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# Influence of Selenium Nanoparticles Synthesized by Co-precipitation Method on Oxidative Stress and Semen Quality of Chilled Rabbit Semen

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CHILLING process has negative impact on sperm function and quality attributes resulting in some modifications at the biochemical and structural levels. Selenium nanoparticles (SeNPs) supplementation could stop or even reverse this impact and improve the quality of cooled rabbit. The present study was designed to evaluate the effects of adding five different concentrations of SeNPs (0.5, 1, 1.5, 2 and 2.5 µg/ml) to protect rabbit spermatozoa from chilling adverse effects. X-ray diffraction (XRD), high resolution transmission electron microscopy (HR-TEM), and scanning electron microscopy (SEM) were used to characterize SeNPs synthesized by co-precipitation method. Semen was collected from ten sexually mature and fertile New Zealand White male rabbits using artificial vagina and pooled semen samples were diluted with tris-egg yolk-fructose extender only (control) or extender supplemented with 0.5, 1, 1.5, 2 and 2.5 µg/ml of SeNPs. Sperm motility, membrane integrity and acrosome defects were measured. To determine antioxidative role of SeNPs, malondialdehyde (MDA) level as a biomarker of lipid peroxidation was measured in seminal plasma. Sperm motility and membrane integrity increased with SeNPs at concentrations 0.5 µg/ml. Likewise, no adverse effect was reported on percentages of a live sperm as well as sperm morphology with all concentrations. MDA levels decreased with concentration 0.5 µg/ml SeNPs. Notably, adding 0.5, 1 and 2 µg/ml SeNPs to extender produced best significant positive effects on semen parameters compared to SeNPs free group. In conclusion, the results confirm that low concentrations of SeNPs fabricated by co-precipitation method could effectively preserve the quality of chilled rabbit semen.

**Keywords:** Semen chilling, Selenium nanoparticles, Oxidative stress, Semen quality, Rabbits.

## Introduction

The use of artificial insemination (AI) in rabbit business has attracted interest on a global scale as a result of substantial advancements made in both productive and financial aspects of rabbit industry [1]. In addition, the demand for stored semen to be used in AI programs of livestock animals [2] and rabbit [3] is increasing. Rabbit semen preservation is one of the main problems for a wide use of AI in rabbitries [4]. Although, rabbit spermatozoa had a minimum capacity to chilling [5] or storage, the freezing technique is more expensive and need more equipment than with chilling technique. During semen preservation processes, sperm function is affected by a number of stress factors, including temperature reduction, increased osmolality, ice formation, and increased oxidative stress [6]. It is recognized that sperms are mainly vulnerable to damage caused by oxidative stress because it is rich in polyunsaturated fatty acids that readily undergo peroxidation, alter redox signaling, making secondary products that can oxidize sperm lipids and proteins [7-8]. It has been reported in several animal species that adding antioxidants to preservation media could prevent oxidative stress and maintain membrane integrity while semen is being stored [9-11]. Accordingly, addition of antioxidants to semen extenders has been applied for sperm preservation to reduce the possible effects of oxidative stress on sperm motility, viability and lipid peroxidation

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during storage process [12]. Selenium (Se) is a necessary trace nutrient for growth and development of humans and animals and also it serves as an essential antioxidant in animal reproduction [13]. Se compromises a necessary part of glutathione peroxidase which is an enzyme responsible for protection of internal structures of the cell from free radicals and subsequently it is considered an excellent antioxidant for lipids of the cellular membrane [14]. With the development of nanotechnology, the elemental nano-Se powder exhibits novel properties different from other Se sources. nano-Se has attracted wide spread attention due to its high bioavailability and low toxicity as nanometer particulates exhibit novel characteristics, such as great specific surface area, high surface activity, a lot of surface-active centers, high catalytic efficiency and strong adsorbing ability [15]. Due to reported low toxicity and high bioavailability as well as antioxidative properties of SeNPs, we hypothesized that the use of Se at nanoscale could protect sperms in a rabbit model from the harmful effects of the chilling process. Therefore, the goal of the current study was to fabricate SeNPs by simple co-precipitation method. Additionally, to investigate the impact of modifying semen extender by using SeNPs in five different concentrations (0.5, 1, 1.5, 2 and 2.5 g/ml) to shield ram spermatozoa from chilling damage.

## Material and Methods

### *Ethical approval*

The Medical Research Ethics Committee (MREC) (Approval Number: 19144) of the National Research Centre - Egypt approved all animal-related procedures and they were all carried out in line with the Guide for the Care and Use of Laboratory Animals (National Research Council [U.S.], 2011.

