







## RESEARCH ARTICLE

# Impact of Vitamin E and Selenium Prior the Ovsynch Synchronization on Reproductive Performance in Friesian Dairy Cows During Hot Season

Al-Moataz Bellah Mahfouz SHAARAWY<sup>1</sup>  Ahmed Mohamed SHEHABELDIN<sup>2</sup>   
Mohamed El-Shafie Abd El-Kader OMAR<sup>1</sup>  Ashraf Ali MEHANY<sup>1</sup>   
Reda Abdel Samee Ahmed REZK<sup>3</sup>  Hamada Mahmoud YOUSIF<sup>3</sup> (\*) 

<sup>1</sup> Agricultural Research Center (ARC), Animal Production Research Institute (APRI), Department of Cattle Breeding Research, 12618, Dokki, Giza, EGYPT

<sup>2</sup> Agricultural Research Center (ARC), Animal Production Research Institute (APRI), Department of Biotechnology Research, 12618, Dokki, Giza, EGYPT

<sup>3</sup> Agricultural Research Center (ARC), Animal Health Research Institute (AHRI), Department of Biochemistry, Toxicology and Feed Deficiency, 12618, Dokki, Giza, EGYPT



(\*) **Corresponding author:** Hamada Mahmoud YOUSIF

Phone: +20 1223074010

Fax: +20 33350030

E-mail: [hamadayousif82@gmail.com](mailto:hamadayousif82@gmail.com)

How to cite this article?

Shaarawy AM, Shehabeldin AM, Omar MEA, Mehany AA, Rezk RAA, Yousif HM: Impact of vitamin E and selenium prior the ovsynch synchronization on reproductive performance in Friesian dairy cows during hot season. *Kafkas Univ Vet Fak Derg*, 30 (5): 595-602, 2024.  
DOI: 10.9775/kvfd.2024.317740

Article ID: KVFD-2024-31740

Received: 05.02.2024

Accepted: 23.06.2024

Published Online: 12.07.2024

## Abstract

Heat stress during hot season enhances oxidative stress, alters hormonal secretion and adversely affects reproductive performance. So, it's necessary to supply antioxidants such as vitamin E and selenium. This work aimed to investigate the impact of vitamin E and selenium administration on reproductive performance before ovsynch synchronization and artificial insemination (AI) in Friesian cows during hot season. Twenty ovsynch-synchronized Friesian cows were divided according to the time of vitamin E and selenium administration into four groups (each 5 cows), G<sub>1</sub> control group without any administration, G<sub>2</sub> administered pre-synchronization, G<sub>3</sub> administered at AI, G<sub>4</sub> administered two doses pre-synchronization and at AI. Follicular functions during synchronization period revealed a significant increase in both follicle number and diameter of G<sub>2</sub> and G<sub>4</sub> compared to G<sub>1</sub> and G<sub>3</sub>. Blood samples were collected from all cows weekly from 0-day to 5<sup>th</sup> week post AI for hormones and antioxidant detection. Serum analysis results revealed increased progesterone, prolactin, CAT, SOD, GSH and total antioxidant capacity (TAC), while decreased cortisol and MDA of G<sub>4</sub> compared to G<sub>1</sub>, G<sub>2</sub> and G<sub>3</sub>. Pregnancy detection was performed at 60 days post-AI. Conception rates were 40, 60, 60, and 80% in G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub> and G<sub>4</sub>, respectively. Therefore, vitamin E and selenium administration improve antioxidant activities and overcome oxidative stress providing a better impact on reproductive performance even in hot season.

**Keywords:** Antioxidants, Conception rate, Heat stress, Hormones, Synchronization, Vitamin E and selenium

## INTRODUCTION

Heat stress during hot season could change the physiological, biochemical, and productive functions of the livestock resulting in reduced fertility and economic losses for the dairy industry. Furthermore, the adverse effect of environmental high temperatures on fertility rates cannot be omitted<sup>[1]</sup>. Exposure of dairy cows to heat stress during early lactation can adversely affect their productivity, fertility, and blood biochemistry in subsequent lactation periods<sup>[2]</sup>. Under such conditions, heat stress can affect the cellular functions of germ cells and directly impact fertility. Heat stress directly affects follicular development, follicular waves, steroid gene activity in both

follicular and granulosa cells, corpus luteum development and functionality via reduced progesterone production<sup>[3]</sup>. Low progesterone secretion during the luteal phase can alter oocyte maturation leading to implantation failure and early embryonic death<sup>[4]</sup>. While, reduced feed intake during heat stress reduces the frequency of luteinizing hormone (LH) and lengthens the follicular waves with the appearance of smaller dominant follicles<sup>[5]</sup>.

Heat stress increases body temperature, pulse rate, and respiration rate leading to reduced feed intake, redistribution of blood flow, weakening of the immune system, and changes in endocrine function, which ultimately affects fertility<sup>[6]</sup>. Environmental heat stress, may



**Table 1.** Average of the daily ambient temperature (°C), relative humidity (%), and temperature humidity index (THI) during the hot season

Month	Ambient Temperature (°C)			Relative Humidity (%)			THI
	Max.	Min.	Average	Max.	Min.	Average	
June	33	26	28.89±0.28	57	35	50.85±0.90	76.82±0.27
July	32	27	29.85±0.21	59	42	51.14±0.62	78.16±0.25
August	33	29	29.95±0.16	60	45	54.04±0.76	78.74±0.16
September	31	26	28.51±0.18	60	47	54.57±0.72	76.92±0.28

Data are expressed as average ± SE

activate the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal axis, both of which mediate hormonal changes. Additionally, it suppresses thyroid and pancreas activity, resulting in decreased secretion of thyroid hormones and insulin, while concurrently enhancing adrenal cortex activity and increasing the secretion of the stress hormone cortisol [7]. Animals exposed to heat stress revealed altered hormones concentrations such as thyroxin, cortisol, and prolactin. Plasma cortisol levels are used as a marker of heat stress, decreasing during heat acclimation to reduce heat production [8]. Furthermore, heat stress reduces antioxidant activity and induces oxidative stress, while it promotes excessive production of free radicals, and reduces antioxidant status by impairing antioxidant defense systems [9].

Vitamin E and selenium (Se), both necessary co-factors of the enzyme glutathione peroxidase (GSH-px), are a vital part of the antioxidant defense system present in various cell types [10], and are playing important roles in animal growth performance, immune function, and reproductive performance through their participation in key enzymatic reactions [11]. The contribution of vitamin E and Se to progesterone production by the corpus luteum improves the resumption of ovarian activity [12]. It has been demonstrated that administration of vitamin E and/or Se increases the pregnancy rates and both together can improve the conception rate of cattle and sheep by decreasing early embryonic deaths [13,14]. Furthermore, vitamin E and Se could improve reproduction, metabolic profiles, and antioxidant capacity in cows under conditions of heat stress.

The purpose of this study was to investigate the impact of vitamin E and selenium administration on the reproductive performance of Friesian cows during the hot summer season.

