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EFFECT OF NANO SELENIUM SUPPLEMENTATION ON SEMEN CHARACTERISTICS STORED AT COOLTEMPERATURE

H. A. El-Kholy *; E. M. El-Siefy*; H. M. El-Kelawy** and M. N. EL- Gaafary **

* *Biotechnology Research Department, Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Egypt.*

****** *Department of Animal and Poultry Production, Faculty of Technology & Development, Zagazig University, Zagazig, Egypt.*

e. mail: *drhassan_2105@yahoo.com*

ABSTRACT

Semen was collected once a week from four sexually mature buffalo bulls raised at International Livestock Management Training Center (ILMTC), Sakha, belonging to the Animal Production Research Institute, to evaluate the effect of addition different concentrations of selenium Nano-particles (SeNPs) on sperm motility, livability, abnormality, plasma membrane and acrosome integrity in semen stored at 5°C for different storage periods. Only ejaculates with mass motility of 70% or more were pooled for each collection day. The collected semen was diluted with Tris-extender and held in a water bath at 37°C, then divided into six portions. Different concentrations of SeNPs were added to the six diluted portions of semen (0 (control), 25, 0.5, 0.75, 1 and 1.25µg/mL /mLTris extender). Thereafter, all portion of semen were stored at 5°C (refrigerator temperature) for 0, 6, 24, 48, 72 and 96 h periods. Percentages of progressive motility, livability, abnormality, plasma membrane and acrosome integrity of spermatozoa were determined at various storage times.

Results revealed that *addition SeNPs improved all semen characteristics during different storage periods compared to control but the differences during the periods of 0, 24 and 48 h of storage were not significant. While the differences were highly (P<0.05) significant among the extenders supplemented with SeNPs compared with control during the periods of 72 and 96 h of storage. Sperm motility, livability, plasma membrane and acrosome integrity decreased (P<0.05) by increasing storage period. However, sperm abnormality was increased affected by storage period,. Semen supplemented with 1.25 µg SeNPs was the highest sperm motility, livability, plasma membrane and acrosome integrity.*

Conclusively, *the present results suggested the possibility of storage buffalo bulls semen at 5°C for 72 h with maintenance of sperm motility, livability, plasma membrane and acrosome integrity percentages above 40% in*

semen supplemented with 1.25µg/mL /mLSeNPs. Accordingly, it can be used for artificial insemination.

Keywords; buffalo sperm, SeNPs, storage, semen characteristics.

INTRODUCTION

Modern dairy industry extensively depends on semen cryopreservation for artificial insemination (AI). Although, chilled semen has a relatively short shelf life, it is technically easier and cheaper than freezing one (Singh *et al*., 2012). Semen storage at 5°C is lowering the spermatozoa metabolic rate, thus, extending cell viability (Vishwanath and Shannon, 2000). Semen extenders must provide suitable media for longer survival of spermatozoa. Animal origin egg yolk based extenders are largely used in frozen and chilled semen preservation (Wall and Foote, 1999 and Stradaioli *et al*., 2007).

During cryopreservation, mammalian spermatozoa are highly susceptible to peroxidative damage and oxidative stress [imbalance between the production of the antioxidant defense system and reactive oxygen species (ROS)], owing to a high polyunsaturated fatty acid ratio. The cryopreservation process may lead to membrane integrity loss, DNA fragmentation, or impaired cell function, all of which may cause a marked reduction in the motility of sperm and fertilization (Sardoy *et al*., 2008 and El-Badry *et al*., 2016,). Mammalian sperm cryopreservation is a complex process; in order to obtain good quality semen for AI several factors come into play (Ferdinand *et al*., 2014) , such as the cryoprotectant or extender types used, the cooling and thawing rates, and the packaging method (Clulow *et al*., 2008 and Abdel-Khalek *et al*., 2018). Semen cryopreservation induces damages in bull spermatozoa (Amirat *et al*., 2004). An alteration in the antioxidant defense system may occur during the semen freezing process (Bilodeau *et al*., 2000) leading to an increase in the ROS production (Ball *et al*., 2001), which induces changes in the membrane function and structure of spermatozoa. Additionally, the freezing process results in a significant glutathione (GSH) content reduction in the frozen semen (Gadea *et al*., 2004). GSH is an essential antioxidant defense in cells and plays a key role in regulating apoptosis versus necrosis. GSH is a substrate for glutathione peroxidase (GPx) to antagonize hydrogen peroxide (H2O2) (Yuan and Kaplowitz, 2009). During freezing, the temperature reduction is associated with oxidative stress that lead to some functional and structural damage to sperm, such as loss of the motility, vitality, plasma membrane integrity, and ultimately the fertility of bull (Stradaioli *et al*., 2007) sperm cells.

