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Fertility Improvement in Cross-Bred Dairy Cows Through Supplementation of Vitamin E as Antioxidant

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A B S T R A C T

Reproductive cyclicity of cross-bred cattle has been reported to be reduced due to stress. This study was conducted to investigate the role of vitamin E in combating stress. The experiment was conducted on 36 lactating dairy cows, comprising indigenous (9 Sahiwal, 9 Achai), 9 cross-bred (Sahiwal x Holstein Frisian) and 9 Holstein Frisian located at two state dairy farms. Vitamin E was supplemented in feed at a dose rate of 1000 IU/ cow/ day for 40 days. Sampling was conducted on day-zero (control), day-20 and day-40 of vitamin E supplementation during the diestrus phase. Concentrations of SOD and GPx increased significantly ($P<0.001$) with vitamin E supplementation. Breeds also varied in SOD and GPx activities ($P<0.05$ and $P<0.001$ respectively). The stress markers (plasma MDA, HSP-70 and serum cortisol) decreased significantly with vitamin E supplementation while breeds also showed variation in these parameters ($P<0.001$). Progesterone concentration increased significantly ($P<0.001$) with vitamin E supplementation showing significant variation ($P<0.05$) among breeds. Number of follicles decreased significantly with vitamin E supplementation ($P<0.01$), highest in cross-bred cows indicating quality graafian follicles with supplementation. The stress markers (cortisol, HSP-70 and MDA) were positively correlated mutually while negatively with antioxidant markers. Progesterone was positively correlated with antioxidant markers. It was concluded that Holstein Frisian and crossbred cows showed more favorable response to vitamin E supplementation in respect of expression of stress and reproductive markers suggesting regular feeding of antioxidant to these breeds for better performance.

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IK and MSQ conceived and designed the study. IK analyzed the data and wrote the article. SA, IA and G helped in experimental work.

Key words
Vitamin E, dairy breed, MDA, HSP-70, stress, fertility, SOD, GPx

INTRODUCTION

Heat stress occurs when heat production (the energy necessary for maintenance, production and reproduction) exceeds heat loss to the surrounding environment (Bernabucci *et al.*, 2010). Cellular response to stress is an essential defense mechanism against different stressors accompanied with the synthesis of heat shock proteins (HSPs) which can be transcribed and translated in high ambient temperatures. The biological role of HSPs is to restore protein homeostasis to the pre-stress state by refolding and repairing damaged proteins (Haque *et al.*, 2012).

Environmental thermal stress coupled with heavy milk production causes stress to the animal resulting into the production of reactive oxygen species (ROS) (Sordillo and Aitken, 2009). In biological system, naturally found major free radicals include hydrogen peroxide, super oxides, hydroxyl radicals and fatty acid radicals. The free oxides produced due to stress are toxic to body hence body developed its own antioxidant system. Superoxide dismutase (SOD) an enzyme containing copper and zinc, converts super oxides to H_2O_2 which is then converted to water by Glutathione Peroxidase enzyme. Both the enzymes efficiently control these free radicals in cytosol (Moyes *et al.*, 2009).

Different managemental practices are used for the reduction of effects of thermal stress. The use of antioxidants to improve production and reproduction in dairy cattle under stress conditions is well documented (BV *et al.*, 2011). Several vitamins and trace minerals have role in antioxidant defense system and deficiency of any of these elements may result into reduce immunity (Spear and Weiss, 2008). In a broad way antioxidants can be defined as substances that delays, prevents or removes oxidative damage to the targeted molecule (Halliwell and Gutteridege, 2007).

Vitamin E is considered as intracellular antioxidant which maintains membrane integrity and phospholipids against peroxidation and oxidative damage (Lawrence *et al*., 2004). It also increases the production of immunoglobulins in the body and increased the killing of intracellular micro organisms by neutrophils during calving (Franklin *et al.*, 2005). Lymphocytes proliferation

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is suppressed directly by the glucocorticoids (immune-suppressive hormones) produced during stress. However, vitamin E stabilizes the membrane of leukocytes because it contains more free fatty acids so they are more susceptible to vitamin E effect than other body cells (Spears and Weiss, 2008). Administration of vitamin E improves fertility in cattle by regulating the free radicals in the ovarian tissues (Wilde, 2006). It has also been reported that vitamin E protects steridogenic enzymes from oxidative enzymes and promotes the release of luteinizing hormone (LH), follicle stimulating hormone (FSH) and adrenocorticotropic hormone (ACTH) (Garnsworthy *et al.*, 2008).

