



RESPONSE TO ANTIOXIDANT OILS ON SEMEN QUALITY OF MANDARAH COCKS

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ABSTRACT: The present study aimed to investigate the effects of olive oil or nigella sativa oil as antioxidant dilutants on semen quality and hatchability upon semen storage at 5°C. A 3x4 factorial experimental designs was performed including three dilutant sources {semen diluted 1: 1 with lake diluent (LD) sered as a control, semen diluted at 1ml semen: 1ml with LD and supplemented with olive oil or with nigella sativa oil (1 ml/100 ml of diluent), respectively} and four storage time (0, 24, 48 and 72 hours at 5°C). A total number of 60 cocks and 180 hens at 38-week-old were used to provide data on sperm assess and hatchability.

The obtained results show significant ($P<0.01$) increased sperm motility (%), and decreased dead spermatozoa (%), abnormal spermatozoa (%) and acrosomal damage (%) of cocks consequently, better hatchability rate of hens treated with diluted semen with nigella sativa oil (NSO) followed by olive oil (OO) compared to the control during different storage time (0, 24, 48 and 72 hours at of 5 °C). Percentage of sperm motility of cocks and hatchability rate of hens were decreased, while dead spermatozoa (%), spermatozoa abnormal (%) and acrosomal damage (%) of cocks were significantly ($P<0.01$) increased with the advancement of storage time. Therefore, Nigella sativa oil and olive oil could be used to improve the semen quality of cocks when stored at 5°C, up to 72 hours, as well as hatchability rate of Mandarah laying hens.

Keywords: Olive oil-Nigella sativa oil- in vitro storage-semen quality- hatchability.

INTRODUCTION

Avian spermatozoa are characterized by high proportions of polyunsaturated fatty acids (PUFAs) especially docosatetraenoic acid (C22:4 n6) arachidonic acid (C20:4n-6) which associated with increased susceptibility to reactive oxygen species (ROS) and lipid peroxidation (Surai and Sparks, 2001). Several studies focused on the potential of ROS as one of the prime mediators of infertility. Production of ROS in the reproductive tract is detrimental not only to the fluidity of the sperm plasma membrane, but also to the integrity of DNA in the sperm nucleus (Cecil and Bakst, 1993).

The use of chilled-stored semen is limited by its relatively short time fertilizing capacity (Aurich et al., 1997). Oxidative stress in chicken semen during storage reduced sperm numbers reduced motility, increased percentage of dead spermatozoa and raised the level of lipid peroxidation; these changes would lead to lower fertility (Eid et al., 2006). The survival of ejaculated sperm in seminal plasma alone is limited to a few hours (Ball et al., 2001).

Evidence suggests that nutritional antioxidants such as olive oil and nigella sativa oil have been used in the herbal medicine of different populations specifically in the Middle East (Siegfried and Hughes, 2012). Giovannini et al. (2004) showed that some biophenols, containing olive oil, may counteract the ROS, mediated cellular damage and related disorders by improving in vivo antioxidant defenses. D'Angelo et al. (2001) demonstrated that olive oil effectively counteracts the cytotoxic effects of ROS in various cellular systems. Masella et al. (2004) found that olive oil antioxidants had the following effects: 1-

