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Original Article

Modulation of Negative Effects of Physiological Stress on Frozen-Thawed Semen with Nutrition of Organic Selenium in Ross 308 Rooster

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Abstract

Current experiment was carried out in factorial 2×2 arrangement to study the effects of stress (with or without dexamethasone administration) and addition of dietary selenium (with or without selenium supplementation in the diet) in male broiler breeder on the quality of frozen-thawed sperm under oxidative stress induced by dexamethasone. A total of 24 broiler breeder roosters with the age of 28 weeks were used based on a completely randomized design with four therapeutic approaches (factorial 2×2) and six birds in each approach. The experimental treatments were: 1) basal diet without selenium supplementation and injection of saline (CON), 2) basal diet with dexamethasone injection (4 mg/kg BW, three times every other day for one week), (DEX), 3) without dexamethasone injection and supplementation with 0.3 mg/kg selenium (Sel-Plex), and 4) dexamethasone injection and basal diet supplemented with 0.3 mg/kg of diet selenium (Sel-Plex+Dex). Sperm samples were collected from roosters. Motility, progressive motility, plasma membrane integrity, viability, malondialdehyde concentration and antioxidant parameters were evaluated in fresh and frozen-thawed semen. In spite of non-significant interaction effects, factorial analysis indicated the significant effect of every factor on different experimental parameters in fresh and frozen-thawed semen (P<0.05); The results revealed that total and progressive motility, plasma membrane integrity and viability were lower in DEX group when compared with other treatments (P<0.05). On the other hand, malondialdehyde concentration was higher in DEX group in comparison with Con, Sel-Plex and Sel-Plex+DEX groups (P<0.05). Moreover, total antioxidant capacity, level of glutathione peroxidase and superoxide dismutase were lower in DEX group as compared with other treatments (P<0.05). Our findings indicated that administration of selenium in dexamethasone-receiving roosters (Sel-Plex+DEX) improved the parameters of fresh and frozen-thawed sperm; but the best results were observed in Sel-Plex treatment. Therefore, selenium supplementation in the diet of roosters without dexamethasone injection improved total motility, progressive motility, membrane integrity, viability, malondialdehyde, total antioxidant capacity, glutathione peroxidase and superoxide dismutase pre- and post-freezing. It can be concluded, selenium in organic forms in stressed and non-stressed rooster's diet might improve all motility and antioxidant parameters in fresh and frozen-thawed sperm.

Keywords: Frozen-thawed sperm, Motility parameters, Physiological stress, Sel-Plex

1. Introduction

Freezing of rooster sperm is essential to access male gametes for artificial insemination. However, sperm cryopreservation reduces sperm viability. Freezing-thawing process increases the production of reactive oxygen species (ROS), which alter sperm antioxidant defense and affecting on sperm quality and fertility (1). The sperm plasma membrane is very prone to lipid

peroxidation due to the high concentration of unsaturated fatty acids (PUFAs) (2). These unsaturated fatty acids assist sperm to move easily and fertilize the ovum. Lipid peroxidation reduces sperm and ovum fertilization by eliminating the fluidity and membrane integrity (2).

Oxidative stress is an essential factor that negatively distresses the fertility potential of spermatozoa by lipid peroxidation. The necessity of selenium has been well known, which has played a crucial role in antioxidant activity and also involved in several important physiological processes (3). Glutathione peroxidase (GPx) as the first selenoenzyme is an essential component of organism's antioxidant system (4).

The selenium deficiency leads to a reduction in glutathione peroxidase activity; as activity of responsible enzymes in antioxidant system is directly related to the amount of selenium consumption, there is a strong association between selenium deficiency and oxidative stress (5). Accordingly, selenium deficiency leads to higher production of free radicals, which causes cell damage. Besides, some researchers have shown that dietary selenium supplementation, as organic selenium is more effective than inorganic form (6).

Studies have shown that the use of dexamethasone produces large amounts of reactive oxygen derivatives and has been suggested to stimulate post-injection physiological stress in animals (7). Previous studies have also demonstrated that glucocorticoids induce physiological stress mechanisms in tissues and bloodstream (8) and consequently negative effects on sperm production is expected in animals but there is no report about its cryopreservability.

Therefore, this is beneficial to test some methods to reduce negative effects of stress on sperm quality and cryopreservability. As roosters are affected by various stressors in farm condition that reduce their fertility and to assess the role of selenium in antioxidative systems, this study aimed to evaluate the effects of dietary supplementation of organic selenium on semen cryopreservability under oxidative stress in broiler breeder rooster.