## MATERIAL AND METHODS

### Ethical Statement

This protocol was approved by the Research Committee of the Animal Health Research Institute and authorized by the Institutional Animal Care and Use Committee

(ARC-IACUC)/Agricultural Research Center (ARC/AHRI/33/24).

### Farm Location and Climatic Conditions

This work was established at Animal Production Experimental Station, Sakha (31°05'17.3" north and 30°56'29.9" east), Kafr El-Sheikh, belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Egypt from June to September 2022. Ambient temperature and relative humidity (RH) during the hot season are shown in Table 1. These parameters were recorded daily for the entire experimental period outside by the National Aeronautics and Space Administration (NASA) Langley Research Centre (LaRC) Prediction of Worldwide Energy Resource (POWER) Project funded through the NASA Earth Science/Applied Science Program. Temperature humidity index (THI) was calculated using the formula proposed by Mader et al. [15]:

$$THI = (0.8 \times \text{ambient temperature}) + [(\% \text{ RH}) / 100] \times (\text{ambient temperature} - 14.4)] + 46.4$$

Armstrong [16] describes the temperature humidity index (THI) for dairy cattle, establishing a spectrum where a THI up to 71 is classified as comfort. Indices ranging from 72 to 79 signify mild thermal stress, 80 to 89 indicate moderate stress, and values exceeding 90 represent severe stress, and a THI surpassing 100 indicates lethal outcomes.

### Experimental Design

Twenty apparently healthy Friesian cows, with an age range 5-6 years, an average body weight 500±45 kg, daily milk yield 18±5 kg, and within 55 to 70 days postpartum, were used in this study. Ovsynch synchronization of all cows was performed according to the following protocol; 2 mL GnRH was injected on day 0, followed by 2 mL PGF<sub>2</sub>α injected on the 7<sup>th</sup> day, and concluding with a second 2 mL GnRH injection on day 9 with artificial insemination (AI) being performed 16 hours post-final injection [17]. The cows were divided into four groups; the 1<sup>st</sup> group (N=5) did not receive any administration used as a control group (G<sub>1</sub>), the 2<sup>nd</sup> group (N=5) received a single dose of E-SELEN® (1 mL/45 kg BW) immediately pre-synchronization only (G<sub>2</sub>), the 3<sup>rd</sup> group (N=5) was administered a single dose of E-SELEN® (1 mL/45 kg BW) at the time of AI only (G<sub>3</sub>), and the 4<sup>th</sup> group (N=5) received a double dose of

E-SELEN<sup>®</sup> (1 mL/45 kg BW) the first dose immediately pre-synchronization and the second dose at the time of AI (G<sub>4</sub>).

The follicular functions were detected on day 0, as well as on the 7<sup>th</sup> and 10<sup>th</sup> day after synchronization using a real-time ultrasound scanning device (Ultrasound scanner, model MyLab 30 Gold ultrasound machine-Esaote-Pie Medical-Holland-Italy) with LV 315 Linear trans-rectal probe. Scans of both ovaries were executed to enumerate follicles and measure their diameters in millimeters. Also, the same real-time ultrasound scanning device was used for pregnancy diagnosis on day 60 post-AI.

### Samples

Blood samples were collected from the jugular vein of all cows on day 0, and 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>-week post-AI. Serum samples were obtained by centrifugation of blood samples at 5000 rpm for 5 min. The clear sera were aliquoted into clean, dry Eppendorf tubes and maintained at 4°C for subsequent hormonal assays, followed by storage at -20°C pending biochemical analyses.

### Chemicals and Medications

Estrumate<sup>®</sup>; Beef and dairy cattle were intramuscularly injected with Prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) (MSD Animal Health Company). Cystorelin<sup>®</sup>; a synthetic GnRH analogue for the release of both luteinizing (LH) and follicle-stimulating hormone (FSH), contains Gonadorelin (GnRH) diacetate tetrahydrate 50 mcg/mL, manufactured by Ceva-Africa Company. E-SELEN<sup>®</sup>; each mL contains 5.48 mg sodium selenite (equivalent to 2.5 mg selenium), 50 mg (68 IU) vitamin E (as α-alpha tocopherol acetate), manufactured by NITA-FARM Company.

### Serum Biochemical Analysis

Serum progesterone concentrations were estimated using ELISA kit (Catalog No. 10005, PerkinElmer) with a sensitivity threshold of 0.2 ng/mL. The serum concentration of tri-iodothyronine (T<sub>3</sub>), thyroxin (T<sub>4</sub>),

and thyroid stimulating hormone (TSH) were assessed using ELISA kit (Immunospec corporation, USA, catalog No. PerkinElmer-10301, PerkinElmer-10302 and PerkinElmer-10304). Serum cortisol levels were evaluated using ELISA kit (PekinElmer-10005; 00000; DBC, Canada; catalog No. CAN-C-270 and SinoGene-Clon-SG-60105). Serum prolactin (PRL) concentration were detected using Bovine Prolactin ELISA kit (SinoGene-Clon Biotech Co., Ltd) with a sensitivity threshold of 0.3 ng/mL.

Commercial kits were used to determine Superoxide dismutase activity (BIODIAGNOSTIC, CAT. No. SD2521) and total antioxidant capacity (TAC) (CAT. No. TA2513). Lipid peroxidation expressed as malondialdehyde (MDA) was detected calorimetrically according to Okhawa et al.<sup>[18]</sup>, reduced glutathione (GSH) levels were detected by the method described by Pleban et al.<sup>[19]</sup>, and catalase (CAT) activity was detected by the method described by Aebi<sup>[20]</sup>.

### Statistical Analysis

Data were statistically analyzed using one-way (ANOVA) by SPSS 22 software to assess the significant differences between groups and times within groups. The results were demonstrated as means ± SE. The results were considered statistically significant at P<0.05.

## RESULTS

The results of follicular functions (*Table 2*) revealed a significant (P<0.05) increase in follicle number (FN) in both G<sub>2</sub> and G<sub>4</sub> groups on the 10<sup>th</sup> day of synchronization compared to the other groups. There was a gradual significant (P<0.05) increase in follicle diameter (FD) from the 7<sup>th</sup> day to the 10<sup>th</sup> day of synchronization in both G<sub>2</sub> and G<sub>4</sub> groups compared to groups G<sub>1</sub> and G<sub>3</sub>.

