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*(Original Article)*

# Cortisol Levels and Milk Yield as Affected Injection of Vitamin E, hCG, and their Combination in Pregnant and non-pregnant Milking Cows Under the Cold Weather (Winter)

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## Abstract

To investigate the impact of VitE, human chorionic gonadotropin (hCG), and VitE+hCG on milk yield and blood cortisol concentrations in pregnancy and non-pregnancy under cold months. Thirty-four Holstein healthy milking cows were assigned randomly into four groups (control, VitE alone, hCG alone, and VitE+hCG). These groups were injected IM with 1.0 ml propylene glycol/animal, 1.39 ml α-tocopherol/animal three times before mating for ten days, 2000 IU hCG/animal once post-mating for one week, and 1.39 ml+ 2000 IU/animal as the same doses and dates as in VitE and hCG groups, respectively. Results revealed that treatments did not influence cortisol concentrations during mating in pregnancy and non-pregnancy. The combination group affected significantly the cortisol than other treatments in pregnant and ($P > 0.05$) in non-pregnant cows. The total cortisol concentrations decreased ($P < 0.05$) in the combination of pregnant and non-pregnant cows. Temperature-Humidity index (THI) was ≥ 46. Milk yield increased significantly in VitE and combination groups during mating and pregnancy phases (not non-pregnancy). The total milk yield was significantly greater in VitE and VitE+HCG of pregnant cows in non-pregnant cows. Conclusively, the cold weather didn't affect cortisol concentrations in mating, pregnancy, and non-pregnancy, and the combination group resulted in low cortisol in pregnancy, probably due to the THI comfortable index ≥ 46 or VitE as a potential antioxidant, leading to high milk yield and low cortisol in pregnancy and non-pregnancy. In conclusion, the treatments by VitE and combination (VitE+hCG) enhanced the milk yield and reduced the cortisol level under the cold weather.

***Keywords***: *Human chorionic gonadotropin, Milk, Cortisol, Cows, Temperature-Humidity Index,*

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## Introduction

It is known that homeothermic animals protect themselves from the cold by increasing heat production and decreasing heat loss. When the body temperature drops, the hypothalamus is responsible for the thermoregulation center through chemical reaction series that can lead to heat production or heat loss prevention. Mant (1964) reported that heat production plays a crucial role in the secretion of stress-response hormones (adrenaline, noradrenaline, and cortisol) that trigger an overall increase in lipid metabolism, particularly ketogenesis. Various nutritional and environmental conditions can compromise the barrier function in the gut, allowing lipopolysaccharides to enter the bloodstream. Thus, excess circulating lipids and low antioxidant status were correlated with oxidative stress when free radicals initiate chain reactions to generate reactive oxygen species (ROS) that promote inflammation (Bradford *et al.* 2015).

Cortisol level affects reproductive behavior. Administering the cortisol during pro-estrus inhibited the pre-ovulatory surge of LH, ovulation, and estrus in dairy heifers (Stoebel and Moberg. 1982b). Also, injection of ACTH delayed the LH surge, ovulation, and estrus when heifers injection was at 12-hour intervals before estrus in heifers (Stoebel and Moberg. 1982b). Similarly, stresses such as confinement and electrical shock elevated serum corticosteroid concentrations and inhibited the LH surge in dairy heifers (Stoebel and Moberg, 1982). Seasonal variation in plasma cortisol concentrations was confirmed in many previous studies, where the highest levels of cortisol occurred in spring (Persson *et al*. 2008, Miller *et al*. 2016), summer (Kanikowska *et al*. 2018), and the lowest levels were in winter (Weitzman *et al*. 1975). Cortisol increased the milk synthesis (Neville *et al*., 2002; Casey and Plaut, 2007. Acute stresses (vaccination farm management practices) such as vaccination, vermifugation, first milking, and transport caused cortisol release and decreased milk yield (Sevi *et al*., 2001a, b; Canaes *et al*., 2009; Caroprese *et al*., 2010). Stress has a negative influence on milk yield, but the relationship between cortisol release and milk yield needs further studies because cortisol is necessary for maintaining homeostasis and promoting animal adaptation (Negrao and Marnet, 2003; Trevisi and Bertoni, 2009; Brown and Vosloo, 2017). Previous human and dairy animal studies showed that cortisol affected protein, glucose, and lipid metabolism. Consequently, the glucose and fatty acids availability increased. These molecules are an essential precursor for milk production (Stewart and Thompson 1984, Shamay *et al.* 2000, Djurhuus *et al*. 2002, Huzzey *et al*. 2012). Cortisol in vivo regulated protein metabolism in the mammary epithelial cells (Lei *et al*. 2013) and affected milk yield depending on the lactation stage (Bomfim *et al. 2022).*

Dairy cows respond differently to stress. It is important to remove harmful elements from the reproductive stages to improve productivity and reproductive performance. Oxidative stress (OS) can damage reproductive organs by attacking cellular membranes through lipid peroxidation. An increase in reactive oxygen species (ROS) disrupts metabolic processes and damages cells in the body Sordillo and Aitken, 2009). This damage and disruption can lead to reproductive disorders, especially during physiological stages such as pregnancy, parturition, and lactation (Sharma *et al*. 2011). Antioxidants play a protective role by affecting ROS in four ways: scavenging, quenching, restoring, and breaking chains (Cherubini *et al*. 2005). Vitamin E, as a potential antioxidant, helps reduce cellular damage caused by free radicals (Kolb *et al*. 1997, Sordillo, 2013). Inadequate essential vitamin and micronutrient supplies can lead to a biological competition between the mother and conceptus, which can be detrimental to the health of both.

Progesterone (P4) is essential for maintaining the pregnancy. The positive association between plasma P4 concentrations during ovarian follicle development and fertility in lactating dairy cows has been found in several studies (Folman *et al*. 1990, Colazo *et al*. 2013, Bisinotto *et al*. 2015). Increasing P4 concentrations in the early di-estrus phase reduced uterine dysfunctions and enhanced embryonic survival. Inducing ovulation with human chorionic gonadotropin (hCG) promoted ovulation in non-cyclic lactating cows and prevented the harmful effects of heat stress on ovulation (De Rensis *et al*. 2010). Heat stress occurs if the cows are under an environmental Temperature-Humidity Index (THI) >72 levels (De Rensis *et al*. 2015), and their reproductive performance is hurt. Regardless of the cool or warm conditions, however, hCG treatment increased the pregnancy rate in multiparous cows *(*Garcia-Ispierto *et al.* 2019). Treatment with hCG increased luteal steroidogenic capacity and P4 secretion (Farin *et al*., 1988; Vergani *et al*., 2020; Rodrigues *et al*., 2022) in addition to the formation of accessory CL due to hCG's LH-like actions (Coleson *et al*., 2015). Therefore, the study aimed to investigate the effect of VitE, hCG, and their combination as beneficial agents for improving reproductive performance and against oxidation and cold stress in milking cows. Therefore, the study aimed to investigate the injection of VitE, hCG, and their combination as beneficial agents against oxidation and cold stress in Holstein milking cows.