Based upon the above discussion the present study was designed with the objectives to find out the role of vitamin E in combating stress and improving fertility in cross-bred cows reflected by different stress reproduction markers during thermal stress. Second, to find out thermal stress, oxidant and antioxidant status of dairy cows after vitamin E supplementation.

MATERIALS AND METHODS

Selection of animal

Thirty six disease free, normal cyclical lactating dairy cows, comprising indigenous (9 Sahiwal, 9 Achai) and cross-bred (9 Sahiwal x Holstein Frisian), located at livestock research and development station Surezai, district Peshawar, Pakistan, while 9 pure-exotic Holstein Friesian (HF) dairy cows located at The University of Agriculture Peshawar Dairy Farm were selected. Managemental conditions were similar for all the dairy cows during the whole study period. Drinking water was provided ad libitum. Green fodder, wheat straw and concentrate were fed as basal diet. Mean daily milk yield and body condition score (BCS) of Sahiwal cows was 6.43±0.28 kg and 3.02, Achai 5.37±0.34 kg and 2.95, cross-bred 7.5±0.46 kg and 3.12 while HF 15.5±0.87 kg and 3.05 respectively.

The BCS of all the experimental cows was recorded at the farm level using the method described by Peters and Ball (1987) observed by the same person throughout the experimental period. According to this method the thickness of fat over the lumber and tail head area was estimated and was assigned a score from 1 (very weak) to 5 (very fat). 1, spine prominent and transverse processes feel sharp with little fat cover; 2, transverse processes can be felt but are rounded with a thin covering of fat; 3, individual transverse vertebral processes can only be felt by firm pressure; 4, the transverse processes cannot be felt; and 5, the transverse processes covered with a thick layer of fat.

Supplementation of vitamin E

All the dairy cows were supplemented with powdered vitamin E (Foodchem International Corporation, China) in feed at a dose rate of 1000 IU/ cow/day. Ten days as adaptation period was given to all the cows. Sampling was conducted at day-zero (control) before vitamin E supplementation, day-20 and day-40 post vitamin E supplementation during the diestrus phase of the estrus cycle.

Blood sampling and analysis

Blood samples were collected on scheduled dates from jugular vein in sterile vacutainer tubes. Five ml blood was collected in sterile vacutainer tube containing EDTA (1 mg/ml) anticoagulant for plasma separation, while another five ml blood was collected in sterile vacutainer tube without EDTA anticoagulant for serum separation. Immediately, the tubes were brought to the laboratory for plasma and serum separation, which was performed by centrifugation at 3000 rpm for 20 min. Separated plasma and serum were stored at -20°C till further chemical analysis.

Changes in oxidative/anti-oxidative status

Plasma malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined to find out changes in oxidative/anti-oxidative status. Plasma MDA, SOD and GPX was determined using kits “Lipid Peroxidation (MDA) Assay Kit”, “Superoxide Dismutase (SOD) Activity Assay Kit” and “Glutathione Peroxidase Activity Assay Kit”, BioVision, USA through colorimetric method.

Changes in reproduction and stress markers

Serum progesterone concentrations and number of follicles were determined as reproduction markers, while serum cortisol and HSP-70 concentrations were determined as stress markers. Progesterone concentrations were determined using enzyme immunoassay kit (Biocheck, Inc), serum cortisol concentrations using kit (Calbiotech, USA) and plasma HSP-70 concentrations using Cusabio Biotech Co., Ltd. through ELISA.

Number of follicles was determined through rectal ovarian ultrasonography under optimized conditions using “esaote Piemedical Aquila Pro ultrasound scanner equipped with a Linear Array 6.0/8.0 MHz Endorectal transducer”. The follicles were identified as dark circular areas. Each ovary was monitored for the presence of follicles. Presence of more than one follicle on the same ovary was viewed in the same image for accurate count after freezing the image on the screen. The diameter of spherical follicles was recorded and the non-spherical

Table I.- Different oxidant and antioxidant parameters (Mean±SE) from different dairy cattle breeds as affected by vitamin E supplementation (n=108).

