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Effect of Chitosan Nanoparticles on Ram Sperm Characterisation after Semen Cryopreservation

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Abstract

THIS STUDY evaluated the possible protective role of chitosan nanoparticles (CNPs) on ram semen after cryopreservation using a Tris-egg yolk-based extender. Chitosan synthesis and characterization were done by transmission electron microscope (TEM), scanning electron microscope (SEM) and energy dispersive analysis of X-ray spectroscopy (EDAX). Semen ejaculates were collected by artificial vagina from five rams with proven fertility (two ejaculates / ram / week) for five weeks. Only good quality ejaculates were pooled and then diluted with tris extender supplemented with different concentrations of *CNPs* (0, 5, 15, 25, 50, and 75 µg/ml). The diluted Samples gradually cooled to 4°C over 90 min, then equilibrated for farther 2hrs in a cold Cabinet (4°C) after that packed in 0.5ml French straws, frozen on liquid nitrogen (LN) vapor for 15 minutes then stored in LN until evaluation. Post-thawed samples were examined by computer-assisted sperm analysis (CASA) recording sperm motion parameters. Additionally, the acrosome defects, membrane integrity, antioxidant activity as well as sperm ultrastructure, and DNA integrity were recorded. The results showed a significant improvement in total, rapid, and progressive motility, in addition to preserving the integrity of the acrosome, the plasma membrane, and the ultrastructure of sperm after adding (15 µg/ml) of chitosan nanoparticles. There was a non-significant increase in antioxidant activity with a decrease in the levels of lipid peroxides and no adverse effect on the DNA. It could be concluded that CNPs cross-linked with dextran sulfate were able to successfully protect the viability of frozen-thawed ram sperm by decreasing cryo-injury.

Keywords: Chitosan Nanoparticles, Cryopreservation, Oxidative Stress, Sperm Ultrastructure, DNA, Ram.

Introduction

Egyptian sheep production is crucial for converting unfit forages into meat and milk, providing essential human dietary protein. They adapt well to various agricultural conditions, especially in reclaimed and desert lands, and are sustainable to climatic changes [1]. Environmental problems brought on by climate change are predicted to get worse in the future years, leading to various issues, especially with food [2]. Because sheep are a vital component of Egypt's food security strategy and can raise daily protein intake, the Egyptian government encourages the cultivation of sheep [1]. To satisfy the growing demand for livestock, reproductive biotechnologies including artificial insemination can improve genetic selection

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and efficiency in sheep breeding programs, improving global food security [3]. It is known that small ruminant spermatozoa are more sensitive to cryopreservation than gametes from other species. It leads to the development of excess reactive oxygen species (ROS) during the freezing and thawing process, which destroys the sperm cell membrane's double carbon chain fatty acids and causes an increase in malondialdehyde (MDA) levels [4]. As a result, semen cryopreservation affects sperm motility, normal morphology, membrane integrity, spermatozoa DNA integrity, and lipid peroxidation [5]. Semen storage time could be prolonged by adding antioxidants to semen extenders to boost their antioxidant capacity and lessen the effects of oxidative stress [6].

The fundamental issue in semen cryopreservation is to prevent spermatozoa from damage induced by freezing and thawing processes. Semen cryopreservation could be enhanced by integrating biocompatible nanomaterials [7]. Chitosan is a safe material used in nanoparticle technology [8]. Chitosan is a naturally occurring amino polysaccharide produced by deacetylation of chitin and is the second most often used natural polymer. Its non-toxic, biocompatible, and biodegradable qualities have sparked substantial research into a variety of uses and are safe for human consumption [9]. Consequently, it offers an enormous array of possible uses. This is where chitosan's unique properties come into play. Chitosan is a biodegradable and biocompatible polymer consisting of alternating repeating units of N-acetyl glucosamine and glucosamine with 1-4- glycosidic linkage [10]. Nanotechnology has enabled the fabrication of nano-sized particles that exhibit higher chemical reactivity, enhanced cellular penetration, and increased biological activity [11]. Active compounds are dispersed more efficiently in media, allowing for more precise cell targeting compared to microparticles. The potential effects of adding chitosan nanoparticles to the egg yolk diluent for freezing ram semen to achieve optimum post-thawing semen quality for artificial insemination have not been extensively previously studied. This research aimed to address this problem by synthesizing chitosan nanoparticles as well as investigating their impact on the post-thaw ram semen quality and determining the suitable concentration for ram semen.

Material and Methods

Synthesis and Characterization of Chitosan Nanoparticles (CNPSs):

Chitosan nanoparticles were processed by the method described by Ribeiro et al. [12]. The morphology and size of chitosan nanoparticles were analyzed using the high-resolution electron microscope (HRTEM, JEOL-JEM2100, Japan) and scanning electron microscope (SEM, QUANTA FEG 250). In short, one drop of ethyl alcohol was added to the nano-particles, the surface was covered with a copper grid, allowed to sit for two minutes, and then the sample was analyzed under an HRTEM. For SEM scanning, the synthesized nanoparticles were deposited on SEM stubs using adhesive tape and evenly coated with gold. Energy dispersive analysis of X-ray spectroscopy (EDAX) microanalysis system connected to SEM was used to perform compositional analysis.

