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Research Article

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

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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_1 control group without any administration, G_2 administered pre-synchronization, G_3 administered at AI, G_4 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_2 and G_4 compared to G_1 and G_3. Blood samples were collected from all cows weekly from 0-day to 5th 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_4 compared to G_1, G_2 and G_3. Pregnancy detection was performed at 60 days post-AI. Conception rates were 40, 60, 60, and 80% in G_1, G_2, G_3 and G_4, 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 [1]. Exposure of dairy cows to heat stress during early lactation can adversely affect their productivity, fertility, and blood biochemistry in subsequent lactation periods [2]. 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 [3]. Low progesterone secretion during the luteal phase can alter oocyte maturation leading to implantation failure and early embryonic death [4]. 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 [5].

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 [6]. 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 × ambient temperature) + [(% RH) /100) × (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 $PGF_2\alpha$ injected on the 7th 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 1st group (N=5) did not receive any administration used as a control group (G_1), the 2nd group (N=5) received a single dose of E-SELEN® (1 mL/45 kg BW) immediately pre-synchronization only (G_2), the 3rd group (N=5) was administered a single dose of E-SELEN® (1 mL/45 kg BW) at the time of AI only (G_3), and the 4th group (N=5) received a double dose of

E-SELEN® (1 mL/45 kg BW) the first dose immediately pre-synchronization and the second dose at the time of AI (G_4).

The follicular functions were detected on day 0, as well as on the 7th and 10th 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 1st, 2nd, 3rd, 4th, 5th-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®; Beef and dairy cattle were intramuscularly injected with Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) (MSD Animal Health Company). Cystorelin®; 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®; 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_3), thyroxin (T_4), 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.[18], reduced glutathione (GSH) levels were detected by the method described by Pleban et al.[19], and catalase (CAT) activity was detected by the method described by Aebi [20].

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_2 and G_4 groups on the 10th day of synchronization compared to the other groups. There was a gradual significant ($P<0.05$) increase in follicle diameter (FD) from the 7th day to the 10th day of synchronization in both G_2 and G_4 groups compared to groups G_1 and G_3.

Serum hormone results *(Table 3)* revealed a gradual increase of progesterone (P4) level in all groups, but there was a significant ($P<0.05$) increase in G_4 from the 1st week to week 5 post-AI compared to G_1 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			
		1st Group (G_1)	2nd Group (G_2)	3rd Group (G_3)	4th Group (G_4)
Follicle Number	0-day	1.80±0.84	2.00±0.71	1.80±0.84	2.00±0.71
	7th day	2.00±0.71	3.00±0.71	2.20±0.84	3.20±0.84
	10th day	2.20±0.84^{b}	3.40±1.14^{a}	2.40±0.55^{b}	3.60±1.14^{a}
Follicle Diameter (mm)	0-day	5.60±0.87	6.26±0.71	5.66±0.64	6.14±0.86
	7th day	6.16±0.66^{bB}	9.40±0.81^{aAB}	6.22±0.74^{bB}	9.62±0.86^{aAB}
	10th day	7.82±0.77^{bA}	10.00±0.8^{aA} 7	7.90±0.8 9^{bA}	10.60±0.80^{aA}

Data are expressed as mean ± SE

^{a,b} *Superscript: Mean significance difference among groups in the same row on $P<0.05$*

