Post-thaw sperm morphokinetics and mitochondrial genes profile of buck semen supplemented with selenium in two different forms

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Background
The quality characteristics of semen is a determinant factor for improving fertility of rabbit.
Objectives
The current investigation was done to evaluate post-thaw sperm morphokinetics and mitochondrial genes expression profile of buck semen supplemented with selenium in two different forms.
Materials and methods
Sexually mature bucks (n=16) that aged three months on average were used for collecting two ejaculates which were evaluated using computer assisted semen analysis (CASA) program. The semen samples of all experimental animals which recorded progressive motility greater than or equal to 70% were pooled for cryopreservation. The pooled semen was divided into three groups that were supplemented with selenium nanoparticles and selenium in normal form in addition to the control group. Morphological characteristics as well as CASA parameters were assessed after freezing for 1 week. Moreover, enzymatic activity assays were performed to measure the antioxidant capacity of cryopreserved buck semen. Transcriptional profile of mitochondrial activity and antioxidant defense regulating genes was conducted using quantitative real-time polymerase chain reaction (PCR).
Results and conclusion
The addition of selenium in normal and Nano forms has significantly ($P \leq 0.05$) enhanced some of CASA parameters such as DCL (μm), DAP (μm), DSL (μm), VCL (μm/s), VAP (μm/s), Amplitude of lateral head (ALH) (μ), BCF (Hz), and VSL (μm/s) during prefreezing period compared with control group. Moreover, the data presented in the present study indicated a significant ($P \leq 0.05$) improvement of post-thaw total and progressive sperm motility in the two groups supplemented with normal and Nano compared with control group. The post-thaw level of total antioxidant capacity (TAC) and percentage of live sperm were higher in the two groups supplemented with selenium and Nano selenium than the control group. The expression profile of candidate genes regulating mitochondrial activity (ATP Synthase F1 Subunit Alpha (*ATP5A1)*, NADH dehydrogenase subunit1 (*ND1)*, NADH dehydrogenase subunit2 (*ND2),* and Carnitine palmitoyltransferase (*CPT2)*) was increased significantly ($P \leq 0.05$) in semen supplemented with selenium in normal and Nano forms compared with the control group. In conclusion, the data of this investigation demonstrated enhancement of CASA parameters during pre-freezing post thaw total and progressive sperm motility in the two groups supplemented with normal and nano selenium. The proportion of sperm viability and the level of total antioxidant capacity were enhanced in the two groups supplemented with selenium which was coupled with up-regulation of mitochondrial transcripts. It seems the both two forms shared the same mechanism on improving post-thaw physical and molecular qualities of rabbit sperm.

Keywords:
genes, mitochondria, motility, rabbit, selenium, spermatozoa

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Introduction

Semen freezing is a good biotechnology tool to preserve rabbit strains under extinction and individuals with good productive genetic merit in addition to reduce inbreeding depression [1]. However, post thaw semen

biophysical traits of rabbit may deteriorate due to either structural or chemical damage such as lipid peroxidation. Indeed, the freezing procedure may form ice crystals that could deform the sperm structure [2–4].

In addition, rabbit semen is well-known to have a rich content of polyunsaturated fatty acids that subsequently led to lipid peroxidation and harmful level of reactive oxygen species(ROS) that induce intracellular oxidative stress [5–9].In addition, it is well-established that the antioxidant cytoplasmic content of mature sperm is low which increase susceptibility lipid peroxidation during freezing [10,11].

Oxidative stress is a harmful condition to sperm which occurs when the level of the intracellular oxidants exceeds the capacity of endogenous antioxidants to scavenge [12]. However, normal physiological level of free radicals are supporting sperm capacitation, and acrosome reaction to get fertilizing ability [13]. On the other hand, high intracellular sperm ROS cause harmful damage to during cryopreservation that reduced its fertilizing capacity [13]. Therefore, supplementation of different antioxidants was proposed to be added to semen extender during cryopreservation in order to alleviate the negative effects of oxidative stress [14]. Indeed, several investigations have confirmed the positive action of antioxidant supplementation on rabbit physiochemical semen quality. The supplementation of glutamine [5], vitamin E [6], amino acid such as cysteine [7], sugar such as trehalas [8] and hormone such as melatonin [9], were investigated in several studies which concluded that antioxidants are vital to semen extender to reduce incident of oxidative stress and enhancing post-thaw rabbit semen quality. A recent study by our research group has also supported the beneficial actions of cysteine, melatonin and L-carnitine on physical and biochemical characteristics of rabbit semen [15].