### *Fabrication and characterization of SeNPs*

$Na_2SeO_3$ (Loba Chemie, India), 2-Mercapto ethanol $C_2H_6OS$ (Carl Roth GmbH, catalog number: 4227), Without additional purification, sodium hydroxide pellets (Merck) and deionized water were utilized as usual. SeNPs were created using the chemical co-precipitation method [16]. XRD patterns were recorded on a PANalytical (Empyrean) X-ray diffraction using Cu Kα1 radiation (wavelength 1.5406 Å) at an accelerating voltage of 40 kV, current of 30 mA, scan angle 5–80° range and scan step 0.02°. Microstructure and morphology were investigated using SEM (QUANTA FEG 250) and HR-TEM (JEOL-JEM2100, Japan).

### *Animals' management and semen collection*

For the purposes of this investigation, ten New Zealand white male rabbits sexually mature and fertile were taken from the same herd on a commercial farm. The initial weight of the 26- to 30-week-old rabbits ranged from 2.3 to 2.9 kilogram. Bucks were kept separately in metal wire mesh cages that had their own food and watering supply. Before collecting ejaculates using an artificial vagina (IMV, France) that had been heated to 40–42° C, bucks were trained to mount teaser female. Semen was gathered once every week. Each ejaculate's initial semen quality was evaluated, and only those that were white milky and contained more than 70% motile spermatozoa were used in the study.

### *Experimental design*

Selected ejaculates were pooled right after semen collection to eliminate individual variations and to get enough volume for each treatment. According to Hozyen *et al.* [17], with a few modifications, five concentrations of SeNPs 0.5, 1.0, 1.5, 2.0, and 2.5 g/ml were used in a Tris-based extender. Chilling was performed for 48 hours at 10° C.

### *Lipid peroxidation*

The principle is based on the reaction of thiobarbituric acid with the malondialdehyde (MDA) in acidic medium at catalytic temperature 95°C for 30 minutes. A pink reactive product is measured at 534 nm [21].

### *Semen quality evaluation*

Incubation at 10° C for 48 h were performed for diluted semen samples. Only forward motility was examined after 2, 24 and 48 hours, while sperm viability, sperm morphology, acrosome and sperm membrane integrities were assessed after 2, and 48 h post-chilling.

### *Sperm motility*

A drop of semen was thrown down onto a slide that had been warmed to 37° C and covered with a cover slip. Using a phase contrast hot stage microscope with a heating plate (37° C) and a magnification of 400 ×, the subjective motility of sperm was measured.

### *Sperm morphology and viability*

A stained smear was created as soon after ejaculation using a 1:4 dilution rate of an eosin nigrosine staining combination [18].

### *Sperm membrane integrity: Hypo-osmotic swelling test (HOST)*

HOST test assesses the spermatozoa membrane's functional integrity. Plasma membrane is considered intact when spermatozoa is swollen with coiling tail [19].

### *Acrosome integrity*

Giemsa was utilized in the current investigation to stain the acrosome a deep purple colour. The following staining method [20] was used:

- The ratio of fresh ejaculate to warm normal saline was 1:5.
- Smearing and air-drying of diluted semen.

- Fixation of smear for 15 minutes in 10% neutral formal saline.
- Fixed smear washing for 20 minutes under running water.
- Overnight, the fixed smear was submerged in the Giemsa working solution.
- Rinsing of stained smear in two changes of distilled water and air-drying.
- In each stained smear, the acrosome integrity of 100 spermatozoa per sample was assessed at a 1000-fold magnification.

### *Statistical analysis*

The analysis of variance (ANOVA) was calculated for the various parameters comparing the control and additive replications using the SAS computerized program version 9.2 [22]. Significant difference between means was calculated using Duncan multiple range test at $P<0.05$.

## Results

### *Characterization of selenium nanoparticles:*

XRD patterns of produced selenium nanoparticles are displayed in Figure (1). The standard JCPDS data (JCPDS card No. 01-086-2246) and all of the diffraction peaks in the 2θ range are in excellent agreement. The diffraction peaks present at 2θ (degrees) of 23.57°, 29.73°, 41.28°, 43.68° and 45.43° and 71.60° corresponds to (100), (101), (110), (012) and (111) planes of selenium. SeNPs micrographs of HR-TEM and SEM are presented in Figures (2) and (3), respectively. Successful synthesis of spherical shape SeNPs with an average diameter of 48 nm is confirmed by the micrographs.