Nanoparticles (NPs), with diameter of 1–100 nm, have become increasingly common in a variety of medical applications (Barkhordari *et al*., 2013). Previous studies showed the benefits of supplementing semen extenders with various Nano-element types (Shahin *et al*., 2020) and natural medicinal herb extracts used as antioxidants (Tvrda *et al*., 2018) in different animal species for cryopreservation and AI. Poor absorption, high metabolic rate, and rapid systemic clearance are the main obstacles for the low bioavailability of the medicinal herbs (Srimal and Dhawan, 1973).

The use of antioxidants, such as nano-zinc oxide, can be important in reducing ROS generation and increasing sperm survival (Barkalina, *et al*., 2014 and Heidari, *et al*., 2018). Using zinc nanoparticles (50g/mL) or selenium nanoparticles (1g/mL) in a SHOTOR extender enhanced morphological characteristics and ultrastructure of camel epididymal spermatozoa after cryopreservation via the reduction of apoptosis and lipid peroxidation (Shahin, *et al*., 2020). In Holstein bulls, supplementing a semen extender with Se-NPs (1.0g/mL) improved post-thaw sperm quality and conception rate and sperm damage (Khalil *et al*., 2019). Moreover, in rams, Hozyen *et al*. 2019 and Nateq *et al*. 2020 indicated that using SeNPs (1g/mL) led to improve the percentages of motility, viability, and membrane integrity, while acrosome defects, DNA fragmentation, and malondialdehyde (MDA) concentrations were reduced.

Therefore, the aim of this study was to investigate the effect of selenium Nano particles addition at different levels in semen extender on quality of buffalo bulls spermatozoa preserved at refrigerator (5°C) temperature for four days.

MATERIALS AND METHODS

This study was carried out at Department of Animal and Poultry Production, Faculty of Technology & Development, Zagazig University, while the experimental trials of this study were conducted at the International Livestock Management Training Center (ILMTC), Sakha, belongs to the Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, during the period from November 2021 to October 2022.

Production of selenium nanoparticles (SeNPs):

The production of SeNPs rich products was carried out according to the method based on the patent (US8003071) by Prokisch and Zommara (2011). SeNPs size ranged from 55-238 nm with an average of 122.6 ± 34.6 (SD) or 122.6 ± 8.6 (SE). SEM (JSM-IT100, JEOL Co. Japan) photos of purified SeNPs (Figure 1) were used for SeNPs size determination according to Nagy *et al*.

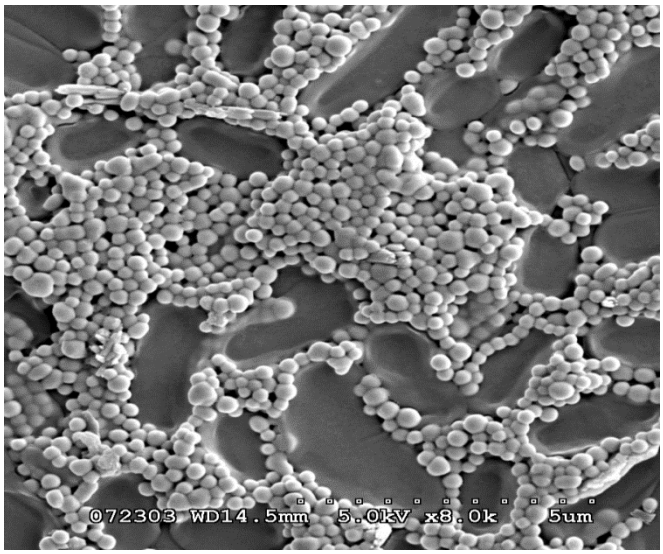

Figure. 1: SEM photograph of a yoghurt culture-SeNPs suspension.