The antioxidant activity of selenium is dependent on its presence as constituent in selenoprotein such as glutathione peroxidase and thioredoxin reductase well know antioxidant enzymes markers of alleviating oxidative stress [16]. Recently, addition of trace minerals such as Se, Zn, Cu to *in vitro* fertilization medium has elevated the number of spermatozoa bound to zonal pellucida confirming crucial role of in acquisition of fertilizing potentiality [17]. However, there is no investigation has compared between the Nano and normal form of these minerals except one that done in camel semen [18].

Therefore, the current study was performed to compare the effect of supplementing selenium either in normal or Nano form on rabbit semen physical, viability rate, level of total antioxidant capacity level, and molecular characteristic of mitochondrial genes.

Materials and methods

Experimental design

The current study was done to compare the effect of supplementing selenium in Nano and normal forms on rabbit semen post thaw quality traits. Therefore, supplementation selenium to the extender of buck semen was done according to recommended concentration 2 μg/ml [19]. Semen was collected from 16 sexually mature bucks and was directly evaluated individually and only ejaculates that had greater than or equal to 70% progressive motility were continued for cryopreservation. These good quality freshly collected were pooled and divided into three biological aliquots and diluted at 20 : 280 (v/v) with rabbit semen extender supplemented with selenium in Nano and normal in addition to control group. The post thaw physical semen traits were measured in addition to molecular profile of genes regulating semen quality was performed using quantitative real-time polymerase chain reaction (PCR).

Management of experimental animals

The current investigation was done at the animal physiology laboratory, Cairo University, Faculty of Agriculture, Animal Production Department, Giza, Egypt, from December 2019 to April 2020. The Institutional Committee of Animal Care and Use, Cairo University (CU-IACUC) has accepted to conduct this investigation (CU-IIF-2722).

The present study has used 16 healthy and sexually mature New Zealand white rabbit males at 6 months of age. The animals have body weight of 3.8 kg at the beginning of the experiment. The bucks were housed in rabbit metal cages individually. The bucks were fed standard pellet (protein 20%) which provide all body nutrient requirements in addition water was available all the time.

Sperm motion characteristics (morpho kinetics)

The semen samples of all experimental groups were stored in liquid nitrogen container at −196°C for 1 week. The frozen samples were thawed by putting the straws in a water bath at 40°C for 15 s. The motion characteristics of semen samples were evaluated using CASA program in 8 biological replicates. The sperm motion parameters of CASA parameters such as: average distance curved line (DCL μm), distance

path (DAP μm), distance straight line (DSL μm), velocity curved line (VCL μm/sec), average velocity path (VAP μm/sec), straightness (STR=VSL/VAP), straight velocity line (VSL μm/sec), beat cross frequency (BCF Hz), linearity (LIN=VSL/VCL), the wobble (WOB= VAP/VCL), and amplitude of lateral head (ALH μm).

The percentage of live and dead spermatozoa

The evaluation of sperm viability of all experimental groups was done by thawing semen straws of all groups in a water bath at 45°C for 15 s then a drop of semen was smeared on a clean prewarmed (37°C) glass slide. The smeared semen sample was stained with Eosin-Nigrosin until dried out at room temperature. The stained slide was evaluated under an inverted microscope using an oil-immersed lens. The proportion of sperm viability was calculated by counting number of live sperm from a total of 200 spermatozoa that evaluated in 8 microscopic fields of each experimental group.

The mesurement of total antioxidant capacity (TAC)

Six frozen straws of each group were warmed at 37°C for 30 s and pooled in 1.5 ml Eppendorf tube then washed three times with 1 ml of dulbecco's phosphate saline (PBS) followed each time with centrifugation at 1030xg for 10 min at 4°C. The pellet of each sample was suspended in 1 ml of distilled. The assay of total antioxidant capacity (TAC) was done using commercial kits (Sunostk SBA 733 plus, Bio diagnostic – Egypt) according to manufacture procedure and finally measured with spectrophotometer at 505 nm wavelength.