2. Materials and Methods

2.1. Animals and Experimental Treatments

In order to perform this experiment, 24 Ross 308 breeder roosters aged 28 weeks were enrolled in a completely randomized design with a 2×2 factorial arrangement consisting of two levels of stress (with or without dexamethasone administration) and two levels of selenium (with or without selenium supplementation) were used based on four treatments with six birds in each treatment.

The roosters received experimental treatments as follows: 1) basal diet (table 1) without selenium supplementation with saline injection (CON), 2) basal diet with dexamethasone injection (4 mg/kg BW), three times every other day for one week), (DEX), 3) without dexamethasone injection and a basal diet supplemented with 0.3 ppm of selenium provided by Sel-Plex (Alltech, USA) as a source of organic selenium (Sel-Plex) For two weeks and, 4) a group receiving dexamethasone with basal diet supplemented plus 0.3 ppm of selenium provided by Sel-Plex (Sel-plex+DEX). Selenium was used as an additive according to Ebeid (6) study.

Roosters were housed under normal condition of 15 hours of light and 9 hours of darkness. Daily feeding was performed at the start of photoperiod (06:00 A.M.) with a basal diet formulated to meet Cobb 500 catalog recommendations (150 g/day) that provided 0.19 ppm (mg/kg) selenium as basal diet which determined by Central Laboratory of Medical Science University of Tabriz. The roosters had free access to water through a nipple drinker. Basal diet composition is presented in table 1.

2.2. Dexamethasone Injection and Sperm Collection

In the present experiment, inducing oxidative stress was performed by injection of dexamethasone (Abureyhan Pharmaceutical Company, Iran) at a rate of 4 mg/kg BW, three times every 2nd day for one week (9). After two weeks of habituation, sperm collection was performed on 24 roosters by dorso-abdominal massaging twice a week for two weeks. Sperm samples were stored at 37 °C to evaluate its primary motility,

concentration, and color. Sperm samples with 75% motility and appropriate concentration were selected and mixed to eliminate the individual effects.

Table 1. Ingredients and the chemical composition of basal diet fed to broiler breeder roosters (DM basis)

Ingredient	Content (%)
Corn	69.18
Soybean	8.5
Wheat bran	19.19
Dicalcium phosphate	1.4
Oyster shell	0.8
Vitamin premix[a]	0.25
Mineral premix[b]	0.25
Sodium chloride	0.32
Sodium bicarbonate	0.05
DL-Methionine	0.11
Total	100
Composition	
Metabolizable Energy (kcal / kg)	2754
Protein (%)	12
Methionine (%)	0.30
Lysine (%)	0.46
Threonine (%)	0.38
Calcium (%)	0.70
Phosphorus (%)	0.35

[a] Supplied per kg diet: vitamin A, 12,000 IU; vitamin D3, 3500
IU; niacin, 50 mg; vitamin E, 100 IU; vitamin K3, 5 mg; riboflavin, 12 mg; thiamin, 3.0 mg; D-pantothenic acid, 13 mg; folic acid, 2 mg; pyridoxine, 6 mg; vitamin B12, 0.03 mg, and biotin, 0.66 mg.
[b] Supplied per kg diet: Fe ($FeSO_4 \cdot H_2O$), 50 mg; Mn ($MnSO_4 \cdot H_2O$), 120 mg; Zn (ZnO), 110 mg; Cu ($CuSO_4 \cdot 5H_2O$), 10 mg; iodine (KI), 2 mg.

2.3. Procedure of Sperm Cryopreservation

Semen samples were mixed to remove individual male effects, diluted by extender in a ratio of 1 to 20, (The final concentration of sperm per ml was 2.5×10^8) and transferred to a refrigerator at 4 °C for two hours. When the temperature of the samples reached 4 °C, the sperm cells were manually drawn into the 0.25 ml straws and sealed with hematocrit paste and the concentration of sperm in each straw was 50×10^6. Sperm straws were placed at a height of 4 cm above nitrogen vapor for 7 minutes and then, transferred to a nitrogen tank (-196 °C) for storage to complete the freezing process (10). The extender in this experiment (table 2) was the modified Beltsvile extender (11), in which all ingredients were prepared from Merck (Merck, Germany), (Table 2). In order to evaluate the sperm quality of experimental treatments, the straws containing frozen sperm were thawed in a hot water bath at 37 ° C for 30 seconds and then drained into the microtubule to evaluate the samples' quality (12).

