Combined effect of vitamin E and selenium on some productive and physiological characteristics of ewes and their lambs during suckling period

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ABSTRACT

Twenty four pregnant ewes (1/2 **Ossimi** X 1/2 Chios) averaged 49.75 ± 2.65 kg were used to evaluate the combined effect of vitamin E and selenium (Se) injection on growth performance and some physiological reactions of these ewes and their lambs. The ewes were randomly divided into two equal groups (12 ewes each). The first group (G1) served as control and injected with 1.0 ml/head of saline solution (0.9 % NaCl), while the second group (G2) were injected intramuscularly with 1.0 ml/head viteselene. Each ml of viteselene contained 150 mg vitamin E acetate and 1.67 mg saodium selenite. The ewes were injected with viteselen biweekly starting at 4 weeks late gestation and during suckling period for 12 weeks.

The results showed that lambs born from treated ewes (G2) recorded higher ($P<0.05$) body weight and daily gain than those born from control ewes (G1). Blood Hb concentrations significantly ($P<0.05$) increased for G2 compared with G1. Lambs born from treated ewes had significantly ($P<0.05$ to $P<0.01$) higher values of red blood cells, hemoglobin, packed cell volume and mean corpuscular hemoglobin concentration than those born from control ewes. The total count of leucocytes increased ($P<0.05$) for treated ewes, lymphocytes and eosinophils percentages increased ($P<0.05$) while neutrophils, basophils and monocytes were insignificantly changes compared with the control ewes. Lambs born from G2 showed insignificant increase in total count of leucocytes accompanied with an increase ($P<0.05$) in lymphocytes and a decrease ($P<0.01$) in neutrophils with no significant changes in eosinophils, basophils and monocytes percentages compared to lambs born from G1. In vitamin E plus Se-injected ewes and their lambs, there were significant ($P<0.05$ to $P<0.01$) increases in plasma total protein and globulin. Changes in plasma albumin, total lipids and glucose concentrations for treated ewes and their lambs were not significant, while plasma T_3 and T_4 levels were higher ($P<0.05$) in treated ewes compared with control ewes.Plasma level of T_3 only was higher ($P<0.05$) in lambs born from treated ewes (G2) than those born from control ewes (G1). These results indicated that administration of vitamin E plus Se to ewes at 4 weeks late gestation and during suckling period improved growth performance, some immune responses and viability of their lambs as a result of favorable signs in their physiological reactions.

Key Words: *Vitamin E plus Selenium, Ewes, Suckling lambs, Physiological Reactions.*

INTRODUCTION

Vitamin E (α-tocopherol) and selenium (Se) have complementary role, as antioxidants, in the protection of cells against the damaging effects of lipid peroxides and free radicals produced during normal metabolism. The multiple functions of both nutrients, at cellular and molecular levels, extend beyond antioxidant protection, as their inclusion in the diet at concentrations above requirements is associated with variable improvements in sheep performance and immune function (Rooke *et al.*, 2004). It has been emphasized that both nutrients should be administered, to sheep, in combination in order to improve their immune competence (Hamam and Abou-Zeina, 2007). A major contributor to non-enzymatic protection against lipid peroxidation is vitamin E, a known free radical scavenger (Rikans *et al.*, 1991). If vitamin E helps to protect membrane integrity of cells, the health status and efficiency of growth and production should be increased by supplemental vitamin E in sheep (Ali *et al.*, 2004). Newborns are susceptible to vitamin E

deficiency and, due to the negligible amount of vitamin E crossing to the fetus in uterus, it is important that colostrum supplies the lamb with sufficient amounts of vitamin E (McDowell et al., 1996). It has been reported that ewes supplemented with vitamin E and Se, during late gestation, had lambs with significantly higher birth weight and reduced mortality compared to lambs of non-supplemented ewes (Kott et al., 1998). Both vitamin E and Se have been shown to improve immune responses (Shinde et al., 2007). In addition, dietary Se supplementation and vitamin E injection to ewes in late gestation and during lactation may provide some improvement in lamb performance and livability (Ali et al., 2004). In sheep, however, the physiological reactions of the combined effect of vitamin E plus Se given to ewes on their lamb performance has not been fully documented. Therefore, the present study aimed to detect the mechanistic aspects through which ewe-vitamin E plus Se injection, at 4 weeks late gestation and during suckling period, might influence the performance of their lambs via monitoring some of their productive and physiological characteristics.