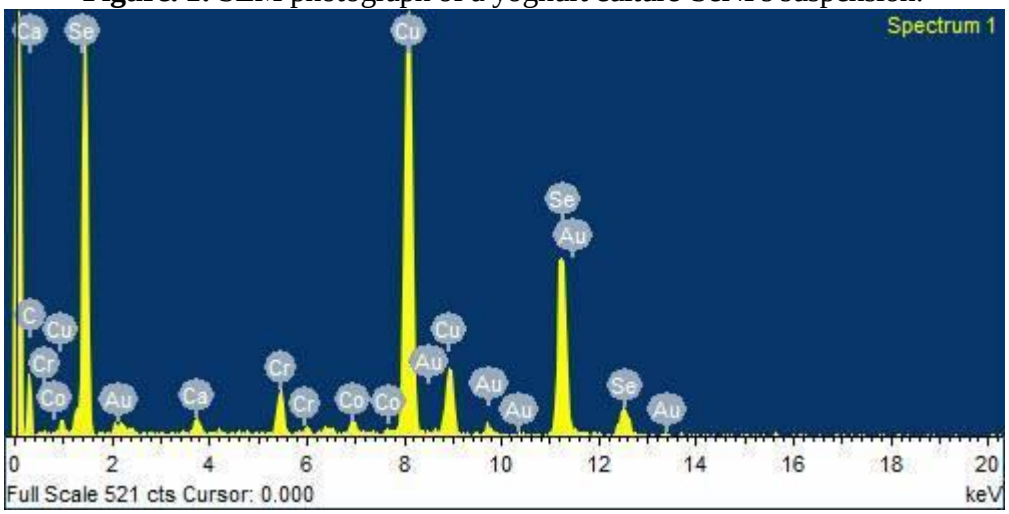

Figure 2. Energy dispersive X-ray spectra of the produced metal spheres.

(2016). Figure 2. showing the energy dispersive X-ray spectra of the produced SeNPs spheres.

Preparation of whey medium

Whey broth media was prepared according to Kar and Misra (1999). Fat-free sweet Ras cheese whey was heated to 80°C then filtered with cheese cloth

and filter paper to separate any coagulated casein particles from the whey and the pH was adjusted to 5.5 with 10% sodium bicarbonate ($NaHCO_3$) solution. After measuring the whey volume, 0.5% glucose, 0.1% skim milk and 1% yeast extract were added (Parente and Zottola, 1991). The pH was adjusted to 5.8 by sodium bicarbonate and the medium was autoclave sterilized.

Preparation of selenium stock solutions:

1000 ppm Na_2SeO_3 [Se (IV)] stock solution was prepared by dissolving 0.219 g of Na_2SeO_3 (MW: 172.94) in 100 mL distilled water.

Lactic acid bacteria (lab) cultures:

Pure lyophilized culture of *c*ommercially available yoghurt starter culture (YC-XII-DVS) consisting of *Streptococcus thermophilus* (*S. thermophiles*) and *Lactobacillus dellbreuckii* subsp. *bulgaricus* (*L. bulgaricus*) at ratio (1:1) were obtained from CHR - Hansen's lab, Copenhagen, Denmark.