## Materials and Methods

### Animals

The experiment was carried out in Alumminium Company Farm - Naga Hammadi – Egypt in the winter from December 2021 to February of 2022. Thirty-four Holstein healthy milking cows (second lactation season) were divided randomly into four groups (unequal numbers). The 1st group (n=8) was i.m. injected with 1.0 ml propylene glycol and considered the control to the treatments. In the 2nd group (n=9), each cow was i.m. injected with 1.39 ml vitamin E (α-Tochopherol ≥ 95.5 %, 1003222740, Sigma-Aldrich, product number 258024, USA) three times for ten days before AI. In the 3rd group (n=9), each cow was i.m. injected with 2000 IU hCG only once after insemination by one week. The 4th group (n=8) was i.m. injected with the combination VitE+hCG with the same doses and dates as in the 2nd and 3rd groups. All cows were synchronized using GnRH and Receptal (GPG protocol). The pregnancy was diagnosed by measuring P4 concentration at 7 days after insemination.

## Feeding

Animals fed a ration contained 16 % crude protein, 2 % crude fat, 14% crude fibers, 65% TDN). The ingredients are yellow corn, wheat brane, uncotryicayed cotton seeds, Soya bean, limestone meal, and salt. Each animal fed a concentrated ration three times a day (8.0 a.m. (5 kg), 4.0 p.m. (3 kg), and 11:30 p.m. (6 kg). Besides, wheat straw (4 kg) and green fodder (14 kg daily) were introduced at noon. Water was available throughput the time.

## Estrous synchronization

All cows exhibited estrous synchronization using the GPG protocol that depends on GnRH and PGF2α analogs (Panjaitan *et al*. 2019). This protocol was used in estrous synchronization in different agricultural animals for regulating ovarian function by modulating follicular waves and corpus luteum life span (Holtz *et al*. 2008) and reducing the intervals to first AI and days open (Tenhagen *et al*. 2004). All cows were injected i.m, with 2.5 mg GnRH (Reciptal, Reg No 2139product of Intervet International GmbH-Germany) on day 0 followed by seven days 2.0 ml PGF2α (Estrumate, Product of Vet Pharma Frissoythe GmbH Germany) injection, the second injection of Reciptal was after nine days of the first injection. All cows were serviced using an AI device on day 10 of the GPG protocol without estrous detection. Each cow was inseminated with 15× 106 sperms and viability 80 %. Semen was purchased from COOPEX company (Extrafin and Humme), Montbelirde.

## Management

All cows were milked using milking machine two times a day (7.0 a.m. and 3.0 p.m.). Cows were housed in semi-open pens under the average air temperature and relative humidity throughout the study (from December 25, 2021, to February 18, 2022) were 14.7 ºC and 47.67 %

## Temperature-Humidity Index (THI)

The Table 1 represents the degree of temperature (ºC) and relative humidity (%) during 3 cold months which cows were exposed to them pre-and post mating and continued until 45-DPM. The Temperature-Humidity Index was calculated from Thomb, (1959) equation

$$\mathbf{(THI) = ( 1.8 \times T+32) - (0.55 - 0.0055) \times RH \ (1.8 \times T-26)}$$

Where: T= air temperature degree. RH= relative humidity

**Table 1 Represents the air temperature degree and Relative humidity during cold months**

| Degree | Months of Temp. and RH | | |
|---|---|---|---|
| | December | January | February |
| **Temp.** | 10.9 | 7.2 | 12.3 |
| **RH %** | 56.8 | 73.2 | 68.5 |
| **THI** | 49.1 | 39.4 | 52 |

## Blood collection

The blood was collected from all cows in pregnancy or non-pregnancy to determine cortisol concentrations and milk yield in pregnant and non-pregnant cows**.** The blood was withdrawn from the jugular vein using an 18-G needle in the intervals of mating, 7, 15, and 45 days post-mating (7, 15, and 45-DPM). Following, the blood was collected in the early morning (7.00 a.m.) before feed intake, centrifuged (*2000 x g)* for getting the serum, and stored in Eppendorf tubes under -20 oC for cortisol hormone determination later.

## Cortisol analysis

Cortisol was assayed by enzyme-linked immune sorbent assay (ELISA) method using kits of cortisol purchased from DiaMetra, Mod. PIS000-1, Italy) and built on the principle of competitive binding with an assay range of 0.05–60 ng/mL and reading at 620–630 nm (Bio Tek Instruments, Inc, Highland Park, Winooski, VT 05404-0998, REF EPOCH, USA**).**

## Statistical analysis

Cortisol concentrations and milk yield data were analyzed using the Statistical Package for the Social Sciences (SPSS, 2007**).** The normality of data distribution was verified using the Kolmogorov-Smirnoff test and expressed as the mean ± standard error. The analysis of variance (ANOVA) was used among groups, and Duncan's multiple range test was used as a post hoc test to determine the significance of the treatment **(**Duncan, 1955**)**. The linear model was: Yij = μ + Tinm + eij was used for computing the univariate analysis of variance, whereas: Yij refers to the value of any experimental observation in this study, μ is the general mean, Ti is the treatment effect, and eij signifies the experimental random errors of observations.

## Results

### Cortisol

Based on the data presented in Table 2**,** the mean cortisol concentrations in pregnant cows during the PM phase were significantly higher in the VitE+HCG group compared to the VitE, hCG, and control groups, while no significant differences between the treatments and the control in non-pregnant cows. No significant differences between the treatments and the control in cortisol values during the mating phase in pregnant and non-pregnant cows (Table 2)**.** The mean cortisol concentrations were significantly lower in the combination (VitE+ hCG) during the 7, 15, and 45-DPM phases of pregnant cows than in VitE, hCG, and control groups. The same low cortisol concentrations occurred during 7-and 45-DPOM phases in non-pregnant cows except for 15-DPM in which the cortisol was lower without significance than the other groups (Table 2)**.** The table also indicated that the total cortisol concentrations in pregnant and non-pregnant cows were lower in the combination group compared with the other two treatments and control groups (Fig. 1).