Breed	Treatment	MDA (nmol/ml)	SOD (IU/ml)	GPx (IU/ml)
Sahiwal	Control	30.23±0.85 [abcd]	2.25±0.35 [def]	30.57±1.10 [efg]
	E-20	25.81±0.57 [efg]	2.79±0.33 [cd]	35.72±1.61 [cd]
	E-40	25.16±1.75 [fg]	3.89±0.39 [ab]	47.51±0.82 [a]
Achai	Control	31.32±2.11 [abc]	2.31±0.39 [def]	29.44±0.75 [fg]
	E-20	26.30±1.45 [def]	3.24±0.39 [bc]	40.22±0.56 [bc]
	E-40	21.83±1.48 [g]	4.36±0.21 [a]	47.74±1.25 [a]
Cross-bred	Control	32.76±1.58 [ab]	1.91±0.21 [ef]	26.01±1.95 [g]
	E-20	28.56±0.58 [bcdef]	2.58±0.26 [cde]	34.69±2.32 [de]
	E-40	27.67±1.81 [cdef]	4.11±0.19 [ab]	45.57±0.69 [a]
Holstein-Friesian	Control	34.08±2.34 [a]	1.48±0.16 [f]	27.02±1.40 [g]
	E-20	31.62±0.51 [abc]	2.23±0.11 [def]	31.62±2.68 [def]
	E-40	30.18±2.13 [abcde]	3.73±0.42 [ab]	43.42±2.45 [ab]
P- Value	Breed	< 0.001	< 0.05	< 0.001
	Vit-E	< 0.001	< 0.001	< 0.001
	Interaction	0.643	0.944	0.477

[a, b, c, d] means with different superscript with in the column are different significantly at p= 0.05
MDA, malondialdehyde; SOD, superoxide dismutase; GPx, glutathione peroxidase.

follicles were measured by averaging the largest and widest diameters. Follicles up to 15 mm diameter were considered as developing and beyond 15mm were considered as graafian follicles.

Statistical analysis

Data was analyzed through SPSS package-16 using General Linear Model. The data obtained in this experiment were presented as mean ± SEM and subjected to analysis of variance (ANOVA). Least significant difference (LSD) test was applied for significant difference between different variables. Associations between various variables were determined using coefficient of correlation (Pearson's correlation).

RESULTS

Changes in oxidative status

MDA concentrations were determined for oxidative changes. Table I shows that plasma MDA concentrations varied with breed and vitamin E supplementation (P<0.001). However, the interaction of breed and vitamin E was not found significant (P>0.05). Concentration of MDA decreased continuously in all breeds with increase in vitamin E supplementation. The decrease was abrupt during early supplementation of vitamin E. Similarly, the decrease in MDA concentration was more prominent upto day-20, though it was non significant up to day-40. The results showed that the intensity of response was higher in indigenous cows than in the crossbred and HF cows.

Changes in antioxidant status

Table I shows effect of vitamin E supplementation (P<0.001) on SOD and GPx activity level in different breed of cows. The indigenous breeds of Achai and Sahiwal showed highest SOD and GPx activities compared to the cross and HF cows. The SOD and GPx activities level increased after vitamin E supplementation in all the breeds. Sahiwal and cross-bred dairy cows performed almost similar, while HF and cross breed lowest SOD and GPx activities.

Stress markers

Table II shows serum cortisol and HSP 70 after different dairy cattle breeds after vitamin E supplementation. The results showed that cortisol and the HSP 70 concentrations were significantly decreased after vitamin E supplementation (P<0.001) in different breeds, the interaction of breed and vitamin E did not show any significant effects (P>0.05). Although the indigenous breeds showed lower concentrations of cortisol and HSP 70, the overall decrease was higher in crossbred and HF cows. It is indicated that improved breeds showed more prominent response to vitamin E supplementation.

Table II shows that concentration of HSP-70 decreased with increase in post vitamin E supplementation days.

Reproductive markers

An increase in progesterone (P_4) concentration was observed with vitamin E supplementation in all breeds (Table II). Significant increase in P_4 concentrations of P_4

Table II.- Different stress and reproductive parameters (Mean±SE) from different dairy cattle breeds as affected by vitamin E supplementation (n=108).