Table 2. Composition of the modified Beltsville extender Ingredient

Chemical compounds	Amount
Sodium-L-glutamate (g/L)	8.67
Potassium citrate tribasic monohydrate (g/L)	0.64
N-[Tris (hydroxymethyl) methyl]-2 (g/L)	3.2
Magnesium chloride anhydrous (g/L)	0.34
Potassium phosphate dibasic trihydrate (g/L)	7.59
Potassium phosphate monobasic (g/L)	0.7
D-(-)-Fructose (g/L)	5
Sodium acetate trihydrate (g/L)	3.2
Glycerol (v/v)	3 %
Lecithin	0.5 %
Purified water (mL)	1000
pH	7.4
Osmolarity (mOsm/kg)	310

2.4. Evaluation of Sperm Motility with CASA

Sperm motility parameters such as TM (total motility), PM (progressive motility), were assessed by using a Computer-Assisted Semen Analysis (CASA) system fitted with the Sperm Class Analyzer (SCA) software (Version 5.1; Microptic, Barcelona, Spain) equipped with a phase contrast microscope (Labomed LX400; Labomed Inc., USA) and a magnification of 100×. For this purpose, three straws from each treatment group were thawed and transferred into microtubes, then ten microliters of diluted semen sample were poured on the pre-warmed chamber and the loaded chamber placed on the thermal plate of the microscope (37◦Celsius) and sperm motility parameters were evaluated using the CASA (13).

2.5. Sperm Viability

Sperm viability was assessed via eosin-negrosin staining containing eosin (1.67 gr), nigrosin (10 gr), and sodium citrate (2.9 gr) in 100 ml of distilled water. For this purpose, ten microliters of diluted semen were

gently mixed with 20 microliters of dye on a clean slide and after preparation of expansion and drying at 37 ° C, the viability of 200 sperm cells was examined by an optical microscope with a magnification of 400×. The sperm cells that had not been stained were considered alive (14).

2.6. Sperm Plasma Membrane Integrity

In this experiment, a hypoosmotic test (HOST) was used to evaluate the integrity of the sperm plasma membrane according to Najafi, Kia (12) method. Briefly, 10 µl of semen were added to 100 µl of hypoosmotic medium containing 9 gr of fructose, 4.9 gr of sodium citrate, and 1000 ml of distilled water with a 100 mOsm. Then, it was incubated for 30 minutes at 37 °Celsius. Afterward, at least three drops of the incubated sample were examined under a microscope with 400× magnification for evaluating the condition of spermatozoa with flat or complex tail. After this experiment, sperms with tied tail were considered as cells with integrated membranes, while cells with smooth tails were considered as non-integrated membranes (12).

2.7. Membrane Lipid Peroxidation Index

To measure malondialdehyde (MDA) concentration as an indicator of lipid peroxidation, 1ml of semen sample (2.5×10^8 sperm/ml) was mixed with 1ml of trichloroacetic acid (TCA) 20% (wt/vol). Then samples were centrifuged at 1500×g for 10 minutes to precipitate protein. After that 250 µl of supernatant was taken and mixed with 1 ml of thiobarbituric acid (0.67%) for 10 minutes at 100 °Celsius. After cooling on ice, the absorption number of supernatant was read via spectrophotometer at 532 nm (15).

2.8. TAC, GPx and SOD Determination

Semen samples were prepared for evaluation of Total Antioxidant Capacity (TAC) and the Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD) activities by centrifuging of samples at 500×g for 5 min. After supernatant removal, in order to separate seminal plasma, samples were centrifuged at 10000×g for 20 min at 4 °C, then stored at -20 °C until assessment of mentioned parameters (12).

GPx activity was assessed by Zellbio kit (Zellbio GmBH, Germany) and reading the absorbance with microplate reader at 412nm according to kit instruction. Assessment of SOD and TAC were carried out by Tebpajohan commercial kits (Tehran, Iran) regarding the manufacturer's instructions. The SOD kit measures SOD activity by utilizing tetrazolium salt, which produces a water-soluble formazan dye upon reduction with superoxide anion. The formazan formation rate is inhibited by presence of SOD in environments and is measurable photometrically at 440 nm using a microplate reader. For measuring of TAC, the kit utilizes a peroxidase chromogenic substrate, which produces a water-soluble chromogen upon oxidation by ferryl myoglobin radicals. The green chromogen formation rate is inhibited by presence of antioxidants in environments and is measurable photometrically at 412 nm using a microplate reader. In the assessment of mentioned parameters, all microplates were read by ELISA reader (TECAN, GmBH, Austria).