completely prevented the oxidation of LDL; 2- counteracted the time dependent variations in intracellular redox balance, inhibiting the production of O₂ - and H₂O₂ and the decrease of glutathione content; 3- restored glutathione reductase and peroxidase activities; and 4- restored the mRNA expression of γ - glutamylcysteine synthetase, glutathione reductase, and glutathione peroxidase to control values. Al-Daraji (2012) cleared that supplementation of the diluent of aged roosters' semen with olive oil can improve semen quality when semen samples in vitro are stored at 5 °C for up to 72 h and olive oil can be used as an efficient tool for improving semen quality during liquid storage of diluted semen. On the other hand, nigella sativa oil has been reported to possess antioxidant activity (Burits and Bucar, 2000). Since nigella sativa oil containing high percentage of unsaturated fatty acids and there are no reports concerning the effect of this oil on male reproductive system or sexual hormones. The fixed oil of nigella sativa oil consists of 50% linoleic acid, 25% oleic acid, 12% palmitic acid, 2.84% stearic acid, 0.34% linolenic acid and 0.35% myristic acid (Cheikh-Rouhou et al., 2007). Tawfeek et al. (2006) demonstrated that beneficial effect of the treatment with Nigella sativa oil as manifested by 85% increase in the percentage of live/dead spermatozoa, and 0.66% decrease in the percentage of morphologically abnormal spermatozoa reflecting its antioxidants effect that counteract the H₂O₂ effect on spermatozoa. The present study aimed to define the effects of olive oil or nigella sativa oil addition to diluted semen in cocks semen quality and hatchability during storage at 5 °C for up to 72 hours

Olive oil-Nigella sativa oil- in vitro storage-semen quality- hatchability.

MATERIALS AND METHODS

The present study was carried out at the Inshas Poultry Research Station, Animal Production Research Institute, Giza, Egypt, during the period from November, 2017 until February, 2018. A 3x4 factorial experimental designs was performed including three dilutant sources {semen diluted 1: 1 with lake diluent (LD) sered as a control, semen diluted at 1ml semen: 1ml with LD and supplemented with olive oil or with nigella sativa oil (1 ml/100 ml of diluent), respectively} and four storage time (0, 24, 48 and 72 hours at 5°C). A total number of 60 cocks and 180 hens at 38-week-old were used to provide data on sperm assess and hatchability. Feed and water were permitted ad libitum. Cocks were habituated to abdominal massage response (3 week period from 38-54 weeks of age) for semen collection according to Zhang and Zheng (2002). Semen characteristics were evaluated every 21 days at 38 and 54 weeks of age. Semen samples were divided into 3 test tubes (1 ml each) to provide 3 replicates pooled samples per each treatment group. This experimental work was planned to study the effects of olive oil or Nigella sativa oil addition to Lake Extender on semen quality and hatchability during storage at 5 °C as the method described by Lake (1960) which consisted of sodium glutamate :1.35 g, potassium citrate: 0.128 g, sodium acetate: 0.51 g, glucose :0.80 g, streptomycin sulphate :0.20 g, penicillin G sodium :0.04 g and distilled water :100 ml.

The final extension rate was 1 ml semen: 1 ml diluent. After each storage (0, 24, 48, 72 h) period, percentages of sperm motility, dead spermatozoa, abnormal spermatozoa and acrosomal damage were recorded according to Lake (1960).

Sperm motility was estimated on a percentage basis according to Ommati et al. (2013). A spermatozoon abnormal was assessed using eosin-nigrosin staining. Abnormal spermatozoa concentrations were recognized as rates of the total (200) sperm. Acrosomal damage of spermatozoa was determined according to Watson (1975). Artificial inseminations were performed at 4 cm depth using 0.05 ml diluent semen / hen at the following periods, 0, 24, 48 and 72 hr and collected 40 eggs/ group, after 21 days hatchability rate was calculated by the following equation:

$$\text{Hatchability rate} = \frac{\text{Chicks hatched}}{\text{Total eggs}} \times 100.$$

A total number of 180 hens of Mandarah strain were used to assess of hatchability. Hens at 38 weeks of age were used and fed on a diet of 2759 kcal ME/kg, and 16.11, 3.29, and 0.39 % Crude protein, calcium, and available phosphorus, respectively in individual cages. Cocks were fed a commercial diet (16.02% CP and 2828 Kcal / kg diet) up to 54 weeks of age according to Feed Composition Tables for Animal and Poultry Feedstuffs used in Egypt (2001) that are shown in Table 1. Every cock received 125 g of diet/day at 38 weeks of age, up to 140 g/d at the end of the experiment. Birds were healthy, examined against diseases and immunizations.