Serum hormone results (*Table 3*) revealed a gradual increase of progesterone (P<sub>4</sub>) level in all groups, but there was a significant (P<0.05) increase in G<sub>4</sub> from the 1<sup>st</sup> week to week 5 post-AI compared to G<sub>1</sub> and a non-significant

**Table 2.** Effects of vitamin E and selenium administration of ovsynch synchronized Friesian cows on follicle number (FN) and diameter (FD) during hot season

Item	Time	Groups			
		1 <sup>st</sup> Group (G <sub>1</sub> )	2 <sup>nd</sup> Group (G <sub>2</sub> )	3 <sup>rd</sup> Group (G <sub>3</sub> )	4 <sup>th</sup> Group (G <sub>4</sub> )
Follicle Number	0-day	1.80±0.84	2.00±0.71	1.80±0.84	2.00±0.71
	7 <sup>th</sup> day	2.00±0.71	3.00±0.71	2.20±0.84	3.20±0.84
	10 <sup>th</sup> day	2.20±0.84 <sup>b</sup>	3.40±1.14 <sup>a</sup>	2.40±0.55 <sup>b</sup>	3.60±1.14 <sup>a</sup>
Follicle Diameter (mm)	0-day	5.60±0.87	6.26±0.71	5.66±0.64	6.14±0.86
	7 <sup>th</sup> day	6.16±0.66 <sup>bb</sup>	9.40±0.81 <sup>aAB</sup>	6.22±0.74 <sup>bb</sup>	9.62±0.86 <sup>aAB</sup>
	10 <sup>th</sup> day	7.82±0.77 <sup>ba</sup>	10.00±0.8 <sup>aA</sup>	7.90±0.8 <sup>9ba</sup>	10.60±0.80 <sup>aA</sup>

Data are expressed as mean ± SE

<sup>a,b</sup> Superscript: Mean significance difference among groups in the same row on P<0.05

<sup>A,B</sup> Superscript: Mean significance difference among administration times in the same column on P<0.05

**Table 3.** Effects of vitamin E and selenium administration of ovsynch synchronized Friesian cows on serum hormones during hot season

Parameters	Groups	Times					
		0-day	Week 1	Week 2	Week 3	Week 4	Week 5
Progesterone (ng/mL)	1 <sup>st</sup> group (G <sub>1</sub> )	0.287±0.05 <sup>C</sup>	1.037±0.411 <sup>bB</sup>	2.580±0.935 <sup>A</sup>	3.301±1.724 <sup>bA</sup>	3.535±1.861 <sup>bA</sup>	4.594±2.201 <sup>bA</sup>
	2 <sup>nd</sup> group (G <sub>2</sub> )	0.311±0.056 <sup>D</sup>	2.376±0.704 <sup>abC</sup>	3.740±1.340 <sup>BC</sup>	5.380±1.81 <sup>aAB</sup>	5.580±1.630 <sup>abAB</sup>	6.499±1.446 <sup>abA</sup>
	3 <sup>rd</sup> group (G <sub>3</sub> )	0.294±0.057 <sup>D</sup>	2.288±0.571 <sup>abC</sup>	3.820±1.150 <sup>BC</sup>	5.420±1.85 <sup>aAB</sup>	5.620±1.650 <sup>abAB</sup>	6.579±1.495 <sup>abA</sup>
	4 <sup>th</sup> group (G <sub>4</sub> )	0.344±0.072 <sup>E</sup>	2.592±0.673 <sup>aD</sup>	4.420±1.336 <sup>CD</sup>	6.420±2.13 <sup>aBC</sup>	7.420±2.023 <sup>aAB</sup>	8.379±1.847 <sup>aA</sup>
T <sub>3</sub> (ng/mL)	1 <sup>st</sup> group (G <sub>1</sub> )	3.034±0.417	2.993±0.21 <sup>b</sup>	2.933±0.201 <sup>b</sup>	2.994±0.171 <sup>b</sup>	3.009±0.193 <sup>b</sup>	3.088±0.271 <sup>c</sup>
	2 <sup>nd</sup> group (G <sub>2</sub> )	3.165±0.334 <sup>B</sup>	3.25±0.233 <sup>abAB</sup>	3.411±0.227 <sup>aAB</sup>	3.63±0.294 <sup>aA</sup>	3.598±0.245 <sup>aA</sup>	3.368±0.275 <sup>bcAB</sup>
	3 <sup>rd</sup> group (G <sub>3</sub> )	3.032±0.385 <sup>C</sup>	3.27±0.329 <sup>abBC</sup>	3.59±0.255 <sup>aAB</sup>	3.687±0.264 <sup>aA</sup>	3.694±0.205 <sup>aA</sup>	3.613±0.232 <sup>abAB</sup>
	4 <sup>th</sup> group (G <sub>4</sub> )	3.294±0.347 <sup>B</sup>	3.452±0.328 <sup>aAB</sup>	3.71±0.268 <sup>aA</sup>	3.763±0.26 <sup>aA</sup>	3.783±0.272 <sup>aA</sup>	3.86±0.225 <sup>aA</sup>
T <sub>4</sub> (µg/dL)	1 <sup>st</sup> group (G <sub>1</sub> )	6.549±0.689	6.233±0.749	6.224±0.632	6.396±0.602	6.414±0.57	6.444±0.63
	2 <sup>nd</sup> group (G <sub>2</sub> )	6.494±0.668	6.362±0.597	6.28±0.579	6.421±0.644	6.542±0.555	6.636±0.532
	3 <sup>rd</sup> group (G <sub>3</sub> )	6.589±0.727	6.433±0.572	6.456±0.694	6.441±0.669	6.58±0.568	6.676±0.583
	4 <sup>th</sup> group (G <sub>4</sub> )	6.629±0.668	6.808±0.637	6.861±0.496	6.804±0.556	7.053±0.479	7.118±0.574
TSH (µU/mL)	1 <sup>st</sup> group (G <sub>1</sub> )	0.871±0.068 <sup>bD</sup>	1.025±0.089 <sup>bc</sup>	1.032±0.058 <sup>C</sup>	1.057±0.061 <sup>BC</sup>	1.175±0.078 <sup>AB</sup>	1.223±0.079 <sup>A</sup>
	2 <sup>nd</sup> group (G <sub>2</sub> )	1.010±0.077 <sup>aD</sup>	1.054±0.091 <sup>abCD</sup>	1.103±0.07 <sup>BCD</sup>	1.177±0.084 <sup>AB</sup>	1.206±0.094 <sup>AB</sup>	1.232±0.115 <sup>A</sup>
	3 <sup>rd</sup> group (G <sub>3</sub> )	0.986±0.087 <sup>aB</sup>	1.131±0.068 <sup>abA</sup>	1.134±0.075 <sup>A</sup>	1.161±0.072 <sup>A</sup>	1.191±0.091 <sup>A</sup>	1.227±0.107 <sup>A</sup>
	4 <sup>th</sup> group (G <sub>4</sub> )	1.076±0.097 <sup>a</sup>	1.173±0.076 <sup>a</sup>	1.133±0.068	1.113±0.056	1.112±0.054	1.133±0.068
Cortisol (µg/dL)	1 <sup>st</sup> group (G <sub>1</sub> )	20.663±2.696 <sup>aA</sup>	22.86±2.965 <sup>aAB</sup>	23.103±2.819 <sup>aB</sup>	23.863±2.965 <sup>aBC</sup>	24.663±2.877 <sup>aBC</sup>	25.623±2.926 <sup>aC</sup>
	2 <sup>nd</sup> group (G <sub>2</sub> )	15.42±1.42 <sup>b</sup>	15.1±1.38 <sup>b</sup>	14.42±1.22 <sup>b</sup>	14.02±1.096 <sup>b</sup>	14.26±1.07 <sup>b</sup>	14.46±1.24 <sup>b</sup>
	3 <sup>rd</sup> group (G <sub>3</sub> )	19.86±1.898 <sup>aB</sup>	15.29±1.27 <sup>bA</sup>	14.07±1.18 <sup>bA</sup>	13.78±1.15 <sup>bA</sup>	13.62±1.12 <sup>bA</sup>	13.55±1.05 <sup>bA</sup>
	4 <sup>th</sup> group (G <sub>4</sub> )	14.90±1.2 <sup>b</sup>	13.7±1.07 <sup>b</sup>	13.48±0.74 <sup>b</sup>	13.26±0.62 <sup>b</sup>	13.09±0.67 <sup>b</sup>	12.77±0.78 <sup>b</sup>
Prolactin (ng/mL)	1 <sup>st</sup> group (G <sub>1</sub> )	241.10±22.07 <sup>c</sup>	236.40±21.87 <sup>c</sup>	235.20±21.65 <sup>c</sup>	238.05±20.09 <sup>c</sup>	242.69±21.44 <sup>c</sup>	247.15±21.1 <sup>c</sup>
	2 <sup>nd</sup> group (G <sub>2</sub> )	310.90±25.8 <sup>ab</sup>	316.70±25.85 <sup>b</sup>	324.30±24.16 <sup>b</sup>	331.16±24.48 <sup>b</sup>	327.34±25.62 <sup>b</sup>	331.35±28.37 <sup>b</sup>
	3 <sup>rd</sup> group (G <sub>3</sub> )	286.6±27.34 <sup>bc</sup>	313.88±25.39 <sup>bBC</sup>	339.10±22.33 <sup>baB</sup>	347.11±24.55 <sup>baB</sup>	352.80±25.58 <sup>baB</sup>	360.54±30.27 <sup>ba</sup>
	4 <sup>th</sup> group (G <sub>4</sub> )	326.940±25.49 <sup>aE</sup>	372.50±27.27 <sup>aD</sup>	406.97±29.03 <sup>aC</sup>	434.30±34.38 <sup>aBC</sup>	459.10±39.47 <sup>aB</sup>	474.62±37.35 <sup>aA</sup>

