of cryopreserved semen from Holstein bulls

Mohammed A. Abdel-Ghani¹, Mohamed E. El-Sharawy², Hadeer M. Elsayed²,
Abd El-Salam M. Metwally², Ahmed M. Shehabeldin³, Sara M. Ali⁴

¹King Faisal University, College of Veterinary Medicine, Department of Clinical Sciences, Al-Ahsa, Saudi Arabia

²Kafrelsheikh University, Faculty of Agriculture, Department of Animal Production, Kafrelsheikh, Egypt

³Agricultural Research Center, Animal Production Research Institute, Giza, Egypt

⁴Assiut National University, Faculty of Medicine, Assiut, Egypt

Received June 8, 2026

Accepted September 7, 2026

Abstract

The purpose of the current study was to investigate the effects of palmitic acid-loaded nanoparticles (PANano) concentrations on Holstein cryopreserved semen. Semen was diluted, equilibrated, and cryopreserved using control extenders and different PANano concentrations (2, 4, and 6 $\mu\text{l/ml}$). Semen was analysed for sperm characteristics and kinetics. The results demonstrated that after dilution, progressive sperm motility (PM), viability, and acrosome integrity percentages did not differ ($P > 0.05$) among the control and different PANano concentrations. After equilibration, although the PM, viability, normal morphology, membrane integrity, and intact acrosome values at 4 $\mu\text{l/ml}$ were consistently greater than those of the control, 2, and 6 $\mu\text{l/ml}$, there were no changes ($P > 0.05$) between the control and different concentrations of PANano. After thawing, compared to the control and 2 and 6 $\mu\text{l/ml}$ groups, the PM, membrane integrity, and intact acrosome values at 4 $\mu\text{l/ml}$ were greater ($P < 0.05$). The mitochondrial potential values were higher at 4 and 6 $\mu\text{l/ml}$ than at the control and 2 $\mu\text{l/ml}$. Curvilinear velocity values were higher at 4 $\mu\text{l/ml}$ ($P < 0.05$) than at 2 and 6 $\mu\text{l/ml}$. Straight line velocity was higher ($P < 0.05$) at 4 $\mu\text{l/ml}$ compared to 2 and 6 $\mu\text{l/ml}$ values. Compared to 2 and 6 $\mu\text{l/ml}$, non progressive motility values were lower ($P < 0.05$) at 4 $\mu\text{l/ml}$. In conclusion, 4 $\mu\text{l/ml}$ PANano enhanced sperm characteristics and kinetics, whereas high doses had an adverse effect on sperm kinetics and traits.

Cattle, reproduction, fertility, sperm characteristics, cryodamage

The structural and biochemical integrity of the sperm membrane plays a pivotal role, as it directly influences sperm morphology, motility, acrosomal reaction, and sperm–oocyte fusion (Safarinejad et al. 2010). Fatty acid (FA) composition of the sperm membrane is especially important, as it affects membrane fluidity, mitochondrial function, and overall sperm viability (Martínez-Soto et al. 2016).

Palmitic acid (PA), a highly abundant saturated fatty acid present in both spermatozoa and seminal plasma (Maulana et al. 2025), has been increasingly studied for its role in sperm function and fertility. For sperm quality and fertility, metabolites in seminal plasma and sperm are essential. Fatty acids dominate the sperm metabolite profile. Palmitic acid is found in seminal plasma, enhancing its defensive and nourishing functions (Maulana et al. 2025). The post-thaw quality of buffalo bull semen was enhanced by adding PA to the extender (Singh et al. 2019). Additionally, it was shown that adding oleic or palmitic acid to the extender enhanced the quality of bulls' sperm (Kiernan et al. 2013).

In a well-controlled boar sperm model, treatment with PA significantly increased progressive motility, straight-line velocity, and intracellular ATP levels (Zhu et al. 2020). Human studies have also indicated that PA is the most abundant saturated FA in normozoospermia, showing positive correlations with motility and sperm concentration. Importantly, antioxidant therapy in patients with asthenozoospermia has been shown to normalize seminal PA concentrations and restore motility, indicating oxidative stress

Address for correspondence:

Mohammed A. Abdel-Ghani
King Faisal University, Saudi Arabia
Mohamed E. El-Sharawy
Kafrelsheikh University, Egypt

E-mails: mataha@kfu.edu.sa; elsharawy78@gmail.com
<http://actavet.vfu.cz/>

as a key mediator of PA depletion and impaired function (Amirjannati et al. 2023). Collectively, these findings underscore the critical, dose- and context-dependent roles of PA in maintaining optimal sperm motility and function.

Nanoparticles have several properties that distinguish them from bulk materials simply by virtue of their size, such as chemical reactivity, energy absorption, and biological mobility (Murthy 2007). The advantages of nanoparticles in contemporary veterinary practices are extensive. Nanotechnology has emerged as a promising approach in reproductive biology, enabling the targeted delivery of antioxidants and bioactive compounds to improve sperm function, protect against cryodamage, and enhance overall semen quality (Abdel-Ghani et al. 2024). In this regard, nanostructured lipid carriers not only enhance the stability and bioavailability of active compounds but also hold significant potential for industrial applications in sperm cryopreservation. Therefore, in the current study, palmitic acid-loaded nanoparticles (PANano) were developed and evaluated for their ability to improve sperm quality at the post-dilution, post-equilibration, and post-thaw stages in Holstein bulls' semen.

Materials and Methods

Ethical approval

All experimental procedures used in the current study were approved by the guidelines of The Institutional Animal Care and Use Committee (ARC-IACUC) protocol number (KFS-IACUC/118/23) Agricultural Research Center, Egypt.

Animals

The International Livestock Management Training Center (ILMTC) of the Animal Production Research Institute (APRI) served as the site of the current investigation. Semen was collected from mature Holstein bulls ($n = 16$, 3–5 years old and average 550–700 kg body weight; 48 replicates in total; 3 replicates in a bull). During the experiment, the bulls were fed the same diet and housed in separate stalls under approved animal care practices.