^{A,B} *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)	1st group (G_1)	0.287 ± 0.05^{C}	1.037 ± 0.411^{bB}	2.580 ± 0.935^{A}	3.301 ± 1.724^{bA}	3.535 ± 1.861^{bA}	4.594 ± 2.201^{bA}
	2nd group (G_2)	0.311 ± 0.056^{D}	2.376 ± 0.704^{abC}	3.740 ± 1.340^{BC}	5.380 ± 1.81^{aAB}	5.580 ± 1.630^{abAB}	6.499 ± 1.446^{abA}
	3rd group (G_3)	0.294 ± 0.057^{D}	2.288 ± 0.571^{abC}	3.820 ± 1.150^{BC}	5.420 ± 1.85^{aAB}	5.620 ± 1.650^{aAB}	6.579 ± 1.495^{abA}
	4th group (G_4)	0.344 ± 0.072^{E}	2.592 ± 0.673^{aD}	4.420 ± 1.336^{CD}	6.420 ± 2.13^{aBC}	7.420 ± 2.023^{aAB}	8.379 ± 1.847^{aA}
T_3 (ng/mL)	1st group (G_1)	3.034 ± 0.417	2.993 ± 0.21^{b}	2.933 ± 0.201^{b}	2.994 ± 0.171^{b}	3.009 ± 0.193^{b}	3.088 ± 0.271^{c}
	2nd group (G_2)	3.165 ± 0.334^{B}	3.25 ± 0.233^{abAB}	3.411 ± 0.227^{aAB}	3.63 ± 0.294^{aA}	3.598 ± 0.245^{aA}	3.368 ± 0.275^{bcAB}
	3rd group (G_3)	3.032 ± 0.385^{C}	3.27 ± 0.329^{abBC}	3.59 ± 0.255^{aAB}	3.687 ± 0.264^{aA}	3.694 ± 0.205^{aA}	3.613 ± 0.232^{abAB}
	4th group (G_4)	3.294 ± 0.347^{B}	3.452 ± 0.328^{aAB}	3.71 ± 0.268^{aA}	3.763 ± 0.26^{aA}	3.783 ± 0.272^{aA}	3.86 ± 0.225^{aA}
T_4 (µg/dL)	1st group (G_1)	6.549 ± 0.689	6.233 ± 0.749	6.224 ± 0.632	6.396 ± 0.602	6.414 ± 0.57	6.444 ± 0.63
	2nd group (G_2)	6.494 ± 0.668	6.362 ± 0.597	6.28 ± 0.579	6.421 ± 0.644	6.542 ± 0.555	6.636 ± 0.532
	3rd group (G_3)	6.589 ± 0.727	6.433 ± 0.572	6.456 ± 0.694	6.441 ± 0.669	6.58 ± 0.568	6.676 ± 0.583
	4th group (G_4)	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)	1st group (G_1)	0.871 ± 0.068^{bD}	1.025 ± 0.089^{bC}	1.032 ± 0.058^{C}	1.057 ± 0.061^{BC}	1.175 ± 0.078^{AB}	1.223 ± 0.079^{A}
	2nd group (G_2)	1.010 ± 0.077^{aD}	1.054 ± 0.091^{abCD}	1.103 ± 0.07^{BCD}	1.177 ± 0.084^{AB}	1.206 ± 0.094^{AB}	1.232 ± 0.115^{A}
	3rd group (G_3)	0.986 ± 0.087^{aB}	1.131 ± 0.068^{abA}	1.134 ± 0.075^{A}	1.161 ± 0.072^{A}	1.191 ± 0.091^{A}	1.227 ± 0.107^{A}
	4th group (G_4)	1.076 ± 0.097^{a}	1.173 ± 0.076^{a}	1.133 ± 0.068	1.113 ± 0.056	1.112 ± 0.054	1.133 ± 0.068
Cortisol (µg/dL)	1st group (G_1)	20.663 ± 2.696^{aA}	22.86 ± 2.965^{aAB}	23.103 ± 2.819^{aB}	23.863 ± 2.965^{aBC}	24.663 ± 2.877^{aBC}	25.623 ± 2.926^{aC}
	2nd group (G_2)	15.42 ± 1.42^{b}	15.1 ± 1.38^{b}	14.42 ± 1.22^{b}	14.02 ± 1.096^{b}	14.26 ± 1.07^{b}	14.46 ± 1.24^{b}
	3rd group (G_3)	19.86 ± 1.898^{aB}	15.29 ± 1.27^{bA}	14.07 ± 1.18^{bA}	13.78 ± 1.15^{bA}	13.62 ± 1.12^{bA}	13.55 ± 1.05^{bA}
	4th group (G_4)	14.90 ± 1.2^{b}	13.7 ± 1.07^{b}	13.48 ± 0.74^{b}	13.26 ± 0.62^{b}	13.09 ± 0.67^{b}	12.77 ± 0.78^{b}
Prolactin (ng/mL)	1st group (G_1)	241.10 ± 22.07^{c}	236.40 ± 21.87^{c}	235.20 ± 21.65^{c}	238.05 ± 20.09^{c}	242.69 ± 21.44^{c}	247.15 ± 21.1^{c}
	2nd group (G_2)	310.90 ± 25.8^{ab}	316.70 ± 25.85^{b}	324.30 ± 24.16^{b}	331.16 ± 24.48^{b}	327.34 ± 25.62^{b}	331.35 ± 28.37^{b}
	3rd group (G_3)	286.6 ± 27.34^{bC}	313.88 ± 25.39^{bBC}	339.10 ± 22.33^{bAB}	347.11 ± 24.55^{bAB}	352.80 ± 25.58^{bAB}	360.54 ± 30.27^{bA}
	4th group (G_4)	326.940 ± 25.49^{aE}	372.50 ± 27.27^{aD}	406.97 ± 29.03^{aC}	434.30 ± 34.38^{aBC}	459.10 ± 39.47^{aAB}	474.62 ± 37.35^{aA}