Data are expressed as mean ± SE

<sup>a, b, c</sup> Superscript: Mean significance difference among groups in the same column on P<0.05<sup>A, B, C, D, E</sup> Superscript: Mean significance difference among administration times in the same row on P<0.05

increase compared to the other two groups, G<sub>2</sub> and G<sub>3</sub>. There was a non-significant change in T<sub>4</sub> levels during the experimental period between all groups. There was a gradually non-significant decrease in T<sub>3</sub> level of G<sub>1</sub> until the week 4. A significant (P<0.05) increase in the T<sub>3</sub> level of G<sub>2</sub>, G<sub>3</sub>, and G<sub>4</sub> from week 2 to week 5 compared to G<sub>1</sub>. TSH levels results revealed a gradual increase in G<sub>1</sub>, G<sub>2</sub>, and G<sub>3</sub> from week 1 to week 5 with only a significant (P<0.05) decrease in G<sub>1</sub> on the 1<sup>st</sup> day post-AI compared to G<sub>2</sub>, G<sub>3</sub>, and G<sub>4</sub> and also a significant (P<0.05) decrease on the week 1 compared to G<sub>4</sub>. There was a significant (P<0.05) increase in cortisol levels of G<sub>1</sub> throughout the experimental period compared to G<sub>2</sub>, G<sub>3</sub>, and G<sub>4</sub> groups. On the other hand, there was a significant (P<0.05) increase in the prolactin level of G<sub>4</sub> throughout the experimental period compared to G<sub>1</sub>, G<sub>2</sub>, and G<sub>3</sub>. In the same time, there was a

significant (P<0.05) increase in prolactin levels of G<sub>2</sub> and G<sub>3</sub> throughout the experimental period compared to G<sub>1</sub>.

The results of oxidative stress biomarkers and antioxidants activities (Table 4) revealed a significant (P<0.05) increase in MDA and a significant (P<0.05) decrease in GSH, CAT, SOD, and TAC activities in G<sub>1</sub> compared with the other groups (G<sub>2</sub>, G<sub>3</sub>, and G<sub>4</sub>) from week 1 to week 5 post-AI. At the same time, there was a significant (P<0.05) decrease in MDA and a significant (P<0.05) increase in GSH, CAT, SOD, and TAC of G<sub>4</sub> compared to the other two groups of vitamin E and selenium administration G<sub>2</sub> and G<sub>3</sub> on the most of the periods post-AI.

The results of pregnancy diagnosis (Table 5) revealed that the conception rates were 2/5 (40%), 3/5 (60%), 3/5 (60%), and 4/5 (80%) in G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub>, and G<sub>4</sub>, respectively.

**Table 4.** Effects of vitamin E and selenium administration of ovsynch synchronized Friesian cows on serum oxidative stress biomarkers during hot season