Preparation of PANano

Palmitic acid nanoemulsion was created utilizing hot solvent diffusion ref technique. Palmitic acid was dissolved in ethanol at 70–80 °C, above its melting point, to form a distinct organic phase. The hot organic phase was then progressively injected into an aqueous phase containing Tween 80 (Sigma-Aldrich, St. Louis, MO, USA) by high-speed homogenization while remaining at the same temperature. The quick diffusion of organic solvent into the aqueous phase produced a fine oil-in-water nanoemulsion. The resultant dispersion was subjected to a brief probe sonication to minimize droplet size before the organic solvent was withdrawn at low pressure (Plate IV, Fig. 1A, B). A stable palmitic acid nanoemulsion was produced by bringing it to room temperature and then storing at 4 °C (Hu et al. 2005).

The nanoparticles were used for subsequent characterization. Particle size, polydispersity index (PDI), and zeta potential were determined by dynamic light scattering. Nanoparticle morphology was further examined by transmission electron microscopy (JEOL JEM-2100, JEOL Ltd., Tokyo, Japan) operating at an accelerating voltage of 160 kV.

Semen collection

Each bull was allowed to ejaculate twice a week using a sterile artificial vagina. After semen collection, the conical tube containing the ejaculate was immersed immediately in a water bath at 37 °C until evaluation in the laboratory within 5–10 min after collection. Following semen collection, the semen quality was evaluated under a light microscope (LEICA Co., Wetzlar, Germany), including sperm waves, motility, viability, concentration, and morphology. Motility was evaluated on a slide at 37 °C. Bulls ejaculated semen with rapid wave motion (mass activity; > +++), > 75% motility, > 80% viability, > 70% normal morphology, and a concentration of $> 1 \times 10^9$ sperm/ml semen, which were used for the experiments.

Preparation of semen extenders

Tris (3.028 g; 1.675 g citric acid and 0.75 g fructose; Sigma) and egg yolk (20%) constituted the basic extender. Following the dissolution of all components in 100 ml of distilled water, the basic extender was mixed with 500 µg of penicillin (Sigma) and 500 µg of streptomycin (Sigma). The extender received a 7% addition of glycerol (Sigma) as a cryoprotectant. To investigate the effects of palmitic acid nanoparticles, PANano (2, 4, or 6 µl/ml) were added to each of three parallel semen samples from the same bull. Semen samples without PANano were used as controls.

Semen dilution and freezing

The ejaculates were divided into four parts and diluted with four freezing extenders at room temperature (20 to 23 °C) after semen evaluation when motility was $\geq 70\%$. Post-dilution was then assessed. After 4 h

of equilibration at 4 °C, the diluted semen was assessed post-equilibration. A high-mobility extender was packaged for cryopreservation in 0.25-ml plastic straws (IMV Technologies, L'Aigle, France) after equilibration. The cooled straws were floated horizontally in liquid nitrogen vapor for 10 min, 5 cm above the liquid nitrogen surface, and then plunged into liquid nitrogen. The straws were thawed by immersion in a 37 °C water bath for 20 s.

Sperm evaluation

The mass activity was measured using a light microscope at a $\times 100$ magnification and 37 °C. The estimated proportion of motility was $\times 400$. The live/dead sperm ratio was calculated using eosin-nigrosine (Sigma) staining on a heated slide. For each semen sample, a total of 200 sperm were counted. Sperm plasma membrane integrity, acrosomal integrity, and mitochondrial membrane potential were assessed as previously described by Kanno (2018). Briefly, 2 μ l of Hoechst 33342 stock solution (Sigma) was mixed with the sample and incubated for 10 min. After incubation, 1 μ l of JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide; mitochondrial membrane potential) solution was added and incubated for 3 min. The sample was combined with 0.5 μ l of propidium iodide (PI; Sigma) solution and 0.5 μ l of fluorescein isothiocyanate-labelled peanut agglutinin (FITC-PNA; Sigma) and incubated for 8 min. All incubations were performed at 37 °C in the dark. Following staining with fluorescent probes, 2 μ l of 10% formaldehyde (v/v) was added to the samples to stabilize the sperm. Next, 5 μ l of the sperm suspension was placed on a slide and covered with a cover glass. More than 200 sperm cells on a glass slide were examined under a fluorescence microscope (Nikon Co., Tokyo, Japan). Sperm were classified as shown in Fig. 2 (Plate V). Briefly, plasma membrane integrity was graded as intact when the sperm head was stained with Hoechst 33342 and damaged when stained with PI (red). When FITC-PNA (green) was used to stain acrosomes, their integrity was compromised. If the acrosome was not discoloured, it was classified as intact. The colour of the JC-1-stained midpiece of the sperm was used to estimate the mitochondrial membrane potential. When the midpiece was stained orange, it indicated a strong mitochondrial membrane potential. Normal sperm were defined as those with an intact plasma membrane, undamaged acrosome, and high mitochondrial membrane potential (Kanno 2018).

Sperm kinetic properties determination

Sperm kinematics were assayed using a computer-assisted sperm analysis (CASA) instrument: SpermVision™ software (Minitüb GmbH, Tiefenbach, Germany) program. Curvilinear velocity (VCL, μ m/s); straight line velocity (VSL, μ m/s); average path velocity (VAP, μ m/s); linearity (LIN%); straightness (STR, VSL/VAP) $\times 100\%$; and wobble coefficient (WOB, VAP/VCL) $\times 100\%$ were measured. In addition, progressive sperm motility (PM%) was evaluated.

Statistical analysis

ANOVA was used to compare means, and Tukey's multiple comparisons test were then applied (JMPpro18; Cary, NC, USA). The different means were significant at $P < 0.05$. All data were expressed as means $\pm$ SD. The figures were created using GraphPad Prism v10 software (GraphPad Software, Inc., San Diego, CA).

Results

Physicochemical characterization of PANano

Dynamic light scattering analysis showed a Z-average diameter of 19.8 nm with a PDI of 0.422, confirming a narrow particle size distribution (Plate IV, Fig. 1C). Zeta potential analysis demonstrated a surface charge of -25.1 mV (Fig. 1D), indicating good colloidal stability and suggesting that the nanoparticles were sufficiently stabilized against aggregation by electrostatic repulsion.

Effect of PANano on bull semen post-dilution

Although the PM, viability, normal morphology, membrane integrity, intact acrosome, and mitochondrial potential values at 4 μ l/ml of PANano were regularly higher than those of the control, 2 and 6 μ l/ml, there were no differences ($P > 0.05$) between the control and different concentrations of PANano after dilution (Fig. 3).

