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Cryopreservation of Frozen Buffalo Bulls Spermatozoa Supplemented with Nano Selenium

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Abstract

The aim of the current study was to evaluate the effect of supplementation of different levels of selenium Nano particles in Tris-egg yolk extenders and to investigate its effect on quality and fertility of frozen-thawed buffalo bulls spermatozoa. Semen was collected once a week from four buffalo mature bulls and ejaculates with 70% progressive motility prior to cryopreservation were pooled and diluted in a tris-yolk glucose extender supplemented with different concentrations of Se NPs at levels 0 (control), 25, 0.5, 0.75, 1 and 1.25µg/ml. Semen was diluted to 80 million sperm/ml packaged into 0.25ml straws, cooled held at 5ºC for 4 h, and then frozen in liquid nitrogen and stored at –196ºC until artificially inseminated. The percentages of progressive sperm motility, live sperm, sperm abnormality, intact sperm acrosome and plasma membrane integrity were evaluated post dilution, post–equilibration and post frozen–thawed processes. The results showed that extender containing 1.25µg/ml SeNPs was more effective on cryopreservation of semen of buffalo spermatozoa than control extender and other levels containing SeNPs extenders. Also, conception rate was considerably higher in 1.25µg/ml SeNPs extender compared with control extender (68.2% vs 57.5%, respectively). In conclusion, 1.25µg/ml SeNPs extender improves freezability and fertility of buffalo bull spermatozoa and can be used as an additive to tis-egg yolk in cryopreservation of buffalo bulls semen.

Keywords: Cryopreservation, SeNPs, extender, conception rate, buffalo bull.

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1. Introduction

Artificial insemination with cryopreserved semen is the most viable biotechnology for faster and increased genetic improvement in many species. The genetic improvement and disease control in domestic animals have primary importance in the success of agriculture and food industry. Moreover, the quality of frozen–thawed semen is one of the most influential factors affecting the conception [1]. Application of AI with frozen– thawed semen has been on a limited scale in buffalo, because the freezability and fertility of buffalo spermatozoa are poor as compared with cattle spermatozoa [2,3,4,5,6,7].

Several researchers found a strong favourable relationship between sperm quality and selenium levels in the plasma of semen. The totality of the possible anti-ROS enzymes, like GSH-PX, is represented by the total antioxidant capacity of the plasma of semen. The enzyme GSH-PX, which uses selenium as one of its components, guards against peroxidative damage to cellular membranes and organelles that contain lipids. Selenium in the form of selenite aids in the detoxification of the media in cell culture to shield the cells from oxidative damage [8,9]. In buffalo bulls, addition of 1 and 2 µg/ml sodium selenite to semen extender improved the percentage of live sperm, progressive motility, the integrity of sperm membrane, and sperm total

antioxidant capability compared to control during different stages of cryopreservation [10].