Animal and Semen Collection:

Five adult Barki rams (2–3.5 years old) that were sexually fertile and in good clinical health were used in the current investigation. Using the artificial vagina, fifty ejaculates (two ejaculates from each ram once a week) were obtained for five weeks. The animals were sheltered in a clean, semi-open shed stall owned by Egypt's Animal Reproduction Research Institute, the Agriculture Research Center in Giza.

Experimental Design:

Ejaculates were delivered to the lab immediately after collection, where their volume, concentration, sperm motility, and morphology were evaluated. Then we pooled the good-quality ejaculates that contained a sperm concentration of at least 3 x 10^9 sperm/ml, motility of at least 70%, and volume of at least 1 ml, diluted with a Tris-based extender at 37^{o}C to a final concentration rate, 200 × 10^6 sperm/ml, divided into six aliquots each supplemented with (0, 5, 15, 25, 50, and 75 µg/ml CNPs respectively. Samples were gradually cooled to 4^{o}C over 90 minutes then left to be equilibrated for another 2hrs, then packed into 0.5 ml French straws (IMV-Technologies France), then frozen on liquid nitrogen vapor (4–5 cm above the liquid nitrogen surface) for 15 minutes after that plunged into liquid nitrogen and finally stored until evaluation.

Post-Thawing Evaluation:

Representative straws from each treatment were thawed in a water bath at 37°C / 30 seconds. The different semen parameters including CASA motility, membrane integrity, acrosome defects, antioxidant activity, MDA together with sperm ultrastructure, and DNA integrity were measured.

Sperm Motility Assessment:

Motility percentages and Sperm kinetics were assessed with the aid of Computer-Assisted Sperm Analysis (CASA) system (Andro Vision® software minitube, Germany) at the Artificial Insemination and IVF Research Department, Animal Reproduction Research Institute.

Plasma Membrane Integrity Assessment:

Plasma membrane integrity was evaluated by the hypoosmotic swelling test (HOST) following the method of Nalley et al. [13]. Briefly, to prepare a

hypo-osmotic solution (150 mOsm/L), dissolve 7.35 g sodium citrate and 13.51 g fructose in 1000 mL of distilled water. Incubate 10 µL of semen in each 2 mL hypo-osmotic solution at 37°C for an hour. Place a drop of the suspension on a glass side covered with a coverslip. Examine the sperm under a microscope at 400 x magnification. Each slide contained around 200 sperm cells, which were counted at random. Determine the percentage of swollen-tailed sperm (sperm that reacted to the HOST).

Acrosomal Membrane Integrity Assessment:

Acrosome integrity was evaluated using Giemsa stain following the method of Chowdhury et al. [14]. The stock Giemsa stain was prepared. Representative straws from each concentration were thawed at (37^0 C / 30 seconds). Air-dried smears of diluted sperm were made on grease-free slides. The sides were fixed in a 5% formaldehyde solution for 30 minutes. Washing with running tap water then air dried. Giemsa's working solution was produced by combining Giemsa's stock (3 ml), SPS (2 ml), and Milli-Q water (45 ml) in a cup container at 37^0 C for 30 minutes. Slides were immersed in the working solution and kept at 37^o C/2 hours. Rewashing with running tap water, followed by air drying. Using an oil immersion Olympus microscope with a magnification of 1000X. The percentage of acrosome intact spermatozoa was estimated by counting at least 200 cells per slide.

Assessment of Sperm Ultrastructure: following the method of Boonkusol et al. [15]. Representative straws for each treatment were thawed at 37^oC/30 sec. and were rinsed three times with phosphate-buffered saline (PBS). To wash the spermatozoa, frozen-thawed semen was prefixed with 2% glutaraldehyde in PBS for 2-3 hours before centrifugation at 1000 g with PBS (pH 7.4) three times for 5 minutes at 4°C. After that, spermatozoa were post-fixed in 1% osmium tetroxide for 1-2 hours at 4°C . Spermatozoa were dehydrated in propylene oxide and then embedded in epon resin. Ultrathin slices were cut with a Leica EM UC6 ultramicrotome and stained with uranyl acetate and lead citrate. Fields were investigated at random using a transmission electronic microscope (JEOL-EM-100 S at TEM lab FA-CURP, at the Faculty of Agriculture Cairo University - Electron Microscopy Unit (Giza, Egypt), and photographed for additional analysis.

Determination of Antioxidant Activity and Lipid Peroxidation in Ram Seminal Plasma:

Five representative semen straws from each concentration were thawed at 37◦C, pooled, and centrifuged at 500 xg for 15 min. Then the collected supernatant was used to determine the total antioxidant capacity (TAC), superoxide dismutase (SOD), and malondialdehyde (MDA) levels by commercial kits (Bio Diagnostic, Egypt) following the manufacturer's instructions.