Production of selenium nanoparticles (senps) by lab:

Whey media supplemented with 200 ppm selenium as sodium selenite (Na_2SeO_3) (Sigma-Aldrich, Switzerland), was inoculated with 3% LAB starter culture about (10^5 cfu/ ml) and incubated at 37ºC for 72 hours. Growth rate was estimated by reduction in pH value (3020 Jenway, England) and absorbance at 650 nm (Ayad *et al*., 2004). The media was centrifuged at 6.000 rpm for 20 min. The supernatant was removed and the obtained sediment containing SeNPs and bacterial cultures were air-dried on laboratory oven at 60 ºC. The dried product was mild to a fine powder using laboratory miller and used for animal feeding experiments in the supplementation to semen extender.

Semen collection and evaluation:

Semen sample was collected once weekly from buffalo bulls using an artificial vagina throughout the experiment. Four sexually mature healthy buffalo bulls with average of 600 kg body weight and 3-4 years old age were used for semen collection. All bulls were healthy and clinically free from external and internal parasites. Palpation of the testicles showed that they were typically normal. The testicular tone was glandular, almost equal in size and movable freely up and down within the scrotal pouches. Immediately after semen collection, ejaculates were held in water bath at 37°C until evaluated. Ejaculates of more than 70% motility were used by pooling to eliminate the effect of bull. On each collection day, after semen evaluation, the pooled fresh semen was split into six equal portions; the first was diluted with tris-20% egg yolk

extender (control) and others five media supplemented with different concentrations of 0.25, 0.50, 0.75, 1.00 and 1.25 µg/mL selenium nanoparticles**.** Tris-citric acid extender was used as a buffer, consisted of 3.025g tris–(hydroxymethyl-aminomethane), 1.675g citric acid, 0.75g glucose and 7.0% glycerol, 20% fresh egg yolk, 0.25 g linco-spectin and 0.005g streptomycin. All contents were dissolved in bi-distilled water up to 100 ml.

Diluted semen were gradually cooled down to 5°C in a refrigerator and stored for four days at the same temperature. At each period of semen storage (0, 24, 48, 72 and 96 h), the semen quality was evaluated.

Semen quality assessment:

Semen characteristics (the percentages of progressive motility, live sperm, sperm abnormality, plasma membrane integrity and acrosome integrity) were estimated at 0, 24, 48, 72 and 96 h of storage.

Progressive motility (%) was estimated according to Amann and Hammerstedt (1980). Live sperm percentage was assessed according to Hancock, (1951). Sperm abnormalities percentage was determined during the examination of live/dead sperm percentage at a high power magnification (400x), according to the classification adopted by Blom (1983).The plasma membrane integrity of spermatozoa was assessed using the hypo-osmotic swelling test (HOST) as described by Jeyendran *et al.* (1984). Acrosome integrity was determined by using a Giemsa stain procedure as described by Watson, (1975). Progressive motility (%) was estimated according to Amann and Hammerstedt (1980). Live sperm percentage was assessed according to Hancock, (1951). Sperm abnormalities percentage was determined during the examination of live/dead sperm percentage at a high power magnification (400x), according to the classification adopted by Blom (1983).The plasma membrane integrity of spermatozoa was assessed using the hypo-osmotic swelling test (HOST) as described by Jeyendran *et al.* (1984). Acrosome integrity was determined by using a Giemsa stain procedure as described by Watson, (1975).

Statistical analysis:

Data were statistically analyzed using general model program (SPSS, 2013). Duncan multiple range test was used to test the differences among means (Duncan, 1955). The percentage values were

subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentages.

RESULTS AND DISCUSSION

The effects of Nano-Selenium supplementation to semen extender on progressive motility cooled of buffalo spermatozoa during storage at 5^0C for 96 h are shown Table 1. Progressive motility in the freshly diluted semen, 24 and 48 h of storage did not show any significant differences among the different extenders used but the best value of progressive motility percentage was obtained in the extender containing 1.25µg/ml Nano-selenium particles (Se-NPs) compared to the control and other Se-NPs extenders. The first significant difference in sperm motility was detected with the advancement of storage time after 72, 96 hours for 1.25µg/ml Se-NPs based extender (P< 0.01). It was noticed that the progressive motility started to reduce with increasing storage time, but this reduction was smaller in 1.25µg/ml Se-NPs extender than that in control (20% EY) and other Se-NPs extenders. The use of 1.25µg/ml Se-NPs gave the highest progressive motility and more efficient on keeping long-term preserved buffalo spermatozoa at 5^0C up to 96.