**Table 2. Effect of VitE, hCG, and their combination (VitE+hCG) on cortisol concentrations during Pre-mating (PM), mating (M), 7, 15, and 45-DPM (day pot-mating) in pregnant and non-pregnant milking cows**

| Phase | Pregnancy | | | | No Pregnancy | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | VitE | VitE+HCG | HCG | Control | VitE | VitE+HCG | HCG |
| PM | 10.5±0.4[b] | 9.6±2.5[b] | 17.4±1.5[a] | 12.1±2.0[ab] | 12.3±3.0 | 14.4±1.2 | 11.8±1.4 | 12.7±1.8 |
| M | 14.1±3.3 | 12.8±0.8 | 11.8±3.2 | 14.0±1.8 | 13.7±1.5 | 15.4±1.3 | 11.2±2.2 | 12.9±1.6 |
| 7-DPM | 12.7±1.7[a] | 8.9±0.6[ab] | 8.3±1.3[b] | 12.7±1.1[a] | 17.1±2.4[a] | 10.3±0.8[b] | 10.5±0.5[b] | 13.1±0.8[ab] |
| 15-DPM | 11.2±1.4[a] | 7.7±1.0 [ab] | 6.0±1.2[b] | 10.9±1.1[a] | 10.6±1.9 | 13.5±1.0 | 10.4±1.0 | 10.7±0.7 |
| 45-DPM | 10.8±0.3[ab] | 8.3±1.3[ab] | 6.4±2.3[b] | 13.8±1.5 | 10.2±0.5[ab] | 8.2±2.2[ab] | 5.1±0.9[b] | 11.1±1.8[a] |
| Total | 59.3±5.7 | 47.4±4.5 | 47.7±5.8 | 63.4±3.5 | 61.5±3.0 | 61.6±0.4 | 49.0±4.2 | 60.6±4.0 |

Mean followed different superscript letter a -b--ab within the same row is significant different at p<0.01or P<0.05

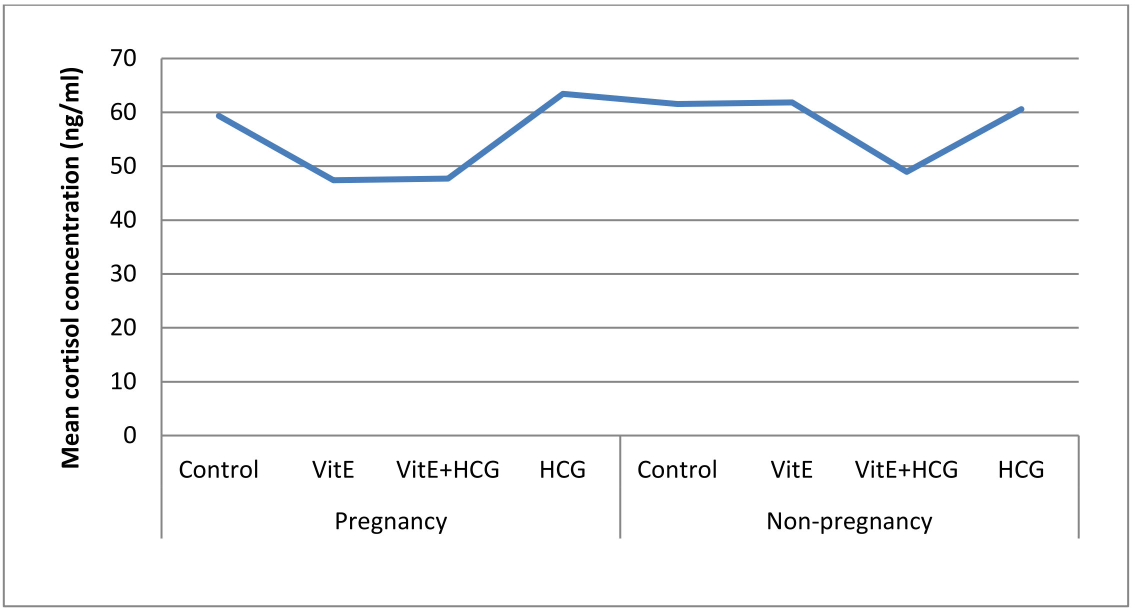


Figure 1. Total cortisol concentration from pre-mating (PM) to 45-DPM (day post-mating) phases in pregnant and non-pregnant milking cows treated with VitE, VitE+HCG, and HCG compared with the control ones.

## Milk yield

Data in Table 3 showed that the mean milk yield in the PM phase of pregnant cows was slightly higher (P<0,05) in the VitE group and lower in the HCG group than in the control. The Table showed that milk yield in pregnant cows increased significantly in VitE and VitE+HCG groups during the phases of mating (M), 7, 15, and 45-DPM, where the higher milk yield occurred in the 15 and 45-DPM phases compared with the control and HCG groups, while the milk yield in non-pregnant cows was not affected by the treatments in all phases (Table 3). The total milk yield from the PM phase to 45-DPM phase of pregnant cows was significantly greater in VitE and VitE+HCG groups than in HCG and control groups (Fig 2), while in non-pregnant milking cows, the total milk yield was not affected by the treatments despite the milk yield was high in the VitE group (Fig 2).

**Table 3. Effect of VitE, hCG, and their combination (VitE+hCG) on milk yield (Liter) in Holstein milking cows**

| Phase | Pregnancy | | | | Nom-pregnancy | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | VitE | VitE+hCG | HCG | Control | VitE | VitE+ hCG | hCG |
| PM | $22.0\pm1.3^{ab}$ | $24.0\pm0.6^{a}$ | $22.9\pm0.8^{ab}$ | $20.6\pm1.5^{b}$ | 21.4±0.4 | 20.3±0.8 | 18.5±4.0 | 20.6±1.8 |
| M | $17.8\pm1.3^{b}$ | $21.9\pm0.7^{a}$ | $20.9\pm0.8^{a}$ | $16.6\pm1.3^{b}$ | 18.0±0.5 | 19.5±0.5 | 17.5±4.0 | 17.8±1.8 |
| 7-DPM | $22.0\pm1.3^{ab}$ | $27.3\pm0.8^{a}$ | $24.7\pm0.9^{ab}$ | $21.9\pm0.8^{b}$ | 21.5±0.5 | 24.0±2.0 | 20.0±4.2 | 20.5±1.9 |
| 15-DPM | $22.2\pm0.7^{3b}$ | $29.0\pm0.7^{a}$ | $26.5\pm1.0^{a}$ | $21.5\pm1.1^{b}$ | 21.8±0.7 | 26.3±0.3 | 21.0±4.0 | 20.6±2.2 |
| 45-DPM | $21.8\pm1.2^{b}$ | $29.4\pm0.8^{a}$ | $27.1\pm1.1^{a}$ | $21.3\pm1.\ 9^{b}$ | 21.9±0.8 | 26.3±0.3 | 22.8±3.8 | 20.9±2.2 |
| Total | 105,8±5.8 | 131,6 ±3.6 | 122,1±4.6 | 101..9±6.5 | 104.6±2.9 | 116.4±3.9 | 99.8±20.0 | 100.4±9.9 |

N= Number of animals within the group
a-b-ab: Values sharing different superscripts in the column are significantly different at $P<0.05$