Effect of PANano on bull semen post-equilibration

There were no differences ($P > 0.05$) between the control and various concentrations of PANano after equilibration, even though the PM, viability, normal morphology, membrane integrity, and intact acrosome values at 4 μ l/ml were consistently higher than those at the control, 2, and 6 μ l/ml concentrations (Fig. 4).

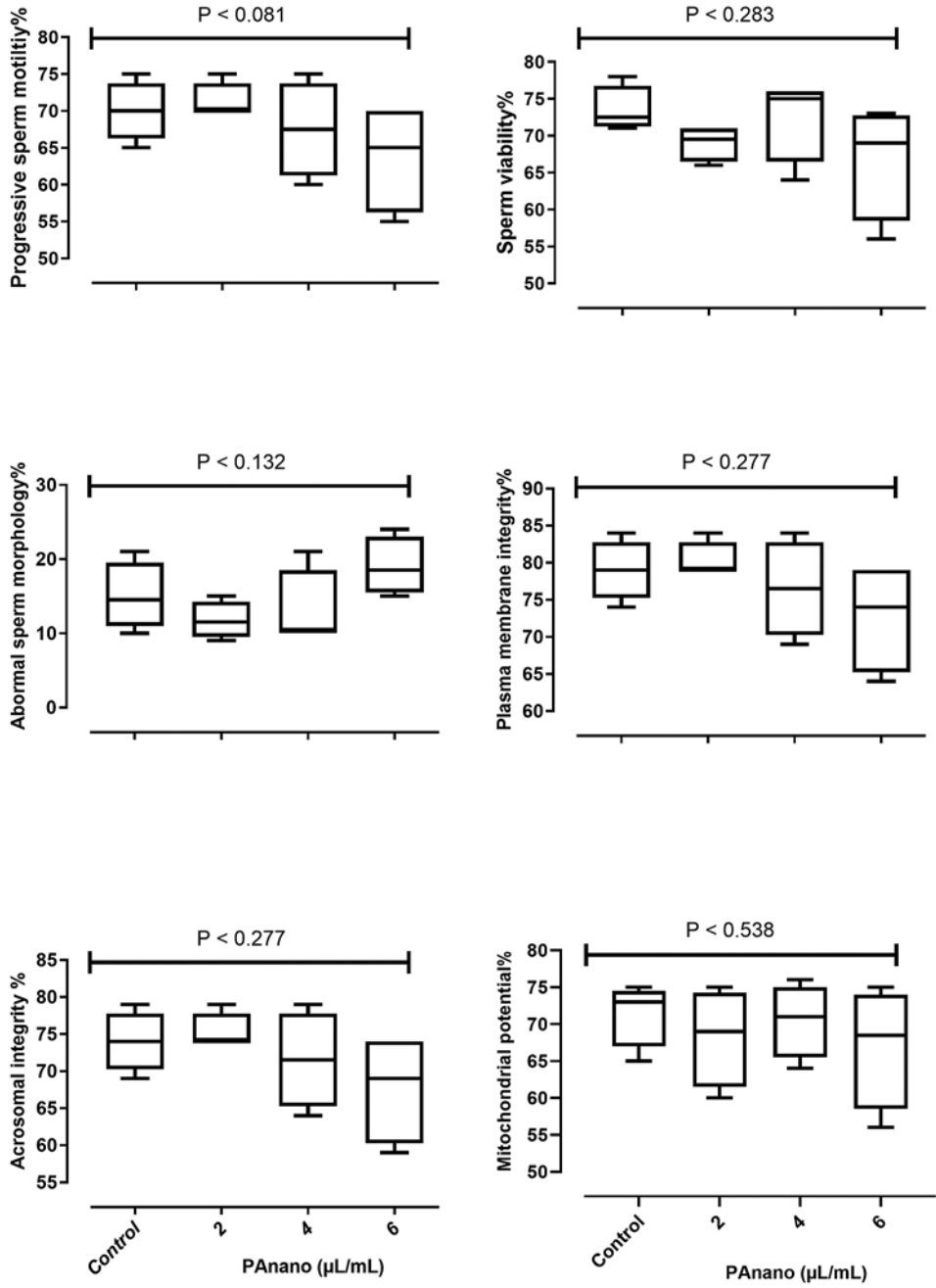


Fig. 3. Sperm characteristics in Holstein bulls' semen after dilution in extender treated with 0, 2, 4, and 6 µL/ml palmitic acid nanoparticles.