The statistical analysis was conducted using SAS® (2003) software program, using factorial experimental designs analyses of variance of GLM procedures considering the replicate as the experimental unit. Mean differences were tested at $P \leq 0.05$ by Student Newman Keuls Test (SAS, 2003) using $P \leq 0.05$. Before analysis, all percentages were arc sin to normalize data distribution. Data were reported based on the main effects

and their interactions. Hatchability rates were analyzed by the Chi-square test.

RESULTS AND DISCUSSION

Data presented in Table 1 shows that a significant ($P<0.01$) increased sperm motility (%), and a significant ($P<0.01$) decreased dead spermatozoa (%), abnormal spermatozoa (%) and acrosomal damage (%) of cocks consequently, better hatchability rate of hens diluted semen added with nigella sativa oil (NSO) followed by olive oil (OO) compared to the control during different storage time (0, 24, 48 and 72 hours at of 5 °C). Percentages of sperm motility, dead spermatozoa, abnormal spermatozoa, and acrosomal damage of cocks and hatchability rate of hens were significantly ($P<0.01$) influenced by interaction between treatments and storage time (Figures 1-5). The positive effect of NSO and OO into semen diluent may be attributed to NSO is rich in fatty acid, (oleic, linoleic and linolenic acid) and carotene which is converted into vitamin A (Al-Jassir, 1992) and active ingredients, like thymoquinone, possess reproducible anti-oxidant effects through enhancing the oxidant scavenger system, a consequently lead to antitoxic effects induced by several insults (Salem, 2005). In addition, some unsaturated fatty acids like linoleic acid (about 60%) and oleic acid (about 20%) present in NSO may also improve of sperm parameters such as sperm count and sperm motility (Kolahdooz et al., 2014). Nigella sativa has been found to be able to improve of sperm quality in rabbit (Riad et al., 2004). Masella et al. (2004) found that olive oil containing antioxidants had the following effects: 1-completely prevented the oxidation of LDL; 2- counteracted the time – dependent variations in intracellular redox balance, inhibiting the

production of O_2 and H_2O_2 and the decrease in glutathione content; 3- restored glutathione reductase and peroxidase activities; and 4- restored the mRNA expression of γ – glutamylcysteine synthetase, glutathione reductase, and glutathione peroxidase to control values. Olive oil contains a large amount of natural antioxidants which provide oxidative stability during storage (Boskou et al., 2005). Enhancing the antioxidant capacity of the diluted semen stored at 5 °C as a result of the addition of OO or NSO improves the sperm preservation capacity of the cocks by two ways defense towards peroxidative harm is crucial to hold the structural integrity of the spermatozoa and minimization of lipid peroxidation will save you any discount inside the concentrations of the functionally important $C_{20} - 22$ polyunsaturated fatty acids of the sperm phospholipids (Al-Daraji, 2012 and Bruits and Bucar, 2000). It is speculated that the development in spermatozoa viability and integrity are a result of antioxidants suppressing or limiting the harmful effects of lipid peroxidation in vitro. Possibly the development to these sperm parameters are at the extent of the membrane, as lipid – and water – and – lipid – soluble antioxidants maintained viability, membrane integrity, and motility of turkey sperm after 48 h in vitro storage, however the water soluble antioxidant examined did not (Donoghue and Donoghue, 1997). Because in their lipid solubility, olive oil can permeate plasma membrane of spermatozoa and suppress free radical harm. However, Donoghue and Donoghue (1997) found that the antioxidant activity in seminal plasma and sperms isn't enough to prevent lipid peroxide harm after extension and in vitro

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storage, and those supplemental antioxidants may want to enhance semen shelf existence.

