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Impact of vitamin E and selenium supplementation on oxidative parameters and reproductive efficiency of Egyptian buffaloes

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Abstract. A total of 17 pregnant buffaloes were divided into 2 groups: treated group (n = 10), supplemented with vitamin E and Se (15 ml of Viteselen 15®), and control group (n = 7); not supplemented with vitamin E and Se. The blood sample was collected 4 weeks before and after parturition. The reproductive performance and blood biochemical analysis were conducted as calcium (Ca), phosphorus (P), magnesium (Mg), and progesterone (P4). Oxidative stressed parameters as malondialdehyde (MDA) and nitric oxide (NO), serum glutathione peroxidase (GSH-px), and superoxide dismutase (SOD) were measured. Our results revealed that, treated group free from any postpartum uterine disorder with earlier ovarian resumption than control. The P4, Ca, P, and Mg was increased in the treated blood. Reduce concentrations of NO and MDA was found in the treated group. Meanwhile, a significant increase in SOD and GSH-px postpartum in the treated group was found compared to the control group. In conclusion, supplementation of buffaloes with vitamin E and Se during the transition period has a vital role, through the changes in different oxidative parameters and enhancing metabolic status, which optimizes Egyptian buffaloes' reproductive efficiency.

Keywords: *Vitamin E, selenium, Egyptian buffaloes, reproductive performance*

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1. Introduction

Reproductive performance is considered a significant role in determining the economic value of buffalo cows. Reproductive efficiency may determine buffalo cows' ability to become pregnant and give vital offspring (Barile, 2005). The reduced reproductive efficiency in domesticated buffaloes may be related to many reasons as inbreeding, nutrition, and health care (Danell, 1987), but the lower fertility of buffaloes seems to be the main problem higher than in cattle (Abol-Roos and Gaffar, 2000).

The long postpartum periods consider as a significant factor of lowered reproductive efficiency in buffaloes. Retention of placental (RP), metritis, or pyometra is considered the major factor responsible for delayed uterine involution and recovery of ovarian activity following parturition (Hadiya and Maini, 2007).

Enhancing the animal's productivity through improving the nutritional and immunological status of animals at the peripartum period has become a significant problem. Nutrition plays a vital role at the beginning of the reproductive cycle in domesticated animals. Besides general nutrition, imbalance in specific nutrients as minerals and vitamins found to have a deleterious effect on reproductive performance, causing infertility (Borghese, 2005).

The appropriate supplementation of vitamin E and Se is essential for optimizing the reproductive efficiency, productivity health of lactating and periparturient buffaloes. Vitamin E and Se are considered as central components of the antioxidant immuno-defense mechanism and play a vital role in maintaining the maximum immune function (Sikka, 2006), also maintain animal's reproductive performance through their required in the critical enzymatic reactions (Maraba et al., 2018), and act as

anti-stress factors (Kahlon et al., 2006). Their demands are higher as compared to the production or reproduction requirements (Weiss, 1998).

Therefore, the objective of our present study is to investigate the impact on intramuscular (i.m.) injections of vitamin E and Se during the transition period on the metabolic status, oxidative stress, and the postpartum reproductive performance of Egyptian buffaloes.

2. Materials and methods

2.1. Ethics

All steps of the present experiment were done regarding the Committee of Local Experimental Animal Care and approved by ethics of the Theriogenology Department institutional committee, Veterinary College, University of Assiut, Egypt.

2.2. Animals and experimental treatment

A total of 17 pregnant healthy, lactating buffalo cows (3-8 years age, 3-5 parity, 330-580 kg weight with an average 17-25 kg daily milk production) and were used in our study from a private dairy farm related to the Abnonb Village at Assiut province, Egypt (February to December 2018), and kept under the same nutritional and management conditions. These pregnant buffalo cows were divided into 2 experimental groups: Treated group (n = 10); supplemented with vitamin E and selenium (intramuscular injection of 15 mL of Viteselen 15®, Adwia Co. S.A.E, Made in Egypt). Supplementation was started from 4 weeks before the predicted time of parturition till 4 weeks after parturition (during the transition period) and control group (n = 7); not supplemented with vitamin E and selenium, follow up started from 4 weeks before the predicted time of calving till 4 weeks after calving.

2.3. Gynecological examination and reproductive parameters

All these pregnant animals in our study were subjected to a rectal and gynecological examination and recorded in a gynecological sheet (the case history, breeding history, nature of previous parturition and condition of appetite, and findings rectal examination). These animals were regularly examined for postpartum events as calving nature, placental expulsion, general health of reproductive genitalia, and postpartum ovarian resumption were recorded.