In order to defend spermatozoa from reactive oxygen species, sperm contains a variety of antioxidants that shield against this reactive oxygen species (ROS) formation [11] The antioxidant defense potential of semen is reduced during processing and cryopreservation, the inclusion of antioxidant in the cooling diluents had a shield against lipid peroxidation, maintaining metabolic function and cellular function [12]. Reactive oxygen species (ROS) and oxidative stress, which can be created in semen, have been demonstrated to negatively impact spermatozoa [13]. On the other hand, spermatozoa's production of ROS is crucial for maintaining the ability to fertilize and for sperm capacitation, among other typical physiological functions [14]. Due to spermatozoa's high vulnerability to oxidative stress and ROS, many damages can be induced, including lipid peroxidation, DNA damage, apoptosis, and decreased sperm motility [15]. By scavenging released free radicals, antioxidants are molecules with the capacity to limit or diminish the oxidative process in other molecules [16]. The two categories of antioxidants are enzymatic and non-enzymatic. Superoxide dismutase, glutathione peroxidase, catalase, and glutathione reductase are examples of enzyme-based antioxidants, whereas vitamins A, C, and E, pyruvate, glutathione, and coenzyme Q are examples of non-enzymatic antioxidants. The antioxidant action of selenium (Se) is mediated by the enzyme glutathione peroxidase. Vitamins E and C, as well as Se and Zn, which are elements of antioxidant systems, are among the antioxidants in semen that are known to enhance sperm quality. The protective antioxidant systems in spermatozoa originate from the cytoplasm, and spermatozoa discard a significant portion of their cytoplasm as a residual body during the final stages of differentiation, so it is likely that these antioxidant agents are insufficient in preventing lipid peroxidation and sperm plasma membrane damage during the freezing-thawing process. Sperm motility and metabolic activity are significantly reduced during freezing and thawing process [17]. During different cryopreservation phases semen is exposed to oxygen which enhances its susceptibility to lipid peroxidation due to increased ROS generation. One of the crucial components changed by cryopreservation is the seminal plasma membrane, which might then have an impact on the function and fertility of the sperm cells [18]. An essential antioxidant and indicator of oxidative stress, selenium is always a part of the glutathione peroxidase enzyme [19]. [10] observed that addition 1 and 2 µg/ml SeNPs to buffalo semen extender dramatically increased sperm survival, motility, membrane integrity, and total antioxidant capability. Additionally, Selenium plays a vital role in male reproduction, however, studies have shown that semen preserved with selenium nanoparticles reduces spermatic membrane lipid peroxidation, oxidative damage, and decreases sperm acrosome membrane damage; consequently, increasing fertility rate [20]. They caused a decline in sperms with DNA damage in frozen-thawed semen. Supplementation semen extender with 1 µg/ml SeNPs increased post-thaw sperm progressive motility, viability and membrane integrity in bull spermatozoa [21]. In Rooster, addition of 5 µg/ml Vit E and 1% of SeNPs improved sperm motility overall, sperm movement with time, live sperm percentage, and sperm membrane integrity after post-thawing process [22]. Addition of 1 µg/ml SeNPs in ram and camel diluents were increased the percentages of total and progressive motility of sperm, plasma membrane integrity, live sperm as a percentage, decreased acrosomal membrane damage and abnormal sperms [23,24].

The present investigation aimed to evaluate the effect of selenium Nano particles supplementation in semen extender on freezability and fertility of buffalo bulls spermatozoa.

2. Materials and Methods

The present study was carried out at Department of Animal Production, Faculty of Technology and 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.

2.1. Experimental Animals

Four sexually mature healthy buffalo bulls with average of 600 kg body weight and 3-4 years old age were used for semen collection. The bulls were housed individually under semi-open sheds and allowed to drink all the day. All bulls were healthy and clinically free of external and internal parasites. Palpation of the external genital showed that they were normal. The testicular tone was glandular all epididymal regions were present in both testes, almost equal in size and moved freely up and down within the scrotal pouches.

2.2. Feeding procedure

The feeding regime was applied according to the live body weight as recommended by [25], every bull was fed by 8 kg concentrate mixture (14.2 % crude protein, 7.66 % crude fiber, 1.6 % ether extract and 12.8% moisture), 6 kg rice straw and 40 kg green berseem (*Alexandria trifolium*) during the green feeding period (winter season).

2.3. Experimental procedure

2.3.1. Semen collection

The bulls were subjected to one false mounts before ejaculation to arise the bulls sexual desire. In the early morning, semen ejaculates were collected from buffalo bulls once weekly using an artificial vagina throughout the experiment. Bull was used as a teaser for mounting.

Collected semen immediately was held in a water bath at 37ºC for examination. Ejaculates having good mass motility 70% or more were pooled for each collection day to obtain sufficient semen for replicates and to eliminate the bull effect.

2.3.2. Semen quality assessment

Semen characteristics (the percentages of progressive motility, live sperm, sperm abnormality,

plasma membrane integrity and acrosome integrity) were estimated during different stages of cryopreservation (after dilution, post equilibration and post-thawing).