Assessment of Sperm DNA Damage Using Comet Assay

The comet assay, also known as single-cell gel electrophoresis (SCGE), was employed to assess the integrity of sperm DNA. With a few changes, we followed Corington et al.'s [16] protocol, where 2 x 10^4 sperm cells in 10 µl PBS were mixed with 90 µl of 1% low melting gel (LMG) and kept at 4 °C until the gel solidified. After overnight incubation in the comet assay lysis buffer, the lysed cell were left in an alkaline unwinding buffer containing 30 mM NaOH and 1 mM EDTA at pH 12.1for 15 minutes. , then neutralized in 1 x TBE at pH 7.4. After 30 minutes of electrophoresis at 20 volts and 10 mA in the neutral 1x TBE buffer, pH 8.0 (Tris-Borate-EDTA) , the comet gels were covered with absolute ethanol for 10 minutes. The stained gels with 20 µg/ml ethidium bromide (50 µl/gel) were examined under a fluorescent microscope (Leica Microsystem, Germany) equipped with 40X objective. The comet parameters of 100 sperm nuclei for each sample were reported using the software Tri-Tek Comet ScoreTM (freeware v1.5.) .

Statistical Analysis:

Data were analyzed statistically using SPSS software (version 18 for Windows, SPSS Inc., Chicago, IL, USA) by one-way analysis of variance (ANOVA). Group means were compared by Duncan's Multiple Range Test at a 5% level of probability. Data were illustrated as the mean ± standard error of the mean (SEM).

Results

Characterization of The Chitosan Nanoparticles:

High-resolution transmission electron microscope (HR-TEM) and scanning electron microscope (SEM) micrographs of the chitosan nanoparticles are shown in Figure (1&2). The micrographs confirm the spherical structure of chitosan nanoparticles with a diameter of less than 100 nm. The elemental composition of the chitosan sample obtained from SEM-EDX analysis is shown in Figure 3. The elemental analysis indicated 41.37 % Carbon, 47.49 % Nitrogen, 10.48 % Oxygen and, 0.66 % Sodium, respectively.

Effect of Chitosan Nanoparticles on Ram Sperm Post-Thawing CASA Parameters:

Table (1) shows that supplementing the freezing extender supplemented with CNPs (15µg/ml) significantly increased the total, progressive, and rapid motility after the freezing/thawing process ($p<$ 0.05). However, the dose of 75 µg/ml resulted in a

significant decrease when compared to the control and the other treated groups. Sperm kinetics didn't differ significantly between the control and the other supplemented groups, except for the rotation parameter where 15 µg/ml showed a significant increase compared to the control and the highest concentration (75 µg/ml).

Effect of Chitosan Nanoparticles on Ram Sperm Post-Thawing Membrane And Acrosome Defects:

As shown in Fig 4 (A&B), supplementation of the freezing extender with CNPs at (5& 15 µg/ml) concentrations, significantly increased the sperm membrane integrities as ($p< 0.05$) compared with the control and other supplemented groups. On the other hand, lowered the acrosome defects after the freezing-thawing process.

Effect of Chitosan Nanoparticles on Ram Sperm Post-Thawing Total Antioxidant Capacity (TAC), Super Oxide Dismutase (SOD) Activity, And Malondialdehyde (MDA) Level.

Figure 6. illustrates that antioxidant enzyme activity and Malondialdehyde level showed no significant differences after supplementation of CNPs to the freezing extender when compared with the non-treated group. Mathematical non-significant elevation of TAC& SOD and decreased level of MDA was recorded after supplementation of the freezing extender with (15 µg/ml) CNPs.

Effect of Chitosan Nanoparticles on Ram Sperm Post-Thawing Sperm Ultrastructure:

The electron microscopy micrographs of the post-thawed ram sperm control group showed varying degrees of mid-piece area alterations, transverse cristae loss, and mitochondrial degeneration. Moreover, signs of a swollen, degenerated, fenestrated, and vacuolated plasma membrane with a swollen acrosome and a non-homogenized nucleus were observed (Fig. 7 A, B, C, and D). Compared to that, the electron micrograph of a sagittal section through the head region from post-thawed ram semen treated with chitosan nanoparticles (15 g/ml) demonstrated intact nuclear content (N) and plasma membrane (PM), and the cross-section through the mid-piece region shown the presence of intact transverse cristae, good mitochondrial dense electron space, and normal mitochondrial organization.

Effect of Chitosan Nanoparticles on Ram Sperm Post-Thawing Sperm DNA Integrity:

The comet assay, which evaluates DNA integrity, demonstrated no significant variations between the control group and the groups supplemented with varying concentrations of CNPS (Table 2 and Fig. 9).