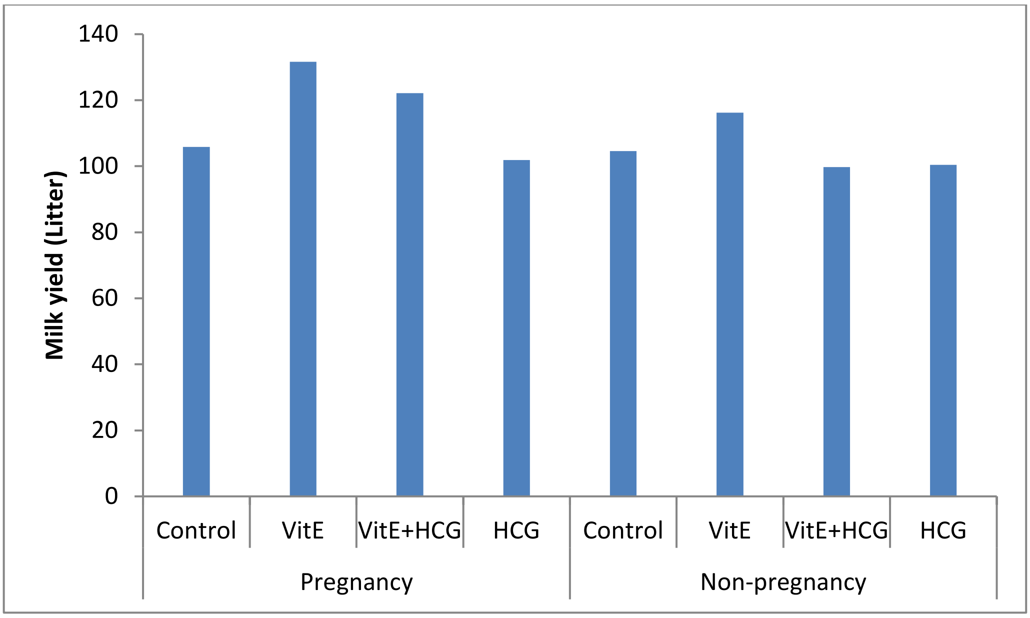


Figure 2. Total milk yield from pre-mating (PM) to 45-DPOM (day post-mating) in pregnant and non-pregnant of milking cows compared with the control ones.

## Discussion

### Cortisol

Little information is present in the literature about the effect of VitE and hCG injection on the stress response in pregnant milking cows recognized by a particular physiological status compared with non-pregnant milking cows. However, previously, supplementation of vitamin E and selenium decreased plasma cortisol levels in pregnant water buffaloes (Dimri *et al.,* 2010), pregnant crossbreed dairy cattle (Gupta *et al.,* 2005), and in dairy cows under abomasal displacement surgery (Mudron and Rehage, 2018). Furthermore, VitE and selenium i.m. injected in high lactating cows resulted in increased antioxidant indicator (erythrocyte glutathione peroxidase activity) *(*Zahrazadeh *et al., 2018).*

The current results indicate that cortisol concentration during 7-DPM in pregnant and non-pregnant cows was significantly greater in the control than VitE and Vit+hCG groups. Previous studies revealed that cortisol is secreted in large quantities during cold stress (Nicholson and Young, 1978, Sakly and Koch, 1982). Similar previous studies showed that exposure to cold air (5 - 15º C) increased serum cortisol levels (Wagner *et al*. 1987, Tikuisis *et al*. 1999). On the contrary, some studies have reported decreased (Leppäluoto *et al*. 1988, Wittert *et al*. 1992) or unchanged serum cortisol levels in response to cold (Galbo *et al*. 1979, Gerra *et al*. 1992). Otherwise, winter does not affect the cortisol level (Maes *et al*. (1997, Takagi (1986). Wilson *et al*. (1970) reported that exposure to cold air (-5 - - 2º C) during the winter decreased cortisol levels more slowly than exposure to a warm summer day. Further, plasma ACTH levels increased if cold exposure was more than 30 min at 4º C (Ohno *et al*. 1987, Vigas *et al*. 1988, Wittert *et al*. 1992). However, seasonal variation in plasma cortisol concentrations has been confirmed in previous studies, where the highest levels of cortisol were in spring (Persson *et al*. 2008, Miller *et al*. 2016), summer (Kanikowska *et al*. 2018), and the lowest levels were in winter (Weitzman *et al*. 1975). However, ROS level elevation harmed the metabolism and health of dairy cows. These harmful effects may be alleviated with enzymatic and non-enzymatic antioxidants (Miller and Brzwzinska-Slebodzinska, 1993). Similarly, Sordillo and Aitken (2009) reported that an increase in ROS impaired the metabolic process and damaged all body organs and tissues which impactcted on the metabolic pathways. Likewise, ROS increases its levels during the physiological stages, such as pregnancy, parturition, and lactation, in which oxidative stress increases (Sharma *et al*. 2011). Antioxidants affect ROS in four ways: scavenger, quencher, restorative, and chain breaker (Cherubini *et al*. 2005). The VitE (potential antioxidant) reduced the cellular damage caused by endogenous free radicals (Kolb *et al*. 1997, Sordillo, 2013).

Any stressor can change animal homeostasis because cattle transported from one place to another resulted in increased activity in enzymes and hormones that impede homeostasis, metabolic processes, and immunity (Adenkola and Ayo, 2010) and increased levels of ROS owing to inflammation with depletion of antioxidant enzyme activity (Piccione *et al*., 2013; Deters and Hansen, 2020). The VitE (potent antioxidant) scavenged free radicals in cattle (Spears, 2000).

**Milk yield**

The current results revealed that milk yield increased in VitE and the combination groups during mating and 45 D-PM when THI levels were between 39.4 and 52. Although the cold stress could reduce milk secretion in lactating cows because of a reduction in mammary blood flow, the treatment with VitE assisted in expanding the maternal blood flows by enhancing the release of prostacyclin (a metabolite of arachidonic acid that inhibits platelet aggregation), which increases blood vasodilation (FNBIM, 2000). A lactating cow requires about five hundred liters of blood flow to the udder to produce one liter of milk (Pandey *et al. 2018,* Pellegrino *et al.* 2021*).* Hence, VitE (α-tocopherol) could be associated with the blood flow and nutrient supply to the mammary gland. Many previous studies confirmed that prostacyclin regulated blood flow between the placenta and fetus and within the fetal *circulation (*de Jong *et al.,* 2000; Walsh, 2004) because the imbalance between prostacyclin and thromboxane led to vasoconstriction (reduced blood flow between fetus and placenta might increase the risk of fetal growth restriction) (de Jong *et al*., 2000; Walsh, 2004). McDowell *et al*. (1996) reported that VitE improved body functions (growth, reproduction, and immunity), prevented diseases, and protected tissues. Previous studies indicated that VitE supplementation during the dry period reduced rates and duration of infections and incidence of mastitis as Indicated by somatic cell counts in the milk (Weiss *et al*. 1997; Politis *et al*. 2012). Vitamin E levels drop strongly during the last weeks of gestation and are the lowest levels at calving before they increase again during the first month of lactation (Stowe *et al*. 1988; Weiss *et al*. 1994). Many found positive effects on immune status with supplementation during late gestation, such as improved functionality of leukocytes neutrophils, and lymphocytes (Hogan *et al*. 1990; Politis *et al*. 2001; Weiss *et al*. 2009; De *et al*. 2014), and reduced indicators of oxidative stress measured in the blood (Bouwstra *et al*. 2009; Aggarwal *et al*. 2013; Aggarwal and Chandra, 2018). A few studies conducted during lactation reported positive effects of VitE supplementation on animal health parameters (Schingoethe *et al*. 1979; Hogan *et al*. 1990; Rahmani *et al*. 2015). However, the increased milk production with VitE supplementation was confirmed in most of the experiments conducted in India (Chatterjee *et al*. 2005; Aggarwal *et al*. 2013; Chandra *et al*. 2015; Singh *et al*. 2020) which are in harmony with the current results.