2.9. Statistical Analysis

All data were analyzed using GLM procedure of SAS software (9.1 Ver.) for the analysis of variance. A 2×2 factorial arrangements with two levels of stress factor (with or without dexamethasone administration) and two levels of selenium inclusion (with or without selenium supplementation) were used in a completely randomized design. Significant differences among treatments were identified at 5% level by Duncan multiple range test.

3. Results

Factorial analysis of our data showed the significant effects of every factor (dexamethasone administration or dietary selenium supplementation) on different experimental parameters ($P<0.05$) in fresh and frozen-thawed semen; while there were no significant interaction effects of mentioned factors on different sperm characteristics in pre- or post-freezing status ($P>0.05$).

In general, dietary supplementation of organic selenium improved the overall quality of sperm cells before and after the freezing and thawing process in

roosters which did not receive dexamethasone (Sel-Plex), compared with Con, Dex, and Sel-Plex+Dex treatment groups (P<0.05). Table 3 indicates the effect of dietary supplementation of selenium on sperm motility parameters pre and post-freezing conditions. This finding showed that use of organic selenium had a positive effect on total and progressive motility (P<0.05). In post-freezing condition, the parameters were almost similar to liquid conditions. It should be noted that in Dex treatment group, all motility parameters were significantly reduced (P<0.05) as compared with other treatments.

Results related to plasma membrane integrity and sperm viability of pre- and post-freezing conditions are presented in table 4. The results showed that dexamethasone injection reduced the sperm integrity and viability (P<0.05). The supplementation of roosters' diets with Sel-Plex under dexamethasone-stress improved plasma membrane integrity and sperm viability parameters compared with Con and Dex treatments (P<0.05). The use of organic selenium in non-stressed roosters (Sel-Plex) lead to the highest membrane integrity and sperm viability in pre- and post-freezing conditions.

Table 3. Effect of dietary organic selenium on motility parameters of broiler breeder rooster sperm in pre and post freezing conditions

Parameters	Treatment[1]					factorial *P*-value		
	Con[2]	Dex	Sel-Plex	Sel-Plex+Dex	SEM	Stress	Selenium	Stress*selenium
Pre freezing								
TM[3] (%)	79.4^{c}	62.20^{d}	96.3^{a}	91.33^{b}	0.77	*	*	NS
PM (%)	42.53^{b}	27.03^{c}	57.06^{a}	36.16^{b}	1.11	*	*	NS
Post freezing								
TM (%)	68.86^{c}	49.16^{d}	85.8^{a}	79.9^{b}	0.98	*	*	NS
PM (%)	26.33^{b}	12.46^{c}	42.36^{a}	32.83^{ab}	1.48	*	*	NS

[1)] Different letters (a, b, c and d) in the same line show statistically differences (P< 0.05), NS: not statistically significant; ∗: P<0.05.
[2)] Con, control; Dex, control with dexamethasone; Sel-Plex, control with organic selenium; Sel-Plex+Dex, organic selenium with dexamethasone.
[3)] Tm, total motility; Pm, progressive motility.

Table 4. Effect of dietary organic selenium on membrane integrity and viability of broiler breeder rooster sperm in pre and post freezing conditions.

Parameters	Treatment[1]					factorial *P*-value		
	Con[2]	Dex	Sel-Plex	Sel-Plex+Dex	SEM	Stress	Selenium	Stress*selenium
Pre freezing								
Membrane integrity (%)	80.78^{c}	71.92^{d}	94.46^{a}	88.10^{b}	1.01	*	*	NS
Viability (%)	78.33^{b}	73.57^{b}	95.08^{a}	88.13^{a}	1.18	*	*	NS
Post freezing								
Membrane integrity (%)	58.5^{c}	50.26^{d}	82.76^{a}	76.57^{b}	0.87	*	*	NS
Viability (%)	63.68^{c}	50.84^{d}	85.2^{a}	79.84^{b}	0.95	*	*	NS

[1)] Different letters (a, b, c and d) in the same line show statistically differences (P< 0.05), NS: not statistically significant; ∗: P<0.05.
[2)] Con, control; Dex, control with dexamethasone; Sel-Plex, control with organic selenium; Sel-Plex+Dex, organic selenium with dexamethasone.