Parameters	Groups	Times					
		0-day	Week 1	Week 2	Week 3	Week 4	Week 5
MDA (nmol/mL)	1 <sup>st</sup> group (G <sub>1</sub> )	1.785±0.139 <sup>a</sup>	1.830±0.134 <sup>a</sup>	1.759±0.14 <sup>a</sup>	1.744±0.136 <sup>a</sup>	1.775±0.125 <sup>a</sup>	1.797±0.154 <sup>a</sup>
	2 <sup>nd</sup> group (G <sub>2</sub> )	1.461±0.128 <sup>b</sup>	1.414±0.108 <sup>b</sup>	1.360±0.093 <sup>b</sup>	1.395±0.097 <sup>b</sup>	1.417±0.099 <sup>b</sup>	1.431±0.091 <sup>b</sup>
	3 <sup>rd</sup> group (G <sub>3</sub> )	1.709±0.123 <sup>aA</sup>	1.394±0.107 <sup>bB</sup>	1.287±0.103 <sup>bBC</sup>	1.206±0.082 <sup>cC</sup>	1.163±0.103 <sup>cC</sup>	1.209±0.102 <sup>cC</sup>
	4 <sup>th</sup> group (G <sub>4</sub> )	1.354±0.128 <sup>bA</sup>	1.226±0.06 <sup>cAB</sup>	1.106±0.071 <sup>cBC</sup>	1.013±0.084 <sup>dC</sup>	0.982±0.055 <sup>dC</sup>	1.1040±0.078 <sup>cC</sup>
GSH (mg/dL)	1 <sup>st</sup> group (G <sub>1</sub> )	2.297±0.179 <sup>c</sup>	2.264±0.196 <sup>d</sup>	2.309±0.122 <sup>c</sup>	2.452±0.141 <sup>d</sup>	2.377±0.116 <sup>d</sup>	2.305±0.113 <sup>d</sup>
	2 <sup>nd</sup> group (G <sub>2</sub> )	4.129±0.362 <sup>cC</sup>	4.448±0.383 <sup>bB</sup>	4.553±0.384 <sup>bA</sup>	4.339±0.363 <sup>cBC</sup>	4.048±0.346 <sup>CD</sup>	3.818±0.277 <sup>cD</sup>
	3 <sup>rd</sup> group (G <sub>3</sub> )	2.900±0.176 <sup>bC</sup>	3.949±0.283 <sup>bB</sup>	4.837±0.326 <sup>bA</sup>	5.049±0.361 <sup>bA</sup>	4.998±0.358 <sup>bA</sup>	4.707±0.321 <sup>bA</sup>
	4 <sup>th</sup> group (G <sub>4</sub> )	4.254±0.324 <sup>aA</sup>	5.094±0.407 <sup>aB</sup>	5.522±0.438 <sup>aC</sup>	5.681±0.449 <sup>aC</sup>	5.767±0.433 <sup>aC</sup>	5.547±0.351 <sup>aC</sup>
CAT (U/mL)	1 <sup>st</sup> group (G <sub>1</sub> )	7.751±0.604 <sup>b</sup>	7.638±0.661 <sup>c</sup>	7.592±0.671 <sup>c</sup>	7.715±0.682 <sup>d</sup>	7.742±0.684 <sup>d</sup>	7.558±0.656 <sup>d</sup>
	2 <sup>nd</sup> group (G <sub>2</sub> )	13.529±1.189 <sup>aB</sup>	14.178±1.218 <sup>bAB</sup>	15.084±1.26 <sup>bA</sup>	14.665±1.199 <sup>cAB</sup>	13.721±1.12 <sup>cAB</sup>	13.020±1.15 <sup>cB</sup>
	3 <sup>rd</sup> group (G <sub>3</sub> )	8.952±0.727 <sup>bC</sup>	13.845±1.192 <sup>bB</sup>	15.636±1.156 <sup>bA</sup>	16.79±1.314 <sup>bA</sup>	16.86±1.439 <sup>bA</sup>	16.216±1.462 <sup>bA</sup>
	4 <sup>th</sup> group (G <sub>4</sub> )	13.901±1.054 <sup>aB</sup>	17.539±1.277 <sup>aA</sup>	18.484±1.355 <sup>aA</sup>	18.719±1.478 <sup>aA</sup>	19.023±1.535 <sup>aA</sup>	18.865±1.423 <sup>aA</sup>
SOD (U/mL)	1 <sup>st</sup> group (G <sub>1</sub> )	13.422±1.046 <sup>c</sup>	13.228±1.144 <sup>c</sup>	13.494±0.714 <sup>c</sup>	14.331±0.826 <sup>c</sup>	13.891±0.681 <sup>d</sup>	13.47±0.66 <sup>d</sup>
	2 <sup>nd</sup> group (G <sub>2</sub> )	26.16±2.587 <sup>aBC</sup>	28.10±2.844 <sup>bAB</sup>	30.448±2.321 <sup>bA</sup>	30.134±2.34 <sup>bA</sup>	26.979±1.819 <sup>cB</sup>	24.074±1.718 <sup>cC</sup>
	3 <sup>rd</sup> group (G <sub>3</sub> )	16.893±1.401 <sup>bC</sup>	27.02±2.03 <sup>bB</sup>	31.63±2.373 <sup>abA</sup>	33.67±2.77 <sup>aA</sup>	32.06±2.18 <sup>bA</sup>	29.81±2.39 <sup>bA</sup>
	4 <sup>th</sup> group (G <sub>4</sub> )	27.77±2.92 <sup>aC</sup>	31.73±2.79 <sup>aB</sup>	34.22±2.66 <sup>aAB</sup>	35.36±2.87 <sup>aA</sup>	36.31±2.42 <sup>aA</sup>	34.63±2.35 <sup>aAB</sup>
TAC (mU/L)	1 <sup>st</sup> group (G <sub>1</sub> )	1.089±0.085 <sup>b</sup>	1.073±0.093 <sup>c</sup>	1.094±0.058 <sup>c</sup>	1.162±0.067 <sup>c</sup>	1.127±0.055 <sup>d</sup>	1.092±0.054 <sup>d</sup>
	2 <sup>nd</sup> group (G <sub>2</sub> )	1.34±0.089 <sup>aB</sup>	1.394±0.129 <sup>bAB</sup>	1.492±0.122 <sup>bA</sup>	1.496±0.114 <sup>bA</sup>	1.391±0.102 <sup>cAB</sup>	1.324±0.108 <sup>cB</sup>
	3 <sup>rd</sup> group (G <sub>3</sub> )	1.185±0.073 <sup>bC</sup>	1.357±0.097 <sup>bB</sup>	1.576±0.128 <sup>bA</sup>	1.636±0.134 <sup>bA</sup>	1.654±0.142 <sup>bA</sup>	1.573±0.126 <sup>bA</sup>
	4 <sup>th</sup> group (G <sub>4</sub> )	1.418±0.122 <sup>aD</sup>	1.531±0.13 <sup>aCD</sup>	1.632±0.129 <sup>aBC</sup>	1.719±0.143 <sup>aAB</sup>	1.762±0.152 <sup>aA</sup>	1.714±0.121 <sup>aAB</sup>

Data are expressed as mean ± SE  
<sup>a, b, c, and d</sup> Superscript: Mean significance difference among groups in the same column on P<0.05  
<sup>A, B, C, and D</sup> Superscript: Mean significance difference among times of administration in the same row on P<0.05

**Table 5.** Effects of vitamin E and selenium administration of ovsynch synchronized Friesian cows on conception rate during hot season of each group (n=5)

Parameter	Rate	Groups			
		1 <sup>st</sup> Group (G <sub>1</sub> )	2 <sup>nd</sup> Group (G <sub>2</sub> )	3 <sup>rd</sup> Group (G <sub>3</sub> )	4 <sup>th</sup> Group (G <sub>4</sub> )
Conception rate	Number	2	3	3	4
	Percent (%)	40	60	60	80

## DISCUSSION

In this study, ovsynch synchronization was provided using a GnRH and PGF<sub>2</sub>α combination protocol, consistent with the previous studies in buffalo [21], and Friesian cows [22]. The results showed decreased follicular number and diameter in both G<sub>1</sub> and G<sub>3</sub> groups that did not receive vitamin E and Se supplementation. This decrease may be attributed to the adverse impact of heat stress during the hot season on reproductive performance and fertility [1,2]. Heat stress at the beginning of ovulation reduces the diameter and volume of the dominant follicle [23]. Additionally, it may directly impair the cellular function

of reproductive cells, influence follicular maturation and waves [3], and result in smaller dominant follicles due to decreased feed intake during the hot season [5]. Heat stress adversely affects fertility by disrupting follicles and oocytes, likely due to oxidative damage [24]. Elevated temperatures in the hot season reduces feed intake that may compromise the energy balance of cattle and/or disrupting the hypothalamic-hypophyseal-ovarian axis. These factors impair the reproductive performance of the cow and compromise the quality of oocytes, and corpora lutea [25]. Vitamin E and selenium administration in group G<sub>2</sub> and G<sub>4</sub> led to increased follicular number and diameter, highlighting their

role in enhancing reproductive performance and follicular development [26,27]. Administration of vitamin E improves fertility in cows by regulating the free radicals within ovarian tissues. It was thought that this fertility improvement is likely attributed to the intracellular antioxidant activities of vitamin E and selenium which protect the cell membranes from oxidative damage by scavenging reactive oxygen radicals [28]. Notably, reactive oxygen species play important roles in ovulation, oocyte maturation, corpus luteum dynamics, implantation, and fetal development [29].

