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Impact of adding nano-selenium on the quality of diluted buck semen preserved by cooling during summer and winter

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ABSTRACT

Bucks reproductive performance can be improved by artificial insemination technique, that affected by many factors the most important one is semen quality, which is affected by the methods of semen preservation cooling and freezing. The goal of this study was to assess the effect of adding different concentrations of antioxidant as Nano-selenium to the diluted buck semen during preservation by cooling in both summer and winter seasons. Eight mature healthy Zaraibi bucks were used in the study. Semen samples were collected by an electro- ejaculator twice per week during summer 2021 (August, September) and winter (January, February). Samples were pooled and diluted by extender then divided to experimental groups: Group 1: Nano-selenium: 100 µL /100 ml and Group 2: 200 µL / 100 ml. Then the groups cooled for measuring some semen parameters and seminal antioxidant activities after 1 h from adding and at 8 hours then every 8 hrs up to 64 hr. Nano selenium 200 µL /100 ml has a favorable impact on diluted buck semen during cooling preservation during summer and winter.

Introduction

The goal of semen preservation is extending the sperm life span while keeping its ability to fertilization. This depends on saving the metabolites and preventing their exhaustion, and keeping the sperm in the dormant state, in which spermatozoa utilize the minimum level of metabolism (Noakes *et al*., 2018). Preservation of the semen can occur at room temperature, cooling and freezing. Each type has its specific storage time (El-Rheem *et al*., 2019). Cooling of spermatozoa is the simplest method that can successfully depress spermatozoa metabolic rate and therefore, prolong sperm survival. Cooling is keeping the semen in its liquid states using the refrigerator in 4°C, it is considered cheaper and easier than freezing (Chenoweth and Lorton, 2014). The cooling rate for each animal depends mainly on studying the sperm cell membrane component and seminal plasma composition to be efficient and to avoid the deleterious effect on both the physical and chemical cellular condition. In modern farms breeding, where AI is widely applied as a tool facilitating extensive utilization of processed semen from genetically superior sires both cooled and frozen.

Semen is extend the time span of the viability of spermatozoa, their metabolic rate must be slowed down thereby reducing the rate at which substrates are used and toxins are produced (Batellier *et al*., 2010). Though chilling semen provides an efficient and successful means of short-term storage, it has yet some adverse effects on the spermatozoa manifested as a depression in viability rate, cooled shock, oxidation, depressed motility, and conception rates (Noakes *et al*., 2018). Antioxidant is defined as substances that can prevent or slow damage to cells caused by free radicals, unstable molecules and to protect the sperm cell from oxidation, there is different ways mostly depend on improving the cellular antioxidant mechanism or delaying the oxidation. The cellular mechanism to control the detrimental effect of reactive oxygen species (ROS) can be by enzymatic and non-enzymatic way (John Aitken *et al*., 2011). The enzymatic antioxidant defense mechanism includes many enzymes such as superoxide dismutase (SOD) and catalase (CAT), the non-enzymatic include many minerals as selenium and zinc oxide and vitamins as vitamin C that act as co-stimulant for these enzymes and some of them act as scavengers of ROS. The aim of this work was to evaluate the effect of adding different concentrations of Nano-particles form as Nano-selenium as antioxidants to diluted buck semen for improving semen quality during preservation by cooling.

Materials and methods

This study was performed at the Theriogenology Department farm of Faculty of Veterinary Medicine at Alexandria University, Alexandria, Egypt. This study was approved by Alexandria university's institutional Animal Care and Use Committee (permit #2021/89). Mature healthy Zaraibi bucks were used in the study. Eight bucks with average age of 3 years and their weights more than 28 Kg were subjected to study. They were kept in an open yard system separated from the females and received a good balanced ration, in addition to mineral blocks and vitamins supplementation, with water ad libitum. Samples of semen were collected by using Electro ejaculator twice weekly during summer 2021 (August, September) and winter 2022 (January, February). Immediately after collection each ejaculate was transferred to a water bath maintained at 27°C prior to evaluation.

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Semen evaluation

Gross examination

Semen Volume was calculated using graduated tubes with normal thick creamy or milky consistency and grayish white color.

Microscopic examination

Sperm motility

The sperm motility was estimated subjectively by using ordinary light microscope (Olympus CKX 41; Olympus Optical Co., Tokyo, Japan) at 40x magnification.

Sperm cell concentration

It was evaluated by using a hemocytometer slide. Semen was diluted, with 2.9% sod. citrates dehydrate solution at rate of 1:200.