Discussion

Nanotechnology has accelerated the field of artificial insemination and methods for improving the quality of sperm by supplementing the cryo-diluent with nanoparticles during semen preservation [17]. The current work examined the effects of adding different doses of synthesized chitosan nanoparticles in a ram semen extender to enhance sperm quality after semen cryopreservation. In the present study, TEM analysis revealed that the chitosan nanoparticles morphology cross-linked with dextran sulfate was spherically shaped with an amorphous nature as Boruah et al [18] reported. The use of antioxidants to neutralize ROS overproduction, either directly in semen extenders or by diet inclusion, has been extensively explored and described by Qamar et al. [19]. Our study revealed that improvement of the seminal plasma antioxidant levels of SOD & TAC and a reduction of MDA of post-thawed ram semen, this improvement may be ascribed to the potent antioxidant properties of chitosan. Chitosan exhibited observable antioxidant capabilities hence protecting against destructive effects caused by free radicals. As such, it can alleviate lipid peroxidation and reduce the malondialdehyde (MDA) level [20&21]. The scavenger properties of chitosan can be ascribed to its amino and several hydroxyl groups, that protect against free radicals. [22]. Chitosan effectively chelates metal ions and scavenges free radicals [23]. After post-thawing, the CNPs concentration of 15 µg/ml was the most effective in maintaining sperm quality ($p < 0.05$). This finding is linked to the antioxidant properties of CNPs, the tendency to Increase in TAC and SOD, and a decrease in MDA levels are consistent with the hypothesis that this finding may be linked to the antioxidant qualities of CNPs. Moreover, the scavenging activity of CNPs appeared with increasing concentrations in semen samples up to (15) and started to decline in the higher doses up to 75 it may be due to a change in diluent osmolality which may affect the sperm membrane. Noteworthy, low CNPs supplementation (10µg/mL) in vitro maturation media (IVM) for river buffalo oocytes increased cumulus cell expansion, maturation rates, BCL2/BAX gene expression ratio, and SOD1 gene expression [24]. Our findings are consistent with the study which reported an improvement in semen parameters in goat bucks supplemented with chitosan in their diet [25]. This improvement may be attributed to chitosan's anti-inflammatory and antioxidant properties. Chitosan and its derivatives have been evaluated using a variety of antioxidant assays, including ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid), DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), FRAP (ferric antioxidant power) assays, peroxide and hydroxyl radical scavenging assays, and the use of macrophage models. [26].

The cryopreservation technique can potentially damage sperm, causing structural and functional changes, and ultimately affecting sperm quality and fertility [27].

Spermatozoa motility and viability are crucial quality-related factors that directly affect fertility.

Regarding sperm motility and membrane integrities, our study revealed that CNPs supplementation to the freezing extender improved sperm motility, and membrane integrity and reduced the acrosome defects where the (15 µg/ml) recorded the greatest effect, these ameliorating effects could be attributed to chitosan antioxidant properties [20]. In contrast to our results, [28] study showed that Chitosan-modified nanoparticles had a transient effect on the membrane integrity, but did not have any influence on cell viability, it may be attributed to the difference between the target cells of each experiment or the preparation method of chitosan nanoparticles.

For the comet assay's testing of the DNA integrity of ram spermatozoa in our study, no significant variation was observed between cryopreserved semen samples in diluent including or missing chitosan nanoparticles. Examination of the DNA integrity of ram spermatozoa revealed no discernible negative influence of chitosan nanoparticles on ram sperm DNA integrity. Even though the differences are not statistically significant, the comet assay parameters seem to be better for the first two chitosan nanoparticle concentrations (5 and 15 mg/ml) than for the higher concentrations. However, others reported a substantial positive impact of chitosan nanoparticles on sperm DNA integrity. The reported improvement in DNA integrity of Boer buck spermatozoa upon the addition of 1 ug/ml chitosan nanoparticle of green tea extract [29] Even though these advantages seem encouraging, the precise effects of chitosan nanoparticles on post-thawed ram semen can differ based on many factors, including concentration, processing method, and experimental setup. For cryopreservation techniques to completely comprehend and optimize the usage of chitosan nanoparticles for increasing the quality of post-thawed ram semen, more investigations are required especially its effect on the conception rate.

Conclusions

Higher quality post-thawing ram semen can be attained by supplementing the egg yolk tris diluent with 15 µg/mL of chitosan nanoparticles before cryopreservation. Optimization of the usage of chitosan nanoparticles for increasing the quality of post-thawed ram semen required more investigations especially its effects on the conception rate.

Authors' contributions:

Magdy Badr compiled ideas and designed the main framework for the research work. Ayat A. Elshamy & Ahmed Monir collected and assessed the ejaculate's quality. prepared the extender and freezing of extended semen, evaluated the post-thawed semen quality interpreted data and statistical analysis: Heba Hozyen Synthesis and characterization of chitosan nanoparticles and antioxidants assays. Mohamed Assi Determined the sperm ultrastructure and critically read and commented on the images. Eman Mohamed Abd El Fattah Assessed the sperm DNA damage using comet assay, all authors shared the writing process and revised the manuscript for intellectual content. Read and approved the final manuscript.