Breed	Treatment	Cortisol (ng/ml)	HSP-70 (ng/ml)	P_4 (ng/ml)	Follicle No
Sahiwal	Control	86.49±8.39 ^{cdef}	4.16±0.28 ^{ab}	3.89±0.42 ^{bcde}	1.55±0.24 ^{bc}
	E-20	75.51±8.17 ^{cdef}	2.98±0.40 ^{de}	3.95±0.55 ^{abcd}	1.22±0.14 ^{c}
	E-40	66.56±6.69 ^{f}	2.38±0.31 ^{e}	4.76±0.54 ^{a}	1.22±0.14 ^{c}
Achai	Control	98.52±13.45 ^{abcd}	4.08±0.54 ^{abc}	3.08±0.39 ^{cde}	1.44±0.17 ^{c}
	E-20	92.93±9.94 ^{bcdef}	2.97±0.48 ^{de}	4.28±0.42 ^{abc}	1.44±0.17 ^{c}
	E-40	68.91±8.30 ^{ef}	2.09±0.24 ^{e}	5.12±0.71 ^{a}	1.22±0.14 ^{c}
Cross-bred	Control	116.62±10.73 ^{ab}	4.48±0.30 ^{ab}	2.69±0.28 ^{e}	2.11±0.26 ^{ab}
	E-20	95.72±12.65 ^{abcd}	3.07±0.03 ^{cde}	3.18±0.83 ^{cde}	1.66±0.23 ^{abc}
	E-40	73.62±8.05 ^{def}	2.43±0.32 ^{e}	4.48±0.42 ^{ab}	1.33±0.16 ^{c}
Holstein-Friesian	Control	120.01±8.53 ^{a}	5.04±0.47 ^{a}	2.51±0.30 ^{e}	2.22±0.32 ^{a}
	E-20	100.79±8.83 ^{abc}	3.86±0.60 ^{bcd}	3.02±0.28 ^{de}	1.55±0.24 ^{bc}
	E-40	82.67±8.42 ^{cdef}	2.95±0.27 ^{de}	4.17±0.45 ^{abcd}	1.44±0.17 ^{c}
P- Value	Breed	0.011	0.025	0.034	< 0.05
	Vit-E	< 0.001	< 0.001	< 0.001	< 0.01
	Interaction	0.902	0.998	0.973	0.671

$^{a, b, c, d}$ means with different superscript with in the column are different significantly at p= 0.05
HSP-70, Heat Shock Protein-70; P_4 , Progesterone

Table III.- Pearson Correlation between different studied parameters of different dairy cattle breeds (n=108).

	MDA	HSP-70	SOD	GPx	Cortisol	P_4	Follicle. No
Vit. E Sig.	-0.430** 0.000	-0.573** 0.000	0.666** 0.000	0.813** 0.000	-0.417** 0.000	0.464** 0.000	-0.320** 0.001
MDA	-	0.371** 0.000	-0.399** 0.000	-0.447** 0.000	0.225* 0.019	-0.460** 0.000	0.174 0.072
HSP-70	-	-	-0.355** 0.000	-0.498** 0.000	0.285** 0.003	-0.348** 0.000	0.282** 0.003
SOD	-	-	-	0.571** 0.000	-0.388** 0.000	0.404** 0.000	-0.293** 0.002
GPX	-	-	-	-	-0.330** 0.000	0.443** 0.000	-0.289** 0.002
Cortisol	-	-	-	-	-	-0.377** 0.000	0.278** 0.004
P_4	-	-	-	-	-	-	-0.104 0.283

*,**. Correlation is significant at the 0.05 and 0.01 level respectively.
For abbreviations, see Tables I and II.

the effect of Vit. E administration is not significant with reference to different breeds of cow was also observed (P<0.05). Highest value of progesterone concentration was observed for Achai followed by Sahiwal and then the two improved dairy breeds. Overall increase was highest in cross-bred and Achai followed by HF and Sahiwal.

Table II also shows that the interaction of breed x vitamin E showed no significant effect on follicular numbers (P>0.05). The follicular numbers decreased with vitamin E supplementation in all the four breeds. However, the cross-bred and HF showed more positive response to vitamin E than the indigenous dairy cows. Sahiwal and HF cows showed abrupt decrease in number of follicles from control to day-20 and then no significant decrease was observed up to day-40 in these two breeds. Achai breed performed opposite to Sahiwal and cross-bred dairy cows showing no significant change from control to day-20 and then a significant decrease in number of follicles was observed up to day-40. However, the HF breed showed a continuous decrease in dominant follicles number from control to day-20 and then day-40.