Sperm livability

Viability of the semen samples were evaluated by means of Nigrosine-Eosin staining.

Sperm acrosomal integrity

It was evaluated using Giemsa stain according to method of Watson (2010).

Plasma membrane integrity

It was evaluated using Hypo-Osmotic Swelling test (HOST). The hypo-osmotic solution (125mOsm/1) was prepared according to method of Fonseca *et al*. (2018).

Enzymatic examination

CAT, SOD and MDA colorimetric assay: This procedure was carried out according to the protocol of Bio-diagnostic Company, Cairo, Egypt.

The requirement of the extenders

Buffer composed of 4.543 g (375 mM) tris (hydroxymethyl aminomethane, lobe chemie Pvt. Ltd., India), 2.382 g (124 mM) and citric acid (Loba Chemie PvT .Ltd., India).
Sugar: 0.75 g (41 mM) Glucose (D-Glucose, Chemjet, Egypt)
Phospholipoprotein: 2.5% egg yolk of the all the diluent. By cleaning and sterilizing the surface of chicken eggs and cracking the surface in half then discard albumin. Removing the reminder of albumin by 12 cm diameter filter paper. The intact egg yolk was then punctured, and the internal yolk was allowed to flow in a sterile beaker.
Antibiotics: 500 µl of Gentamicin 5% (aniMedica GmbH, Germany)

Nanoantioxidants diluents with different concentration of Nano-selenium (Nano selenium, Nano Gate , Egypt): group 1: Nano-selenium 100 µL / 100 ml and group 2: 200 µL / 100 ml. antioxidant according to each diluent was added and mixed with a gentle swirling motion until mixture appears homogenous then mixture was poured through a funnel fitted with a folded milk filter (Ø230 mm, Hygia Favorit, Universal Dairies GmbH, Germany) to remove any clumps. The extenders were stored at 5°C.

Dilution and cooling

Semen samples were collected in different falcon tubes and pooled into one falcon tube to eliminate the buck effect then diluted at rate of 1:10, the semen parameters as motility, viability, acrosomal integrity, cell membrane integrity, enzymatic CAT, SOD and MDA were assessed as a control sample then the diluted semen were fractionated with different concentrations of Nano-selenium at different tubes. The tubes containing antioxidants diluted semen were divided into Eppendorf tubes with 1 ml capacity then placed in a glass beaker containing 200 ml water which transferred to a refrigerator (5°C) to achieve steady cooling rate, stored and examined after 1, 8, 16, 24, 32, 40, 48, 56 and 64 h from cooling.

Statistical analysis

Data were analyzed by two-way analysis of variance (ANOVA) using SAS (2009). Duncan's multiple range test of the same software was used to compare treatment mean.

Results

The effect of adding different concentrations of Nano selenium on diluted semen parameters in both summer and winter during preservation by cooling

Nano selenium groups and the control in both summer and winter, there were no significance difference ($p<0.05$) in the sperms progressive motility, livability, acrosome integrity and cell membrane integrity between 0h and 1h, but there were a significant decrease in semen parameters ($p<0.05$) from 1h till 56 h. in both summer and winter. At 1 h there was no significance difference in semen parameters ($p<0.05$) among Nano selenium groups and the control. In both summer and winter from 8 h till 56 h Nano selenium 200 µL was significantly higher than Nano selenium 100 µL and Nano selenium 100 µL was significantly higher in semen parameters than the control.

Effect of adding different concentrations of Nano-selenium on MDA, SOD and CAT of diluted buck semen in both summer and winter

Nano selenium groups showed a significant increase in seminal MDA from 0 h till 24 h but at 48 h the Nano-selenium 100 µL group was significantly ($p<0.05$) decreased than all groups during summer and winter seasons. The control group showed no significant difference between 0h and 1h and showed a significant decrease at 24 h in both summer and winter. From 1 h till 48 h Nano selenium 200 µL was highly significant more than Nano selenium 100 µL and the control. Nano selenium groups showed a significant ($p<0.05$) decrease in seminal SOD at 1 h but the control group showed no significance difference between 0h and 1h during summer and winter seasons. At 48 h the SOD was significantly increased at Nano-selenium groups in both seasons. Both Nano selenium groups had no significant difference of SOD concentrations during 1 h, 24 h and 48 h. Nano selenium groups showed a significant increase in seminal CAT at 1 h and 24 h in both summer and winter. Nano selenium 200 µL group was highly significant ($p<0.05$) more than Nano selenium 100 µL and the control from 1 h to 48 h (Table 2).