Sperm motility of cocks and hatchability rate of hens were decreased while, dead spermatozoa (%), abnormal spermatozoa (%) and acrosomal damage of cocks were significantly ($P<0.01$) increased with the increase in storage time of diluted semen from 0 to 72 at 5°C (Table 1). These results are in agreement with those results of El-Saadany (2002) who reported that increasing storage time of diluted semen from local chicken breed for 0, 24, 48 and 72 hr significantly ($P<0.01$) decreased the fertility rate (FR) by 5.12, 9.55 and 12.53%, respectively. Siudzinska and Lukaszewick (2008) found that during the time of storage, a decrease in live, morphologically normal spermatozoa and an increase of dead spermatozoa and spermatozoa with bent necks were observed. Also, Hudson et al. (2016) found that the percent of dead spermatozoa and abnormal spermatozoa significantly increased with increasing storage periods. Brown et al. (1972) observed that at 5°C, motility of sperm lasted for longer time period; due to the fact the vital substances wished for power metabolism are conserved. The maintenance of motility during storage was decreased when no energy substrate was present in the extender.

Percentages of sperm motility, dead spermatozoa, abnormal spermatozoa, acrosomal damage of cocks and hatchability rate of hens were significantly ($P<0.01$) influenced by interaction between semen diluted supplemented with or without olive oil or Nigella sativa oil and storage time. Sperm motility (%), dead spermatozoa (%),

abnormal spermatozoa (%) and acrosomal damage of cocks and hatchability rate of hens were significantly ($P<0.01$) improved with NSO followed by OO compared to control semen for all storage periods. The advancement of storage time was significantly ($P<0.01$) lower the percentage of sperm motility and hatchability rate in extender semen either with NSO, OO or the control. While, significantly ($P<0.01$) higher the percentages of (%), dead spermatozoa (%), abnormal spermatozoa (%) and acrosomal damage of spermatozoa either in the extender semen added with NSO, OO or the control. These results are in agreement with that of Al-Daraji, (2012) who showed that supplementation the diluent of roosters' semen with olive oil can improve semen quality when semen samples in vitro stored at 5 °C for up to 72 hr. Similarly, Eid et al. (2006) reported that chicken semen during storage reduced sperm numbers reduced motility, increased percentage of dead spermatozoa and raised the level of lipid peroxidation; these changes would lead to lower fertility.

IN CONCLUSION

addition of nigella sativa oil or olive oil to diluted semen cocks showed increased sperm motility (%) and decreased dead spermatozoa, abnormal spermatozoa, acrosomal damage of spermatozoa, consequently higher hatchability rate than the control cocks spermatozoa (free-nigella sativa or olive oil medium). There, it could be recommended to addition of nigella sativa or olive oil at a level of 1 ml/100 ml extender to extender cock, during at 5 °C to enhance of hatchability of hens.

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Table (1): Ingredients composition and calculated analysis of the basal experimental diet.

Composition (per 100 Kg)	Diet	
	Cocks	Hens
Yellow corn	67.10	65.36
Soybean meal (44% CP)	18.15	18.75
Corn gluten meal (60% CP)	1.25	2.85
Wheat bran	9.71	3.05
Dicalcium phosphate	1.75	1.50
Limestone	1.25	7.60
Nacl	0.40	0.40
Vit. & Min. Premix**	0.30	0.30
DL-Methionine	0.09	0.19
Total	100	100
Calculated analysis:**		
Crude protein (CP); %	16.02	16.11
ME; kcal/kg	2828	2759
Ether extract	2.85	2.84
Crude fiber	3.83	3.12
Calcium	0.94	3.29
Av. Phosphorus	0.45	0.39
Lysine	0.73	0.72
Methionine	0.36	0.47
Methionine + cystine	0.66	0.77