Ethics statement

This research protocol was approved by The Institutional Animal Care and Use Committee IACUC (ARC- ARRI 45-24) -Agricultural Research Center

Disclosure statement

No potential conflict of interest was reported by the authors

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TABLE 1. Effect of chitosan nanoparticles on ram sperm post-thawing CASA parameters:

	Control	5µg/mL	15µg/mL	25µg/mL	50µg/mL	75µg/mL
Tota Motility	58.14±1.90^{ac}	63.68±3.45^{ab}	67.65±3.56^{b}	60.87±2.13^{abc}	54.69±3.46^{c}	44.93±3.24^{d}
Progressive motility	57.77±1.87^{ac}	63.25±3.42^{ab}	67.34±3.52^{b}	60.57±2.13^{abc}	54.32±3.42^{c}	44.61±3.23^{d}
Rapid motility	27.30±1.76^{ad}	33.66±2.35^{bc}	37.34±2.51^{b}	32.57±1.78^{bc}	29.00±2.00^{ac}	22.01±1.67^{d}
DCL (µm)	23.89±2.96	28.11±1.67	30.09±3.35	25.98±0.97	23.83±0.95	20.04±0.65
DSL (µm)	8.85±1.46	10.75±0.63	13.82±2.49	10.77±1.35	9.07±0.81	7.21±0.55
DAP (µm)	11.28±1.64	13.54±0.81	16.14±2.35	13.11±1.21	11.28±0.68	9.26±0.46
VCL (µm/s)	78.17±8.41	95.46±6.23	89.54±11.45	85.79±2.01	77.21±9.15	66.86±4.28
VSL (µm/s)	32.16±4.17	40.03±3.10	42.39±3.94	38.14±2.99	31.69±3.42	27.31±0.60
VAP (µm/s)	38.75±4.58	47.78±3.45	48.40±4.66	44.52±2.42	37.70±4.13	32.82±0.82
Radius(µm)	2.55±0.40	3.09±0.19	3.85±0.63	3.06±0.34	2.59±0.20	2.05±0.10
Rotation	0.29±0.03^{abd}	0.36±0.02^{bc}	0.39±0.03^{c}	0.33±0.02^{bc}	0.29±0.03^{abd}	0.25±0.01^{d}
BCF(Hz)	15.83±1.35	18.18±0.21	21.45±1.36	17.99±1.45	16.81±1.36	15.38±0.68
ALH (µm)	0.79±0.06	0.88±0.05	0.80±0.09	0.74±0.03	0.79±0.07	0.64±0.05
HAC	0.20±0.01	0.24±0.01	0.22±0.03	0.22±0.01	0.20±0.02	0.18±0.02
STR	0.83±0.01	0.84±0.01	0.88±0.02	0.85±0.02	0.84±0.02	0.83±0.01
LIN	0.41±0.02	0.42±0.01	0.48±0.03	0.45±0.03	0.42±0.02	0.41±0.03
WOB	0.50±0.01	0.50±0.01	0.55±0.02	0.52±0.03	0.49±0.02	0.49±0.02

Data expressed as Mean ± SEM

Superscript letters (a, b, c &d) indicate significant differences ($p < 0.05$) within the same row.

ALH, amplitude of lateral head displacement; BCF, Beat cross frequency DAP, length of average path; DCL, length of the curvilinear path; DSL, length of straight path; LIN, Linearity STR, Straightness, VAP, average path velocity; VCL, curvilinear velocity; VSL, straight line velocity.

Table 2. Effect of chitosan nanoparticles on ram sperm post-thawing DNA integrity

Group	%DNA in Head	%DNA in Tail	Tail Moment	Olive Moment
control	96.23±0.54	3.78±0.54	4.40±0 .90	6.05±1.10
5µg/ml	97.02±0.47	2.98±0.47	3.48± 1.19	4.93±1.13
15µg/ml	97.11±0.38	2.89±0.38	3.05±0.80	5.27±0.82
25µg/ml	96.74±0.49	3.26± 0.49	4.30± 0.82	6.10± 1.00
50µg/ml	96.17±0.58	3.83±0.59	5.55±1.42	7.17±1.28
75µg/ml	96.21±0.58	3.79±0.58	5.22±1.65	6.53±1.34

Data expressed as Mean ± SEM

No significant difference among groups was observed. ($p \geq 0.05$).

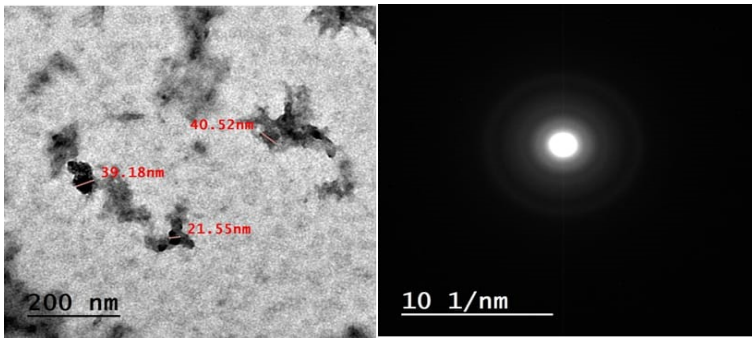

Fig. 1. HR-TEM micrograph of Chitosan nanoparticles.