Ribonucleic acid (RNA) isolation

RNA extraction was done from three biological replicates of four pooled straws of each experimental group. The semen samples were centrifuged at 1030 xg at 4°C for 12 min to get the pellet which was washed with PBS two times followed by centrifugation at 1030 xg at 4°C for 5 min. The extraction of RNA was done by applying the GeneJET RNA isolation kits manufacture instructions (Thermofisher Scientific, Vilnius, Lithuania). The lysis buffer was prepared by adding B-mercaptoethanol (20 uL per each 1 ml). The semen pellet was suspended with 600 μl of lysis buffer and mixed by vortex for 10 s at room temperature then centrifuged at 14000 xg for 5 min. The supernatant was directly pipetted into a new tube and 360 μl of absolute ethanol (100%) was added to each sample tube and mixed well by pipetting. A volume of 700 μl of lysate was transferred into the GeneJET RNA purification columns inserted in collection tubes followed by centrifugation for at greater than or equal to 12000 xg1 min. The flow-through was discarded, and the purification column was placed back into the collection tube. The purification columns were washed twice with 600 μl of both wash buffer 1 and wash buffer 2 provided with the kit. The elution of RNA was performed by adding 30 μl of nuclease-free water directly into the purification column followed by centrifugation for 1 min at greater than or equal to 12000 xg. The digestion of DNA contaminant was performed by applying the DNase I enzyme according to the protocol provided by the company (Thermo Scientific, California, USA). The concentration and purity of extracted RNA was recorded using Nano-drop 2000C instrument (Thermo Scientific, Wilmington, DE, USA). The samples were stored at −80°C until cDNA synthesis.

The reverse transcription of RNA

The reverse transcription reaction was done according to manufacturer instructions of the kit (Thermo Scientific, California, USA). The final amount of RNA of all experimental samples was modified in order to get the same value. The reverse transcription reaction was done by adding the following for each sample: 1 μl of oligo dt18 primer, 2 μl of dNTPs, 4 μl of reaction buffer, 1 μl of reverse transcriptase, and 1 μl of RNase inhibitor and 11 μl of each RNA sample that mixed well by pipetting. The reverse transcription reaction was incubated at 42°C for

Table 1 The list of the forward and reverse primers that used for Q-polymerase chain reaction analysis

Gene Name	Gene bank accession number	Primer sequence	Fragment size (bp)
ATP5F1A	XM_002713512.3	F: 5′-CTCTTGAGTCGTGGTGTGCG-3′ R: 5′-CCTGATGTTGGCTGATAACGTG-3′	184
CPT2	XM_017346301.1	F: 5'-CCGAGTATAATGACCAGCTC-3' R: 5'-GCGTATGAATCTCTTGAAGG-3'	152
ND1	XM_051849998.1	F: 5'-ACTAATGGGCACAAGGAAAA-3' R: 5'-TCAATGTTCTCCAAACCCTT-3'	122
ND2	AJ012536.1	F: 5'-ATTAATCCACAGCTCAGCAA-3' R: 5'-ATGAGTGTTGGCATGATGAT-3'	192
GAPDH	NM_001082253.1	F: 5'-AGGTCGGAGTGAACGGATTC-3' R: 5'-GGAAGATGGTGATGGCCTTT-3'	219

Abbreviations: bp, base pair; PCR, polymerase chain reaction.

60 min, then for 5 min at 70°C, and finally cooled at 4°C. The reaction was done in a thermocycler instrument (Thermo Scientific, California, USA). The cDNA product of all samples was stored at −20°C until the Quantitative real-time PCR.

Real-time polymerase chain reaction

The primers that target the genome sequence of rabbits (*Oryctolagus cuniculus*) were designed (Table 1) from the GenBank database of gene sequences (www.ncbi.nlm.nih.gov). The Primer3 program (http://primer3.wi.mit.edu//) was used for primer designing of all genes. The real-time PCR reaction was done using a Step OnePlus instrument (Applied Biosystems, California, USA). The PCR reaction was composed of 12 µl of SYBR Green Master Mix (Thermofisher Scientific, California, USA), 0.2 µl forward primer, 0.2 µl reverse primer, 5.6 µl molecular biology grade water, and 2.0 µl cDNA. The PCR settings were as follows: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C, 20 sec at 60°C, and 30 s at 72°C and finally annealing for 1 min at 60°C. The method of delta Ct was used to analyze the data of expression profile after normalization of the target genes (ATP Synthase F1 Subunit Alpha (*ATP5A1)*, NADH dehydrogenase subunit1 (*ND1)*, NADH dehydrogenase subunit2 (*ND2),* and Carnitine palmitoyltransferase (*CPT2)*) to the housekeeping gene (*GAPDH*) according to our previous publication.