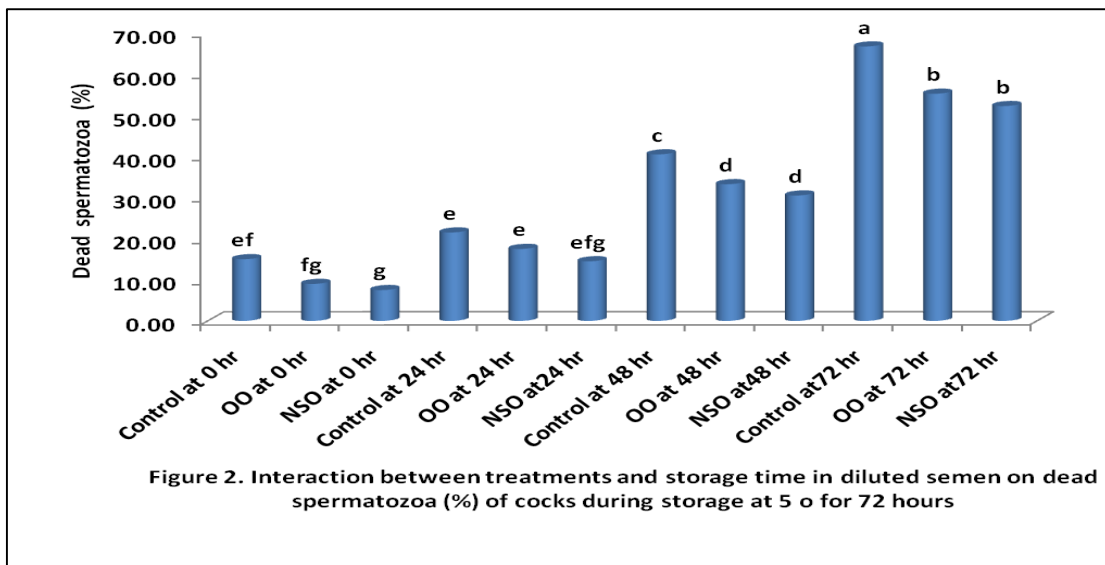
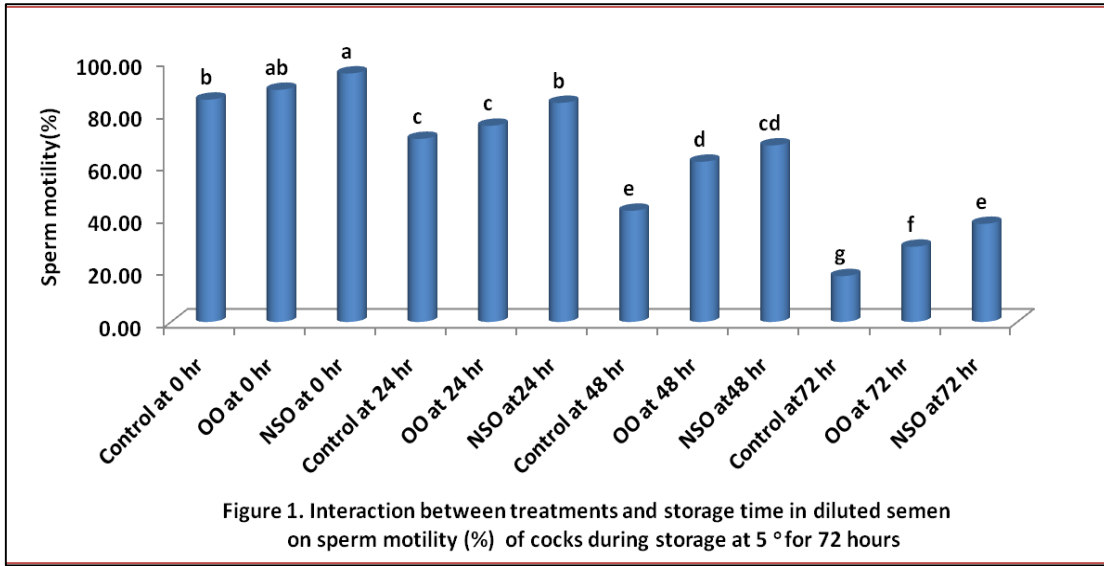
* Vitamins and mineral premix provides per 3kg: Vit. A 12000 IU; Vit. D₃ 2000 IU; Vit. E. 10mg; Vit. K₃ 2mg; Vit.B₁ 1mg; Vit. B₂4mg; Vit. B₆ 1.5 mg; Pantothenic acid 10mg; Vit.B₁₂ 0.01mg; Folic acid 1mg; Niacin, 20mg; Biotin, 0.05mg; Choline chloride (50% choline) 500 mg; Zn, 55mg; Fe, 30mg; I 1mg; Se, 0.1mg; Mn, 55mg; Ethoxyquin 3000 mg.