## Conclusion

Finally, it was resulted from the study that the cold exposure didn't affect cortisol concentrations in mating, pregnancy, and non-pregnancy, and the combination group resulted in low cortisol in pregnancy, probably due to THI comfortable index or VitE as a potential antioxidant that led to ROS reduction and increasing the milk yield and lower cortisol in pregnant and non-pregnant milking cows. Generally, the treatments by VitE and combination (VitE+hCG) enhanced the milk yield and reduced the cortisol level under cold weather.

### References

Adenkola, A.Y., and Ayo J.O. (2010). Physiological and behavioural responses of livestock to road transportation stress: A review. Afr. J. Biotechnol. 9:4845–4856

Aggarwal, A. and Chandra, G. (2018). The effect of additional supplementation of Vitamin E and Zinc on endocrine and metabolic changes in dairy cows during peripartal period. Vet Arh. 88(6):733–748. doi:10.24099/vet.arhiv.0059.

Aggarwal, A., Ashutosh, Chandra, G. and Singh, A.K. (2013). Heat shock protein 70, oxidative stress, and antioxidant status in periparturient crossbred cows supplemented with α-tocopherol acetate. Trop Anim Health Prod. 45(1):239–245. doi:10.1007/s11250-012-0196-z.

Bisinotto, R.S., Lean, I.J., Thatcher, W.W. and Santos, J.E.P. (2015). Meta-analysis of progesterone supplementation during timed artificial insemination programs in dairy cows. J Dairy Sci;,98:2472e87.

Bomfim, G.F.; Merighe, G.K.F.; de Oliveira, S.A. and Negrao, J.A. (2022). Acute and chronic effects of cortisol on milk yield, the expression of key receptors, and apoptosis of mammary epithelial cells in Saanen goats. J. Dairy Sci. 105, 818–830.

Bouwstra, R.J., Nielen, M., Van Werven, T. (2009). Comparison of the oxidative status of vitamin E-supplemented and non-supplemented cows under field conditions. Tijdschr Diergeneeskd. 134(16):656–661.

Bradford, B.J.; Yuan, K.; Farney, J.K.; Mamedova, L.K. and Carpenter, A.J. (2015). Invited review: Inflammation during the transition to lactation: New adventures with an old flame. J. Dairy Sci., 98, 6631–6650.

Brown, E.J., and Vosloo A. (2017). The involvement of the hypothalamopituitary-adrenocortical axis in stress physiology and its significance in the assessment of animal welfare in cattle. Onderstepoort J. Vet. Res. 84:e1–e9.

Canaes, T.S., Negrao J.A., Paiva F.A., Zaros M., and Delgado T.F.G. (2009). Physiologic and productive responses of Alpine goats submitted to transportation to a new dairy location. Arq. Bras. Med. Vet. Zootec. 61:935–940

Casey, T.M., and Plaut, K. (2007). The role of glucocorticoids in secretory activation and milk secretion, a historical perspective. J. Mammary Gland Biol. Neoplasia 12:293–304.

Caroprese, M., Albenzio, M., Marzano, A., Schena, L., Annicchiarico, G., and Sevi, A. (2010). Relationship between cortisol response to stress and behavior, immune profile, and production performance of dairy ewes. J. Dairy Sci. 93:2395–2403.

Chandra, G., Aggarwal, A., Singh, A.K. and Kumar, M. (2015). Effect of vitamin E and zinc supplementation on milk yield, milk composition, and udder health in Sahiwal cows. Anim Nutr Feed Technol. 15(1):67–78. doi:10.5958/0974-181X. 2015.00008.6.

Chatterjee, P.N., Kaur, H., Kewalramani, N. and Tyagi, A.K. (2005). Influence of duration of prepartum and postpartum vitamin E supplementation on mastitis and milk yield in crossbred cows. Indian J Anim Sci. 75:503–507.

Cherubini, A., Ruggiero, C., Polidori, M.C. and Mecocci, P. (2005). Potential markers of oxidative stress in stroke. Free Radical Biology & Medicine. 39:841-852. DOI: 10.1016/j.freeradbio med. 06.025

Colazo, M.G., Dourey, A., Rajamahendran, R. and Ambrose, D.J. (2013). Progesterone supplementation before timed AI increased ovulation synchrony and pregnancy per AI, and supplementation after timed AI reduced pregnancy losses in lactating dairy cows. Theriogenology;79:833e41.

Coleson, M.P., Sanchez, N.S., Ashley, A.K., Ross, T.T. and Ashley, R.L. (2015). Human chorionic gonadotropin increases serum progesterone, number of corpora lutea and angiogenic factors in pregnant sheep. Reproduction 150: 43–52. https://doi.org/10.1530/REP-14-0632

De, K., Pal, S., Prasad, S. and Dang, A.K. (2014). Effect of micronutrient supplementation on the immune function of crossbred dairy cows under semi-arid tropical environment. Trop Anim Health Prod. 46(1):203–211. doi:10.1007/s11250- 013-0477-1.

de Jong, C.L.D., Paarlberg, K.M., van Geijn, H.P., van Kamp, G.J., van Dis, H., Dekker, G.A., (2000). Maternal thromboxane and prostacyclin levels in relation to fetal birth weight. Eur. J. Obstet. Gynecol. Reprod. Biol. 93: 65–69. https://doi.org/10.1016/s0301-2115(00)00266-9.

De Rensis, F., Garcìa-Ispierto, I. and Lopez-Gatius, F. (2015). Seasonal heat stress: clinical implications and hormone treatments for the fertility of dairy cows. Theriogenology;84:659e66.

De Rensis, F.; Lopez-Gatius, F.; Garcia-Ispierto, I. and Techakumpu, M. (2010). Clinical use of human chorionic gonadotropin in dairy cows: An update. Theriogenology, 73, 1001–1008.