Discussion

Selenium is an essential and vital trace element of mammalian cells, which influences important physiological functions (Lukusa, 2019). This kind of Nano-particles can modify the expression of many seleno-proteins such as (glutathione peroxidase) GPXs, which have the potential to regulate the physiological cell functions by acting as antioxidants (Boroumand *et al*., 2019). In this study, the results indicate that adding antioxidant Nano selenium 200 µL/100 ml diluent to the buck semen during short term preservation by cooling is beneficial for improving the semen parameters as Motility, livability, acrosomal integrity and cell membrane integrity and improve the seminal antioxidants enzymes as SOD and CAT. The best-known role of selenium as antioxidant is attributed to its pres

Table 1. The effect of adding different concentrations of Nano selenium on diluted semen parameters in both summer and winter during preservation by cooling.

	Season																			
	Summer										winter									
	0 h	1 h	8 h	16 h	24 h	32 h	40 h	48 h	56 h	64 h	0 h	1 h	8 h	16 h	24 h	32 h	40 h	48 h	56 h	64 h
Motility %																				
Nano Selenium 100 µL / 100 ml		86.65±1.12^{Aa}	76.65±1.25^{Bb}	64.58±1.54^{Cb}	53.12±1.69^{Db}	41.25±1.78^{Eb}	27.15±1.87^{Fb}	18.78±1.24^{Gb}	12.25±1.50^{Hb}	*		88.98±1.74^{Aa}	85.78±1.78^{Ba}	70.54±1.98^{Cb}	60.54±1.85^{Db}	50.78±1.78^{Eb}	38.87±1.51^{Fb}	30.87±1.85^{Gb}	17.85±1.80^{Hb}	*
Nano Selenium 200 µL / 100 ml	87.20±1.60^{A}	86.30±1.25^{Aa}	83.54±1.45^{Ba}	73.36±1.54^{Ca}	63.36±1.87^{Da}	53.35±1.24^{Ea}	43.36±1.87^{Fa}	30.25±1.87^{Ga}	21.69±1.45^{Ha}	11.54±1.54^{I}	90.50±1.87^{A}	89.57±1.68^{Aa}	85.74±1.98^{Ba}	76.87±1.78^{Ca}	68.87±1.73^{Da}	55.98±1.45^{Ea}	45.87±1.78^{Fa}	35.98±1.78^{Ga}	22.52±1.87^{Ha}	13.57±1.57^{I}
Control		85.58±1.49^{Aa}	60.25±1.69^{Bc}	35.30±1.69^{Cc}	25.58±1.69^{Dc}	14.69±1.87^{Ec}	*	*	*			88.39±1.78^{Aa}	62.54±1.36^{Bb}	40.36±1.10^{Cc}	28.57±1.85^{Dc}	17.8±1.87^{Ec}	*	*	*	*
Livability %																				
Nano Selenium 100 µL / 100 ml		90.23±1.12^{Aa}	80.78±1.83^{Bb}	67.58±1.24^{Cb}	55.25±1.69^{Db}	44.68±1.85^{Eb}	29.75±1.85^{Fb}	21.58±1.25^{Gb}	13.58±1.47^{Hb}	*		94.58±1.47^{Aa}	85.65±1.78^{Bb}	72.69±1.29^{Cb}	64.58±1.78^{Db}	55.25±1.25^{Eb}	45.87±1.69^{Fb}	33.36±1.58^{Gb}	18.87±1.40^{Hb}	*