Progressive motility (%)was estimated according to [26]. Live sperm percentage was assessed according to [27]. 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 [28]. The plasma membrane integrity of spermatozoa was assessed using the hypo-osmotic swelling test (HOST) as described by [29]. Acrosome integrity was determined by using a Giemsa stain procedure as described by [30]. Progressive motility (%) was estimated according to [26]. Live sperm percentage was assessed according to [27]. 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 [28]. The plasma membrane integrity of spermatozoa was assessed using the hypo-osmotic swelling test (HOST) as described by [29]. Acrosome integrity was determined by using a Giemsa stain procedure as described by [30].

2.4. Production of selenium nanoparticles (SeNPs)

The production of SeNPs rich products was carried out according to the method based on the patent (US8003071) by [31]. 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 (Fig. 1) were used for SeNPs size determination according to [32]. Fig. (2) showing the energy dispersive X-ray spectra of the produced SeNPs spheres.

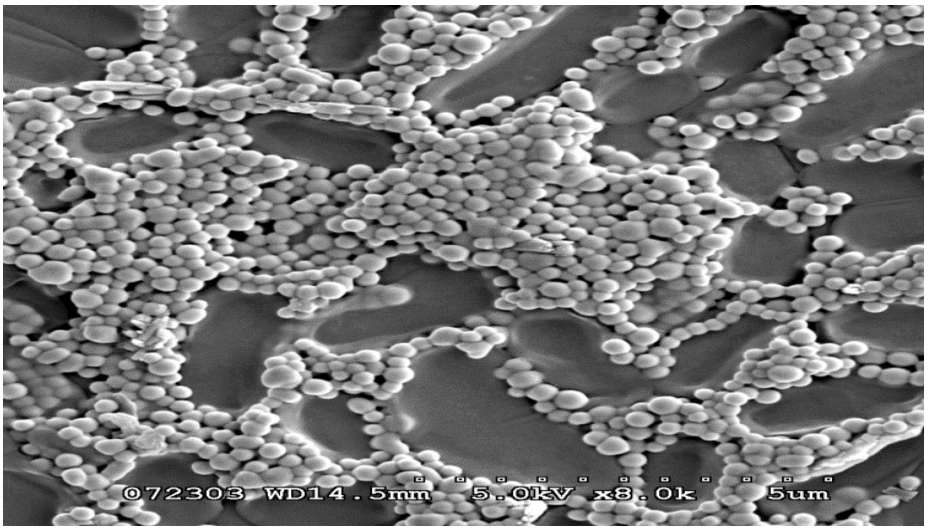

Fig.1: SEM photograph of a yoghurt culture-SeNPs suspension.

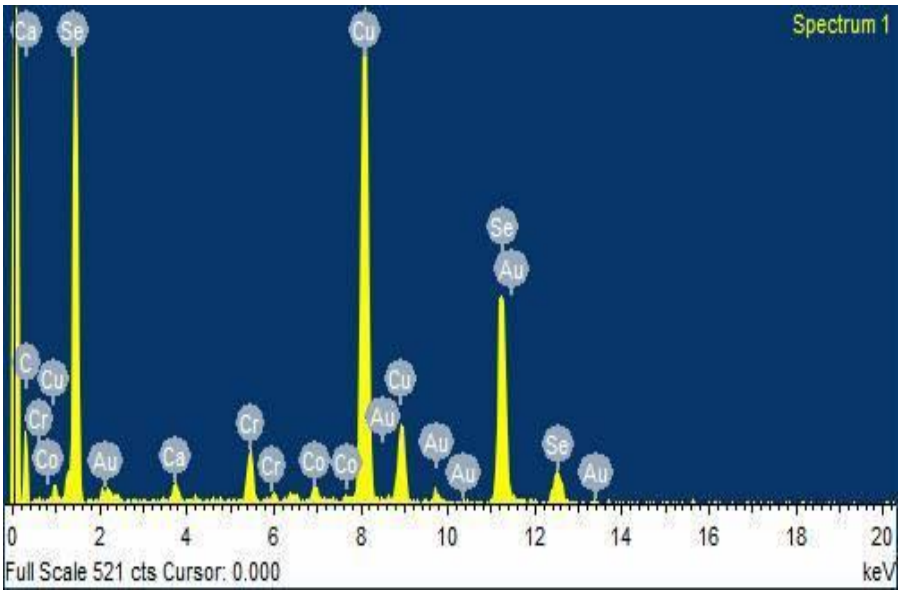

Fig. 2: Energy dispersive X-ray spectra of the produced metal spheres.