Data are expressed as mean ± SE

[a, b, c] *Superscript: Mean significance difference among groups in the same column on P<0.05*

[A, B, C, D, E] *Superscript: Mean significance difference among administration times in the same row on P<0.05*

increase compared to the other two groups, G_2 and G_3. There was a non-significant change in T_4 levels during the experimental period between all groups. There was a gradually non-significant decrease in T_3 level of G_1 until the week 4. A significant (P<0.05) increase in the T_3 level of G_2, G_3, and G_4 from week 2 to week 5 compared to G_1. TSH levels results revealed a gradual increase in G_1, G_2, and G_3 from week 1 to week 5 with only a significant (P<0.05) decrease in G_1 on the 1st day post-AI compared to G_2, G_3, and G_4 and also a significant (P<0.05) decrease on the week 1 compared to G_4. There was a significant (P<0.05) increase in cortisol levels of G_1 throughout the experimental period compared to G_2, G_3, and G_4 groups. On the other hand, there was a significant (P<0.05) increase in the prolactin level of G_4 throughout the experimental period compared to G_1, G_2, and G_3. In the same time, there was a significant (P<0.05) increase in prolactin levels of G_2 and G_3 throughout the experimental period compared to G_1.

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_1 compared with the other groups (G_2, G_3, and G_4) 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_4 compared to the other two groups of vitamin E and selenium administration G_2 and G_3 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_1, G_2, G_3, and G_4, 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)	1st group (G_1)	1.785±0.139^{a}	1.830±0.134^{a}	1.759±0.14^{a}	1.744±0.136^{a}	1.775±0.125^{a}	1.797±0.154^{a}
	2nd group (G_2)	1.461±0.128^{b}	1.414±0.108^{b}	1.360±0.093^{b}	1.395±0.097^{b}	1.417±0.099^{b}	1.431±0.091^{b}
	3rd group (G_3)	1.709±0.123^{aA}	1.394±0.107^{bB}	1.287±0.103^{bBC}	1.206±0.082^{cC}	1.163±0.103^{cC}	1.209±0.102^{cC}
	4th group (G_4)	1.354±0.128^{bA}	1.226±0.06^{cAB}	1.106±0.071^{cBC}	1.013±0.084^{dC}	0.982±0.055^{dC}	1.1040±0.078^{cC}
GSH (mg/dL)	1st group (G_1)	2.297±0.179^{c}	2.264±0.196^{d}	2.309±0.122^{c}	2.452±0.141^{d}	2.377±0.116^{d}	2.305±0.113^{d}
	2nd group (G_2)	4.129±0.362^{aC}	4.448±0.383^{bB}	4.553±0.384^{bA}	4.339±0.363^{cBC}	4.048±0.346 ^{CD}	3.818±0.277^{cD}
	3rd group (G_3)	2.900±0.176^{bC}	3.949±0.283^{cB}	4.837±0.326^{bA}	5.049±0.361^{bA}	4.998 ±0.358^{bA}	4.707±0.321^{bA}