Nano Selenium 200 µL / 100 ml	91.81±1.80^{A}	89.45±1.96^{Aa}	84.57±1.74^{Ba}	71.25±0.98^{Ca}	64.61±1.61^{Da}	54.20±1.37^{Ea}	45.69±1.69^{Fa}	32.36±1.85^{Ga}	24.98±1.36^{Ha}	13.25±1.36^{I}	96.58±1.87^{A}	95.57±1.18^{Aa}	89.65±1.78^{Ba}	77.87±1.65^{Ca}	69.64±1.58^{Da}	62.58±1.47^{Ea}	52.65±1.78^{Fa}	39.57±1.87^{Ga}	28.39±1.55^{Ha}	14.58±1.80^{I}
Control		89.56±1.87^{Aa}	67.88±1.75^{Bc}	40.15±1.58^{Cc}	30.25±1.78^{Dc}	17.66±1.87^{Ec}	*	*	*	*		94.69±0.53^{Aa}	70.50±1.70^{Bc}	54.55±1.82^{Cc}	40.58±0.40^{Dc}	19.87±1.87^{Ec}	*	*	*	*
Acrosome integrity %																				
Nano Selenium 100 µL / 100 ml		93.25±1.25^{Aa}	84.58±1.69^{Bb}	70.58±1.64^{Cb}	57.98±1.85^{Db}	40.64±1.36^{Eb}	32.25±1.74^{Fb}	25.85±1.78^{Gb}	14.58±1.20^{Hb}	*		94.58±1.45^{Aa}	82.47±1.12^{Ba}	76.87±1.69^{Cb}	68.47±1.36^{Db}	61.87±1.12^{Eb}	48.54±1.98^{Fb}	35.87±1.69^{Gb}	22.58±1.52^{Hb}	*
Nano Selenium 200 µL / 100 ml	95.87±1.88^{A}	94.58±1.69^{Aa}	88.87±1.57^{Ba}	74.69±1.98^{Ca}	67.98±1.64^{Da}	47.98±1.35^{Ea}	48.52±1.30^{Fa}	34.82±1.67^{Ga}	27.61±1.33^{Ha}	16.45±1.58^{I}	95.68±1.58^{A}	93.36±1.58^{Aa}	91.58±1.69^{Aa}	81.57±1.11^{Ba}	73.98±1.87^{Ca}	65.81±1.54^{Da}	56.98±1.17^{Ea}	42.69±1.15^{Fa}	34.87±1.19^{Ga}	17.25±1.69^{H}
Control		94.56±1.69^{Aa}	67.88±1.54^{Bc}	47.21±1.58^{Cc}	31.66±1.80^{Dc}	20.87±1.58^{Ec}	*	*	*	*		92.57±1.20^{Aa}	63.57±1.87^{B}	56.33±1.12^{Cc}	42.33±0.31^{Dc}	22.24±1.40^{Ec}	*	*	*	*
Cell membrane integrity %																				
Nano Selenium 100 µL / 100 ml		90.20±1.57^{Aa}	79.65±1.87^{Ba}	66.85±1.36^{Cb}	54.69±1.88^{Db}	37.87±1.30^{Eb}	28.68±1.47^{Fb}	20.22±1.58^{Gb}	9.24±1.58^{Hb}	*		91.15±1.36^{Aa}	83.36±1.57^{Bb}	70.90±1.36^{Cb}	61.25±1.57^{Db}	50.36±1.57^{Eb}	38.69±1.87^{Fb}	28.14±1.69^{Gb}	18.58±1.47^{Hb}	*
Nano Selenium 200 µL / 100 ml	91.36±1.25^{A}	90.36±1.70^{Aa}	78.25±1.45^{Ba}	72.65±1.77^{Ca}	59.98±1.77^{Da}	44.69±1.87^{Ea}	33.87±1.33^{Fa}	25.58±1.65^{Ga}	13.58±1.47^{Ha}	18.74±1.58^{I}	94.87±1.40^{A}	93.74±1.65^{Aa}	87.98±1.47^{Ba}	75.84±1.22^{Ca}	66.25±1.58^{Da}	55.36±1.57^{Ea}	45.87±1.97^{Fa}	33.69±1.20^{Ga}	25.90±1.57^{Ha}	14.58±1.58^{I}
Control		89.98±1.25^{Aa}	66.30±1.69^{Bb}	46.36±1.95^{Cc}	26.33±1.30^{Dc}	19.57±1.58^{Ec}	*	*	*	*		93.54±1.87^{Aa}	64.29±1.11^{Bc}	48.97±1.36^{Cc}	28.98±1.98^{Dc}	23.64±1.78^{Ec}	*	*	*	*