Table 1. Effect of Nano-Selenium supplementation to semen extender on sperm progressive motility (%) of buffalo bulls during different periods of storage at 5^0C.

Storage times	Control	Sperm progressive motility (%)					Sig.
		Nano-Selenium concentrations (µg/mL)					
		0.25	0.50	0.75	1.00	1.25	
Zero Time	62.88±1.62	65.00 ±1.53	62.78±1.60	63.70±1.78	63.65±2.02	65.00±2.05	NS
24h	49.07±1.87	48.52 ±2.41	46.11 ±2.35	46.30 ±2.38	50.00±2.86	50.00±2.86	NS
48h	41.85±2.15	43.70 ±2.25	39.26 ± 2.26	39.63 ±2.18	42.59±2.58	45.18±3.01	NS
72h	34.42^{ab}±2.14	39.23^{a}±1.56	31.54^{b} ± 2.20	31.73^{b}±2.30	36.54^{ab}±2.20	40.19^{a}±2.62	**
96h	25.68^{b}±2.51	31.36^{ab}±2.19	25.68 ±2.70	25.23^{b}±2.12	30.91^{ab}± 2.62	35.68^{a}±2.89	**
Reduction rate%	59.2	60.5	59.0	60.4	51.4	45.0	

NS= Not significant and **= P< 0.01.

a,b and c Means in the same raw with different superscript , differ significantly P < 0.05).

Data of live sperm percentage preserved at 50C for different storage periods are included in Table 2. Addition of different concentrations of Se-NPs to semen extenders led to improve the live sperm percentage compared to control but the differences were not significant during different storage stages (from 0 up to 96 h) of cooled at 50C. in mean time, the percentage of live sperm in all Se-NPs extenders compared to control, being the best in semen extender supplemented with 1.25µg/ml Se-NPs. Thereafter, all percentages of live spermatozoa reduced by increasing storage time. It is meaning that live buffalo spermatozoa were preserved for longer time when stored in Se-NPs extenders for up to 4 days at 5^0C compared to control. Also, It was noticed that live sperm percentage started to reduce with increasing storage time, but this reduction was smaller in 1.25µg/ml Se-NPs extender compared to control and other Se-NPs extenders.

Table 2. Effect of Nano-Selenium supplementation to semen extender on live spermatozoa (%) of buffalo bulls during different periods of storage at 5^0C.

Storage times	Control	Live spermatozoa (%)					Sig.
		Nano-Selenium concentrations (µg/mL)					
		0.25	0.50	0.75	1	1.25	
Zero Time	74.48 ± 1.46	74.67 ± 1.02	73.59± 1.02	74.40± 1.30	73.61± 1.50	76.00± 1.23	NS
24h	66.52 ± 1.23	66.52 ± 1.78	65.04± 1.55	65.92± 1.92	66.44± 2.09	67.96± 2.17	NS
48h	60.48± 1.46	62.15 ± 1.64	59.70 ± 1.66	59.41± 2.01	60.55± 2.20	62.70± 2.50	NS
72h	54.96± 1.94	58.38± 1.013	55.04 ± 1.51	54.23± 2.00	55.65± 1.74	58.73± 2.24	NS
96h	50.95 ± 1.56	53.27 ± 1.46	50.45 ±1.86	50.09± 2.06	51.64± 1.78	54.64± 2.41	NS
Reduction rate%	31.6	28.7	31.4	32.6	29.8	28.0	

NS= Not significant.