2.4.1. Preparation of whey medium

Whey broth media was prepared according to [33]. 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 [34]. The pH was adjusted to 5.8 by sodium bicarbonate and the medium was autoclave sterilized.

2.4.2. 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.

2.4.3. 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.

2.4.4. 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 **[35].** 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.

2.4.5. Selenium determination

Selenium determination was carried out according to the method previously described by [36]. One ml Se containing medium or five ml of medium supernatant (after centrifugation at 7000 rpm for 20 min.) samples were used for total selenium determination in heat-resistant glass digestion tubes. To each tube, 10ml of 65% nitric acid were added and heated at 60°C for 30 min. using digestion block (KJELDATHERM®, Gerhardt, Germany). Then, 3 ml of 30% hydrogen peroxide (Merck, Germany) were added and digestion was continued at 120 °C for 90 min. and then cooled to room temperature. Samples were diluted with Milli-Q water, filtered using filter paper (Macherey-Nagel, Germany) and quantitatively transferred to 25 ml volumetric flasks. Selenium concentration in the diluted digested samples was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (Prodigy 7, Teledyne Leeman Labs, USA).

2.5. Preparation of experimental extender

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.

2.6. Experimental design

Evaluation of the cryoprotective effect of different concentrations of selenium nanoparticles (Se-NPs) (1.0 µg/mL) on freezability and fertility of buffalo bulls spermatozoa.

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, 1.0 and 1.25 µg/mL selenium nanoparticles**.**

2.7. Semen processing and freezing procedure

Ejaculates processing more than 70 % mass motility were pooled. Pooled semen was diluted at 37°C in an experimental extenders, [Tris-20% EY (control), Tris basic extender + 0.25, 0.50, 0.75, 1.0 and 1.25 µg/mL of selenium nanoparticles (Se-NPs). Each ml of extended semen contains approximately $80x10^6$ motile spermatozoa before freezing.

The vial contained the extended semen were placed in a water bath at 37°C for 10 min. subsequently, freshly diluted semen parameters were estimated (the percentages of progressive motility, live sperm, sperm abnormality, acrosome integrity and plasma membrane integrity), after that, extended semen was placed into a refrigerator at 5°C for 4 h. for gradual cooling and also for semen equilibration in the same time and used for filling and freezing in the straws. The cooling rate of semen was adjusted at level of 1°C decrease/4 minutes then evaluated cooling semen before freezing.

At the end of equilibration period, the extended semen was loaded in 0.25ml French straws using a semen filling machine, during filling in the straws extended semen was kept in ice water bath to keep its temperature at 5^0C. Straws were transferred into processing canister and located horizontally in static nitrogen vapor 4cm above the surface of liquid nitrogen for 10 minutes, then the straws were placed vertically in a metal canister and immersed completely in liquid nitrogen container for storage at –196ºC. Frozen semen was thawed by dipping the frozen straws into a water bath at 37ºC for 30 seconds.

2.8. Fertility trial

Buffalo cows owned by small and medium scale breeding holder in different villages in Kafrelsheikh and Gharbia Governorates were artificially inseminated by experienced inseminators with random frozen doses from various extenders.

In the fertility trial, 156 buffalo cows were used. They were divided into 6 groups which were inseminated

with preserved diluted semen supplemented with 0.025, 0.5, 0.075, 0.1 and 1.25 SeNPs.