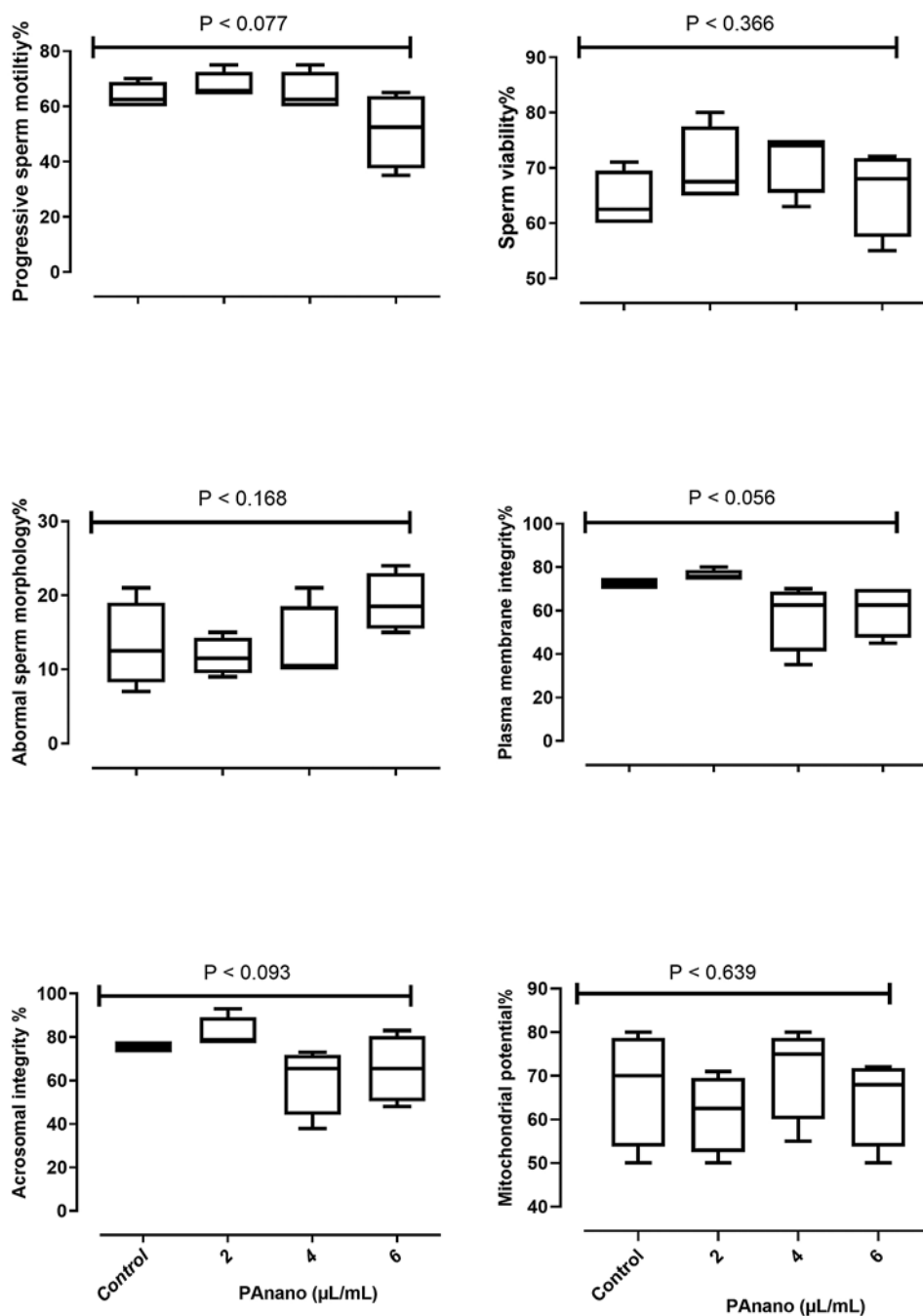


Fig. 4. Sperm characteristics in Holstein bulls' semen after equilibration in extender treated with 0, 2, 4, and 6 µl/ml palmitic acid nanoparticles.

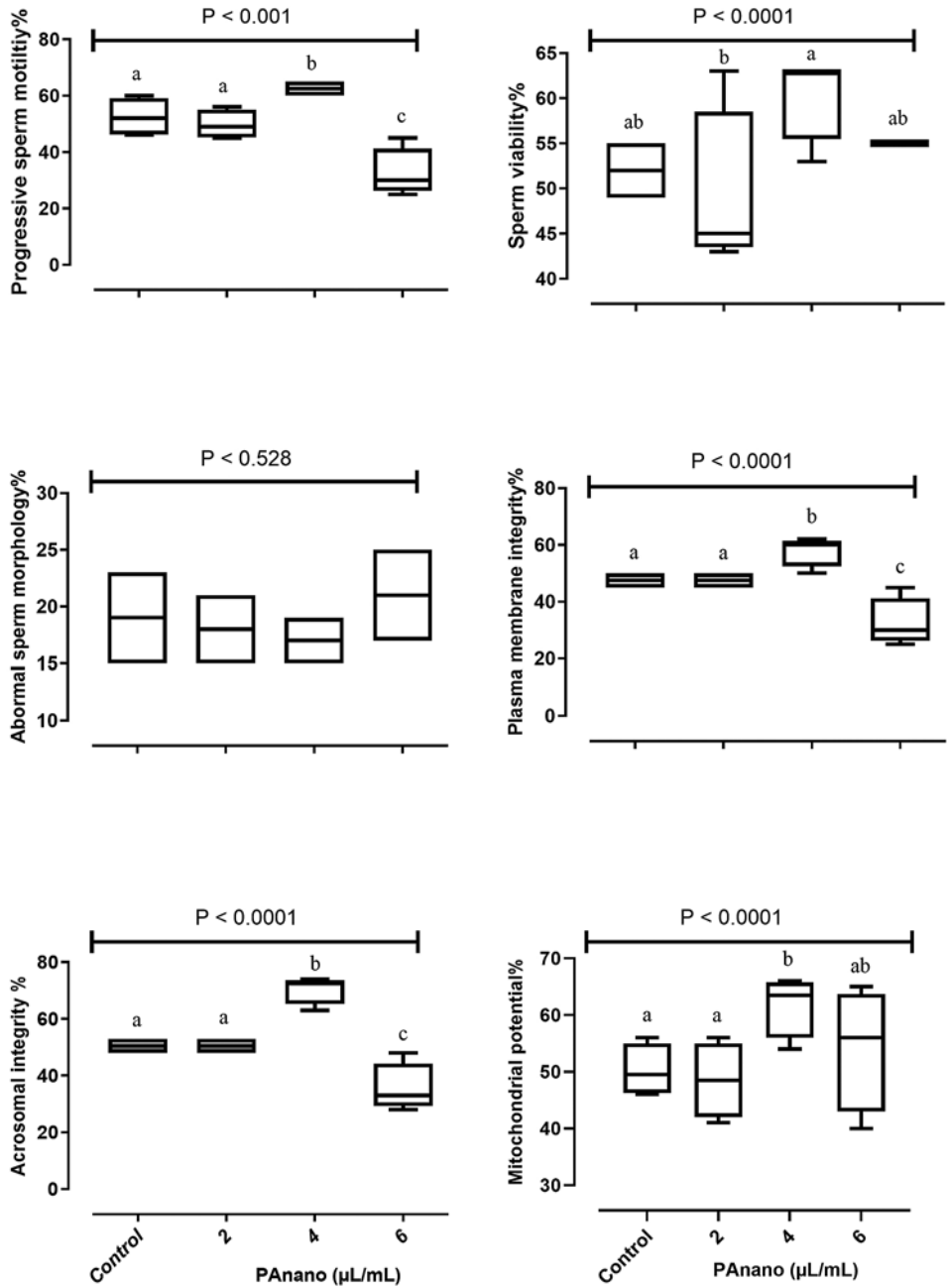


Fig. 5. Sperm characteristics in Holstein bulls' semen after thawing in extender treated with 0, 2, 4, and 6 µl/ml palmitic acid nanoparticles.