Statistical data analysis

The data of this investigation was analyzed using one-way analysis of variance and was expressed as Mean ±SEM. In this regard, SAS software package was applied using the general linear model (v. 9.3, SAS Inst. In., Cary, NC, USA, 2011). The main effect was treatment (S, NS and Control). The traits examined were total motility, progressive motility, CASA parameters such as DCL (µm), DAP (µm), VAP (µm/s), DSL (µm), VSL (µm/s), VCL (µm/s), LIN %, STR %, ALH (µ), BCF (Hz), and WOB (µm/s). The following model was used:

$$Y_{jk} = \mu + T_j + e_{ijk}$$

Where:

µ: The overall mean

T j: The effect of the j th treatment group.

e jk: Random error.

Results

Semen physical quality parameters

The addition of selenium in normal and nano forms has significantly ($P \leq 0.05$) enhanced the sum of CASA parameters such as DCL (µm), DAP (µm), VAP (µm/s), DSL (µm), VSL (µm/s), ALH (µ), BCF (Hz), and VCL (µm/s), during prefreezing period compared with control group as presented in Table 2. However, there was no significant effect of supplementing the two forms of selenium on total and progressive motility in addition to other CASA parameters such as STR %, LIN %, and WOB (µm/s) as shown in Table 2.

The data presented in the present study indicated a significant ($P \leq 0.05$) improvement of post-thaw total and progressive sperm motility in the two groups supplemented with normal and nano compared with control group (Table 3). On contrast, the data of this investigation demonstrated no significant influence of selenium supplementation of both two forms on all CASA paramours at post-thaw evaluation as presented in Table 2.

Table 2 Precryopreservation semen physical quality parameters and computer assisted semen analysis parameters of buck semen supplemented with two different forms of selenium

	Pre freezing			
Trait	C Mean±SE	NS Mean±SE	S Mean±SE	*P**value of F test
Total Motility (%)	71.34[a]±1.6	75.16[a]±1.63	72.48[a]±1.62	0.2354
Progressive motility (%)	58.76 [a]±2.17	62.04[a]±2.22	59.68 [a]±2.19	0.5565
DAP (µm)	28.42[b]±0.65	32.85 [a]±0.67	32.19 [a]±0.66	<0.0001**
DCL (µm)	59.18 [b]±1.12	68.94 [a]±1.15	68.70 [a]±1.14	<0.0001**
DSL (µm)	21.74 [b]±0.60	24.41 [a]±0.6	24.38 [a]±0.6	0.0017**
VAP (µm/s)	62.83 [b]±1.4	73.25 [a]±1.43	70.49 [a]±1.41	<0.0001**
VCL (µm/s)	130.27 [b]±2.35	153.06 [a]±2.4	149.68 [a]±2.37	<0.0001**
VSL (µm/s)	48.06 [b]±1.25	54.64 [a]±1.27	53.60 [a]±1.26	0.0005**
STR %	0.76 [a]±0.01	0.74 [a]±0.01	0.76 [a]±0.01	0.0889
LIN %	0.36 [a]±0.005	0.35 [a]±0.01	0.35 [a]±0.01	0.2387
WOB (VAP/VCL) (µm/s)	0.48 [a]±0.005	0.47 [a]±0.005	0.46 [a]±0.005	0.1901
ALH (µ)	4.32±0.09	4.83 [a]±0.09	4.29 [b]±0.09	<0.0001**
BCF (Hz)	31.40 [b]±0.60	31.48 [b]±0.6	33.77 [a]±06	0.0059**

Table 3 Postcryopreservation semen physical quality parameters and computer assisted semen analysis parameters of buck semen supplemented with two different forms of selenium