Deters, E.L., and Hansen, S. L. (2020). Invited Review: Linking road transportation with oxidative stress in cattle and other species. Appl. Anim. Sci. 36:183–200. https://doi.org/10.15232/aas.2019 -01956

Dimri, U., Ranjan R., Sharma M.C., and Varshney V. P. (2010). Effect of vitamin E and selenium supplementation on oxidative stress indices and cortisol level in blood in water buffaloes during pregnancy and early postpartum period. Trop. Anim. Health Prod. 42:405–410. https://doi.org/10.1007/s11250-009-9434-4.

Djurhuus, C.B., Gravholt, C.H., Nielsen, S., Mengel, A., Christiansen, J.S., Schmitz, O.E. and Møller, N. (2002). Effects of cortisol on lipolysis and regional interstitial glycerol levels in humans. Am. J. Physiol.-Endocrinol. Metab., 283, E172–E177.

Duncan, D.B. (1955): Multiple ranged and multiple F test. Biometrics., 11: 1-42.

FNBIM (Food and Nutrition Board, Institute of Medicine. (2000). Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids. National Academy Press, Washington, DC.

Farin, C.E., Moeller, C.L., Mayan, H., Gamboni, F., Sawyer, H.R. and Nisewender, G.D. (1988). Effect of luteinizing hormone and human chorionic gonadotrophin on cell populations in the ovine corpus luteum. Biol. Reprod. x:413-421.

Folman, Y., Kaim, M., Herz, Z. and Rosenberg, M. (1990). Comparison of methods for the synchronization of estrous cycles in dairy cows. 2. Effects of progesterone and parity on conception. J Dairy Sci; 73:2817e25.

Galbo, H., Houston, M.E. and Christensen, N.J. (1979). The effect of water temperature on the hormonal response to prolonged swimming. Acta Physiol Scand; 105: 326-337.

Garcia-Ispierto, De Rensis F., Casas, X., Caballero, F., Serrano-Perez, B. and Lopez-Gatius, F. (2019). Inducing ovulation with hCG in a five-day progesterone-based fixedtime AI protocol improves the fertility of anestrous dairy cows under heat stress conditions. Theriogenology 124: 65-68.

Gerra, G., Volpi, R. and Delsignore, R. (1992). Sex related responses of beta-endorphin, ACTH, GH and PRL to cold exposure in humans. Acta Endocrinol (Copenh); 126(1): 24- 28.

Gupta, S., Kumar, H. and Soni, J. (2005). Effect of Vitamin E and selenium supplementation on concentrations of plasma cortisol and erythrocyte lipid peroxides and the incidence of retained fetal membranes in crossbred dairy cattle. Theriogenology64:1273–1286. https://doi.org/10.1016/j.theriogenology.2005.03.008.

Hogan, J.S., Smith, K.L., Weiss, W.P., Todhunter, D.A. and Schockey, W.L. (1990). Relationships among Vitamin E, selenium, and bovine blood neutrophils. J Dairy Sci. 73:2372–2378. doi:10.3168/jds.S0022-0302(90)78920-5.

Holtz, W.; Sohnrey, B.; Gerland, M. and Driancourt, M.-A. (2008). Ovsynch synchronization and fixed-time insemination in goats. Theriogenology, 69: 785–792

Huzzey, J.M., Nydam, D.V. and Grant, R.J. (2012). The effects of overstocking Holstein dairy cattle during the dry period on cortisol secretion and energy metabolism. J Dairy Sci; 95:4421–33.

Kanikowska, D.; Roszak, M.; Rutkowski, R.; Sato, M.; Sikorska, D.; Orzechowska, Z.; Breborowicz, A.; Witowski, J. (2018). Seasonal differences in rhythmicity of salivary cortisol in healthy adults. J. Appl. Physiol., 126: 764–770.

Kolb, E., KasKousb S., and Scchawer J. (1997). Nutritional aspects of the importance, utilization, metabolism and the use of vitamin E and selenium in sheep. Berliner und Münchener Tierärztliche Wochenschrift.110: 178–184.

Lei, J.; Feng, D.; Zhang, Y.; Dahanayaka, S.; Li, X.; Yao, K.; Wang, J.; Wu, Z.; Dai, Z. and Wu, G. (2013). Hormonal regulation of leucine catabolism in mammary epithelial cells. Amino Acids, 45: 531–541.

Leppäluoto, J., Korhonen, I., Huttunen, P. and Hassi, J. (1988). Serum levels of thyroid and adrenal hormones, testosterone, TSH, LH, GH and prolactin in men after a 2-h stay in a cold room. Acta Physiol Scand; 132: 543- 548.

Nicholson, M.L. and Young, D.A. (1978). Effect of glucocorticoid hormones in vitro on the structural integrity of nuclei in corticosteroid-sensitive and -resistant lines of lymphosarcoma P1798. Cancer Res.; 38(11 Pt 1):3673–80. PMID: 581273

Maes, M., Mommen, K. and Hendrickx, D. (1997). Components of biological variation, including seasonality, in blood concentrations of TSH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers. Clin Endocrinol (Oxf); 46(5): 587-598.

Mant A. (1964). Some post-mortem observations in accidental hypothermia. Med Sci Law.; 4: 44–6. https://doi.org/10.1177/002580246400400111 PMID: 14106767

McDowell, L.R., Williams, S.N., Hidiroglou, N., Njeru, C.A., Hill, G.M., Ochoa, L. and Wilkinson, N.S. (1996). Vitamin E supplementation for the ruminant. Anim Feed Sci Technol. 60:273–296. doi:10.1016/0377-8401(96)00982-0.

Miller, J.K. and Brzezinska-Slebodzinska, E. (1993). Oxidative stress, antioxidants, and animal function. Journal of Dairy Science, 76: 2812-2823.

Miller, R.; Stalder, T.; Jarczok, M.; Almeida, D.M.; Badrick, E.; Bartels, M.; Boomsma, D.I.; Coe, C.L.; Dekker, M.C.J. and Donzella, B. (2016). The CIRCORT database: Reference ranges and seasonal changes in diurnal salivary cortisol derived from a meta-dataset comprised of 15 field studies. Psychoneuroendocrinology, 73: 16–23.

Mudron, P. and Rehage J. (2018). Effects of vitamin E and selenium supplementation on blood lipid peroxidation and cortisol concentration in dairy cows undergoing omentopexy. J. Anim. Physiol. Anim. Nutr. (Berl.) 102:837–842. https://doi.org/10.1111/jpn .12897.

Negrao, J.A., and Marnet, P.G. (2003). Cortisol, adrenalin, noradrenalin and oxytocin release and milk yield during first milkings in primiparous ewes. Small Rumin. Res. 47:69–75.

Neville, M.C., McFadden, T.B. and Forsyth, I. (2002). Hormonal regulation of mammary differentiation and milk secretion. J. Mammary Gland Biol. Neoplasia 7:49–66.

Ohno, H., Yahata, T., Yamashita, K. and Kuroshima, A. (1987). Effect of acute cold exposure on ACTH and zinc concentrations in human plasma. Jpn J Physiol; 37: 749-755.