**Calculated analysis was, according to Feed Composition Tables for Animal and Poultry Feedstuffs used in Egypt (2001).

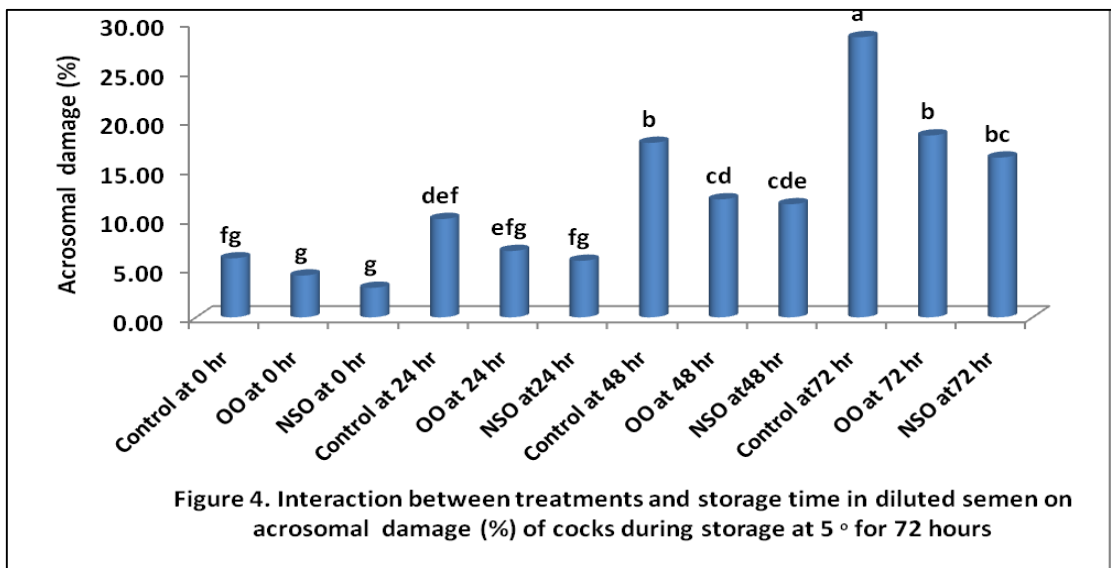
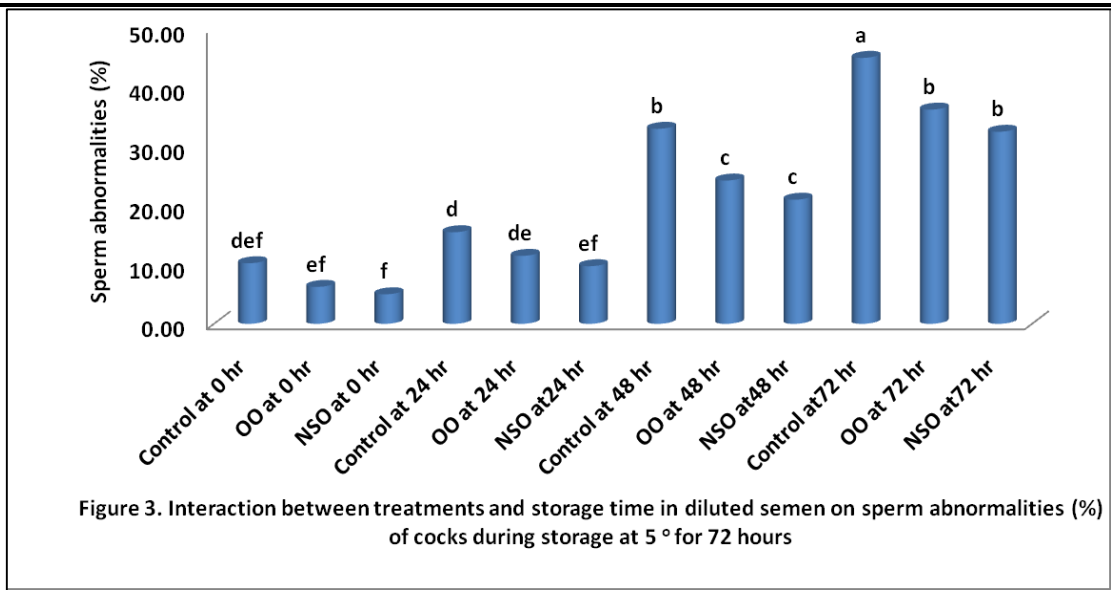
Table (2): Semen characteristics and hatchability % ($\bar{X} \pm SE$) of Mandarrah cocks and hens as affected by olive oil and Nigella sativa oil and their interactions at 38-54 weeks of age.