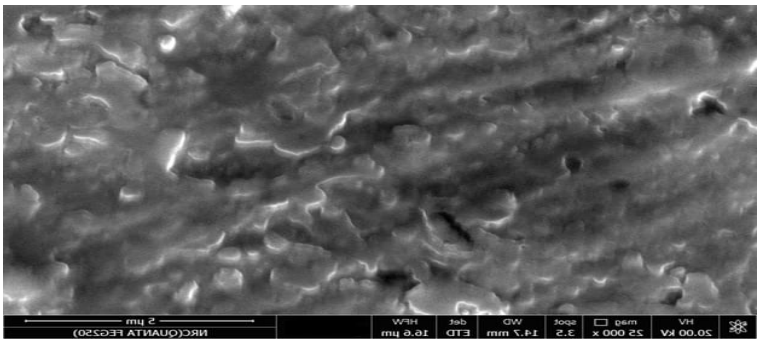

Fig.2. SEM micrograph of chitosan nanoparticles.

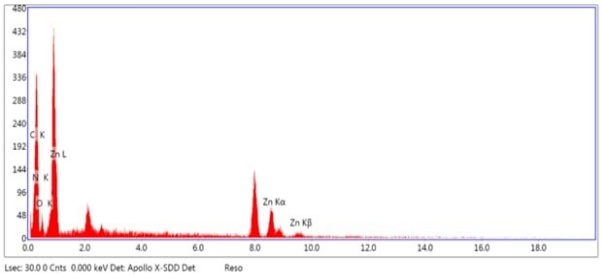

Fig.3. SEM-EDX elemental composition analysis of chitosan nanoparticles.

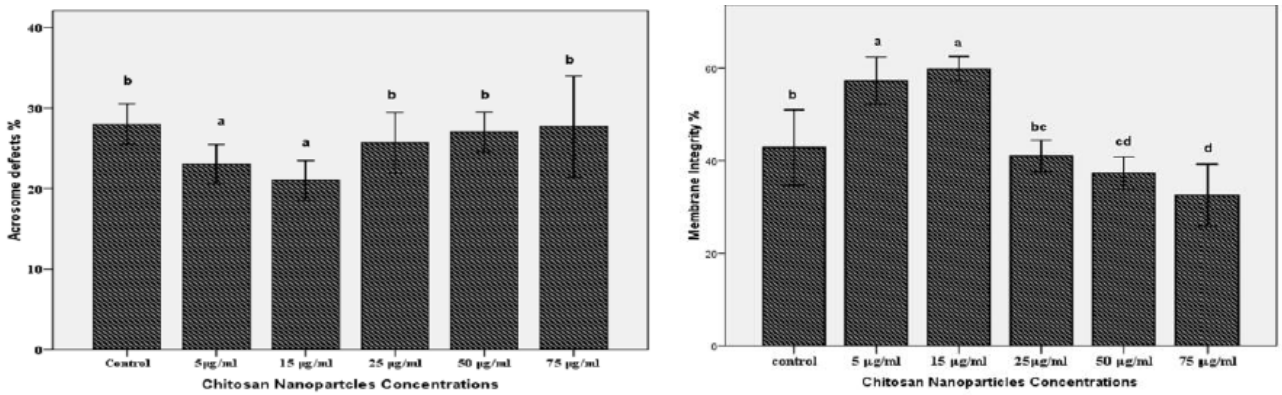

Fig. 4. Post-thawing membrane integrity% and acrosome defects%

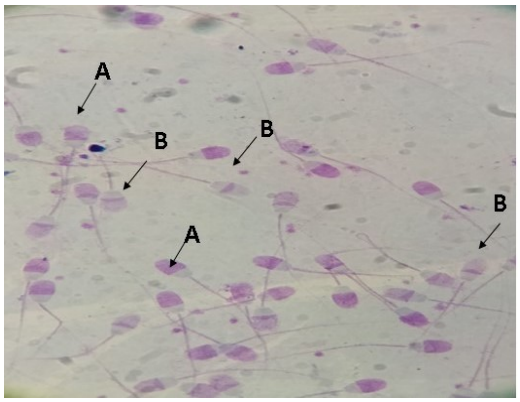

Fig.5. Post-thawed ram sperm stained with Giemsa stain (A) sperm with intact acrosome, (B) sperm with detached acrosome.