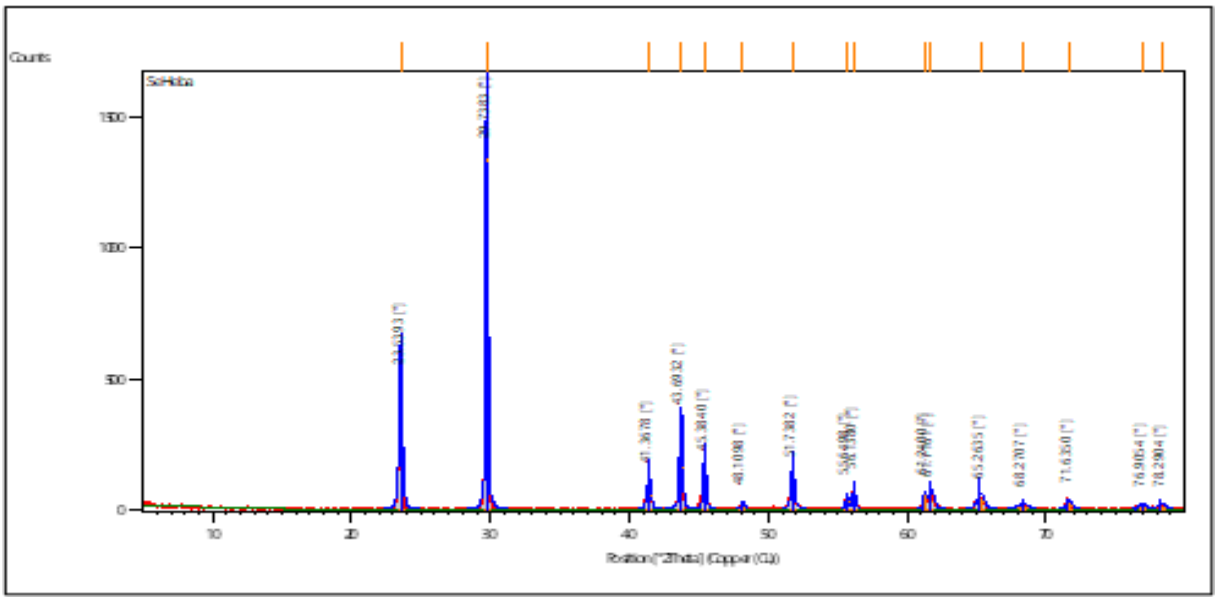


**Fig. 1. XRD patterns of fabricated selenium nanoparticles.**

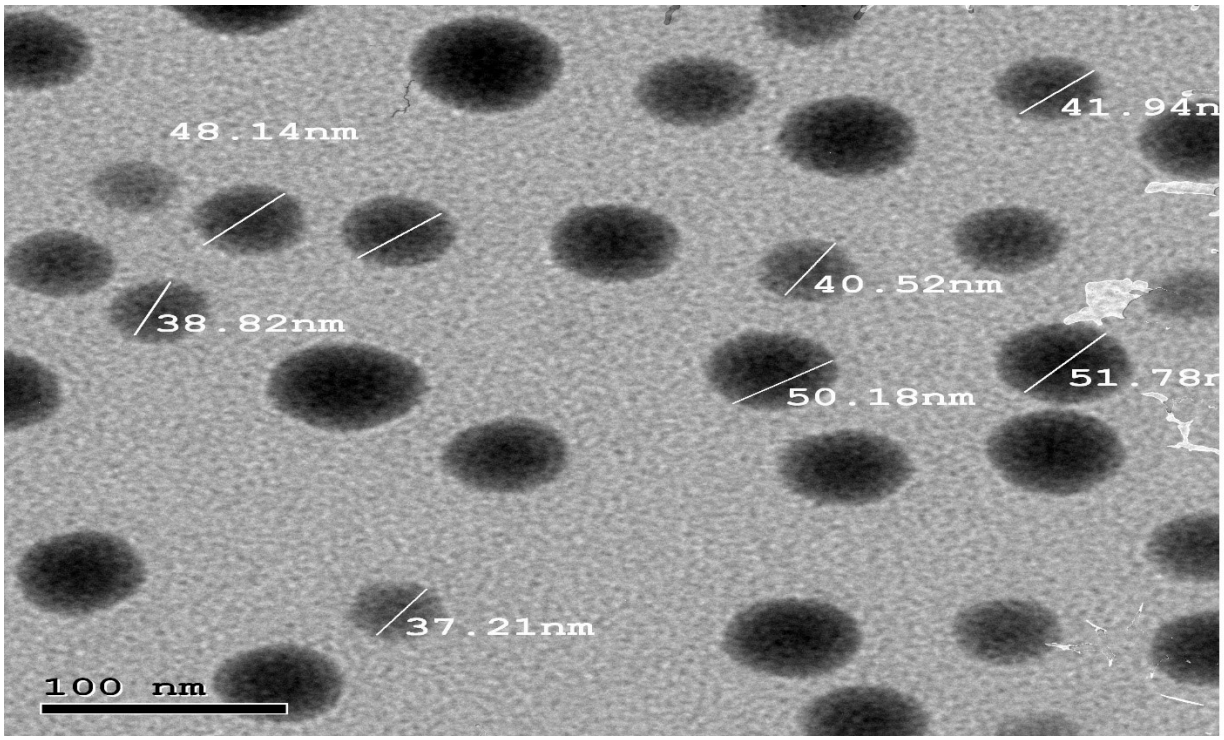


**Fig. 2. High resolution transmission electron microscope micrograph of fabricated selenium nanoparticles.**

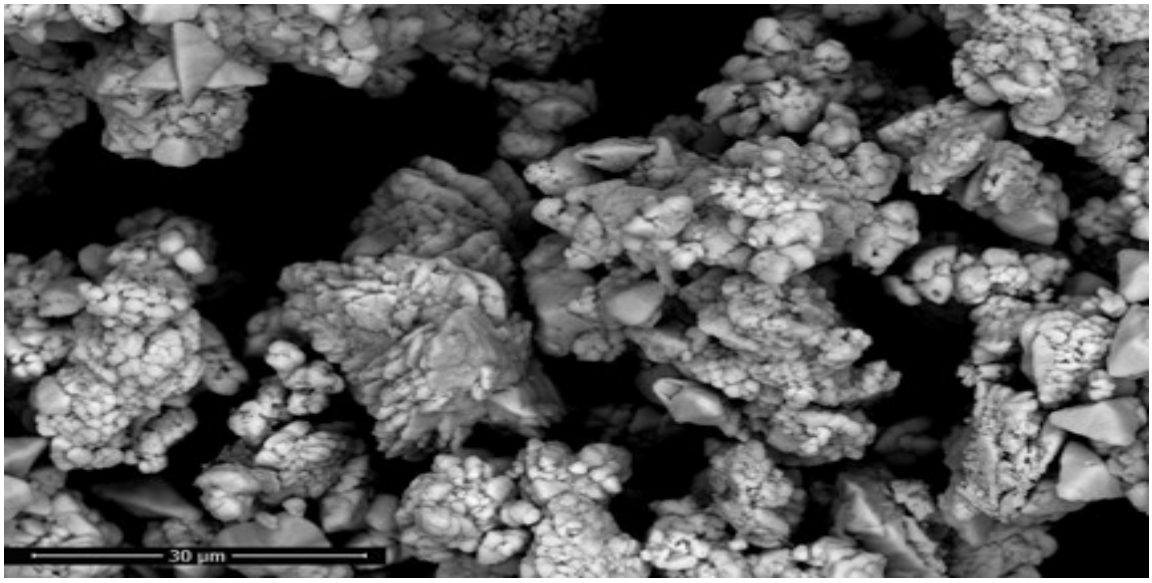


**Fig. 3. Scanning transmission electron microscope micrograph of fabricated selenium nanoparticles.**

***Effect of nano-selenium different concentrations on lipid peroxidation in chilled rabbit semen***

Concerning the effect of nano Se on lipid peroxidation, data output in Table (1), showed that the concentration of 2.5 µg/ml revealed the highest lipid peroxidation of the sperm membrane while the lowest lipid peroxidation was at 0.5 µg/ml. On the other hand, the time also had an effect on the lipid peroxidation which was higher after 48 hours than 2 hours.

**TABLE 1. Effect of nano-selenium different concentrations on lipid peroxidation in chilled rabbit semen**

| SeNPs (µg/ml) | Time (hours) | | Overall mean | F-cal | Sign |