	Post freezing			
Traits	C	NS	S	*P*-value
Total Motility (%)	39.78 [b]±1.86	44.35 [ab]±1.98	46.96[a]±2.02	0.0310*
Progressive motility (%)	22.47 [b]±1.49	25.96[ab]±1.56	29.21 [a]±1.62	0.0103*
DAP (μm)	23.39 [a]±0.87	21.37 [a]±0.92	22.62 [a]±0.94	0.2822
DCL (μm)	51.62 [a]±2.49	46.47 [a]±2.66	49.22 [a]±2.71	0.3723
DSL (μm)	16.15 [a]±0.56	14.95 [a]±0.6	15.54 [a]±0.61	0.3495
VAP (μm/s)	53.14 [a]±1.89	48.35 [a]±2.01	52.82 [a]±2.05	0.1685
VCL (μm/s)	115.78 [a]±5.53	103.64 [a]±5.9	114.13[a]±6.01	0.2801
VSL (μm/s)	36.85 [a]±1.28	34.08 [a]±1.36	36.73 [a]±1.39	0.2659
STR %	0.69 [a]±0.01	0.71 [a]±0.01	0.70 [a]±0.01	0.5687
LIN %	0.32 [a]±0.01	0.35 [a]±0.01	0.34 [a]±0.01	0.2101
WOB (VAP/VCL) (μm/s)	0.47 [a]±0.01	0.49 [a]±0.01	0.48 [a]±0.01	0.2149
ALH (μ)	4.98 [a]±0.16	4.46[b]±0.17	4.69 [ab]±0.17	0.0841
BCF (Hz)	22.63 [a]±0.69	20.68 [a]±0.73	22.10 [a]±0.75	0.1430

Interestingly, all studied parameters including CASA, total and progressive sperm motility were reduced significantly ($P \leq 0.05$) at post thaw evaluation compared with prefreezing period as shown in Table 4.

Sperm viability (%)

The post thaw percentage of sperm viability was significantly increased in the two groups supplemented with normal (79.5±3.4) and nano selenium (77.8±3.4) compared with control group (65.3±3.4) as presented in Fig. 1.

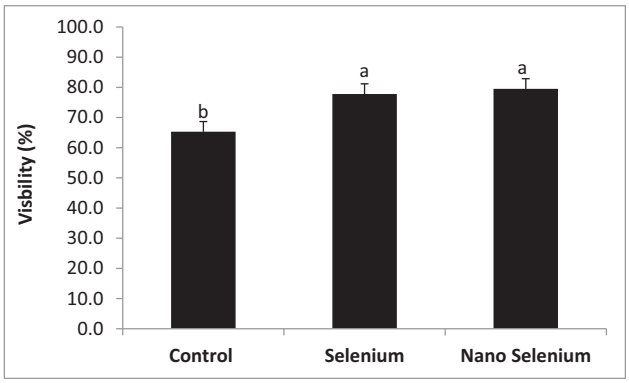
The level of total antioxidant capacity (TAC)

The post-thaw level of TAC in sperm of the two groups supplemented with selenium and nano selenium was higher ($P \leq 0.05$) than that of control group as shown in Fig. 2.

Table 4 Pre and postcryopreservation semen physical quality parameters and computer assisted semen analysis parameters of buck semen supplemented with two different forms of selenium