^{a-c} Different superscripts indicate a significant difference between the experimental groups ($P < 0.0001$).

Effect of PAnano on bull semen post-thawing

As shown in Fig. 5, the PM, membrane integrity, and intact acrosome values at 4 $\mu\text{l/ml}$ were higher than those at the control, 2, and 6 $\mu\text{l/ml}$ ($P < 0.05$). At 6 $\mu\text{l/ml}$, PM, membrane integrity, and intact acrosome were lower than those at 2 $\mu\text{l/ml}$ and control ($P < 0.05$). Furthermore, no variations ($P > 0.05$) were observed between the control and 2 $\mu\text{l/ml}$ after thawing. The mitochondrial potential values were higher at 4 and 6 $\mu\text{l/ml}$ than at control and 2 $\mu\text{l/ml}$.

Kinetic properties of post-thawed bull semen treated with PAnano

The kinetics of bull semen treated with PAnano are shown in Table 1. Although the rapid progressive motility values at 4 $\mu\text{l/ml}$ were higher than those at the control, 2, and 6 $\mu\text{l/ml}$, there were no differences ($P > 0.05$) between the control and different concentrations of PAnano after thawing. Non-progressive motility values were lower ($P < 0.05$) at 4 $\mu\text{l/ml}$ than at 0, 2, and 6 $\mu\text{l/ml}$ of PAnano treatment. Additionally, non-progressive motility at 2 and 6 $\mu\text{l/ml}$ was lower ($P < 0.05$) than that of the control group. At 0, 2, and 6 $\mu\text{l/ml}$, the VCL had comparable values ($P > 0.05$); however, the mean values of VCL were higher at 4 $\mu\text{l/ml}$ ($P < 0.05$) than at 0, 2, and 6 $\mu\text{l/ml}$. The VSL was higher ($P < 0.05$) at 4 $\mu\text{l/ml}$ compared to 2 and 6 $\mu\text{l/ml}$. The VAP was greater ($P < 0.05$) at 4 $\mu\text{l/ml}$ than at 6 $\mu\text{l/ml}$. The WOB was lower ($P < 0.05$) at 4 and 6 $\mu\text{l/ml}$ compared to 0 and 2 $\mu\text{l/ml}$ treatments. No variations ($P > 0.05$) were observed in the STR values between 0, 2, 4, and 6 $\mu\text{l/ml}$. The abnormal head and tail were all decreased at 4 $\mu\text{l/ml}$ compared to 0, 2, and 6 $\mu\text{l/ml}$ concentrations. Compared to 2 $\mu\text{l/ml}$, the abnormal neck was lower at 4 $\mu\text{l/ml}$.

Table 1. Computer-assisted sperm analysis showing effect of palmitic acid nanoparticles (PAnano) supplementation to semen extender of Holstein bulls post thawing.

Indicator (%)	PAnano concentration ($\mu\text{l/ml}$)			
	0	2	4	6
Rapid progressive motility	17.3 $\pm$ 3.4	14.9 $\pm$ 1.7	25.9 $\pm$ 5.1	15.5 $\pm$ 3.8
Slow progressive motility	19.7 $\pm$ 1.5 ^{ab}	17.0 $\pm$ 0.3 ^b	24.9 $\pm$ 3.4 ^a	20.6 $\pm$ 2.5 ^{ab}
Total progressive motility	36.9 $\pm$ 3.5	31.9 $\pm$ 1.7	50.8 $\pm$ 8.3	36.0 $\pm$ 6.1
Progressive motility	50.3 $\pm$ 2.4 ^b	51.9 $\pm$ 1.7 ^b	67.5 $\pm$ 3.3 ^a	49.6 $\pm$ 1.7 ^b
Non-progressive	32.1 $\pm$ 2.2 ^a	26.0 $\pm$ 1.3 ^b	14.8 $\pm$ 0.2 ^c	24.4 $\pm$ 1.6 ^b
Immotile sperm	21.0 $\pm$ 4.7	25.5 $\pm$ 2.3	17.7 $\pm$ 3.0	22.9 $\pm$ 1.1
VCL ($\mu\text{m/s}$)	44.2 $\pm$ 3.4 ^b	41.2 $\pm$ 1.3 ^b	70.6 $\pm$ 1.9 ^a	41.8 $\pm$ 2.4 ^b
VSL ($\mu\text{m/s}$)	17.7 $\pm$ 1.0 ^{ab}	15.2 $\pm$ 0.7 ^b	20.1 $\pm$ 1.7 ^a	14.5 $\pm$ 1.1 ^b
VAP ($\mu\text{m/s}$)	55.7 $\pm$ 7.8 ^{ab}	54.9 $\pm$ 2.5 ^{ab}	67.0 $\pm$ 3.6 ^a	41.8 $\pm$ 2.7 ^b
LIN (%)	32.5 $\pm$ 2.2 ^b	46.4 $\pm$ 0.7 ^a	15.7 $\pm$ 2.4 ^c	45.7 $\pm$ 0.0 ^a
STR (%)	32.7 $\pm$ 5.0	28.0 $\pm$ 4.4	30.5 $\pm$ 4.6	34.7 $\pm$ 0.1
WOB (%)	124.6 $\pm$ 14.2 ^a	133.8 $\pm$ 16.4 ^a	95.3 $\pm$ 13.4 ^b	99.9 $\pm$ 13.6 ^b
Normal form (%)	52.7 $\pm$ 6.9	52.6 $\pm$ 0.7	58.5 $\pm$ 7.9	50.8 $\pm$ 4.2
Abnormal head (%)	33.3 $\pm$ 0.0 ^a	42.9 $\pm$ 2.4 ^a	20.6 $\pm$ 1.9 ^b	42.7 $\pm$ 7.2 ^a
Abnormal neck (%)	19.7 $\pm$ 7.9 ^{ab}	29.8 $\pm$ 5.5 ^a	11.0 $\pm$ 1.5 ^b	26.3 $\pm$ 3.1 ^{ab}
Abnormal tail (%)	16.5 $\pm$ 5.4 ^a	26.7 $\pm$ 7.4 ^a	3.6 $\pm$ 0.8 ^b	20.6 $\pm$ 2.5 ^a
Mono deformation (%)	27.1 $\pm$ 0.7	15.3 $\pm$ 7.4	23.8 $\pm$ 3.5	23.2 $\pm$ 1.3
Dual deformation (%)	18.2 $\pm$ 8.8	12.5 $\pm$ 2.3	8.4 $\pm$ 2.0	11.6 $\pm$ 2.7
Tri deformation (%)	2.0 $\pm$ 1.2 ^b	19.7 $\pm$ 4.5 ^a	2.6 $\pm$ 0.8 ^b	14.4 $\pm$ 5.6 ^{ab}