|---|---|---|---|---|---|
| | 2 | 48 | | | |
| **Control** | 198.48 ± 5.41[1] | 212.42 ± 1.83[1] | 205.45[a] | | |
| **0.5** | 182.12 ± 2.21[1] | 193.03 ± 5.72[1] | 187.58[b] | | |
| **1.0** | 198.18 ± 3.37[1] | 201.82 ± 8.65[1] | 200.00[ab] | 2.52 | 0.0392 |
| **1.5** | 197.27 ± 7.28[1] | 203.64 ± 11.00[1] | 200.46[ab] | | |
| **2.0** | 190.61 ± 4.46[1] | 221.51 ± 1.57[1] | 206.06[a] | | |
| **2.5** | 202.42 ± 11.38[1] | 218.18 ± 10.55[1] | 210.30[a] | | |
| **Overall mean** | 194.85[B] | 208.43[A] | | Time x concentration = 0.94 | |
| **F-cal** | 11.20 | | | Sign = P<0.4640 | |
| **Sign** | 0.0014 | | | | |

- Using the Duncan multiple range test, different superscripts (A, B) represent the difference between the time overall averages (P<0.05).
- Using the Duncan multiple range test, different superscripts (a, b, etc.) represent the difference between the concentration overall averages (P<0.05).