Relationship among various parameters

Correlation among different studied parameters is given in Table III. The stress markers were positively correlated with each other while negative with antioxidant markers. Antioxidant markers were positive correlated with progesterone concentration. MDA had positive correlation with HSP-70 (r = 0.371, P<0.001) and cortisol (r = 0.225, P<0.01). HSP-70 also had strong positive correlation with cortisol (r = 0.285, P<0.001) and follicular numbers (r = 0.282, P<0.001). Positive correlation was observed between cortisol and follicular numbers (r = 0.278, P<0.01). Progesterone was negatively correlated with all stress markers (cortisol; r = -0.377, P<0.001, HSP-70; r = -0.348, P<0.001, MDA; -0.460, P<0.001). SOD and GPx showed positive effect on fertility by having negative correlation with follicular number (SOD; r = -0.293, P<0.01, GPx; r-0.289, P<0.01).

DISCUSSION

In dairy cattle, imbalance between ROS production and reduced availability of antioxidants may lead to oxidative stress (Gitto *et al*., 2002). GPx act as a catalyst in the reduction of lipid peroxides, hydrogen peroxide and organic hydroperoxide while SOD catalyzes the superoxide anion dismutation into H_2O_2 and molecular oxygen. Thus both SOD and GPx protect the cells from normal metabolism oxidative damage (Descalzo *et al*., 2007). Thermal stress at cellular level leads to oxidative stress and the concentrations of MDA overwhelm the concentrations of antioxidants in the body cells. Therefore, performance of high yielding dairy cattle can be enhanced by supplementing the optimum levels of micronutrients with antioxidant capabilities (Sordillo and Aitken, 2009).

In the present study, a continuous decrease was observed in MDA concentrations with increase in days post vitamin E supplementation in all breeds, more prominent in indigenous than crossbred and HF cows. The decrease in MDA concentration with supplementation of vitamin E is in corroboration with earlier reports (Gupta *et al*., 2005; Vani *et al*., 2010). It appears that supplementations of vitamin E destroyed hydroxyperoxide, reduce the oxidative damage to sensitive membranes (Turk *et al*., 2004), maintain the membrane integrity and improves the ability to resist infections in periparturient cows (Besharati *et al.,* 2013).

In this study, superoxide dismutase (SOD) activity significantly increased with vitamin E supplementation (P<0.001). The indigenous Sahiwal and Achai exhibited higher SOD activity followed by crossbred cows while HF. Descalzo *et al*. (2007) found higher level of SOD activities in vitamin E supplemented group than non supplemented group when trailed pasture versus grain fed cross-bred steers. The positive correlation (r = 0.666; P<0.001) between SOD activity and vitamin E supplementation in our study revealed that vitamin E has an optimistic effect on SOD activity. These results are in consistent with the works of Gatellier *et al.* (2004) and Vani *et al.* (2010) who found synergistic effect of vitamin E on SOD activity.

Vitamin E supplementation increased GPx activity significantly in different breeds more prominent in local than exotic and crossbred cows (P<0.001). Glutathione Peroxidase has vital role in cell protection against oxidative damage by converting reduced GSH to oxidized GSSG and reduced lipid hydroperoxides to their related alcohol and H_2O_2 to water (Mehdi *et al.*, 2013). Several other research workers also found positive effect of antioxidants on GPx activity (Gatellier *et al.*, 2004). When ROS production overwhelms antioxidant defences, oxidative stress occurs, which may deeply threaten the anatomical and functional integrity of the genital tract (Rizzo *et al.*, 2012). It has also been reported that vitamin E increase GPx activity in the cell and thus helping the cell from oxidative damage by reducing the free radicals (Descalzo *et al*., 2007; Vani *et al*., 2010). The findings of these authors are in consistent with our results. The results of present study clearly support the function of GPx interacting with membrane peroxides for scavenging lipid hydroperoxides. Therefore, the increased biochemical activity of GPx with vitamin E might be attributed to increase the levels of α-tocopherol in RBC, neutrophils and plasma (Vani *et al*., 2010).

The serum cortisol concentration of different dairy cattle breeds was significantly decreased by vitamin E supplementation (P<0.001) with more prominent in cross and HF breeds (P<0.001) than local breeds of Achai and Sahiwal. A continuous decrease in cortisol concentrations were observed with increase in days post vitamin E supplementation. These findings are similar with the results of Gupta *et al.* (2005), who found reduction in cortisol concentration after administration of single dose of vitamin E and Se at three weeks before parturition. Hence it can be concluded that vitamin E supplementation might have reduced metabolic ROS and other free radicals by reducing oxidative stress and cortisol level in blood (Gupta *et al*., 2005). Cortisol showed a negative correlation with supplementation of vitamin E (r= -0.417, P<0.001) and progesterone (r= -