VCL, curvilinear velocity; VSL, straight line velocity; VAP, average path velocity; LIN, linearity; STR, straightness (VSL/VAP $\times$ 100%); WOB, wobble coefficient (VAP/VCL $\times$ 100%)

* Three ejaculates were collected from a bull (48 replicates in total; 3 replicates in a bull).

^{a-b} Superscripts indicate a significant difference between the experimental groups in the same column ($P < 0.05$). Data are represented as mean $\pm$ SD.

Discussion

One major difficulty in cryopreservation is the formation of intracellular ice crystals during the freezing process. Cryodamage from cryopreservation causes structural and molecular alterations in spermatozoa. Damage from oxidative, thermal, and osmotic stress can change the DNA integrity, motility, viability, and fluidity of the plasma membrane of spermatozoa (Kopeika et al. 2015). To reduce cryodamage, cryoprotectants and, in some clinical trial situations, antioxidants that could improve the quality of post-thawed sperm are added to the freezing medium (Yáñez-Ortiz et al. 2022). This study aimed to investigate the quality of cryopreserved Holstein bull semen (motility, viability, morphology, plasma membrane integrity, vigor, and progression kinetics) after dilution, equilibration, and post-thawing using PAnano.

After dilution among all concentrations evaluated, 4 $\mu\text{l/ml}$ PAnano showed higher but non-significant progressive motility, viability, membrane integrity, and acrosome integrity than the other groups. These findings suggest that 4 $\mu\text{l/ml}$ PAnano has an immediate positive impact on sperm physiology after dilution.

Sperm samples were kept at 4 °C during the post-equilibration phase to allow gradual cooling and cryoprotectant adaptation. This step is essential to reduce cold shock damage prior to freezing. According to the data, among the treatment groups, 4 $\mu\text{l/ml}$ had sperm with the greatest quality, with the highest acrosome integrity and progressive motility despite a minor overall drop in sperm performance compared to the post-dilution stage.

The largest stress-induced reduction in sperm quality was observed during the post-thawing phase, which represents the valuable reproductive potential of cryopreserved semen. Despite this, the 4 $\mu\text{l/ml}$ again showed the strongest protective effect, whereas the progressive sperm motility, acrosome integrity, and sperm membrane integrity were significantly improved compared to those in the control and at various concentrations of PAnano.

The lipid component of the spermatozoon membrane affects the properties of the membrane that are necessary for the sperm to approach and fuse with the egg. Furthermore, polyunsaturated fatty acids, which comprise 30–50% of the total fatty acid content in the membranes of mammalian spermatozoa, are necessary for reproductive activity and enhance acrosomal responsiveness (Van Tran et al. 2017).

Palmitic acid (PA) makes up 20–30% of the total fatty acids in adipose triacylglycerols and membrane phospholipids (Carta et al. 2015). It has a wide spectrum of pharmacological properties, including lipid metabolism control, analgesia, and antiviral and anti-inflammatory effects (Librán-Pérez et al. 2019). Kiernan et al. (2013) examined the effects of PA at doses ranging from 10 to 100 μM on bull spermatozoa that were kept *in vitro* for up to seven days. Particularly on Days 1 and 3, when the formation of reactive oxygen species (ROS) was at its maximum in alternative therapies, PA maintained progressive linear motion and viability at levels higher than the control and reduced ROS levels. Zhu et al. (2020) examined whether PA was advantageous to boar sperm during liquid storage. The authors showed that the addition of fatty acids to the extender increased boar sperm quality during liquid storage, and that PA could be used by sperm as an energy substrate for ATP synthesis to shield sperm from energy stress *in vitro* because fatty acids are readily integrated into the cell membrane (Jaswal et al. 2011). According to Beecharry et al. (2004), PA can increase oxidative stress by producing ROS, which in turn increases apoptosis. Moreover, poor sperm quality and high oxidative stress in men have been associated with dietary imbalances or high amounts of saturated fatty acids, including PA (Andersen et al. 2020). This may explain the adverse effects of high concentrations of PAnano on sperm characteristics. Based on motility properties, sperm are categorized as having either progressive or non-progressive motility (Iranpour et al. 2000). In terms

of fertility, only the percentage of progressively motile sperm is associated with conception rates. Progressive motility is commonly expressed as a percentage of sperm motility, which is a measure of sperm fertility and indicates proper spermatogenesis and sperm maturation during epididymal transport (Cabrillana et al. 2017).

A link between the success of pregnancy and all post-thaw values of VCL, VSL, and VAP for artificial insemination was found when sperm kinematic features were evaluated in relation to the fertilizing capacity in bulls (Larsen et al. 2000). Three kinematic characteristics that have the strongest correlation with fertility are VCL, VSL, and VAP. Of these, VAP is the most useful semen motility feature with clinical value for predicting the fertility of post-thawed bull semen (Nagy et al. 2015).

The CASA-based evaluation of post-thaw semen demonstrated that supplementation at 4 $\mu\text{l/ml}$ yielded the most favourable improvements in sperm motility, kinematics, and morphology compared to the control and other treatment groups. Progressive motility showed a significant improvement, whereas non-progressive motility exhibited a significant reduction, indicating improved forward progression. The kinematic properties of sperm movement were also positively influenced by 4 $\mu\text{l/ml}$; VCL improved significantly. Moreover, VSL and VAP were enhanced, suggesting better linear progression. Regarding morphology, the 4 $\mu\text{l/ml}$ treatment led to significant reductions in abnormal head, as well as abnormal tails, indicating superior structural preservation after freezing and thawing. The most common indicators for evaluating semen are sperm motility and morphology, which are used as criteria for choosing which ejaculates to analyse further after collection. Overall, 4 $\mu\text{l/ml}$ PANano appeared to be the optimal concentration for improving cryopreserved semen quality, as evidenced by enhanced motility and reduced morphological abnormalities assessed using CASA. According to the current study, spermatozoa with forward motility and a straight linear path may travel farther in a shorter amount of time based on velocity characteristics linked to progressive motility.