***Effect of nano-selenium different concentrations on the chilled rabbit sperm motility %***

Data output from Table (2), concerning the sperm motility %, showed that nano Se at a concentration of 0.5 µg/ml revealed the highest significant (P<0.0001) overall mean compared to the control and other nano Se concentrations. Meanwhile, time has its regressive significant (P<0.0001) effect on sperm motility after 2, 24 and 48 hours of cooling incubation. After 2 hours, the overall mean motility was significantly (P<0.0001) higher than after 24 and 48 hours. The time x concentration interaction showed that there was no significance between means within cells after 2 hours of cooling storage, while after 24 and 48 hours, the nano Se concentration of 0.5 µg/ml was the highest significant (P<0.0001) motility % compared to the other motility % within 24-hour column and 48-hour column.

**TABLE 2. Effect of nano-selenium different concentrations on the chilled rabbit sperm motility %**

| SeNPs (µg/ml) | Time (hours) | | | Overall mean | F-cal | Sign |
|---|---|---|---|---|---|---|
| | 2 | 24 | 48 | | | |
| **Control** | 88.33 ± 1.67[1] | 50.00 ± 2.65[2,3] | 30.00 ± 2.522,3 | 56.11b[c] | | |
| **0.5** | 85.00 ± 2.89[1] | 72.33 ± 1.45[1] | 51.67 ± 1.671 | 69.67[a] | | |
| **1.0** | 85.00 ± 2.891 | 45.00 ± 2.893 | 28.33 ± 1.672,3,4 | 52.78[c] | 32.55 | 0.0001 |
| **1.5** | 85.00 ± 2.891 | 55.00 ± 2.892 | 35.00 ± 2.892 | 58.33[b] | | |
| **2.0** | 75.00 ± 2.891 | 45.00 ± 2.893 | 25.00 ± 1.733,4 | 48.33[d] | | |
| **2.5** | 85.00 ± 2.891 | 36.67 ± 2.034 | 21.67 ± 1.674 | 47.78[d] | | |
| **Overall mean** | 83.89A | 50.67B | 31.94C | | Time x concentration = 7.27 | |
| **F-cal** | 687.83 | | | | | |
| **Sign** | 0.0001 | | | | Sign = P<0.0001 | |

- Using the Duncan multiple range test, different superscripts (A, B) represent the difference between the time overall averages (P<0.05).
- Using the Duncan multiple range test, different superscripts (a, b, etc.) represent the difference between the concentration overall averages (P<0.05).

***Effect of nano-selenium different concentrations on the chilled rabbit alive sperm %***

Data output in Table (3), concerning alive sperm%, showed that there were no significant differences between concentration overall means, time overall means and the time x concentration interaction alive sperm % means.

***Effect of nano-selenium different concentrations on the chilled rabbit sperm morphology***

The results in Table (4), showed significant difference only concerning time effect (P<0.0013). The overall means after 48 hours showed more abnormal sperms defects than after 2 hours.

***Effect of nano-selenium different concentrations on the chilled rabbit sperm acrosome***

Table (5) output showed that time effect had a significant (P<0.0026) difference between overall means after 2 and 48 hours. Sperm acrosome integrity was higher after 2 hours than after 48 hours. Meanwhile, time × concentration interaction showed significant (P<0.0150) difference between means within row but not in column.

***Effect of nano-selenium different concentrations on the chilled rabbit sperm hypo-osmotic swelling***

Table (6) data output showed that the overall mean of the concentration 0.5 µg/ml nano Se was the highest significant (P<0.0253) sperm membrane integrity compared the control, 1 and 1.5 µg/ml nano Se. While, the time effect has its significant (P<0.0001) shadows on the difference between overall means of sperm membrane integrity after 2 hours and 48 hours.

**TABLE 3. Effect of nano-selenium different concentrations on the chilled rabbit alive sperm %**

| SeNPs (µg/ml) | Time (hours) | | Overall mean | F-cal | Sign |
|---|---|---|---|---|---|
| | 2 | 48 | | | |
| **Control** | $89.33 \pm 1.33^{1}$ | $89.33 \pm 2.91^{1}$ | $89.33^{a}$ | | |
| **0.5** | $89.00 \pm 2.08^{1}$ | $86.00 \pm 3.06^{1}$ | $87.50^{a}$ | | |
| **1.0** | $90.67 \pm 0.67^{1}$ | $88.67 \pm 0.67^{1}$ | $89.67^{a}$ | 1.34 | 0.2810 |
| **1.5** | $90.00 \pm 0.67^{1}$ | $89.33 \pm 0.67^{1}$ | $90.00^{a}$ | | |
| **2.0** | $88.00 \pm 1.15^{1}$ | $86.00 \pm 1.15^{1}$ | $87.00^{a}$ | | |
| **2.5** | $87.33 \pm 1.33^{1}$ | $87.33 \pm 1.33^{1}$ | $87.33^{a}$ | | |
| **Overall mean** | $89.17^{A}$ | $87.78^{A}$ | | Time x concentration = 0.27 | |
| | | | | Sign = P<0.9245 | |
| **F-cal** | 2.18 | | | | |
| **Sign** | 0.1530 | | | | |

- Using the Duncan multiple range test, different superscripts (A, B) represent the difference between the time overall averages (P<0.05).
- Using the Duncan multiple range test, different superscripts (a, b, etc.) represent the difference between the concentration overall averages (P<0.05).