0.377, P<0.001). Research demonstrates that both cortisol and progesterone are synthesized from cholesterol (Walker *et al.*, 2008). Under stressful conditions, body converts its total bodily system from rest to emergency mode to maintain homeostasis and favor higher cortisol level. Therefore higher concentration of cortisol may result in low level of progesterone as shown in our results. These findings are in continuation with the results of Besharati *et al.* (2013). A positive correlation (r =0.225, P<0.01) was noted between cortisol and MDA indicating that either stress parameters are directly proportional to stress. Several researchers also reported increased lipid peroxidation under stressful conditions that stimulates the stress axis (HPA axis), causing a rise in cortisol concentration (Gupta *et al*., 2004). Therefore, the decrease in lipid peroxidation (MDA) following vitamin E supplementation in this study could have reduced cortisol concentration.

Environmental high temperature has negative effects on the production and reproduction of high genetic merit cattle. HSP-70 expression also increases with parasitization (Wang *et al.,* 2016). HSP-70 is released from intracellular to extracellular peripheral circulatory system during thermal stress (Basiricò *et al*., 2011). In this study, concentration of HSP-70 decreased with vitamin E supplementation, varied among breeds (P<0.001). Indigenous dairy breeds tolerated thermal stress more efficiently by showing lower concentrations of HSP-70 than crossbred and HF cows. It may be due to higher genetic support and adaptation of these indigenous breeds to the local tropical environment (McManus *et al.*, 2009). *Bos Indicus* cows usually have better performance in tropical and subtropical regions than *Bos Taurus* cows, presumably due to their better adaptation to tropical environments (Camargoa *et al.*, 2007).

The effects of heat stress on animal's health and performance can be minimized by supplementation of some micronutrients. Sivakumar *et al.* (2010) studied the effects of vitamin C and vitamin E with selenium and reported reduced heat stress in goats supplemented with antioxidants as shown in our results. Supplementation of vitamin E has positive effect on antioxidants level in periparturient heifers (Dobbelaar *et al.*, 2010). Reducing heat stress effect of antioxidants was observed in buffaloes by Kumar *et al.* (2011). In our study, the vitamin E has reduced MDA and HSP-70 concentrations showing a negative correlation with HSP-70 (r = -0.573, P<0.001) and MDA (r = -0.430, P<0.001). HSP-70 and MDA were positively correlated with each other (r =0.371, P<0.001). Therefore, reduction in heat and oxidative stress may be attributed to supplementation of vitamin E.

In this experiment, heat stress reduced the quality of selected dominant follicle and increased the number of development follicles accompanied with low progesterone concentrations. Altered endocrine functions reduce follicular activity and modify the ovulatory mechanism, leading to a decrease in oocyte quality (Rensis and Scaramuzzi, 2003). During the luteal phase of the pre-conception estrous cycle, low progesterone concentrations can affect follicular development leading to abnormal oocyte maturation and early embryonic death. In this study, vitamin E supplementation showed a beneficial effect on progesterone concentration during the diestrus period of the estrus cycle, which is in consistent with the works of Wiltbank *et al.* (2006). Highest progesterone concentration was observed for Achai; however, highest response to vitamin E was observed in Cross-bred dairy cows (39.95%). It seems that vitamin E supplementation increased efficiency of luteal cells for progesterone production (Shehab-El-Deen *et al*., 2010).

Heat stress delays follicle selection and lengthens the follicular wave and thus has potentially adverse effects on the quality of oocytes and follicular steroidogenesis (Roth *et al*., 2001). Summer heat stress reduces the degree of dominance of the dominant follicle and more medium-size subordinate follicles survive that result into summer sterility (Qureshi, 2004.). When individual follicular dominance is reduced, more than one dominant follicle can develop (Rensis and Scaramuzzi, 2003). Vitamin E supplementation has shown positive effect on follicular development in this study. However, it appears that time period of vitamin E administration, dose concentration and nutritional status of the experimental animal can show some obvious difference in the results. Therefore, the administration of the antioxidant during summer need long term supplementation to have a beneficial effect on fertility in lactating cows (Shehab-El-Deen *et al*., 2010).

CONCLUSION

Holstein Frisian and crossbred cows showed favorable response to vitamin E supplementation suggesting regular feeding of vitamin E to high producing dairy cows. Effect of heat stress on reproduction may be minimized through vitamin E supplementation as modulated by progesterone concentration and follicular dynamics in the present experiment.

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Conflict of interest

The authors declare that they have no conflict of interest.

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