At observed estrus buffalos were rectal examined to clarify the occurrence of heat. Each female buffalo was inseminated with a single insemination dose (0.25ml French straw 20×10^6 motile spermatozoa) 8-14h after estrus behavior had begun. At the time of insemination, the frozen semen was thawed at 37^0C for 30 seconds. Using recto-vaginal technique and the universal insemination gun, the semen was deposited just next to the anterior end of the cervix. Conception rate was calculated on the basis of pregnancies confirmed by rectal palpation 45-50 days after insemination as following:

Conception rate (%) = (No. of conceived buffaloes /No. of inseminated buffaloes) x100

2.9. Statistical analysis

Data were statistically analyzed using general model program [37]. Duncan multiple range test was used to test the differences among means [38]. 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.

3. Results

3.1. Progressive motility

Data presented in Table (1) showed that semen supplemented with 0.25 and 1.25µg/ml Nano-selenium particles (Se-NPs) recorded slight increase in the percentage of progressive motility of buffalo bulls spermatozoa, compared to the control. While, semen supplemented with 1.0 µg/ml Se-NPs showed the lower value than the other extenders, the differences among treatments were not significant. After equilibration period, addition of µg/ml Se-NPs to semen extender led to improve sperm progressive motility compared to the control and other extenders. Also, the differences among different extenders were not significant. The percentage of progressive motility of frozen-thawed buffalo bulls spermatozoa were significantly (P<0.01) higher in extenders supplemented with 1.25 µg/ml Se-NPs followed by 1.0 and 0.75µ Se-NPs compared to the control and 0.25, 0.5 µg/ml Se-NPs extenders, being the best in semen supplemented with 1.25 Se-NPs. The lowest value was obtained in semen extended by 0.25 µg/ml Se-NPs.

Table 1: Effect of Nano-Selenium supplementation to semen extender on progressive motility (%) of buffalo bulls during different stages of cryopreservation

Items	Control	Nano-Selenium concentrations (µg/ml)					Sig.
		0.25	0.50	0.75	1.0	1.25	
Post dilution	62.96 ± 1.54	68.09 ± 1.22	63.15 ± 1.54	63.33 ± 1.69	55.18^{ab}±1.70	65.00 ± 2.05	NS
After equilibration	55.37^{ab} ± .69	56.30^{ab}± 0.66	54.23^{ab} ±1.7	55.55^{ab}±1.63	55.18^{ab}±1.70	60.00^{a} ±1.48	NS
Post thawing	38.70^{c}± 1.48	33.89^{d}± 1.52	36.48^{cd}±1.83	44.07^{b}± 0.76	45.18^{b}± 1.01	50.00^{a}± 1.0	**

NS= Not significant and **= P< 0.01.
a, b and c means in the same raw with different superscript, differ significantly (P < 0.05).

3.2. Live spermatozoa

Data presented in Table 2 show that supplementation of semen extenders with Se-NPs slightly improved the live sperm percentage compared to control but the differences were not significant, in post dilution period. In Post equilibration phase, the addition of 1.25 µg/ml Se-NPs significantly (P<0.05) improved the percentages of live spermatozoa compared to the control, while the differences between control and other Se-NPs extenders were not significant. Post freezing and thawing, live sperm percentages in 1.25 µg/ml Se-NPs extender were significantly (P<0.01) higher compared to the control extender, the differences among other Se-NPs extenders and control were not significant.

3.3. Plasma membrane integrity

Sperm plasma membrane integrity percentage was significantly (P<0.05) higher in extender containing 1.25 µg/ml Se-NPs when compared with control and other concentrations of Se-NPs extenders at all stages of cryopreservation (Table,4). It was observed also, addition of 1.25 µg/ml Se-NPs significantly (P<0.01) improved the post thawed plasma membrane integrity percentage as compared to the control and all Se-NPs extenders, while the differences among control and 0.25, 0.5, 0.75 and 1.0 µg/ml Se-NPs did not significant.