In conclusion, sperm motility is important for transportation to the fertilization site in the oviducts. Palmitic acid nanoparticles present an intriguing therapy (additive) for increased sperm motility, plasma membrane, and acrosome integrity after cryopreservation. While sperm kinetics and features were improved by 4 $\mu\text{l/ml}$ of PANano, sperm kinetics and attributes were negatively impacted by high doses. One limitation of this study is the absence of a free palmitic acid group due to its poor aqueous solubility. Future studies will explore cyclodextrin-complexed PA for a direct comparison.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgement

This work was supported by Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, (KFU 264543).

References

- Abdel-Ghani MA, El-Sharawy ME, Zaid EK, Shehabeldin AM, Dessouki SM, Moustapha ME, Metwally AESM, El-Shamaa IS 2024: Low concentrations of soybean lecithin nanoparticles had a positive impact on Holstein bulls' cryopreserved semen. *Reprod Domest Anim* **59**: e14613
- Amirjannati N, Henkel R, Hosseini E, Choopanlian P, Moghadasfar H, Arjmand B, Asgharpour SL, Haji PA, Gilany K 2023: The amino acid profile in seminal plasma of normozoospermic men: A correlation analysis with spermogram parameters and total antioxidant capacity. *J Reprod Infert* **24**: 257-268
- Andersen JM, Rønning PO, Herning H, Bekken SD, Haugen TB, Witczak O 2016: Fatty acid composition of spermatozoa is associated with BMI and with semen quality. *Andrology* **4**: 857-865
- Beeharry N, Chambers JA, Green IC 2004: Fatty acid protection from palmitic acid-induced apoptosis is lost following PI3-kinase inhibition. *Apoptosis* **9**: 599-607

- Cabrillana ME, Monclus MA, Lancellotti TES, Boarelli PV, Vincenti AE, Fornés MM, Sanabria EA, Fornés MW 2017: Thiols of flagellar proteins are essential for progressive motility in human spermatozoa. *Reprod Fertil Dev* **29**: 1435-1446
- Carta G, Murru E, Lisai S, Sirigu A, Piras A, Collu M, Batetta B, Gambelli L, Banni S 2015: Dietary triacylglycerols with palmitic acid in the sn-2 position modulate levels of N-acyl ethanolamides in rat tissues. *PLoS ONE* **10**: e0120424
- Hu FQ, Jiang SP, Du Y Z, Yuan H, Ye Y Q, Zeng S 2005: Preparation and characterization of stearic acid nanostructured lipid carriers by solvent diffusion method in an aqueous system. *Colloids Surf B: Biointerfaces* **45**: 167-173
- Iranpour FG, Nasr-Esfahani MH, Valojerdi MR, Al-Taraihi TMT 2000: Chromomycin A3 staining as a useful tool for evaluation of male fertility. *J Assist Reprod Genet* **17**: 60-66
- Jaswal JS, Keung W, Wang W, Ussher JR, Lopaschuk GD 2011: Targeting fatty acid and carbohydrate oxidation – A novel therapeutic intervention in the ischemic and failing heart. *Biochim Biophys Acta* **1813**: 1333-1350
- Kano C 2018: Effects of semen preservation procedure in egg yolk-tris based extender on bull sperm characteristics. In: Evaluation of bovine semen quality based on sperm organelle functions and motility subpopulation structures. Hokkaido University, Hokkaido, Japan, Chapter II; pp 18-29
- Kiernan M, Fahey AG, Fair S 2013: The effect of the *in vitro* supplementation of exogenous long-chain fatty acids on bovine sperm cell function. *Reprod Fertil Dev* **25**: 947-954
- Kopeika J, Thornhill A, Khalaf Y 2015: The effect of cryopreservation on the genome of gametes and embryos: Principles of cryobiology and critical appraisal of the evidence. *Hum Reprod Up* **21**: 209-227
- Larsen L, Scheike T, Jensen TK, Bonde JP, Ernst E, Hjøllund NH, Zhou Y, Skakkebaek NE, Giwercman A 2000: Computer-assisted semen analysis parameters as predictors for fertility of men from the general population. The Danish First Pregnancy Planner Study Team. *Hum Reprod* **7**: 1562-1567
- Librán-Pérez M, Pereiro P, Figueras A, Novoa B 2019: Antiviral activity of palmitic acid via autophagic flux inhibition in zebrafish (*Danio rerio*). *Fish Shellfish Immunol* **95**: 595-605
- Martínez-Soto JC, Domingo JC, Cordobilla B, Nicolás M, Fernández L, Alberio P, Gadea J, Landeras J 2016: Dietary supplementation with docosahexaenoic acid (DHA) improves seminal antioxidant status and decreases sperm DNA fragmentation. *Syst Biol Reprod Med* **62**: 387-395
- Maulana T, Said S, Fidriyanto R, Arifiantini RI, Hasbi H, Jakaria J, Gunawan A 2025: A comparative study of seminal plasma and sperm metabolome profiles in Toraya buffalo bulls. *Vet Med Int* **2025**: 9987127
- Murthy AK 2007: Nanoparticles in modern medicine: State of the art and future challenges *Int J Nanomed* **2**: 129-141
- Nagy Á, Polichronopoulos T, Gáspárdy A, Solti L, Cseh S 2015: Correlation between bull fertility and sperm cell velocity parameters generated by computer-assisted semen analysis. *Acta Vet Hung* **63**: 370-381
- Safarinejad MR, Hosseini SY, Dadkhah F, Asgari MA 2010: Relationship of omega-3 and omega-6 fatty acids with semen characteristics, and anti-oxidant status of seminal plasma: a comparison between fertile and infertile men. *Clin Nutr* **29**: 100-105
- Singh AK, Kaur H, Cheema RS, Kumar A, Honparkhe M, Singh P 2019: Palmitic acid supplementation in tris extender can improve the freezability of buffalo bull semen. *Ind Vet J* **96**: 14-16
- Van Tran L, Malla BA, Kumar S, Tyagi AK 2017: Polyunsaturated fatty acids in male ruminant reproduction - a review. *Asian-Australas J Anim Sci* **30**: 622-637
- Yáñez-Ortiz I, Catalán J, Rodríguez-Gil JE, Miró J, Yeste M 2022: Advances in sperm cryopreservation in farm animals: Cattle, horse, pig and sheep. *Anim Reprod Sci* **246**: 106904
- Zhu Z, Li R, Feng, C, Liu, R, Zheng Y, Hoque SAM, Wu D, Lu H, Zhang T, Zeng W 2020: Exogenous oleic acid and palmitic acid improve boar sperm motility via enhancing mitochondrial B-oxidation for ATP generation. *Animals (Basel)* **10**: 591