The hormonal profile revealed a gradual increase in progesterone concentration across all groups, especially after insemination which correlated with embryonic development [30]. The groups administered vitamin E and selenium showed a significantly higher progesterone concentration compared to group G1 (control group), consistent with previous research [31,32]. These results are likely related to the role of vitamin E and selenium in improving the resumption of ovarian activity by contributing to progesterone production by the corpus luteum [12].

Thyroid hormones revealed a gradual decline in  $T_3$  levels alongside a corresponding elevation of TSH in group  $G_1$  starting from the 1<sup>st</sup> week post-AI. These findings were consistent with previous studies in pregnant cows [33,34]. Reduced  $T_3$  levels during the hot season could be attributed to the negative impact of elevated temperature on thyroid function and thyroid hormone levels [7]. This decrease in thyroid hormones may aid animals in acclimatizing to heat stress, as reduced thyroid concentration leads to decreased cellular oxygen consumption and metabolic heat production [35]. The significant increase of  $T_3$  in the groups which received vitamin E and selenium was consistent with Shakirullah et al. [36] who reported enhanced thyroid hormone production in sheep exposed to combined vitamin E and Se supplementation during heat stress. Selenium is involved in the metabolism of thyroid hormones. The enzyme 5-iodothyronine de-iodinase is a seleno-dependent [37], so co-administration of vitamin E and selenium may promote  $T_3$  levels by facilitating the de-iodination of  $T_4$  into its active form  $T_3$ .

The increased cortisol level in  $G_1$  are in agreement with previous studies, which have reported increased serum cortisol levels during heat stress in the hot season [38]. This finding shows that the cows experience heat stress during such periods. The higher serum cortisol level may be attributed to thermal stress activating the hypothalamic-pituitary-adrenal cortical axis (HPA) leading to enhanced secretion of the stress hormone cortisol [7]. The reduction of cortisol levels observed in the groups administered vitamin E and selenium was consistent with previous studies in sheep [36], and dairy cows [7]. These results

highlight the potential role of vitamin E and selenium in heat stress acclimation, acting as anti-stress factors.

The decrease of prolactin level observed in  $G_1$  compared to the other administered groups may be attributed to the exposure of animals to heat stress, which alters the concentration of hormones such as thyroid hormones, cortisol, and prolactin. Hence, these hormone levels could be used as indicators of stress in animals [8]. Additionally, heat stress reduces feed intake, and also leads to blood flow redistribution, immune system depression and alterations in endocrine functions ultimately affecting the productivity and reproductive performance in cattle [6]. The significant increase in prolactin levels observed in groups administered vitamin E and selenium might be related to the role of selenium and vitamins in stimulating prolactin synthesis and secretion [39]. Current results provide the impact of vitamin E and selenium administration in stimulating prolactin secretion and improving milk yield even during the hot season [26,27].

Despite the adverse effects of heat stress on fertility, including follicle and oocyte disruption potentially induced by oxidative damage, it was essential to assess oxidative stress markers in this study. An increase in MDA activity, as well as decrease in GSH, CAT, SOD and TAC activities within  $G_1$  compared to the administered groups, could be attributed to heat stress induced reduction in antioxidant activity, leading to oxidative stress [9,24,40,41]. Heat stress provides overproduction of free radicals and reactive oxygen species (ROS), disrupting the steady-state concentrations of free radicals, leading to both cellular and mitochondrial oxidative damage. Additionally, the decline in glutathione levels (glutathione insufficiency) is characteristic of reduced glutathione synthesis [42]. Decreased serum TAC concentration may be referred to thermal stress, suggesting that the antioxidant potential as a free radical scavenger has become depleted. Additionally, decreased SOD levels may reveal also endogenous antioxidant mobilization to neutralize free radicals [43]. Lipid peroxidation, mainly involving polyunsaturated fatty acids produces lipid peroxides, with MDA being the most prevalent among them [44]. Improving antioxidant activities in groups administered vitamin E and selenium were in accordance with previous studies in sheep [36], dairy cows [26], buffalos [45], which are in corroboration with the current findings. This condition indicates the positive effects of vitamin E and selenium, which have antioxidant roles. They are important components of the antioxidant defense system, contributing significantly to immune function and reproductive success through their involvement in essential enzymatic reactions [11]. Vitamin E acts as an intra-cellular antioxidant, and scavenging ROS thereby protecting cellular membranes from oxidative damage. Selenium acts as a co-factor in the glutathione

peroxidase enzyme system responsible for extracellular detoxification of free radicals [46].

Pregnancy diagnosis revealed an improved conception rate in groups administered vitamin E and selenium, consistent with findings from previous studies [26,32,47] reporting administration of vitamin E and selenium during late pregnancy improved conception rates in subsequent seasons. This provides the role of vitamin E and selenium to minimize postpartum disorders and improve cow's reproductive efficiency. Hemingway [13] who reported that supplementation of vitamin E plus selenium before mating improved the conception rate of cattle and sheep by decreasing early embryonic deaths. Also, administration of selenium and/or vitamin E combinations has been associated with increased pregnancy rates [14].

In conclusion, heat stress during hot season adversely affects reproductive performance by suppression of reproductive hormones production and enhancement of oxidative stress. Vitamin E and selenium administration reduces the effects of heat stress, acting as potent antioxidant activators, which involvement in crucial enzymatic reactions significantly contributes to reproductive success. Administering two doses of vitamin E and selenium, before synchronization and at artificial insemination, provides more impact on the conception rate compared to a single dose administered either before synchronization or pre artificial insemination.

## DECLARATIONS

**Availability of Data and Materials:** The data sets analyzed during the current study are available from the corresponding author H. M. Yousif on reasonable request.

**Financial Support:** There was no financial support.

**Conflict of Interest:** The authors declared that there is no conflict of interest.

**Declaration of Generative Artificial Intelligence (AI):** This article and/or tables were not written or created by AI and AI-assisted technologies.

**Author Contributions:** A. M. Shaarawy, A. M. Shehabeldin, M. E. A. Omar and A. A. Mehany were participated in the research design and collect the samples. H. M. Yousif and R. A. A. Rezk were performed the experimental duties of this study and serum biochemical analysis. All authors participate in writing and approved the final version of the manuscript.