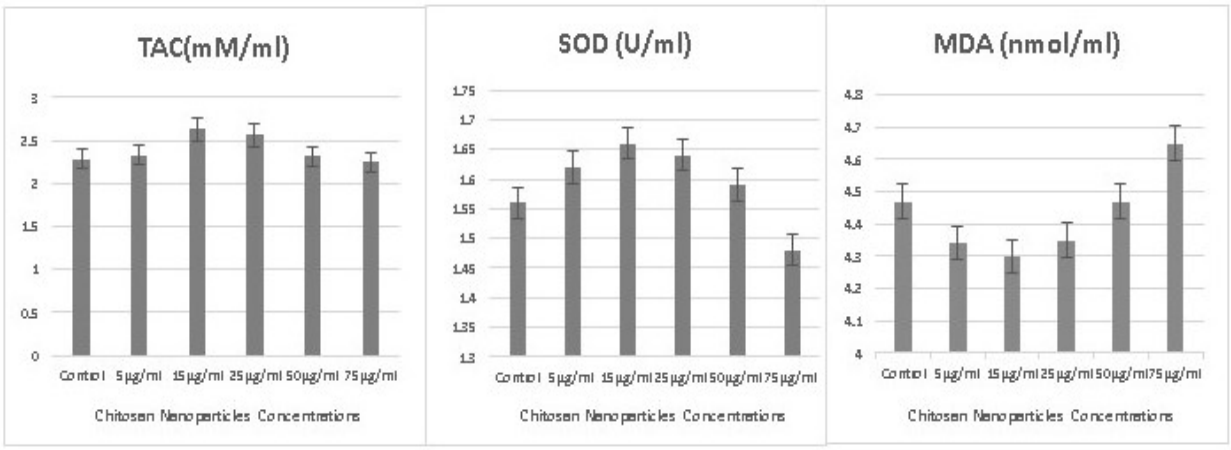

Fig.6. Effect of chitosan nanoparticles on ram sperm post-thawing total antioxidant capacity (TAC) , Super oxide dismutase (SOD) activity, and malondialdehyde (MDA) level.

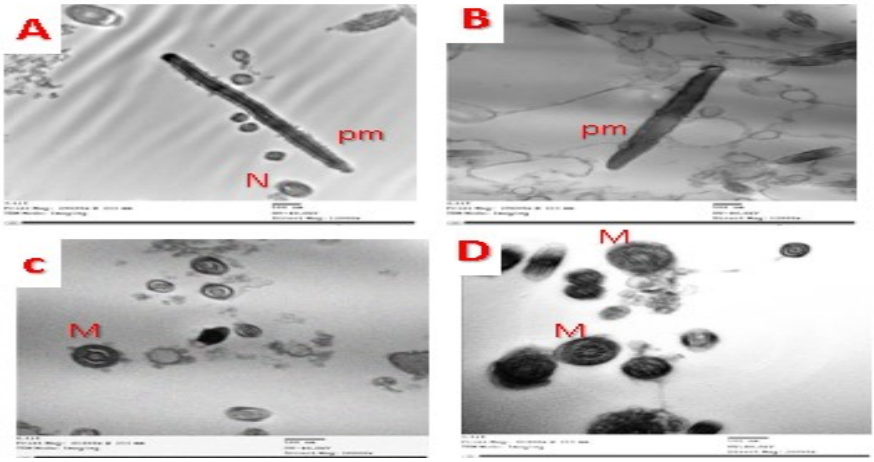

Fig.7. An electron micrograph from a post-thawed ram sperm from the control group:

A&B: Sagittal section in the sperm head illustrates swollen, degenerated and fenestrated plasma membrane (PM) also swollen acrosome and non-homogenized nucleus is recognized (N) (X12000).

C&D: The mid-piece region showing different degrees of mitochondrial degeneration (M) also loss and damaged of the transverse cristae can be noticed (X20000).

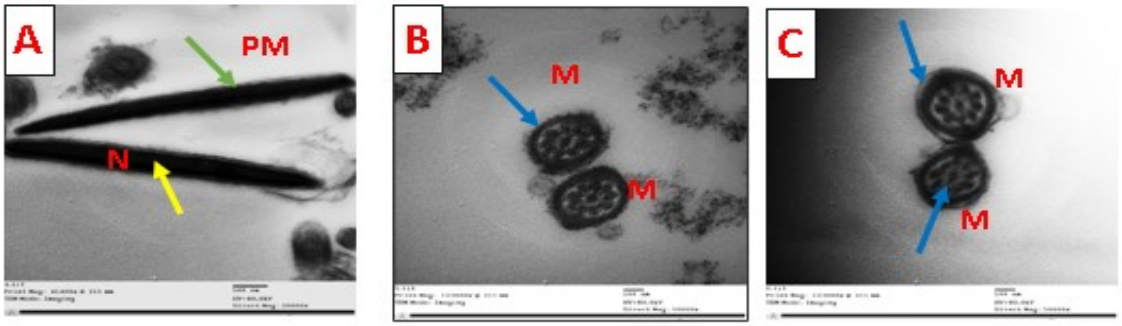

Fig. 8. Electron micrograph of a sagittal- section through the head region from post-thawed ram semen treated with chitosan nanoparticles (15 g/ml) illustrates
A: Intact plasma membrane (PM) and nuclear content (N). in the electron density (X20000)
B&C: Cross-section through the mid-piece region illustrates the normal mitochondrial organization and good mitochondrial dense electron space moreover, the transverse cristae appeared intact (X50000).