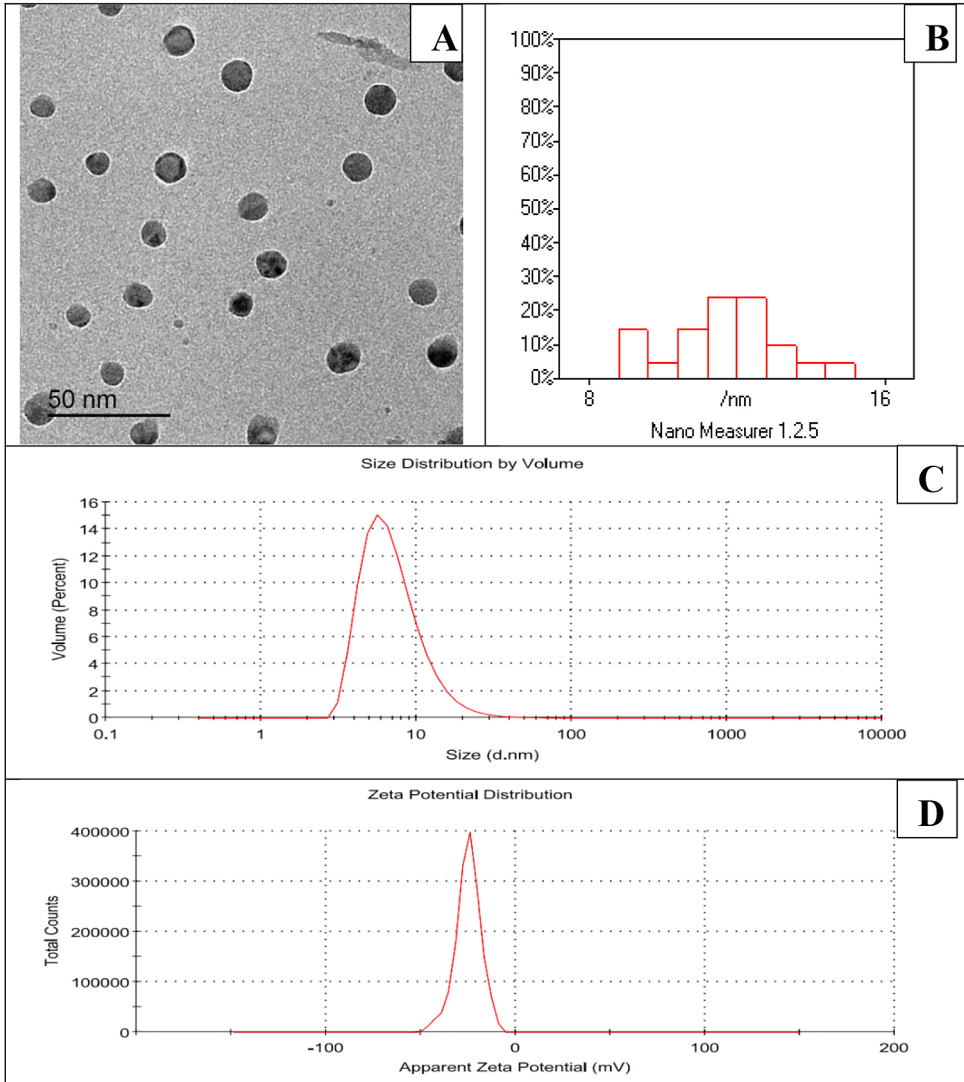


Fig. 1. (A) The morphology of palmitic acid nano emulsion by transmission electron microscopy showing nearly spherical particles. (B) Histogram shows that most particles cluster around 8 to 16 nm, with a good distribution. (C) Zeta size distribution by volume. (D) Zeta potential distribution. The values of Z-average size, polydispersity index, and zeta potential were found to be 19.8 nm, 0.422, and -25.1 mV, respectively.

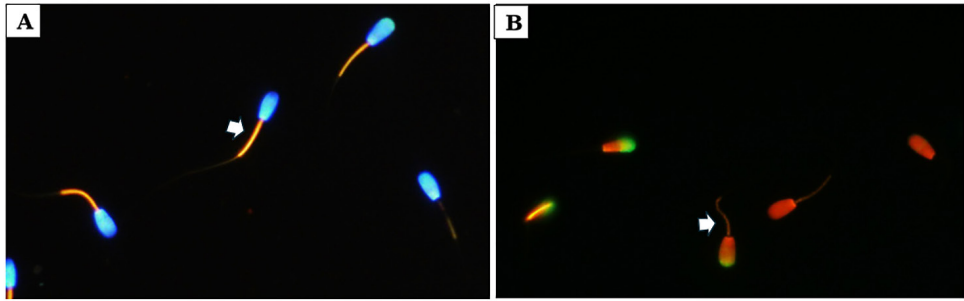


Fig. 2. The classification of sperm traits assessed through fluorescent staining. The sperm heads stained with Hoechst 33342 were assessed as having an intact plasma membrane (A). The sperm heads stained with propidium iodide (red) were considered to have a compromised plasma membrane (B). Acrosome labelled (green) with fluorescein isothiocyanate-labelled peanut agglutinin was considered damaged (B). The orange-dyed midpiece was evaluated as sperm exhibiting a high mitochondrial membrane potential (A and B; white arrow).