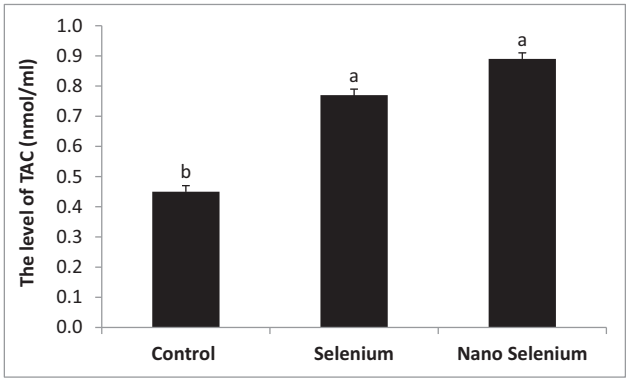
		Groups			
Traits	Time	C	NS	S	*P*-value
Total Motility (%)	Prefreezing	71.34±1.6	75.16±1.63	72.48±1.62	<0.0001**
	Postfreezing	39.78±1.86	44.35±1.98	46.96±2.02	
Progressive motility (%)	Prefreezing	58.76±2.17	62.04±2.22	59.68±2.19	<0.0001**
	Postfreezing	22.47±1.49	25.96±1.56	29.21±1.62	
DAP (μm)	Prefreezing	28.42±0.65	32.85±0.67	32.19±0.66	<0.0001**
	Postfreezing	23.39±0.87	21.37±0.92	22.62±0.94	
DCL (μm)	Prefreezing	59.18±1.12	68.94±1.15	68.70±1.14	<0.0001**
	Postfreezing	51.62±2.49	46.47±2.66	49.22±2.71	
DSL (μm)	Prefreezing	21.74±0.60	24.41±0.6	24.38±0.6	<0.0001**
	Postfreezing	16.15±0.56	14.95±0.6	15.54±0.61	
VAP (μm/s)	Prefreezing	62.83±1.4	73.25±1.43	70.49±1.41	<0.0001**
	Postfreezing	53.14±1.89	48.35±2.01	52.82±2.05	
VCL (μm /s)	Prefreezing	130.27±2.35	153.06±2.4	149.68±2.37	<0.0001**
	Postfreezing	115.78±5.53	103.64±5.9	114.13±6.01	
VSL (μm/s)	Prefreezing	48.06±1.25	54.64±1.27	53.60±1.26	<0.0001**
	Postfreezing	115.78±5.53	103.64±5.9	114.13±6.01	
STR %	Prefreezing	0.76±0.01	0.74±0.01	0.76±0.01	<0.0001**
	Postfreezing	0.69±0.01	0.71±0.01	0.70±0.01	
LIN %	Prefreezing	0.36±0.005	0.35±0.01	0.35±0.01	0.0783
	Postfreezing	0.32±0.01	0.35±0.01	0.34±0.01	
WOB (VAP/VCL) (μm/s)	Prefreezing	0.48±0.005	0.47±0.005	0.46±0.005	0.2222
	Postfreezing	0.47±0.01	0.49±0.01	0.53±0.02	
ALH (μ)	Prefreezing	4.32±0.09	4.83±0.09	4.29 [b]±0.09	0.0009**
	Postfreezing	4.98±0.16	4.46[b]±0.17	4.69[b]±0.17	
BCF (Hz)	Prefreezing	31.40 [b]±0.60	31.48 [b]±0.6	33.77±06	<0.0001**
	Postfreezing	22.63±0.69	20.68±0.73	22.10±0.75	

Figure 1



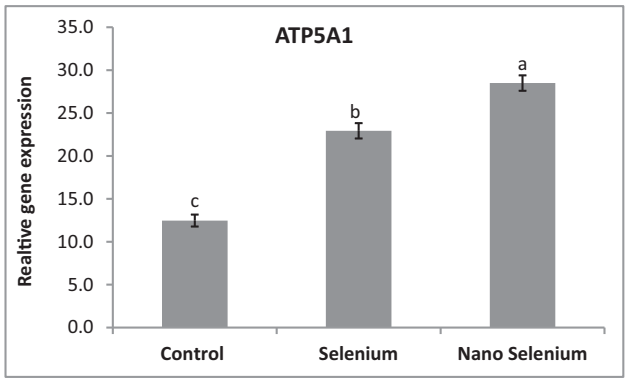
The postthaw viability rate of buck semen cryopreserved with two different forms of selenium.

Figure 2



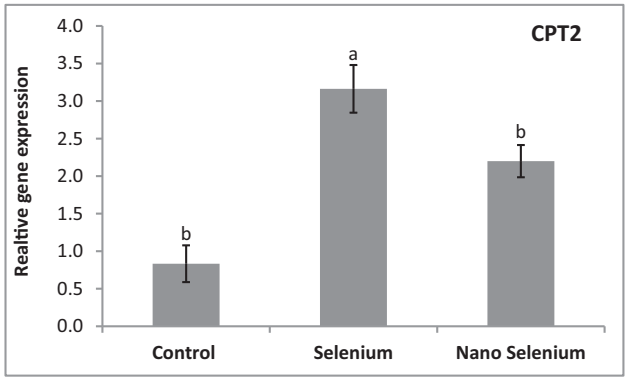
The post thaw level of total antioxidant capacity of buck semen cryopreserved with two different forms of selenium.

Figure 3



Gene expression profile of ATP5A1 gene in buck semen cryopreserved with two different forms of selenium.

Figure 4



Gene expression profile of CPT2 gene in buck semen cryopreserved with two different forms of selenium.