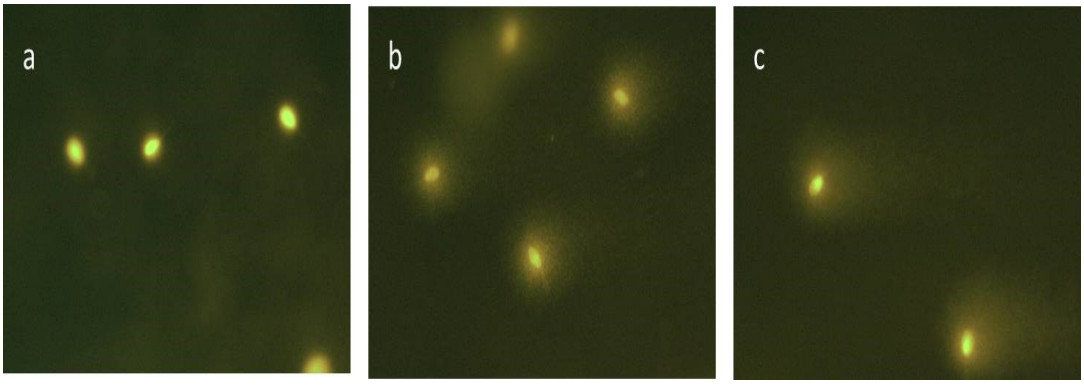

Fig.9. Shows the effect of chitosan nanoparticles addition to the semen diluent on the DNA integrity of frozen / thawed ram spermatozoa using comet assay, representative images for ram sperm nucleoid stained with ethidium bromide visualized by Epifluorescent microscope with 40 X objectives: (a) normal nucleoid, (b, c) DNA damaged nucleoid.

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تأثير جزيئات الشيتوزان النانوية على خصائص الحيوانات المنوية للكباش، ونشاط مضادات الأكسدة، والتركيب الدقيق للحيوانات المنوية، وسلامة الحمض النووي بعد حفظ السائل المنوي بالتجميد

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الملخص

تهدف الدراسة تقييم الدور الوقائي المحتمل لجزيئات الشيتوزان النانوية على السائل المنوي للكباش بعد عملية حفظه بالتجميد باستخدام مخفف التريس مع صفار البيض. تم تخليق ثم توصيف جزيئات الشيتوزان النانوية عن طريق المجهر الإلكتروني الماسح (SEM) والمجهر الإلكتروني النافذ (TEM). تم جمع قذفات السائل المنوي عن طريق المهبل الاصطناعي من خمسة كباش معلومة الخصوبة. تم خلط الجمعات عالية الجودة فقط لاستخدمها فى التجربة ثم تخفيفها بمخفف تريس صفار البيض المدعم بتركيزات مختلفة من جزيئات الشيتوزان النانوية بالتركيزات التالية (0، 5، 15، 25، 50، 75 ميكروجرام/مل). بعد التبريد عند 4 درجات مئوية خلال 90 دقيقة، تم تجميد قصيبات السائل المنوي على بخار النيتروجين السائل لمدة 15 دقيقة وتخزينه حتى التقييم. تم فحص العينات بعد الاسالة عن طريق تحليل حركة الحيوانات المنوية باستخدام جهاز تحليل السائل المنوى (CASA, Androvision®) لتسجيل مؤشرات حركة الحيوانات المنوية. بالإضافة إلى فحص تشوهات القلنسوة وسلامة الغشاءالبلازمى. تم تسجيل نشاط مضادات الأكسدة وكذلك التركيب الدقيق للحيوانات المنوية وسلامة الحمض النووي. وقد اوضحت نتائج الدراسة حدوث تحسن معنوى لكل من مؤشرات الحركة الكلية و الحركة السريعة و الحركة التقدمية الامامية، بالاضافة الى حفظ سلامة غشاء القلنسوة و الغشاء البلازمى للحيوانات المنوية بعد إضافة (15 ميكروغرام / مل) من جزيئات الشيتوزان النانوية . وكان هناك زيادة غير معنوية فى نشاط مضادات الأكسدة مع انخفاض غير معنوى فى مستويات بيروكسيدات الدهون وعدم وجود تأثير سمى على التركيب الدقيق والحمض النووي. يمكن أن نستنتج أن جزيئات الشيتوزان النانوية المرتبطة بكبريتات الدكستران التي تم توليفها كانت قادرة على حماية الحيوانات المنوية المسالة بعد تجمدها بنجاح عن طريق حميتها من التلف الناتج عن عملية التجميد.

الكلمات الدالة: جزيئات الشيتوزان النانوية، الحفظ باتجميد، الإجهاد التأكسدي، التركيب الدقيق للحيامن ، الحمض النووي، الكباش.