Items	Sperm motility (%)	Dead spermatozoa (%)	Abnormal spermatozoa (%)	Acrosomal damage (%)	Hatchability/total eggs (%)
Treatments	**	**	**	**	**
Semen diluted (LD) (Control)	53.75±6.83 ^b	35.94±5.29 ^a	25.94±3.70 ^a	15.57±2.40 ^a	46.42±7.21 ^b
Olive oil (OO)	63.44±5.86 ^b	28.75±4.65 ^b	19.57±3.08 ^b	10.38±1.53 ^b	56.82±5.55 ^a
Nigella sativa oil (NSO)	70.94±5.65 ^a	26.19±4.60 ^b	17.07±2.85 ^b	9.13±1.48 ^b	61.13±5.24 ^a
Storage time (Hours)	**	**	**	**	**
0 hr	87.92±1.57 ^a	10.5±1.19 ^d	7.17±0.96 ^d	4.42±0.52 ^d	80.97±1.39 ^a
24 hr	76.25±1.96 ^b	17.84±1.13 ^c	12.25±0.89 ^c	7.50±0.78 ^c	70.02±2.47 ^b
48 hr	57.09±3.72 ^c	34.75±1.78 ^b	26.09±1.64 ^b	13.75±1.20 ^b	45.25±2.29 ^c
72 hr	27.92±2.79 ^d	58.09±2.65 ^a	37.92±2.21 ^a	21.09±2.1 ^a	22.93±4.13 ^d
Interaction between treatments and storage time	**	**	**	**	**

Means having different letters in the same column are significantly (P<0.05) different