**TABLE 4. Effect of nano-selenium different concentrations on the chilled rabbit sperm morphology**

| SeNPs (µg/ml) | Time (hours) | | Overall mean | F-cal | Sign |
|---|---|---|---|---|---|
| | 2 | 48 | | | |
| **Control** | $16.00 \pm 1.53^{1}$ | $21.00 \pm 2.08^{1}$ | $18.50^{a}$ | | |
| **0.5** | $15.33 \pm 1.45^{1}$ | $18.67 \pm 0.67^{1}$ | $17.00^{a}$ | | |
| **1.0** | $16.33 \pm 0.88^{1}$ | $18.67 \pm 0.67^{1}$ | $17.50^{a}$ | 1.82 | 0.1468 |
| **1.5** | $16.00 \pm 1.15^{1}$ | $18.00 \pm 1.15^{1}$ | $17.00^{a}$ | | |
| **2.0** | $18.00 \pm 0.00^{1}$ | $20.00 \pm 0.00^{1}$ | $19.00^{a}$ | | |
| **2.5** | $19.33 \pm 1.76^{1}$ | $20.67 \pm 1.76^{1}$ | $20.00^{a}$ | | |
| **Overall mean** | $16.83^{B}$ | $19.50^{A}$ | | Time x concentration = 0.54 | |
| | | | | Sign = P<0.7456 | |
| **F-cal** | 13.24 | | | | |
| **Sign** | 0.0013 | | | | |

- Using the Duncan multiple range test, different superscripts (A, B) represent the difference between the time overall averages (P<0.05).
- Using the Duncan multiple range test, different superscripts (a, b, etc.) represent the difference between the concentration overall averages (P<0.05).

**TABLE 5. Effect of nano-selenium different concentrations on the chilled rabbit sperm acrosome**

| SeNPs (µg/ml) | Time (hours) | | Overall mean | F-cal | Sign |
|---|---|---|---|---|---|
| | 2 | 48 | | | |
| **Control** | 93.33 ± 1.67[1] | 68.00 ± 6.24[1] | 80.67[a] | | |
| **0.5** | 84.67 ± 3.71[1] | 82.00 ± 3.06[1] | 83.33[a] | | |
| **1.0** | 83.33 ± 2.40[1] | 80.67 ± 2.91[1] | 82.00[a] | 0.35 | 0.8757 |
| **1.5** | 82.67 ± 2.40[1] | 80.00 ± 4.16[1] | 81.33[a] | | |
| **2.0** | 81.33 ± 3.71[1] | 77.33 ± 3.71[1] | 79.33[a] | | |
| **2.5** | 81.33 ± 2.40[1] | 78.67 ± 2.40[1] | 80.00[a] | | |
| **Overall mean** | 84.44[A] | 77.78[B] | | Time x concentration = 3.56 | |
| | | | | Sign = P<0.0150 | |
| **F-cal** | 11.32 | | | | |
| **Sign** | 0.0026 | | | | |

- Using the Duncan multiple range test, different superscripts (A, B) represent the difference between the time overall averages (P<0.05).
- Using the Duncan multiple range test, different superscripts (a, b, etc.) represent the difference between the concentration overall averages (P<0.05).

**TABLE 6. Effect of nano-selenium different concentrations on the chilled rabbit sperm hypo-osmotic swelling**

| SeNPs (µg/ml) | Time (hours) | | Overall mean | F-cal | Sign |
|---|---|---|---|---|---|
| | 2 | 48 | | | |
| **Control** | 65.00 ± 5.00[1] | 60.00 ± 5.29[1] | 62.50[b] | | |
| **0.5** | 84.00 ± 3.06[1] | 64.00 ± 3.06[1] | 74.00[a] | | |
| **1.0** | 72.00 ± 4.00[1] | 49.00 ± 4.73[1] | 60.50[b] | 3.15 | 0.0253 |
| **1.5** | 76.00 ± 5.77[1] | 44.67 ± 2.84[1] | 60.33[b] | | |
| **2.0** | 74.67 ± 4.81[1] | 56.00 ± 3.06[1] | 65.33[ab] | | |
| **2.5** | 77.33 ± 3.71[1] | 56.67 ± 2.40[1] | 67.00[ab] | | |
| **Overall mean** | 74.83[A] | 55.06[B] | | Time x concentration = 2.15 | |
| | | | | Sign = P<0.0939 | |
| **F-cal** | 69.22 | | | | |
| **Sign** | 0.0001 | | | | |

- Using the Duncan multiple range test, different superscripts (A, B) represent the difference between the time overall averages (P<0.05).
- Using the Duncan multiple range test, different superscripts (a, b, etc.) represent the difference between the concentration overall averages (P<0.05).