3.4. Acrosome integrity

Means of acrosome integrity percentage of sperm subjected to extender with different concentrations of Se-NPs are presented in Table, 5. In post dilution, supplementation of Se-NPs to semen extender significantly (P<0.05) improved acrosome integrity percentage in comparison with control group, the differences among Se-NPs extenders were not significant except 0.5µg. The percentage of acrosome integrity was dramatically improved by increasing of Se-NPs to semen extenders in both equilibration and post-thawing stages, supplementation of 1.25 µg/ml Se-NPs significantly (P<0.01) improved acrosome integrity % compared with control group and all Se-NPs diluents. While the differences among 0.25, 0.50, 0.75 µg/ml Se-NPs extenders and control were not significant. The best value was obtained in the extender containing 1.25 µg/ml Se-NPs during different stages of cryopreservation.

3.5. Conception rate

The effect of different concentration of Nano-Selenium extenders and Tris–egg yolk extender on

conception rate are presented in Table 6. All semen extended with Se-NPs improved the conception rate except the extender contained 0.25µg/ml Se-NPs compared to control–Tris egg yolk extender. The highest value of conception rate was found in extender contained 1.25 µg/ml Se-NPs (68.2%) compared to control (57.9%).

Table 2: Effect of Nano-Selenium supplementation to semen extender on live spermatozoa (%) of buffalo bulls during different stages of cryopreservation

Items	Control	Nano-Selenium concentrations (µg/ml)					Sig.
		0.25	0.50	0.75	1	1.25	
Post dilution	68.81 ± 0.84	70.67 ± 1.24	69.22± 0.87	68.81± 0.98	70.44 ± 1.24	71.22± 1.41	NS
After equilibration	66.41^{b}±0.79	67.14^{b}±0.84	65.58^{b}±0.85	67.22^{b}±0.87	66.92^{b}±1.03	70.18^{a}± 1.47	*
Post thawing	64.11^{b}±0.87	64.48^{b}±0.66	63.85^{b}±0.87	66.03^{ab}±0.73	64.92^{b} ±0.95	68.19^{a} ±1.34	**

NS= Not significant * = P< 0.05 and **= P< 0.01.

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

Table 3: Effect of Nano-Selenium supplementation to semen extender on abnormal sperm (%) of buffalo bulls during different stages of cryopreservation

Items	Control	Nano-Selenium concentrations (µg/ml)					Sig.
		0.25	0.50	0.75	1	1.25	
Post dilution	11.70^{a} 0.43	11.28^{a} ±0.42	11.93^{a}± 0.40	7.92^{b}± 0.17	8.40^{b}± 0.22	7.78^{b} ± 0.12	**
After equilibration	13.37^{a}± 0.48	13.52^{a} ±0.44	13.50^{a}± 0.44	9.89^{c}± 0.16	10.93^{b} ±0.18	10.48^{bc}±0.13	**
Post thawing	15.96^{a}± 0.61	14.20^{b} ±0.38	15.56^{a}±0.55	12.81^{c} ±0.31	12.74^{c} ±0.30	11.85^{c} ±0.42	**

**= P< 0.01.

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

Table 4: Effect of Nano-Selenium supplementation to semen extender on plasma membrane integrity (%) of buffalo bulls during different stages of cryopreservation

Items	Control	Nano-Selenium concentrations (µg/ml)					Sig.
		0.25	0.50	0.75	1	1.25	
Post dilution	68.19^{c} ± 0.71	69.10^{ab}±0.79	67.59^{c}±0.79	68.11^{bc}±0.82	70.70^{ab}±1.07	71.25^{a}± 1.15	*
After equilibration	66.55^{ab}±0.72	66.18^{b} ±0.62	65.00^{b} ±0.78	68.11^{bc}±0.82	67.26^{ab}±0.90	69.22^{a}± 1.36	*
Post thawing	65.37^{b}± 0.72	64.26^{b}±0.62	64.11^{b} ±0.71	63.74^{b}±0.69	65.07^{b}±0.90	68.23^{a}± 1.36	**

* = P< 0.05 and **= P< 0.01.