	4th group (G_4)	4.254±0.324^{aA}	5.094±0.407^{aB}	5.522±0.438^{aC}	5.681±0.449^{aC}	5.767±0.433^{aC}	5.547±0.351^{aC}
CAT (U/mL)	1st group (G_1)	7.751±0.604^{b}	7.638±0.661^{c}	7.592±0.671^{c}	7.715±0.682^{d}	7.742±0.684^{d}	7.558±0.656^{d}
	2nd group (G_2)	13.529±1.189^{aAB}	14.178±1.218^{bAB}	15.084±1.26^{bA}	14.665±1.199^{cAB}	13.721±1.12^{cAB}	13.020±1.15^{cB}
	3rd group (G_3)	8.952±0.727^{bC}	13.845±1.192^{bB}	15.636±1.156^{bA}	16.79±1.314^{bA}	16.86±1.439^{bA}	16.216±1.462^{bA}
	4th group (G_4)	13.901±1.054^{aB}	17.539±1.277^{aA}	18.484±1.355^{aA}	18.719±1.478^{aA}	19.023±1.535^{aA}	18.865±1.423^{aA}
SOD (U/mL)	1st group (G_1)	13.422±1.046^{c}	13.228±1.144^{c}	13.494±0.714^{c}	14.331±0.826^{c}	13.891±0.681^{d}	13.47±0.66^{d}
	2nd group (G_2)	26.16±2.587^{aBC}	28.10±2.844^{bAB}	30.448±2.321^{bA}	30.134±2.34^{bA}	26.979±1.819^{cB}	24.074±1.718^{cC}
	3rd group (G_3)	16.893±1.401^{bC}	27.02±2.03^{bB}	31.63±2.373^{abA}	33.67±2.77^{aA}	32.06±2.18^{bA}	29.81±2.39^{bA}
	4th group (G_4)	27.77±2.92^{aC}	31.73±2.79^{aB}	34.22±2.66^{aAB}	35.36±2.87^{aA}	36.31±2.42^{aA}	34.63±2.35^{aAB}
TAC (mU/L)	1st group (G_1)	1.089±0.085^{b}	1.073±0.093^{c}	1.094±0.058^{c}	1.162±0.067^{c}	1.127±0.055^{d}	1.092±0.054^{d}
	2nd group (G_2)	1.34±0.089^{aB}	1.394±0.129^{bAB}	1.492±0.122^{bA}	1.496±0.114^{bA}	1.391±0.102^{cAB}	1.324±0.108^{cB}
	3rd group (G_3)	1.185±0.073^{bC}	1.357±0.097^{bB}	1.576±0.128^{bA}	1.636±0.134^{bA}	1.654±0.142^{bA}	1.573±0.126^{bA}
	4th group (G_4)	1.418±0.122^{aD}	1.531±0.13^{aCD}	1.632±0.129^{aBC}	1.719±0.143^{aAB}	1.762±0.152^{aA}	1.714±0.121^{aAB}

Data are expressed as mean ± SE

$^{a, b, c, \text{and } d}$ *Superscript: Mean significance difference among groups in the same column on P<0.05*

$^{A, B, C, \text{and } D}$ *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			
		1st Group (G_1)	2nd Group (G_2)	3rd Group (G_3)	4th Group (G_4)
Conception rate	Number	2	3	3	4
	Percent (%)	40	60	60	80

Discussion

In this study, ovsynch synchronization was provided using a GnRH and $PGF_2\alpha$ 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_1 and G_3 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_2 and G_4 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 1st 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 T3 levels by facilitating the de-iodination of T4 into its active form T3.

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 anti-oxidant 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.

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

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