## Discussion

To enhance sperm quality during preservation, an appropriate extender and cryoprotectant are essential [23]. Therefore, recent research has looked into several kinds of nanoparticles (NPs) as reactive oxidant species (ROS) scavengers to preserve animal sperm cell viability during cooling processes [24]. Selenium nanostructures has attracted the interest of many researchers owing to their low toxicity as well as their higher bioactivity [25]. The present study was designed to evaluate the role of nano-protectant additives in extended rabbit semen using different concentrations (0, 0.5, 1, 1.5, 2 and 2.5 µg/ml) of SeNPs. According to Dumore and Mukhopadhyay [26], synthesis conditions including selenium precursor, reductant and temperature could potentially affect both shape and size of synthesized nanoparticles. Successful fabrication of SeNPs by co-precipitation method was achieved in the current work via reduction of sodium selenite with mercaptoethanol at room temperature. According to Albanese et al. [27], materials at the nanoscale (10−9 – 10−7m) are those with at least one dimension generally ranging between 1 and 100 nm. In accordance with our results, Li *et al.* [28] (2010) confirmed the successful preparation of selenium nano structures employing co-precipitation method.

XRD pattern of fabricated selenium nanoparticles showing no other diffraction peaks of impurities indicates production of highly crystalline and pure nano Se [29]. Importantly, physical properties and antioxidant properties of formerly synthesized SeNPs are affected by clean-up procedure. Commonly, nanoparticles are isolated and purified from surrounding liquid by centrifugation at different speeds followed by washing with water [30]. Morphology and size of synthesized nano selenium as characterized by TEM and SEM indicated dispersed spherical shape SeNPs with an average diameter of about 48 nm.

SeNPs are highly biologically active [31] and have been reported to have antioxidant activity against free radicals [32]. Spermatozoa are exposed to different types of stress during cooling process [33]. In the current work, MDA levels were significantly reduced by 0.5 g/ml nano Se when compared to the control group. The fact that Se supplementation improves storage and reduces the release of lipids from the sperm cell during prolonged storage emphasizes the importance of Se [13]. Moreover, Gao et al. [16] has reported the anti-hydroxyl radicals' properties of selenium nanoparticles. ROS are potent stimulators of lipid peroxidation and since it is impossible to measure free radicals directly in vivo, it is necessary to rely on the quantification of their reaction products such as lipid peroxidation products. Prime targets of free radicals are polyunsaturated fatty acids (PUFA) in cell membrane leading to a chain of chemical reactions called lipid peroxidation. Fatty acid breakdown results in the formation of various oxidatively modified products which are toxic to cells and finally converted into stable end products [34]. When a free radical attacks PUFA, the PUFA undergoes oxidation and create a fatty acid radical that quickly combines oxygen to produce a fatty acid peroxyl radical which is the catalyst for a chain reaction. Additional PUFA molecules could be oxidized by free radicals to create lipid hydroperoxide that could then decompose into yet more reactive radical species including lipid peroxyl, lipid alkoxyl and aldehydes like MDA. Lipid peroxidation is the term for this process of oxidative destruction of PUFAs and the metabolic byproducts it produces can either directly destroy membrane structure or, more commonly, harm other cellular components including DNA, RNA, proteins and enzymes via aldehydes like MDA [35]. According to Ashrafi *et al.* [36], the high concentration of PUFA in sperm plasmatic membranes, which makes cells susceptible to cold shock and lipid peroxidation in the presence of low temperatures, has been linked to the sensitivity of sperms to low temperatures in the presence of ROS [37]. These findings support the theory that one of the most advantageous effects of antioxidants supplementation during semen preservation is the decrease in lipid peroxidation of membranes.