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

Table 5: Effect of Nano-Selenium supplementation to semen extender on acrosome integrity (%) of buffalo bulls during different stages of cryopreservation

Items	Control	Nano-Selenium concentrations (µg/ml)					Sig.
		0.25	0.50	0.75	1	1.25	
Post dilution	71.55^{b}± 0.90	73.38^{ab}±1.07	71.18^{b} ±0.89	72.85^{ab}±1.02	74.48^{ab}±1.16	75.22^{a} ± 1.28	*
After equilibration	70.37^{d}± 0.84	68.92^{d}± 0.84	68.42^{d}± 1.01	74.44^{c} ± 0.65	76.89^{b}± 0.68	79.59^{a}± 0.83	**
Post thawing	67.81^{b}± 0.95	67.07^{b} ± 0.85	67.00^{b} ± 0.93	69.30^{b}± 0.64	72.40^{a} ± 0.82	73.80^{a}± 0.73	**

* = P< 0.05 and **= P< 0.01.

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

Table 6: Effect of Nano-Selenium supplementation to semen extender on conception rate of buffalo bulls spermatozoa.

Items	Control	Se-NPs levels (µg/ml)				
		0.25	0.50	0.75	1.0	1.25
No. of inseminated animals	19	16	23	21	33	44
Conceived animals	11	9	15	14	22	30
Conception rate %	57.9	56.3	65.2	66.7	66.7	68.2

4. Discussion

Normal function of mammalian cells is dependent upon maintaining an intricate balance between the production and elimination of reactive oxygen species [39]. Under cryopreservation and thawing conditions, reactive oxygen species (ROS) increases and antioxidants decrease [40]. ROS had negative effect on semen quality and fertility [41]. The balance of the antioxidant defense system affects male fertility in an intricate manner [42].

The findings observed that supplementation of selenium Nano particles in semen extender improved cryopreserved spermatozoa of buffalo bulls spermatozoa [21]. The present results indicated that the extender containing 1.25µg/ml Se-NPs significantly improved the percentages of progressive motility, live sperm, plasma membrane integrity, intact acrosome and decreased sperm abnormality of buffalo spermatozoa in comparison the control egg yolk extender during varies cryopreservation stages. These results are in agreement to that reported by [21] who found that inclusion of SeNPs up to 1.0 µg/ml to Tris-yolk fructose extender significantly improved motility and viable sperm percentage and decreased apoptotic sperm percentage in frozen/thawed bull semen. [43] observed that detrimental effects associated with cryopreservation may be as a result of ROS production causing a series of events that reduce phosphorylation of axonemal protein and result in sperm immobilization. SeNPs positive effects in respect to motility could be attributed to the role of selenium in reducing lipid peroxidation. Additionally, enhancements in sperm motility may be attributed to enhanced antioxidant enzymes including glutathione peroxidase activity suggesting that supplementation of SeNPs could improve the ability of seminal plasma to diminish oxidative stress [21]. In addition, [44] reported positive correlation between sperm quality and selenium concentration in seminal plasma. Cryopreservation exposes spermatozoa to severe osmotic stress [45]. The obtained results of the present study revealed that supplementation of extender of frozen buffalo spermatozoa with SeNPs improved semen characteristics. Effect of Nano- selenium on the reduction of oxidative stress is documented by [46,47] confirmed that the antioxidant properties allow spherical SeNPs. This might be attributed to much smaller size of SeNPs which allow more surface area to react with free radicals and offers plenty of space to absorb oxygen [22]. It has been reported that acrosomal integrity of ram sperm greatly affected by the cryopreservation process specially freezing thawing steps [48]. In the present study, conception rate in buffaloes inseminated with semen cryopreserved in extenders containing different concentrations of SeNPs were higher than control but the best conception rate was obtained in 1.25µg/ml Se-NPs compared to control (68.2 vs 57.5%, respectively).

It could be concluding that supplementation of selenium Nano particles in tris-extender improved semen quality of cryopreserved spermatozoa and fertility of buffalo bulls spermatozoa. It is note that buffalo bull spermatozoa and fertility were the best in extender containing 1.25µg/ml SeNPs.

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