The findings of the biochemical parameters of sperm in pre- and post-freezing conditions are presented in table 5. The results indicated that the mentioned parameters are similar in both pre- and post-freezing conditions. Dexamethasone injection increased lipid peroxidation and produced a higher amount of MDA as an indicator of lipid peroxidation compared with other treatments (P<0.05).

Hereby, dietary organic selenium supplementation in dexamethasone-receiving roosters reduced MDA, but the lowest amount of MDA production was observed in Sel-Plex treatment (P<0.05), However, there were no significant differences between Con, Sel-Plex and Sel-Plex+Dex treatments in the mentioned parameter (P>0.05). Total antioxidant capacity and glutathione peroxidase had similar conditions in both pre- and post-freezing stages. Treatment of Sel-Plex presented the best improvement (P<0.05), while Sel-Plex+Dex treatment showed no significant difference with Con treatment (P>0.05); On the other hand, in comparison with Dex treatment, use of dietary organic selenium in Sel-Plex+Dex group was able to compensate for the destructive effect of dexamethasone. Subsequently, the amounts of SOD as another antioxidant was not statistically different between Sel-Plex and Sel-Plex+Dex treatments, but demonstrated a significant difference in comparison with Con and Dex treatments (P<0.05).

Table 5. The effect of dietary organic selenium on biochemical parameters of broiler breeder rooster sperm in pre and post freezing conditions.

Parameters	Treatment[1]					factorial *P*-value		
	Con[2]	Dex	Sel-Plex	Sel-Plex+Dex	SEM	Stress	Selenium	Stress*selenium
Pre freezing								
MDA[3] (nmol/ml)	3.88^{b}	5.40^{a}	3.69^{b}	4.14^{b}	0.42	*	*	NS
TAC (U/ml)	1.26^{b}	1.05^{c}	1.55^{a}	1.39^{b}	0.16	*	*	NS
GPx (U/ml)	65.27^{b}	52.51^{c}	85.91^{a}	71.60^{b}	1.35	*	*	NS
SOD (U/ml)	121.78^{b}	108.62^{c}	149.65^{a}	142.13^{a}	1.21	*	*	NS
Post freezing								
MDA (nmol/ ml)	6.94^{b}	9.11^{a}	3.88^{d}	5.29^{c}	0.45	*	*	NS
TAC (U/ml)	1.16^{b}	1.01^{c}	1.62^{a}	1.28^{b}	0.15	*	*	NS
GPx (U/ml)	55.69^{c}	46.16^{d}	80.81^{a}	71.12^{b}	1.14	*	*	NS
SOD (U/ml)	111.72^{b}	103.45^{b}	142.5^{a}	134.2^{a}	1.35	*	*	NS

[1)] Different letters (a, b, c and d) in the same line show statistically differences (P< 0.05), NS: not statistically significant; ∗: P<0.05.
[2)] Con, control; Dex, control with dexamethasone; Sel-Plex, control with organic selenium; Sel-Plex+Dex, organic selenium with dexamethasone.
[3)] MDA, malondialdehyde; TAC, total antioxidant capacity; GPx, glutathione per-oxidase; SOD, superoxide dismutase.

4. Discussion

Selenium supplementation increases the enzyme activity using ATP and its reductase pathways in sperm, in which affecting on sperm motility and oxygen consumption (16). Changes in the antioxidant defense system of rooster sperm and semen plasma during cryopreservation may affect semen quality and sperm fertility. Factorial analysis of our data showed the significant effects of dexamethasone administration which decreased the sperm quality regarding evaluation of parameters in fresh or frozen-thawed semen. In accordance with the results of the present research, there is evidence that use of dexamethasone at a dose of 4 mg/kg body weight decreased sperm motility compared with the control treatment in roosters (17).

Increased sperm motility is demonstrated in roosters after diet supplementation with organic selenium (Sel-plex) with 0.3 mg/kg of feed in farm condition (6). One study found that when 0.2 mg/kg of organic selenium was added to the rooster's diet, the percentage of normal sperm increased to 98.7% (18). Similarly, in the present experiment, an increase in sperm motility of

rooster occurred by supplementation with organic selenium (selenomethionine). Another experiment by evaluating the effect of organic and inorganic forms of selenium on the quality of turkey sperm in turkeys, supplemented with organic selenium presented the highest sperm motility (19). Reversely, Feeding pigs with selenium-deficient diet reduced spermatozoon adenosine triphosphate (ATP) concentrations and caused structural abnormalities in the midsection of sperm (16). Subsequently, motility parameters might be closely related to sperm motility.