Pandey, Y.; Taluja, J.S.; Vaish, R.; Pandey, A.; Gupta, N. and Kumar, D. (2018). Gross anatomical structure of the mammary gland in cow. J. Entomol. Zoo. Stud., 6: 728–733

Panjaitan, B.; Dewi, A.; Nasution, F.F.; Adam, M.; Siregar, T.N.; Thasmi, C.N. and Syafruddin, S. (2019). Comparison of estrous performance and progesterone level of kacang goats induced by PGF2α versus ovsynch protocol. In Proceedings of E3S Web of Conferences, Banda Aceh, Indonesia, 15–17 October 2019; p. 01045.

Pellegrino, L.; Marangoni, F.; Muscogiuri, G.; D'Incecco, P.; Duval, G.T.; Annweiler, C. and Colao, A. (2021). Vitamin D fortification of consumption cow's milk: Health, nutritional and technological aspects. A multidisciplinary lecture of the recent scientific evidence. Molecules, 26: 5289.

Persson, R.; Garde, A.H.; Hansen, Å.M.; Österberg, K.; Larsson, B.; Ørbæk, P. and Karlson, B. (2008). Seasonal variation in human salivary cortisol concentration. Chronobiol. Int., 25, 923–937.

Piccione, G., Casella S., Giannetto C., Bazzano M., Giudice E., and Fazio F. (2013). Oxidative stress associated with road transportation in ewes. Small Rumin. Res. 112:235–238. https://doi.org/10.1016/ j.smallrumres.2012.11.001.

Politis, I.; Theodorou, G.; Lampidonis, A.D.; Kominakis, A. and Baldi, A. (2012). Short communication: Oxidative status and incidence of mastitis relative to blood α-tocopherol concentrations in the postpartum period in dairy cows. J. Dairy Sci., 95, 7331–7335.

Politis, L., Hidiroglou, N., Cheli, F. and Baldi, A. (2001). Effects of vitamin E on urokinase-plasminogen activator receptor expression by bovine neutrophils. Am J Vet Res. 62(12):1934–1938. doi:10.2460/ajvr.2001.62.1934.

Rahmani, M., Dehghan-Banadaky, M. and Kamalyan, R. (2015). Comparison between feeding rumen-protected choline and vitamin e on milk yield and blood metabolites in early lactation dairy cows. Anim Prod Sci. 55:752–757. doi:10. 1071/AN14429.

Rodrigues, J.N.D., Guimarães, J.D., Oliveira, M.E.F., Dias, J.H., Arrais, A.M., de Sousa, M.A.P., Bastos, R., Ahmadi, B., Bartlewski, P.M. and Fonseca, J.F. (2022). Human chorionic gonadotropin affects original (ovulatory) and induced (accessory) corpora lutea, progesterone concentrations, and pregnancy rates in anestrous dairy goats. Reprod. Biol. 22, 100591. https://doi.org/10.1016/J.REPBIO.2021.100591.

Sakly, M. and Koch, B. (1982). Physicochemical analysis of glucocorticoid receptor in the immature pituitary gland: evidence for lability and aggregation of the heat-activated form. Mol Cell Endocrinol.; 28(1):27–35. https://doi.org/10.1016/0303-7207(82)90038-7 PMID: 7128920

Sevi, A., Annicchiarico, G., Albenzio, M., Taibi, L., Muscio, A. and Dell'Aquila, S. (2001a). Effects of solar radiation and feeding time on behavior, immune response and production of lactating ewes under high ambient temperature. J. Dairy Sci. 84:629–640.

Sevi, A., Taibi L., Albenzio M., Muscio A., Dell'Aquila S., and Napolitano F. (2001b). Behavioral, adrenal, immune, and productive responses of lactating ewes to regrouping and relocation. J. Anim. Sci. 79:1457–1465.

Schingoethe, D.J., Parsons, J.G., Ludens, F.C., Schaffer, L.V., Shave, H.J. (1979). Response of lactating cows to 300 mg of supplemental vitamin E daily. J Dairy Sci. 62:333–338. doi:10.3168/jds.S0022-0302(79)83244-0.

Shamay, A., Mabjeesh, S. J., and Shapiro, F. (2000). Adrenocorticotrophic hormone and dexamethasone failed to affect milk yield in dairy goats: Comparative aspects. Small Rumin. Res. 38:255–259.

Sharma, N., Singh, N., Singh, O., Pandey, V. and Verma, P. (2011). Oxidative Stress and Antioxidant Status during Transition Period in Dairy Cows. Asian-Australas. J. Anim. Sci.; 24:479–484. doi: 10.5713/ajas.2011.10220.

Singh, A.K., Bhakat, C., Kumari, T., Mandal, D.K., Chatterjee, A., Karunakaran, M., Dutta, T.K. (2020). Influence of pre and postpartum alpha-tocopherol supplementation on milk yield, milk quality, and udder health of Jersey crossbred cows at tropical lower Gangetic region. Vet World. 13:2006–2011. doi:10.14202/vetworld.2020.2006-2011.

Sordillo, L.M. (2013). Selenium-dependent regulation of oxidative stress and immunity in periparturient dairy cattle.Veterinary Medicine International2013:1–8.

Sordillo, L.M. and Aitken, S.L. (2009). Impact of oxidative stress on the health and immune function of dairy cattle. Vet. Immunol. Immunopathol., 128: 104–109.

Spears, J.W. (2000). Micronutrients and immune function in cattle. Proc. Nutr. Soc. 59:587–594. https://doi.org/10.1017/ S0029665100000835.

SPSS (2007). Statistical package for social sciences. Release 16.0 SPSS Inc., Chicago, USA.

Stewart, H.J., and Thompson G.E. (1984). Adrenocorticotrophic hormone stimulation of mammary secretion in lactating goats independent of increased mammary uptake of glucose. J. Endocrinol. 101:203–211

Stoebel, D.P. and Moberg, G.P. (1982a). Effect of adrenocorticotropin and cortisol on luteinizing hormone surge and estrous behavior of cows. Journal of Dairy Science, 65(6), 1016–1024. https://doi.org/10.3168/ jds.S0022-0302(82)82303-5 V

Stoebel, D.P., and Molberg G.P. (1982b). Effect of adrenocorticotropin and cortisol on luteinizing hormone surge and estrous behavior of cows. J. Dairy Sci. 65:1016.

Stowe, H.D., Thomas, J.W., Johnson, T., Marteniuk, J.V., Morrow, D.A, Ullrey, D.E. (1988). Responses of dairy cattle to longterm and short-term supplementation with oral selenium and vitamin E. J Dairy Sci. 71:1830–1839. doi:10.3168/ jds.S0022-0302(88)79752-0.

Takagi, T. (1986). Longitudinal study on circadian rhythms of plasma hormone levels during Japanese Antarctic Research Expedition. Hokkaido Igaku Zasshi; 61: 121-133.