2.4. Blood samples

Blood samples were collected from both groups at 2 weeks' intervals, starting from 4 weeks before parturition till 4 weeks postpartum. Jugular vein-punctures took the weekly sampling in two Wisterman tubes**.** The first blood samples were taken on plain glass tubes, left to coagulate, then centrifuged to separate serum; the second blood samples were taken on a vacutainer containing heparin. These blood samples were centrifuged at 3000 rpm for 15 minutes, and the obtained serum and plasma were stored at -20 °C in a deep freezer.

2.5. Biochemical analysis

Calcium (Ca) was measured using test kits supplied to Spectrum Diagnostics (Egyptian Company for Biotechnology Phosphorus, Magnesium). Magnesium (Mg) kits were obtained from Spectrum Company (Germany). Based on the manufacturer's instructions, phosphorus (P) (Catalog no. 294001) was estimated by a commercially available kit (Egyptian Company for Biotechnology, Cairo, Egypt).

2.6. Oxidative stress parameters and steroid hormone

NO, MDA, SOD, and GSH-px using test kits supplied from Biodiagnostics Company. Progesterone (P4) levels were measured using a linked immunosorbent assay kit provided by Bio Check, Inc., South San Francisco, USA (Catalog number; BC-1113). The intra- and inter-assay coefficients of variation (CVs) for P4 were ≤10.2%.

2.7. Statistical analysis

The packaged SPSS program for Windows (version 16, SPSS Inc., Chicago) was used for statistical analysis. Data were expressed as mean ± standard error of the mean (SEM). An independent sample T-test was used to determine the significant differences between the groups. Differences were considered statistically significant at $p \leq 0.05$.

3. Results and discussion

3.1. Gynecological examination

According to the nature of parturition for each buffalo in both groups, the control group has two cases that suffered from dystocia; one of them was also suffered from placental retention (RP). At the same time, the treated group not suffered from any dystocia. Also, there were no abnormal clinical signs observed on treated buffaloes, either prepartum or postpartum.

These findings may indicate the importance of prepartum supplementation of vitamin E and Se in improving the reproductive health status of pregnant animals, especially after parturition, and these findings were in harmony with Mohsen et al. (2020), who cited that Se supplementation can improve the health status of dairy cattle and prevent the increase of the temperature of the rectum, uterine disorders postpartum and retention of placenta. Also, Kalasariya et al. (2017) found that the intramuscular injection of micro-minerals, especially before parturition, may appreciably lower the incidence of RP and significantly ($p \leq 0.05$) reduced the time of placental expulsion over the non-injected controls. The faster of uterine involution with the early appearance of postpartum estrus with higher conception rates have been related to the combined effect of minerals, protein, and vitamins supplementation due to their significant effect on the steroid genesis and the release, or follicular growth and signs of ovulatory estrus (Srivastava, 2008). Khan et al. (2015) observed that the treated buffaloes with vitamin E and mineral supplementation during the periparturient period not suffered from any clinical metabolic disorders or reproductive problems.

3.2. Biochemical parameters

3.2.1. Metabolic profile

The metabolic adaptation to the reproductive stress during the transition period of the Egyptian buffaloes was shown in (Table 1). The current study showed that postpartum corpus luteum were rectally firstly appeared in the treated group (32.3 ± 11.9 days) than the control one (46.3 ± 14.8 days), and this confirmed by the significant elevation in progesterone (P4) levels postpartum in the treated group than in control group. These findings indicate that the earlier ovarian resumption and first ovulation were recorded in the treated group than the control group. These results were similar to those reported by Abdel-Raheem et al. (2020). Moreover, Yildiz et al. (2015) found a significant increase in P4 level ($p \leq 0.05$) in dairy cows treated with antioxidants than the control group.

Table 1 also shows that the mean values of the measured metabolic parameters of blood serum of Ca, P were significantly elevated in the treated group comparing with the control group ($p \leq 0.05$; postpartum), but the elevation in Mg was not significant. Our presented findings were in harmony with Shi et al. (2017), who declared that plasma metabolites and other biochemical parameters were higher in cows receiving vitamin E, Se, and zinc supplements. Moreover, the same results were obtained by Alhidary et al. (2015).

Table 1. Mean values (±SE) of blood serum P4, calcium, phosphorus, and magnesium in treated and control of Egyptian buffaloes during the transition period.

Transition period	P4 (ng/ml)		Calcium (mg/dl)		Phosphorus (mg/dl)		Magnesium (mg/dl)	
	Treated	**Control**	**Treated**	**Control**	**Treated**	**Control**	**Treated**	**Control**
A	1.91±0.14	1.84±0.06	8.36±0.33	8.07±0.34	4.77±0.10	4.51±0.20	2.21±0.11	2.17±0.26
B	1.79±0.06	1.70±0.14	8.22±0.20	7.54±0.67	4.81±0.32	4.32±0.17	2.18±0.09	2.07±0.10
C	0.96± 0.07	0.91± 0.11	7.34±0.28	7.12±0.39	4.33±0.17	4.08±0.12	1.96±0.02	1.77±0.11
D	0.84±0.01[a]	0.73±0.02	8.27±0.20	8.12±0.47	4.44±0.04[a]	4.38±0.27	2.02±0.09	1.84±0.07
E	1.79±0.01[a]	1.53±0.05	9.99±0.36[a]	8.89±0.35	4.85±0.10[a]	4.78±0.14	2.08±0.06	1.99±0.08

A: 4 weeks prepartum, B: 2 weeks prepartum, C: day of parturition, D: 2 weeks postpartum, and E: 4 weeks post-partum.
a = significant difference between control and treated cows where $p \leq 0.05$.