The effect of Nano-Selenium supplementation to semen extender on abnormal spermatozoa percentage of buffalo spermatozoa during different periods of storage at 5^0C are presented in Table 3. Addition of different concentrations of Se-NPs to semen extenders led to reduce the abnormality of sperm compared to control but the differences were not significant during different storage stages of buffalo bull spermatozoa

Table3. Effect of Nano-Selenium supplementation to semen extender on abnormal spermatozoa (%) of buffalo bulls during different periods of storage at 5^0C.

Storage times	Control	Abnormal spermatozoa (%)					Sig.
		Nano-Selenium concentrations (µg/mL)					
		0.25	0.50	0.75	1	1.25	
Zero Time	13.92± 0.30	13.85± 0.50	13.92± 0.45	12.92± 0.28	14.04± 0.64	13.59 ± 0.60	NS
24h	14.26± 0.29	14.52± 0.32	14.67± 0.25	14.26± 0.33	15.11± 0.25	14.37 ± 0.32	NS
48h	16.41± 0.38	17.35± 0.34	16.37± 0.24	15.89± 0.33	16.04± 0.28	15.85 ± 0.35	NS
72h	17.35± 0.31	17.35±0. 34	17.96± 0.31	17.85± 0.50	17.58± 0.37	17.27 ± 0.42	NS
96h	18.27± 0.35	19.23± 0.45	18.27± 0.29	19.45± 0.59	19.36± 0.50	17.36 ± 0.59	NS
increasing rate%	31.3	38.8	31.3	50.5	39.8	27.7	

NS= Not significant.

cooled at 5^0C. in mean time, the lowest value of sperm abnormalities was found in semen extender supplemented with 1.25µg/ml Se-NPs compared to control. Thereafter, all percentages of abnormal spermatozoa was reduced by increasing storage time. It was noticed that abnormal sperm percentage was increased with long storage time, the percentage of increasing rate was lower in extender supplemented with 1.25µg/ml Se-NPs compared to control and other Se-NPs extenders.

According to the results presented in Tables (4 and 5), the addition of 1.25µg/ml Se-NPs in semen extender had the best protective effect to sperm cells that was shown in the highest plasma membrane and acrosome integrities compared to control or other extenders which supplemented with 0.25, 0.50, 0.75 and1.0 Se-NPs cooled at 5 ^{0}C during different stages of storage time but the differences among all extenders did not differ significantly in plasma membrane integrity or intact acrosome percentage. Also, It was observed that plasma membrane and acrosome integrities started to reduce with increasing storage time, but this reduction were lower in the extender containing 1.25µg/ml Se-NPs than that obtained in control extender and other Se-NPs extenders.

Table 4. Effect of Nano-Selenium supplementation to semen extender on plasma membrane integrity (%) of buffalo bulls during different periods of storage at 5^0C.

Storage times	Control	Plasma membrane integrity (%) Nano-Selenium concentrations (µg/mL)					Sig.
		0.25	0.50	0.75	1.00	1.25	
Zero Time	72.88 ±1.18	73.82 ± 1.06	72.04± 1.09	74.00± 1.32	73.42± 1.43	75.26 ± .24	NS
24h	68.00 ±1.17	68.78 ± 0.89	66.30± 1.43	68.48± 1.52	68.59± 1.58	70.48 ± 1.31	NS
48h	62.96 ±1.08	63.92 ± 0.87	61.15± 1.50	63.37± 1.41	63.33± 1.45	64.41 ± 1.59	NS
72h	58.73 ±1.08	59.35 ± 0.84	56.85± 1.39	57.61± 1.53	58.61± 1.54	59.58 ± 1.65	NS
96h	53.32 ±1.42	55.32± 1.13	53.36± 0.99	53.86± 1.09	54.36± 1.57	56.50 ± 1.31	NS
Reduction rate%	26.8	25.1	25.9	27.2	26.0	24.9	

NS= Not significant.

Table 5. Effect of Nano-Selenium supplementation to semen extender on acrosome integrity (%) of buffalo bulls during different periods of storage at 5^0C.