In the current study oral source of selenium was able to moderate the negative effects of dexamethasone. Min, Niu (9) reported that the immune response of dexamethasone-treated roosters tended to an steady reduced oxidative stress causing alterations in gene expression, mutations, and cell death. So, the administration of synthetic glucocorticoid (dexamethasone) was presented by an increased stress phenomena in broilers such as increasing in body temperature and respiration rate, but reducing the growth rate (20).

Selenium is a major component of glutathione peroxidase (GPx) and selenoproteins, in which increases the sperm viability and improves protection against reactive oxygen substances. The results of the present study are consistent with others in showing the application of dexamethasone at a dose of 4 mg/kg of body weight, in increasing the percentage of dead sperm (17).

Besides, malondialdehyde is a product of lipid peroxidation that produces during the action of oxidizing agents on membrane lipids and can be considered as a diagnostic tool for infertility in males (21). Peroxidation causes undesirable alterations in the structure of the acrosomal portion of sperm and thus, reduces the sperm motility and viability. Also, it is reported that stress increases the lipid peroxidation in semen plasma as well as spermatozoa membrane (17).

Sperm has antioxidant systems such as glutathione peroxidase and superoxide dismutase that act as defense mechanisms against ROS and lipid peroxidation in the semen of animal. In an experiment on pigs, the highest glutathione peroxidase activity was observed in the treatment of organic selenium in comparison with the inorganic selenium and control treatments (22). Glutathione peroxidase is mainly accumulated in the mitochondrial capsule of spermatozoa (middle part of spermatozoa) and as the sperm motility system is located in the middle part of sperm; so, the reduction in motility can be attributed to decline in glutathione peroxidase activity (22).

A well-known effect of selenium deficiency is the lack of optimal function of intermediate part of sperm, which leads to impaired sperm motility (1). Due to the activity of glutathione peroxidase (contains selenium) in semen plasma, this enzyme plays a vital role in detoxifying any lipid peroxide that appears in rooster sperm (23). In one study, consumption of selenium in the male diet elevated the glutathione peroxidase-dependent activity that was associated with greater protection against lipid peroxidation in semen (4). In roosters fed with organic selenium, the increased activity of the semen glutathione peroxidase was observed (6). Glutathione peroxidase is an antioxidant enzyme (resemble to superoxide dismutase), in which affects the sperm functions by inhibiting lipid peroxidation in the sperm membrane and by improving the sperm motility (2). Glutathione peroxidase also plays an important role in sperm production, maturation, and fertility, but absence of this enzyme leads to decrease in the sperm fertility (4).

SOD is the most crucial and the first antioxidant enzyme in all aerobic organisms that is directly involved in the reduction of active oxygen derivatives (24). Subsequently, SOD plays a critical role in cell defense against oxidative damage. The SOD enzyme catalyzes O_2 dismutation to H_2O_2 (25). Consistent with the present study results on decreasing of SOD concentration, oxidative stress induced by dexamethasone injection reduced SOD production by reducing mRNA expression (9).

Our results initially indicated that organic selenium contained positive effects on quality of fresh sperm traits in dexamethasone-treated roosters, such as sperm motility, biochemical enzyme activity and viability, but it seems there is similar effect on frozen-thawed sperm. It means that organic selenium can reduce the induced-effects of freezing process as well as dexamethasone injection.

5. Conclusion

Oxidative stress in poultry through dexamethasone administration in roosters reduced the antioxidant activity, plasma membrane integrity, viability, and motility parameters, as well as increased the malondialdehyde production. However, application of selenium in organic form in stressed and non-stressed rooster's diet improved all motility and antioxidant parameters in fresh and frozen-thawed sperm.

Authors' Contribution

Study concept and design: A. K.
Acquisition of data: N. K.
Analysis and interpretation of data: A. K.
Drafting of the manuscript: M. N. and A. K.
Critical revision of the manuscript for important intellectual content: A. K. and R. M.
Statistical analysis: N. K.
Administrative, technical, and material support: R. M.

Ethics

All experimental and animal housing procedures were supervised by the Institutional Animal Care and Use Committee in the University of Tabriz, Tabriz, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

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