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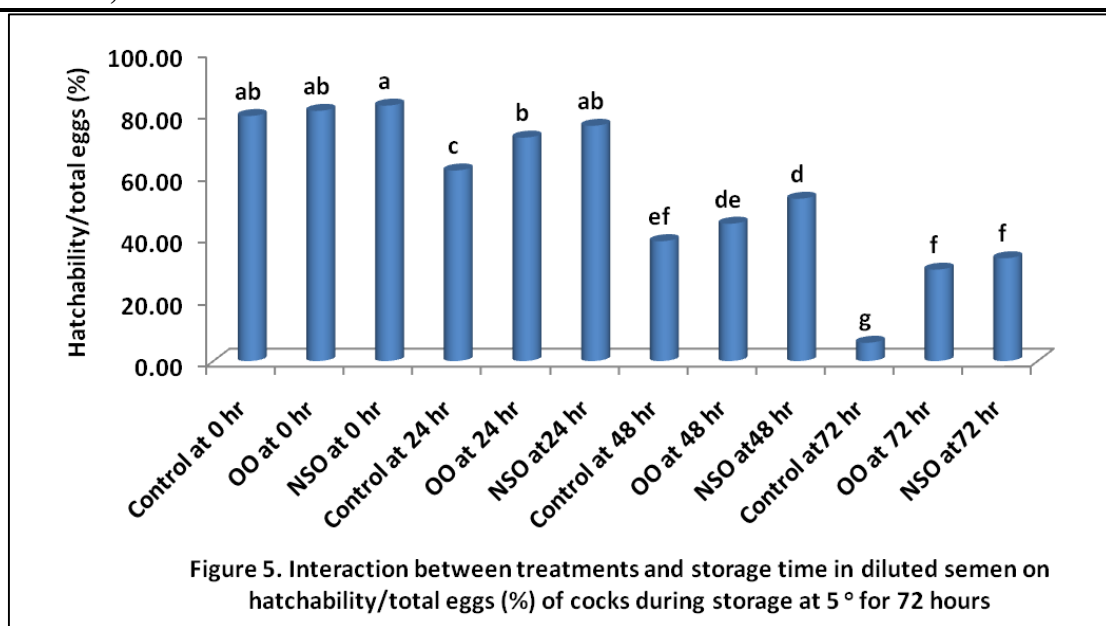


Figure 5. Interaction between treatments and storage time in diluted semen on hatchability/total eggs (%) of cocks during storage at 5 ° for 72 hours

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الملخص العربي

تأثير إضافة مضادات الأكسدة الطبيعية علي كفاءة حفظ السائل المنوي لديوك سلالة المندرية

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الخلاصة: تهدف هذه الدراسة إلى دراسة تأثير إضافة زيت الزيتون أو زيت حبة البركة كمخفف مضادات للأكسدة علي جودة الحيوانات المنوية والخصوبة أثناء التخزين في 5 درجة مئوية. شملت هذه الدراسة تجربة عاملية 4×3 تضمنت ثلاث مصادر {السائل المنوي الذي تم تخفيفه بنسبة 1:1 بمخفف Lake (LD) من دون أي إضافة (كنترول)، السائل المنوي الذي تم تخفيفه بنسبة 1:1 بمخفف LD مع إضافة زيت الزيتون وزيت حبة البركة بنسبة 1 مل/100 مل من المخفف علي التوالي}، 4 توقيتات لحفظ السائل المنوي (0 و 24 و 48 و 72 ساعة تحت درجة حرارة 5 درجة مئوية). تم استخدام 60 ديك ، 180 دجاجة عمر 38 أسبوعاً في هذه الدراسة لتوفير بيانات عن جوده الحيوانات المنوية ونسبة الفقس.

وقد أظهرت النتائج المتحصل عليها زيادة معنوية ($P < 0.01$) في حركة الحيوانات المنوية (%) وانخفاضاً معنوياً ($P < 0.01$) للحيوانات المنوية الميتة (%) والمشوهة (%) وتشوه الأكرسوم (%) الديوك وبالتالي تحسن نسبة الفقس للدجاج مع السائل المنوي المخفف بزيت حبة البركة ثم بزيت الزيتون مقارنة مع بالسائل المنوي المخفف أثناء فترات التخزين المختلفة (صفر، 24، 48، 72 ساعة عند 5 م⁵).

وانخفض معنوياً ($P < 0.01$) النسبة المئوية لحركة الحيوانات المنوية للديوك ومعدل الخصوبة للدجاج، في حين زادت النسبة المئوية للحيوانات المنوية الميتة والمشوهة وتشوه الأكرسوم مع زيادة فترة تخزين السائل المنوي المخفف.

ولذلك ، يمكن استخدام زيت حبة البركة أو زيت الزيتون لتحسين صفات جوده السائل المنوي للديوك عند الحفظ عند 5 م⁵ من صفر إلي 72 ساعة وكذلك معدل فقس دجاج المندرية البياض.