Storage times	Control	Acrosome integrity (%) Nano-Selenium concentrations (µg/mL)					Sig.
		0.25	0.50	0.75	1.00	1.25	
Zero Time	78.08± 1.18	78.89± 0.96	77.18± 1.07	78.74± 1.28	78.35± 1.45	80.63± 1.17	NS
24h	73.55± 1.11	74.33± 0.90	72.30± 1.42	73.81± 1.48	73.59± 1.58	75.78 ± 1.32	NS
48h	68.00± 1.25	69.67 ±0.83	67.00± 1.37	68.70± 1.44	68.48± 1.58	69.81 ± 1.58	NS
72h	64.08± 1.20	65.15 ± 0.84	62.69± 1.31	63.19± 1.57	63.85± 1.54	64.54 ± 1.71	NS
96h	59.09± 1.36	61.09± 1.14	59.82 ± 1.50	60.14 ± 1.29	59.82 ± 1.50	65.82 ± 1.35	NS
Reduction rate%	24.3	22.6	22.5	23.6	23.7	18.4	

NS= Not significant.

The higher percentage of plasma membrane integrity and acrosome integrity were obtained in semen extender with 1.25µg/ml Se-NPs. The short-term preservation of buffalo spermatozoa could enhance

the development of assisted reproductive technologies. In the present study, we have investigated the effect of addition different concentrations of SeNPs to tris-extender of buffalo bull semen stored at 5 °C up to 4 days. The findings revealed that the extender supplemented with SeNPs can improve the quality of semen characteristics during different storage periods. Sperm cells have a high level of unsaturated fatty acids in their membranes, and they lack an essential cytoplasmic component that contains antioxidants that make them vulnerable to lipid peroxide, H_2O_2, and oxidative stress (Storey 1997 and Bansal and Bilaspuri 2011). Mammalian sperm contains low levels of glutathione peroxidase and glutathione reductase that are the key components of reduced glutathione (GSH) formation as a defensive mechanism against oxidative stress (El-Harairy *et al*., 2016). Sperm used for in vitro fertilization or artificial insemination exposed to high levels of oxygen, light and radiation during different stages of cryopreservation that could generate ROS and sperm plasma membrane damage (Foote *et al*., 2002). The protective antioxidant systems in spermatozoa are primarily of cytoplasmic origin. However, spermatozoa discard most of their cytoplasm and lack the significant cytoplasmic component containing antioxidants that counteract the harmful effects of ROS and lipid peroxidation during the final stages of differentiation (Storey, 1997). Antioxidant supplementation can prevent this process (Sikka, 2004). The findings was in agreement with those reports of decreased sperm motility after 48 h of goat buck spermatozoa stored at 4 °C (Kilian *et al*., 2000). Also, Mir *et al*., (2012) observed that declining motility was recorded after 48 h of 4 °C of storage. Also, Our results showed that the addition of SeNPs to semen extender improved progressive motility, live sperm, plasma membrane integrity and acrosome integrity whereas sperm abnormality was decreased, and the best results were observed with SeNPs at a level of 1.25µg/ml. In the same context, Shahin *et al*. (2020) reported that supplemented SHOTOR extender with vitamins (C, E) and minerals (Se, Zn) either in organic or Nano form showed a positive effect on frozen/thawed camel epididymal spermatozoa. Also, The findings agreed with the researcher Dorostkar *et al*.(2012) who observed that buffalo semen extender supplemented with 2 mg/mL of sodium selenite enhanced sperm motility, livability, membrane functionality, and reduced DNA damage and abnormality. Moreover, addition 1.0 µg/mL of SeNPs to Tris-egg yolk extenders increased the

percentage of viable sperm and decreased early apoptotic, and necrotic sperm in post-thawed bull semen (Khalil *et al*., 2019).

Selenium-containing enzymes (Selenozymes) such as glutathione peroxidase which protect cellular membrane lipids from oxidative stress (Marin-Guzman *et al*., 2000). There are a positive relationship between the concentration of glutathione in seminal plasma and semen quality (Eskiocak *et al*., 2005). Handy *et al*. (2008) reported that the proportion of atoms on the surface is significantly greater than the proportion of atoms in the interior of the particle, due to the small size and the high surface area of these nanoparticles relative to the larger equivalents of the same substance.