In the current study, addition of nano Se at concentration 0.5 µg/ml resulted in improved motility compared to the control group. However, percentages of a live sperm and sperm morphology did not differ significantly between different groups. Rabbit semen stored at 5o C showed a gradual decline in motility, functional integrity of sperm membranes and fertility [38]. It may be as a result of ROS production during preservation leading to a cascade of events that result in a reduction of axonemal protein phosphorylation and sperm immobilization, both of which are combined with a reduction of membrane fluidity [39]. Nano Se exerted significant positive effects with respect to sperm parameters in comparison to the control group in this study. This may be contributed to the role of SeNPs in decreasing lipid peroxidation. The role of nano Se in decreasing the level of the lipid peroxidation has been confirmed by MDA levels of supplemented groups compared to the control one. Sperm motility is an important and the most used parameter for assessing fertilizing potential of sperms [40]. It is an indication of the active metabolism and integrity of membranes [41]. These results are consistent with that reported by Hozyen and El Shamy [42] in rams where supplementation of Tris-yolk fructose extender with 0.5 mg/ml nano Se before cooling had enhanced viable sperm percentage and motility. Likewise, addition of bulk selenium to extender improved motility in equilibrated semen and enhanced motility, viability, membrane integrity and DNA damage in post thawed frozen buffalo semen [43]. In order to fertilize an egg in vivo, spermatozoa must have both intact plasma membranes and acrosomes [44]. However, the preservation procedure exposes spermatozoa to extreme osmotic stress [45] that results in membrane and acrosome damage and reduces their ability to fertilize eggs [46]. Interestingly, our results show that 0.5 µg/ml nano Se preserved sperm membrane integrity of chilled rabbit semen. This could be contributed to free radical scavenging function of SeNPs and its ability to reduce oxidative stress [16].

The reason for selenium at nanoscale to show enhanced properties from their conventional counterparts is owing to two considerable smaller size of SeNPs and larger surface-to-volume ratio which provide plenty of space to absorb oxygen and increased surface area to react with free radicals [10]. No doubt that sperm acrosomal integrity is greatly affected by the preservation processes [47]. Supplementation of extender with nano Se in the current work did not exert harmful effects with respect to acrosome integrity. For sperm functions and male fertility, use of nanoparticles based on their antioxidative properties can be very beneficial [24]. In agreement with our findings, it has been reported supplementation of extender with nano Se during cooling [41] and freezing [17] could have membrane protective function.

## Conclusion

The use of SeNPs as nano-protectant additives in semen extender during semen chilling process protected rabbit spermatozoa from lipid peroxidation and preserved motility as well as integrity of the sperm membrane. Rabbit semen quality was the best in extender supplemented with low concentrations of SeNPs.

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*Conflicts of interest*

The authors declare that they have no conflict of interest.

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## تأثير إضافة النانو سيلينيوم المحضر بطريقة الترسيب على الإجهاد التأكسدي وجودة السائل المنوي المبرد للأرانب

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على الرغم من التأثير السلبى لعمليات تبريد السائل المنوى على وظيفة الحيوانات المنوية ونوعيتها إلا أنه يمكن لمكملات جسيمات النانو سيلينيوم أن تحد أو توقف هذا التأثير. ولذلك تم تصميم الدراسة الحالية لتقييم آثار إضافة خمسة تركيزات مختلفة من جسيمات النانو سيلينيوم (٠,٥، ١، ١,٥، ٢ و ٢,٥ ميكروغرام / مل) لحماية الحيوانات المنوية للأرانب من التأثيرات الضارة لحفظ السائل المنوى بالتبريد. وقد تم إستخدام الأشعة السينية، والمجهر الإلكتروني عالي الدقة والمجهر الإلكتروني الماسح لتوصيف جسيمات النانو سيلينيوم التي تم تصنيعها بطريقة الترسيب. تم جمع السائل المنوي من عشرة أرانب ذكور مهبل اصطناعي و من ثم تم تخفيف عينات السائل المنوي المجمعة وإضافة التركيزات المختلفة لجسيمات النانو سيلينيوم. ولقد زادت حركة الحيوانات المنوية وكذلك سلامة الغشاء مع ٠,٥ ميكروغرام / مل تركيز من جسيمات النانو سيلينيوم. وبالإضافة إلى ذلك لم يتم ملاحظة أي تأثيرات سلبية لجميع تركيزات جسيمات النانو سيلينيوم على النسب المئوية للحيوانات المنوية الحية وكذلك شكل الحيوانات المنوية. وتم تسجيل إنخفاض معنوى فى مستويات المالوندايلديهايد مع تركيز ٠,٥ ميكروغرام / مل من جسيمات النانو سيلينيوم. والخلاصة فإن النتائج تؤكد النتائج أن التركيزات المنخفضة لجسيمات النانو سيلينيوم المصنعة بطريقة الترسيب يمكن أن تحافظ بشكل فعال على جودة السائل المنوي للأرانب أثناء عمليات التبريد.

**الكلمات الدالة:** تبريد السائل المنوى ، جسيمات النانو سيلينيوم، الإجهاد التأكسدى ، جودة السائل المنوى ، الأرانب.