Semen molecular profile

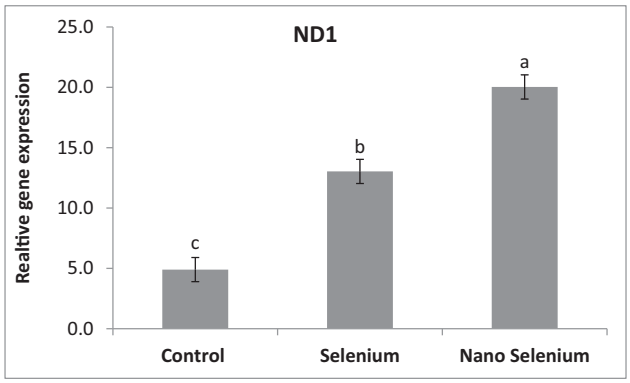
The expression profile of candidate genes regulating mitochondrial activity (ATP5A1, ND1, ND2, and CPT2) was increased significantly ($P \leq 0.05$) in semen supplemented with selenium in normal and nano forms compared with the control group as shown in Figs 3–6. In addition, the transcriptional profile of ATP5A1 and ND1 was greater in semen supplemented with nano than normal selenium (Figs 3 and 5). Moreover, the expression of CPT2 gene was greater in semen supplemented with normal than nano selenium (Fig. 4).

Discussion

Oxidative stress is well-known as one of crucial factors that compromises semen quality and sperm viability [20]. In addition, rabbit semen is well-known to have a rich content of polyunsaturated fatty acids that subsequently led to lipid peroxidation and production of reactive oxygen species and finally cause oxidative stress ROS) [5–9]. In an investigation done in four different breeds of rabbit such as British Spot, Chinchilla, New Zealand White and Fauve de Bourgogne there was variation in resistance to oxidative stress among these breeds [21].

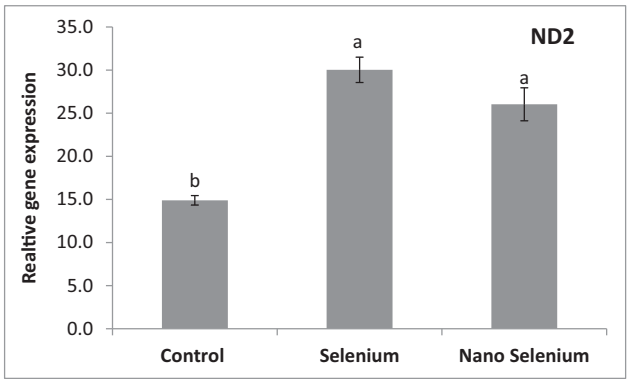
Our results demonstrated enhancement of the sum of CASA parameters in the two groups supplemented with normal and nano during pre-freezing period. Domosławska *et al.*, [22] have indicated improvement of viability, progressive motility and kinematic parameters of dog sperm (VAP, VSK, VCL, ALH, BCF, and RAPID) after supplementation of diet with Se and vitamin E. In addition, supplementation of 1 μg/ml Nano-Se to extender improved the post thawing viability, total and progressive motility of ram spermatozoa [23,24]. However, high concentration of Se in serum (65.9 μg/L) and seminal plasma (52.2 μg/l) was negatively correlated with CASA parameters (VAP, VSL,

Figure 5



Gene expression profile of ND1 gene in buck semen cryopreserved with two different forms of selenium.

Figure 6



Gene expression profile of ND2 gene in buck semen cryopreserved with two different forms of selenium.

STR, and LIN) of male cat sperm [25]. Noteworthy, the data of the current study indicated a significant ($P \leq 0.05$) improvement of post thaw total and progressive sperm motility on the two groups supplemented with normal and nano compared with control group.

The data of this investigation revealed that the post-thaw percentage of sperm viability was significantly increased in the two groups supplemented with normal and nano selenium compared with control group. Similarly, supplementation of bull semen extender with Se-NPs at concentrations of 1.0 µg/ml has improved the proportion of viable sperm and reduced the proportion of apoptotic sperm after freeze-thaw process [26]. Moreover, it was reported that addition of vitamin E to freezing diluent containing Se-NPs improved the post-thaw viability, total and progressive sperm motility and oxidative status of rooster semen [27]. Therefore, it seems that selenium either in normal or nano form exert positive action to improve sperm viability after thawing.