Mean ± SEM in same row with different capital letters are significantly different ($p<0.05$); Mean ± SEM in same column with different small letters are significantly different ($p<0.05$); Dots (-) indicate unmeasured.

Table 2. Effect of adding different concentrations of Nano-selenium on MDA, SOD and CAT of diluted buck semen in both summer and winter

	Season							
	Summer				Winter			
	0 h	1 h	24 h	48 h	0 h	1 h	24 h	48 h
MDA nmol / ml								
Nano Selenium 100 µL / 100 ml		8.10 ± 0.025^{Bb}	6.80 ± 0.031^{Cb}	4.54 ± 0.047^{Db}		8.55 ± 0.047^{Ba}	7.14 ± 0.50^{Cb}	5.14 ± 0.036^{Db}
Nano Selenium 200 µL / 100 ml	6.41 ± 0.087^{A}	11.25 ± 0.078^{Ba}	8.75 ± 0.028^{Ca}	6.60 ± 0.087^{Da}	6.85 ± 0.025^{A}	11.40 ± 0.029^{Ba}	8.81 ± 0.022^{Ca}	7.75 ± 0.044^{Da}
Control		6.33 ± 0.070^{Ac}	5.31 ± 0.048^{Bc}	*		6.87 ± 0.027^{Ac}	4.78 ± 0.087^{Bc}	*
SOD U/ml								
Nano Selenium 100 µL / 100 ml		39.54 ± 0.27^{Bb}	51.67 ± 0.39^{Cb}	63.85 ± 0.75^{Da}		56.50 ± 0.47^{Bb}	63.32 ± 0.41^{Cb}	71.87 ± 0.98^{Da}
Nano Selenium 200 µL / 100 ml	55.20 ± 0.36^{A}	37.85 ± 0.74^{Bb}	48.10 ± 0.20^{Cb}	60.52 ± 0.78^{Da}	60.47 ± 0.87^{A}	54.14 ± 0.78^{Bb}	60.35 ± 0.14^{Cb}	68.57 ± 0.44^{Da}
Control		55.87 ± 0.36^{Aa}	66.54 ± 0.78^{Ba}	*		63.47 ± 0.97^{Aa}	65.87 ± 0.21^{Ba}	*
CAT U/L								
Nano Selenium 100 µL / 100 ml		2.805 ± 0.045^{Bb}	2.392 ± 0.087^{Cb}	1.785 ± 0.047^{Db}		3.361 ± 0.036^{Bb}	3.181 ± 0.073^{Cb}	2.849 ± 0.087^{Db}
Nano Selenium 200 µL / 100 ml	1.751 ± 0.025^{A}	2.980 ± 0.046^{Ba}	2.580 ± 0.074^{Ca}	1.867 ± 0.047^{Da}	1.951 ± 0.025^{A}	3.384 ± 0.074^{Ba}	3.272 ± 0.088^{Ca}	2.902 ± 0.075^{Da}
Control		1.438 ± 0.044^{Bc}	1.899 ± 0.077^{Cc}	*		1.981 ± 0.033^{Bc}	2.283 ± 0.022^{Cc}	*

Mean ± SEM in same row with different capital letters are significantly different ($p<0.05$); Mean ± SEM in same column with different small letters are significantly different ($p<0.05$); Dots (-) indicate unmeasured.

ence in GPx and Seleno-proteins (Aziz *et al*., 2019) which plays a role in the antioxidant defense system of sperm against ROS that induced by the freezing and thawing process. Nateq *et al.* (2020) demonstrated the same finding in which 2 µg/ml Nano-Selenium significantly improve the quality of semen and decreased acrosome membrane damaged and abnormal sperms compared to 1 µg/ml Nano-Selenium and control group. Khalil *et al.* (2019) said that enrichment of semen extender with Nano selenium at a concentration of 1.0 µg/ml improved post-thaw sperm quality of Holstein bulls, and consequently reducing lipid peroxidation and sperm damage occurring by cryopreservation. Hozyen *et al.* (2019) demonstrated that the invitro Nano selenium supplementation minimized the freeze thaw induced damage to ram spermatozoa 2 and 3 µg/ml selenium and improved seminal parameters. The addition of selenium and its Nano form play important role in improving the enzymatic antioxidants that control the ROS produced from lipid peroxidation of the spermatozoa cell membranes, they activate the GSP and CAT so reduce the oxidative stress and improve the semen quality (Aziz *et al*., 2019). Moreover, Khalil *et al*. (2019) demonstrated that supplementation of bull semen extender with Nano selenium at different levels was evaluated for inhibiting the harmful effect of ROS production, which destroy sperm cells, particular plasma membrane during freezing process due to the activation of GSP of the sperms in defensing against the ROS produced. Also Nateq *et al.* (2020) demonstrated that semen enrichment with Nano selenium would decrease spermatic membrane lipid peroxidation and oxidative damage to sperm. Also decrease sperm acrosome membrane damage and subsequently sperm quality properties increase such as motility, viability, plasma membrane integrity and during freezing.

Conclusion

In this study, the results indicate that adding Nano selenium 200 µL /100 ml diluent to the buck semen during short term preservation by cooling is beneficial for improving the semen parameters as motility, livability, acrosomal integrity and cell membrane integrity and improve the seminal antioxidants enzymes as SOD and CAT during summer and winter seasons.

Conflict of interest

The authors declare that they have no conflict of interest.

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