3.2.2. Oxidative stress parameters

The metabolic changes relating to the reproductive stress during the transition period of the Egyptian buffaloes are due to changes of anti-oxidative/ pro-oxidative parameters were shown in Table 2. The mean values of lipid peroxidation product (MDA) and measured oxidants (NO) were significantly lower, shows the mean values of lipid peroxidation product (MDA) and measured

oxidants (NO) were significantly lower in the treated buffaloes in comparison to control buffaloes. Meanwhile, mean values of antioxidant enzyme activities (SOD and GSH-px) showed a significant increase mainly to treated group postpartum.

The present study indicated that pregnant buffaloes were exposed to an increased risk of oxidative stress during the transition period, as noted by the increased NO and MDA concentrations in the control group. Reduce concentrations of NO, MDA in the treated group, especially 2 weeks after calving compared with control buffaloes, suggested that vitamin E has a significant role in the protection against oxidative stress. Our presented results are in agreement with the previous findings reported by Bouwstra et al. (2008). However, the significant increased postpartum levels of SOD and GSH-px was noticed in the treated group as compared with the control may concise with results of Abdel-Raheem et al. (2020), who suggest that the maternal supplementation of vitamin E and Se during the transition period can improve the antioxidant status of Ossimi ewes. Moreover, Gong and Xiao (2016) reported that the body's antioxidant defense mechanism has high SOD and GSH-px in the Se and zinc supplemented group than the control. Therefore, the endogenous antioxidant defense status in cows can beat the harmful effects of reactive oxygen species (ROS) (Kassab et al., 2020). GSH may consider as a selenium-dependent antioxidant enzyme (Gong and Xiao, 2016). Additionally, Aghwan et al. (2016) denoted that vitamin E and Se are considered as essential components of the antioxidant defense mechanism and play a critical role in the growth, the function of the immune system, and improve the productive activity of animal's reproduction via their share in the critical enzymatic reactions (Maraba et al., 2018).

Table 2. Mean values (±SE) of blood serum MDA, NO, SOD, and GSH-pX in treated and control of Egyptian buffaloes during the transition period.

Transition period	MDA (nmol/ml)		NO (umol/L)		SOD (U/mg Hb)		GSH-px (mU/mL)	
	Treated	**Control**	**Treated**	**Control**	**Treated**	**Control**	**Treated**	**Control**
A	4.88±0.61	4.98±0.18	22.38±2.34[a]	33.51±2.93	38.24±2.62[a]	27.33±1.76	247.27±39.50[a]	215.87±18.22
B	5.24±0.28[a]	6.64±0.35	24.16±1.63[a]	38.32±2.94	32.26±2.60[a]	21.46±1.44	230.33±40.96[a]	19513±36.28
C	6.08±0.47[a]	11.07±0.13	26.16±1.59[a]	42.47±2.46	28.63±2.02[a]	16.52±1.42	208.71±27.01[a]	187.70±61.14
D	5.13±0.16[a]	12.45±0.28	23.32±2.60[a]	34.63±2.02	39.93±1.96[a]	26.32±3.18	289.00±57.73[a]	201.23±37.66
E	4.57±0.29[a]	10.67±0.18	21.12±1.66[a]	31.08±2.06	44.33±2.33[a]	31.21±1.96	292.79±35.73[a]	211.38±87.32

A: 4 weeks prepartum, B: 2 weeks prepartum, C: day of parturition, D: 2 weeks postpartum, and E: 4 weeks post-partum.
a = significant difference between control and treated cows where $p \leq 0.05$.

4. Conclusion

Finally, it can be concluded that supplementation of buffaloes with antioxidants, like vitamin E and Se during the transition period, has a beneficial strategy through the changes in different oxidative stress parameters. The level of lipid peroxidation product (MDA) and measured oxidants (NO) were significantly lower. Meanwhile, mean values of antioxidant enzyme activities (SOD and GSH-px) showed a significant increase, especially postpartum. Enhancing blood biochemical parameters (Ca, P and Mg) and significantly increased P4 levels with the earlier resumption of postpartum ovarian activity optimizes Egyptian buffaloes' reproductive efficiency.

Conflicts of interest. There are no conflicts of interest.

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