Remarkably, the data of our investigation has clearly demonstrate that post thaw total and progressive motility of rabbit sperm was improved in the group that supplemented with normal selenium compared with nano form. This the first study that compared between Norma and nano fome of selenium during rabbit seemn cryopreservation. In line with our results that indicated that nanoprticles supplementation to semen extender sometimes has partial improvement to sperm quality or even though harmful effects [28]. A recent study investigated the effect of supplementing diet with selenium nanoparticles on semen quality of heat-stressed (HS) rabbit bucks and results revealed amelioration of heat stress negative impact in addition to improvement of post-thaw sperm viability, concentration, integrity of acrosome membrane [29]. Asri-Rezaei *et al*., [30] have reported more beneficial effect of selenium nanoparticles administration compared with its bulk counterpart (selenite sodium) on mice sperm viability, motility, antioxidant status and subsequent *in vitro* fertilizing ability. Noteworthy, our results clearly revealed enhancement of post thaw TAC level in sperm of the two groups supplemented with selenium and nano selenium.

Indeed, intracellular trace elements is crucial for supporting the functionality of the endogenous defense system of sperm for examples selenium works as a co-factor of glutathione peroxidase enzyme [31,32]. In addition, selenium has a protective role against sperm DNA damage induced by oxidative stress which reflected in improvement of sperm viability and motility viability [33]. Therefore, the positive effect selenium supplementation of post thaw sperm motility could be due to enhanced intracellular defense system that reduced incident of oxidative stress. Recently, Hosny *et al.,* [34] reported improvement in antioxidant capacity level and reduction of malondialdehyde (MDA) level on Rabbits fed the OSe diet than those fed the control diet.

The nanoparticles are well known to have good bioavailability and adsorption characteristics [35]. Addition of nano-selenium in the diet of male Boer goats have enhanced the integrity of sperm membrane and their enclosed mitochondria [35]. A recent study investigated the effect of supplementing diet with selenium nanoparticles on semen quality of heat-stressed (HS) rabbit bucks and results revealed

amelioration of heat stress negative impact in addition to improvement of post-thaw sperm viability, concentration, integrity of acrosome membrane [29]. However, selenium oral supplementation to rams did not affect the sperm characteristics such as volume, motility, count, and integrity of membrane while it decreased the percentage of sperm defects [36]. In parallel, our data showed improvement of spam physical traits in both two forms of selenium.

Mitochondria have a crucial role in intracellular metabolic activity required for movement and fertilizing ability of mammalian spermatozoa [37]. Two recent publication of our group demonstrated increased transcriptional profile of two key mitochondrial genes (*CPT2* and *ATP5F1A*) that required for spermatozoa metabolic activity of antioxidants supplemented groups in semen of male rabbit and goat [38]. The data of the current investigation demonstrated up-regulation of candidate genes regulating mitochondrial activity (*ATP5A1*, *ND1*, *ND2,* and *CPT2*) in semen supplemented with selenium in normal and nano forms compared with the control group. A recent publication of our research group [15]. indicated higher transcriptional profile of two mitochondrial genes (*CPT2* and *ATP5F1A*) in spermatozoa supplemented with different antioxidants (LC+M, Cys, M+Cys, and LC+Cys).The transcript abundance of two mitochondrial proteins (*ATP5A1* and *ATP5B*) was increased in boar spermatozoa with high post thaw motility and viability [39]. Additionally, the quality of semen (motility, acrosome intactness, viability and mitochondrial functionality) following cryopreservation was improved in groups treated with selenium at concentration of 0.6 and 0.9 ppm [40]. Moreover, the level of lipid peroxidation was reduced and overall fertility was enhanced [40].

Conclusions

The data of this investigation demonstrated enhancement of some of physical parameters of post-thawed buck semen in the two groups supplemented with normal and nano selenium. In this regard, there was a significant improvement of post-thaw total and progressive sperm motility in the two groups supplemented with normal and nano selenium. The proportion of sperm viability and the level of TAC were enhanced in the two groups supplemented with selenium which was coupled with up-regulation of mitochondrial transcripts. It seems the both two forms shared the same mechanism on improving post-thaw physical and molecular qualities of rabbit sperm.

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Conflicts of interest

All authors confirmed that there was no conflict of interest.

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