Tenhagen, B.A., Drillich, M., Surholt, R. and Heuwieser, W. (2004). Comparison oftimed AI after synchronized ovulation to AI at estrus: reproductive and economic considerations. J Dairy Sci.; 87:85-94.

Tikuisis, P., Ducharme, M.B., Moroz, D. and Jacobs, I. (1999). Physiological responses of exercised-fatigued individuals exposed to wet-cold conditions. J Appl Physiol; 86: 1319-1328

Thom, E.C. (1959). The discomfort index. Weatherwise12:57-59.

Trevisi, E., and Bertoni G. (2009). Some physiological and biochemical methods for acute and chronic stress evaluation in dairy cows. Ital. J. Anim. Sci. 8:265–286.

Vergani, G.B., Fonseca, J.F., Trevizan, J.T., Pereira, V.S.A., Garcia, A.R., Esteves, S.N., Souza-fabjan, J.M.G. and Oliveira, M.E.F. (2020). Luteotropic effects of human chorionic gonadotropin administered 7.5 days after synchronous estrous induction in Morada Nova ewes. Anim. Reprod. Sci. https://doi.org/10.1016/j.anireprosci.2020.106644

Vigas, M., Martino, E., Bukovska, M. and Langer, P. (1988). Effect of acute cold exposure and insulin hypoglycemia on plasma thyrotropin levels by IRMA in healthy young males. Endocrinol Exp; 22(4): 229-234.

Wagner, J.A., Horvath, S.M., Kitagawa, K. and Bolduan, N.W. (1987). Comparisons of blood and urinary responses to cold exposures in young and older men and women. J Gerontol A Biol Sci Med Sci; 42(2): 173 - 179.

Walsh, S.W., (2004). Eicosanoids in preeclampsia. Prostaglandins Leukot. Ess. Fat. Acids 70, 223–232. https://doi.org/10.1016/j.plefa.2003.04.010.

Weiss, W.P., Hogan, J.S. and Wyatt, D.J. (2009). Relative bioavailability of all-rac and RRR vitamin E based on neutrophil function and total {alpha}-tocopherol and isomer concentrations in periparturient dairy cows and their calves. J Dairy Sci. 92:720–731. doi:10.3168/jds.2008-1635.

Weiss, W.P., Hogan, J.S., Todhunter, D.A. and Smith, K.L. (1997). Effect of vitamin E supplementation in diets with a low concentration of selenium on mammary gland health of dairy cows. J Dairy Sci. 80:1728–1737. doi:10.3168/jds. S0022-0302(97)76105-8.

Weiss, W.P., Hogan, J.S., Smith, K.L. and Williams, S.N. (1994). Effect of dietary fat and vitamin E on α-tocopherol and βcarotene in blood of peripartum cows. J Dairy Sci. 77:1422–1429. doi:10.3168/jds.S0022-0302(94)77080-6.

Weitzman, E.D.; DeGraaf, A.S.; Sassin, J.F.; Hansen, T.; Godtlibsen, O.B.; Perlow, M. and Hellman, L. (1975). Seasonal patterns of sleep stages and secretion of cortisol and growth hormone during 24 hour periods in northern Norway. Acta Endocrinol., 78, 65–76.

Wilson, O., Hedner, P., Laurell, S., Nosslin, B., Rerup, C. and Rosengren, E. (1970). Thyroid and adrenal response to acute cold exposure in man. J Appl Physiol; 28(5): 543-548.

Wittert, G.A., OR, H.K., Livesey, J.H., Richards, A.M, Donald RA, Espiner EA. (1992). Vasopressin, corticotrophin-releasing factor, and pituitary adrenal responses to acute cold stress in normal humans. J Clin Endocrinol Metab; 75(3): 750-755.

Zahrazadeh, M., Riasi, A., Farhangfar, H., and Mahyari, S.A. (2018). Effects of close-up body condition score and selenium-vitamin E injection on lactation performance, blood metabolites, and oxidative status in high-producing dairy cows. J. Dairy Sci. 101:10495– 10504. https://doi.org/10.3168/jds.2017-14196

**شـــكل هورمون الكورتيزول ومحصـــول اللبن المتـأثران بحقن فيتـامين هـــــ، وهورمون الجونـادوتروبين الكوريونى، واندماجهما معا في الأبقـار الحلابـة الحـامل والغير حـامل تحت الطقس البارد**

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**الملخص**

يتحرى هذا البحث تأثير فيتامين هـــ، وهورمون الجونادوتروبين الكوريونى ـــ واندماجهما معا على محصـول اللبن وتركيزات هورمون الكورتيزول في الأبقار الحلابة الحامل والغير حامل تحت الشـهور الباردة. 34 بقرة حلابة صـحية قسـمت إلى أربعة مجاميع غير متسـاوية في العدد. كنترول (حقنت بالبروبيلين جليكول)، مجموعة فيتامين هــــ (حقنت 1.39 ملى ألفانوكوقيرول 3 مرات قبل التزاوج لمدة 10 أيام، مجموعة هورمون الجونـادوتروبين الكوريونى (حقنت 2000 وحدة دولية) مرة واحدة بعد التزاوج بأسبوع، مجموعة الاندماج (حقنت بنفس الجرعات والفترات الزمنية الموجودة في مجموعتي فيتامين هـ ـ وهورمون الجونادوتروبين الكوريونى).

بينت النتائج ان المعاملات ليس لها تأثير معنوي على تركيزات الكورتيزول اثناء التزاوج في الحمل وغير الحمل لكن التركيزات كانت منخفضـة معنويا في مجموعة الاندماج عن المجاميع الأخرى في الأبقار الحامل ومنخفضـة بدون معنوية في الابقار الغير حامل. تركيزات الكورتيزول الكلية كانت منخفضـة في مجموعة الاندماج في الابقار الحامل والغير حامل عن المجاميع الأخرى عند دليل الحرارة والرطوبة 46 ≤ محصول اللبن زاد معنويا في مجموعتي فيتامين هــ والاندماج أثناء التزاوج ومراحل الحمل بينما في الابقار الغير حامل ليس للمعاملات تأثيرات معنويا على محصـول اللبن، ومحصـول اللبن الكلى للأبقار الحامل كان أعلى معنويا في مجموعتي فيتامين هـ والاندماج عن المجاميع الأخرى في الابقار الغير حامل. الخلاصــة الطقس البارد لم يوثر في تركيزات الكوراتيزول اثناء التزاوج والحمل والغبر حمل ومجموعة الإندماج نتج عنها انخقاض في الكورتيزول ربما هذا ناشئ عن دليل الحرارة والرطوبة 46 ≥المريح للحيوان أو ألي فيتامين هــــ كمضـاد أكسده قوى الذي أدى إلى زيادة محصـول اللبن وانخفاض الكورتيزول في الأبقار الحلابة الحامل والغير حامل.