## REFERENCES

1. Turk R, Podpečan O, Mrkun J, Flegar-Meštrić Z, Perkov S, Zrimšek P: The effect of seasonal thermal stress on lipid mobilization, antioxidant status and reproductive performance in dairy cows. *Reprod Domest Anim*, 50 (4): 595-603, 2015. DOI: 10.1111/rda.12534
2. Stefanska B, Sobolewska P, Fievez V, Pruszyńska-Oszmałek E, Purwin C, Nowak W: The effect of heat stress on performance, fertility, and adipokines involved in regulating systemic immune response during lipolysis of early lactating dairy cows. *J Dairy Sci*, 107 (4): 2111-2128, 2024.

DOI: 10.3168/jds.2023-23804

3. Wolfenson D, Sonego H, Bloch A, Shaham-Albalancy A, Kaim M, Folman Y, Meidan R: Seasonal differences in progesterone production by luteinized bovine theca and granulosa cells. *Domest Anim Endocrinol*, 22 (2): 81-90, 2002. DOI: 10.1016/s0739-7240(01)00127-8
4. Guzeloglu A, Ambrose JD, Kassa T, Diaz T, Thatcher MJ, Thatcher WW: Long term follicular dynamics and biochemical characteristics of dominant follicles in dairy cows subjected to acute heat stress. *Anim Reprod Sci*, 66 (1-2): 15-34, 2001. DOI: 10.1016/s0378-4320(01)00082-3
5. Sartori R, Rosa GJ, Wiltbank MC: Ovarian structures and circulating steroids in heifers and lactating cows in summer and lactating and dry cows in winter. *J Dairy Sci*, 85 (11): 2813-2822, 2002. DOI: 10.3168/jds.S0022-0302(02)74368-3
6. Al-Samawi KA, Al-Hassan MJ, Swelum AA: Thermoregulation of female Aardi goats exposed to environmental heat stress in Saudi Arabia. *Indian J Anim Res*, 48 (4): 344-349, 2014. DOI: 10.5958/0976-0555.2014.00453.1
7. Kalmath GP, Swamy-Narayana M: Effect of antioxidants supplementation on plasma hormonal profiles in Hallikar cattle during different seasons. *Indian J Anim Res*, 53 (1): 28-32, 2019.
8. Sivakumar AVN, Singh G, Varshney VP: Antioxidants supplementation on acid base balance during heat stress in goats. *Asian-Australas J Anim Sci*, 23 (11): 1462-1468, 2010. DOI: 10.5713/ajas.2010.90471
9. Alhidary IA, Shini S, Al Jassim RAM, Abudabos AM, Gaughan JB: Effects of selenium and vitamin E on performance, physiological response and selenium balance in heat-stressed sheep. *J Anim Sci*, 93 (2): 576-588, 2015. DOI: 10.2527/jas.2014-8419
10. Scherf H, Machlin LJ, Frye TM, Krautmann BA, Williams SN: Vitamin E bio-potency: Comparison of various natural derived and chemically synthesized  $\alpha$ -tocopherol. *Anim Feed Sci Technol*, 59 (1-3): 115-126, 1996. DOI: 10.1016/0377-8401(95)00892-6
11. Maraba KP, MLambo V, Yusuf AO, Marume U, Hugo A: Extra dietary vitamin E-selenium as a mitigation strategy against housing-induced stress in Dohne Merino lambs: Effect on growth performance, stress biomarkers, and meat quality. *Small Ruminant Res*, 160, 31-37, 2018. DOI: 10.1016/j.smallrumres.2018.01.009
12. Kamada H, Hodate K: Effect of dietary selenium supplementation on the plasma progesterone concentration in cows. *J Vet Med Sci*, 60 (1): 133-135, 1998. DOI: 10.1292/jvms.60.133
13. Hemingway RG: The influences of dietary intakes and supplementation with selenium and vitamin E on reproduction diseases and reproductive efficiency in cattle and sheep. *Vet Res Commun*, 27 (2): 159-174, 2003. DOI: 10.1023/a:1022871406335
14. Zanella R, Bondan C, Soares JCM, Zanella EL, de-Lima MR: Use of antioxidants to improve the pregnancy rate in beef cattle submitted to a synchronization protocol with progesterone (P4). *Ci Anim Bras*, 11 (3): 477-481, 2010. DOI: 10.5216/cab.v11i3.3848
15. Mader TL, Davis MS, Brown-Brandl T: Environmental factors influencing heat stress in feedlot cattle. *J Anim Sci*, 84 (3): 712-719, 2006. DOI: 10.2527/2006.843712x
16. Armstrong DV: Heat stress interaction with shade and cooling. *J Dairy Sci*, 77 (7): 2044-2050, 1994. DOI: 10.3168/jds.S0022-0302(94)77149-6
17. Jena D, Das S, Patra BK, Biswal SS, Mohanty DN, Samal P: Certain hormonal profiles of postpartum anestrous jersey crossbred cows treated with controlled internal drug release and ovsynch protocol. *Vet World*, 9 (10): 1102-1106, 2016. DOI: 10.14202/vetworld.2016.1102-1106
18. Okhawa H, Ohishi N, Yagi K: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*, 95 (2): 351-358, 1979. DOI: 10.1016/0003-2697(79)90738-3
19. Pleban PA, Munyani A, Beachum J: Determination of selenium concentration and glutathione peroxidase activity in plasma and erythrocytes. *Clin Chem*, 28 (2): 311-316, 1982. DOI: 10.1093/clinchem/28.2.311
20. Aebi H: Catalase *in vitro*. *Methods Enzymol*, 105, 121-126, 1984. DOI: 10.1016/s0076-6879(84)05016-3
21. Roza E, Aritonang SN, Susanti H, Sandra A: Synchronization of GnRH and PGF2 $\alpha$  on estrus response, pregnancy, progesterone hormones in