In conclusion, the results revealed that addition 1.25µg/ml selenium Nano particles (SeNPs) in tris-extender improved semen quality of buffalo bulls spermatozoa stored at 5 °C up to 96 h. We recommend that inclusion of Nano-selenium to diluents to improve semen characteristics for longer periods under cool storage conditions.

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تأثير إضافة النانوسلنيوم علي خصائص السائل المنوى لطلائق الجاموس المخزن والمبرد على ٥ °م.

حسن عبد القادر الخولى* - الشناوى محمد الصيفى* – حسن محمود الكيلاوى- *محمد ناجى الجعفرى***

*قسم بحوث التكنولوجيا الحيوية- معهد بحوث الانتاج الحيوانى- مركز البحوث الزراعية

**قسم الانتاج الحيوانى والداجني- كلية التكنولوجيا والتنمية- جامعة الزقازيق

تم جمع السائل المنوى مرة أسبوعيا باستخدام المهبل الصناعى من أربعة طلائق جاموس ناضجة جنسيا. تم خلط السائل المنوى فى يوم الجمع وتم استخدام عينات السائل المنوى التى لا تقل حيويتها عن ٧٠ %. وضع السائل المنوى فى حمام مائى على درجة ٣٧° م . بعد ذلك قسمت إلى ٦ معاملات. المعاملة الأولى كانت بدون إضافة (كنترول) بينما الخمس معاملات الأخرى كانت مضاف إليها تركيزات مختلفة من النانوسلنيوم بمستويات ٠,٢٥ ، ٠,٥٠ ، ٠,٧٥ ، ١.٠٠، ١,٢٥ ميكروجرام علي التوالي / مل من مخفف الترس. تم تخزين السائل المنوى لكل معاملة لمدة صفر، ٢٤،٤٨، ٧٢، ٩٦ ساعة على درجة حرارة ٥° م. تم تقدير النسب المئوية للحركة التقدمية للحيوانات المنوية والحيوانات المنوية الحية والشاذة وسلامة الاكروسوم وسلامة الغشاء البلازمي خلال فترات التخزين المختلفة.

أشارت النتائج إلى ما يلى:

كان تأثير إضافة النانوسلنيوم الى مخفف الترس ادى الى تحسين جميع صفات السائل المنوى موضع الدراسة مقارنة الكنترول لكن لم يكن هناك اختلافات معنوية خلال فترات التخزين ٠، ٢٤، ٤٨ ساعة بينما كانت الإختلافات عالية المعنوية عند مستوى ٥% بين المخففات التى اضيف اليها النانوسلنيوم مقارنة بالكنترول خلال فترات التخزين ٧٢ و ٩٦ ساعة. انخفضت النسبة المئوية للحركة التقدمية والحيوانات المنوية الحية وسلامة الاكروسوم وسلامة الغشاء البلازمي مع طول فترة التخزين ووصلت إلى أدنى قيمة بعد ٩٦ ساعة. ومن ناحية أخرى فإن نسبة الحيوانات المنوية الشاذة زادت مع زيادة فترة التخزين. المخفف المضاف اليه ١,٢٥ ميكروجرام كان اعلى معنويا وحسن جميع صفات السائل المنوى مقارنة بالكنترول او المخففات الأخرى.

التوصية : يمكن تخزين السائل المنوى لطلائق الجاموس على درجة حرارة٥° م لمدة ٧٢ ساعة بإضافة النانوسلنيوم بتركيز١,٢٥ ميكروجرام ، حيث كانت النسبة المئوية للحيوية والحيوانات المنوية الحية وسلامة الاكروسوم وسلامة الغشاء البلازمي أكبر من ٤٠% و وبناء عليه يمكن استخدامه عينات السائل المنوي المعاملة في التلقيح الإصطناعى.