- crossing of Swamp Buffalo and Water Buffalo in West Sumatra, Indonesia. *Biodiversitas*, 20 (10): 2910-2914, 2019. DOI: 10.13057/biodiv/d201019
22. **González-Maldonado J, Rangel-Santos R, Rodríguez-de Lara R, Ramírez-Valverde G, Bribiesca JER, Vigil-Vigil JM, García-Espinosa MF:** Effects of injecting increased doses of vitamins C and E on reproductive parameters of Holstein dairy cattle. *Rev Mex de Cienc Pecuarías*, 10 (3): 571-582, 2019.
23. **Badinga L, Thatcher WW, Diaz T, Dorst M, Wolfenson D:** Effect of environmental heat stress on follicular development and steroidogenesis in lactating Holstein cows. *Theriogenology*, 39 (4): 797-810, 1993. DOI: 10.1016/0093-691x(93)90419-6
24. **Roth Z:** Effect of heat stress on reproduction in dairy cows: Insights into the cellular and molecular responses of the oocyte. *Annu Rev Anim Biosci*, 5, 151-170, 2017. DOI: 10.1146/annurev-animal-022516-022849
25. **De-Rensis F, Lopez-Gatius F, García-Ispierto I, Morini G, Scaramuzzi RJ:** Causes of declining fertility in dairy cows during the warm season. *Theriogenology*, 91, 145-153, 2017. DOI: 10.1016/j.theriogenology.2016.12.024
26. **Kassab AY, Hamdon H, Senosy W, Daghsh H, Soliman A:** Impact of antioxidants supplementation on metabolic status and reproductive performance of Aberdeen Angus cows during seasonal thermal stress in arid subtropical regions. *Egyptian J Anim Prod*, 57 (1): 1-11, 2020. DOI: 10.21608/EJAP.2020.92753
27. **Chen YH, Chen YM, Tu PA, Lee KH, Chen JY, Hsu JT:** Effect of supplementing vitamin E, selenium, copper, zinc, and manganese during the transition period on dairy cow reproductive performance and immune function. *Vet Sci*, 10 (3): 1-15, 2023. DOI: 10.3390/vetsci10030225
28. **Kaya S, Kocak G, Demir MC, Kacar C:** The effect of vitamin e-trace mineral treatments on reproductive performance in Morkaraman sheep during the breeding season. *Kafkas Univ Vet Fak Derg*, 29 (2): 161-169, 2023. DOI: 10.9775/kvfd.2022.28901
29. **Musa SI, Bitto II, Ayoade JA, Oyedipe OE:** Effects of vitamin E and selenium on fertility and lamb performance of Yankasa sheep. *Open J Vet Med*, 8 (9): 167-173, 2018. DOI: 10.4236/ojvm.2018.89015
30. **LemLey CO, Vonnahme KA, Tager LR, Krause KM, Wilson ME:** Diet-induced alterations in hepatic progesterone (P4) catabolic enzyme activity and P4 clearance rate in lactating dairy cows. *J Endocrinol*, 205 (3): 233-241, 2010. DOI: 10.1677/JOE-10-0042
31. **Kamada H:** Effects of selenium-rich yeast supplementation on the plasma progesterone levels of postpartum dairy cows. *Asian-Austral J Anim Sci*, 30 (3): 347-354, 2017. DOI: 10.5713/ajas.16.0372
32. **Damarany AI:** Effect of treatment with vitamin e and selenium during late gestation period on mastitis, retained placenta and postpartum reproductive parameters in Egyptian Baladi cows. *Egyptian J Anim Prod*, 58 (2): 47-56, 2021. DOI: 10.21608/ejap.2021.73169.1014
33. **Steinhoff L, Jung K, Meyerholz MM, Heidekorn-Dettmer J, Hoedemaker M, Schmicke M:** Thyroid hormone profiles and TSH evaluation during early pregnancy and the transition period in dairy cows. *Theriogenology*, 129, 23-28, 2019. DOI: 10.1016/j.theriogenology.2019.01.023
34. **Fazio E, Bionda A, Chiofalo V, Crepaldi P, Lopreiato V, Medica P, Liotta L:** Adaptive responses of thyroid hormones, insulin, and glucose during pregnancy and lactation in dairy cows. *Animals*, 12 (11): 1395, 2022. DOI: 10.3390/ani12111395
35. **Shido O, Sakurada S, Hashimoto T, Fujikake K, Nagasaka T:** Increase in plasma thyroid levels during the previous heat exposure time in heat-acclimated rats. *Can J Physiol Pharmacol*, 71 (12): 904-907, 1993. DOI: 10.1139/y93-137
36. **Shakirullah, Qureshi MS, Akhtar S, Khan RU:** The effect of vitamin E and selenium on physiological, hormonal and antioxidant status of Damani and Balkhi sheep submitted to heat stress. *App Biol Chem*, 60 (6): 585-590, 2017. DOI: 10.1007/s13765-017-0313-9
37. **Mehdi Y, Dufrasne I:** Selenium in cattle: A review. *Molecules*, 21 (4):545, 2016. DOI: 10.3390/molecules21040545
38. **Alameen AO, Abdelatif AM:** Metabolic and endocrine responses of crossbred dairy cows in relation to pregnancy and season under tropical conditions. *American-Eurasian J Agric Environ Sci*, 12 (8): 1065-1074, 2012. DOI: 10.5829/idosi.ajeaes.2012.12.08.6619
39. **Jia Y, Li Q, Burris WR, Aiken GE, Bridges PJ, Matthews JC:** Forms of selenium in vitamin-mineral mixes differentially affect serum prolactin concentration and hepatic glutamine synthetase activity of steers grazing endophyte-infected tall fescue. *J Anim Sci*, 96 (2): 715-727, 2018. DOI: 10.1093/jas/skx068
40. **Mutwedu VB, Nyongesa AW, Oduma JA, Kitaa JM, Mbaria JM:** Thermal stress causes oxidative stress and physiological changes in female rabbits. *J Therm Biol*, 95:102780, 2021. DOI: 10.1016/j.jtherbio.2020.102780
41. **Saleh MA, Rateb MH, Abd-Allah EAA, Mohamed GA:** Effect of hot dry environment on the oxidative stress indices in male Barki lambs. *Assiut Vet Med J*, 68 (174): 88-95, 2022. DOI: 10.21608/AVMJ.2022.143473.1065
42. **Habeeb AAM:** Symptoms of heat stress in tropical and subtropical regions on farm animals. *Euro J Agri Food Sci*, 2 (6): 1-5, 2020. DOI: 10.24018/ejfood.2020.2.6.107
43. **Ellamie AM, Fouda WA, Ibrahim WM, Ramadan G:** Dietary supplementation of brown seaweed (*Sargassum latifolium*) alleviates the environmental heat stress-induced toxicity in male Barki sheep (*Ovis aries*). *J Therm Biol*, 89:102561, 2020. DOI: 10.1016/j.jtherbio.2020.102561
44. **Gutteridge JMC, Halliwell B:** Mini-review: Oxidative stress, redox stress or redox success? *Biochem Biophys Res Commun*, 502 (2): 183-186, 2018. DOI: 10.1016/j.bbrc.2018.05.045
45. **Hassan MS, Abd-Allah EA:** Impact of vitamin E and selenium supplementation on oxidative parameters and reproductive efficiency of Egyptian buffaloes. *J Multidiscip Sci*, 3 (1): 16-20, 2021.
46. **Speckmann B, Grune T:** Epigenetic effects of selenium and their implications for health. *Epigenetics*, 10 (3): 179-190, 2015 DOI: 10.1080/15592294.2015.1013792
47. **Khalili M, Chamani M, Amanlou H, Nikkha A, Sadeghi AA, Dehkordi FK, Rafiei M, Shirani V:** The effect of feeding inorganic and organic selenium sources on the hematological blood parameters, reproduction and health of dairy cows in the transition period. *Acta Sci Anim Sci*, 42 (1):e45371(1-10), 2020. DOI: 10.4025/actascianimsci.v42i1.45371