MATERIALS AND METHODS

Twenty four pregnant ewes (1/2 Ossimi X 1/2 Chios) averaged 49.75 ± 2.65 kg and 2.5 years old were used in this experiment. The animals were apparently healthy and proved to be free from internal and external parasites. The ewes were randomly divided into two equal groups (12 ewes each). The first group (G1) served as control and injected with 1.0 ml/head of sterile saline solution (0.9 % NaCl), while the second group (G2) injected intramuscularly with 1.0 ml/head viteselen contained 0.05 mg selenium + 10.7 IU vitamin E/head/day. Each ml of viteselen contained 150 mg vitamin E acetate and 1.67 mg sodium selenite (eq. to 0.762 mg Se). The ewes were injected biweekly with viteselen starting at 4 weeks before gestation and during the suckling period for 12 weeks. Animals were fed on concentrate feed mixture and bean straw to cover their nutrient requirements according to their live body weight (NRC, 1985). The concentrate mixture contained 30 % yellow corn, 45 % wheat bran, 17 % decorticated cotton seed meal, 5 % molasses, 2 % limestone and 1 % common salt. The calculated feeding values were 66.15 % TDN and 17.14 % crude protein. The calculated concentrations of Se and vitamin E in the concentrate mixture fed were 0.17 ppm and 17.78 IU/kg DM, respectively. The NRC (1985) requirements of ewes in late gestation and during suckling period for Se and vitamin E are between 0.1-0.2 ppm and 24-36 IU/kg DM, respectively with consideration of suckling twins. Feed was offered twice a day at 8 am and 2 pm and mineral blocks and drinking water were available to the animals all times.

Parameters were collected in the morning before animals access to feed or water. Body weights of ewes in G1 and G2 groups were recorded at the beginning of vitamin E and Se injection and biweekly thereafter. Body weights of 6 male lambs born from ewes in each group were recorded within 24 hours from birth then at 2 weeks intervals during suckling period. Average daily gain of lambs were calculated and recorded. After parturition and at 2 weeks intervals, heparinized blood samples (5 ml) were collected biweekly form 6 ewes and 6 of their male lambs in each group from the jugular vein of each animal before feeding and drinking. Whole blood samples were analyzed shortly for blood hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC) and leucocyte counts using conventional methods. Mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the formulas proposed by Schalm (1965). Stained blood smears with Lieshman's stain were prepared for the differential leucocytes count (Dacie and Lewis, 1991). Plasma samples were obtained by centrifugation of blood samples at 3000 rpm for 10 minutes and stored at -20°C until assayed for biochemical analysis. Plasma total protein, albumin, total lipids and glucose were measured spectrophotometrically using standard commercial kits supplied from Bio-Merieux (Marcy-1, Etolie Charbonnieres- Les Bains, France) and Bio-Analytics kits (USA). Globulin was calculated mathematically by subtracting

the difference between total protein and albumin. Plasma triiodothyronine (T_3) and thyroxin (T_4) concentrations were determined by a direct solid-phase I^{125} radioimmunoassay techniques using (coat-A-count TKT_3) RIA kits purchased from Diagnostic Products Corporation (DPC, LA, CA, 90045-559, USA).

The data were analyzed by least square means analysis of variance using General Linear Models (GLM) procedure of the statistical analysis system (SAS, 1992). The model used to analyze the different traits studied for ewes or lambs was as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where: Y_{ij}= ij^{th} observation, μ = Population mean; T_i = Effect of i^{th} treatment (1=control and 2= vitamin E and Se treatment and e_{ij}=Random error particular to the ij^{th} observation and assumed to be independently and randomly distributed (0, X^2). Duncan's Multiple Range test was used to detect the differences between means of the experimental groups (Duncan, 1955).

RESULTS

Animal performance:

The results showed no significant differences in averages body weight (BW) between vitamin E plus Se-injected ewes and control ewes (Table 1). However, average BW of ewes tended to increase by 7.69, 6.44 and 5.2 % at 4, 8 and 12 weeks post-partum due to vitamin E plus Se injection. Data in Table (2) show no significant differences in BW averages at birth between lambs born from treated ewes (G2) and those born from control ewes (G1), while treated lambs recorded 11.7, 10.8 and 12.2 % higher BW averages (P<0.05) (at 4, 8 and 12 weeks of age respectively) than lambs born from control ewes. Average daily gain weights of treated lambs were 13.9, 10.7 and 15.4 % greater (P<0.05) (at periods 4 , 4-8 and 8-12 weeks from birth)than lambs of control group (Table, 2).

Hematological parameters

Data presented in Table 3 show no significant changes in blood parameters (RBC, PCV, MCH, MCV and MCHC) for treated ewes, but there was a significant (P<0.05) increase by 8.5% in concentrations of their blood Hb compared with control ewes. Lambs born from treated ewes (G2) had significantly (P<0.05 to P<0.01) 10.9, 17.6, 9.3 and 8.1 % higher blood RBCs, Hb, PCV and MCHC values than those born from control ewes (G1). While in ewes values of RBCs and PCV (%) showed insignificant increases (more by 4.0 and 4.6 % only) for treated than control ones.

Data presented in Table (4) indicate that treated ewes showed increase of 13.8 % in total count of leucocytes (P<0.05), increase in lymphocytes and eosinophils percentages amounted by 6.8 and 8.5 % (P < 0.05) and no significant changes in neutrophils, basophils and monocytes compared with the control ewes. Lambs born from treated ewes showed 9.7 % increase in lymphocytes (P<0.05) and 21.1 % decrease in neutrophils (P<0.01) while no significant changes in eosinophils, basophils and monocytes percentages and total count of leucocytes compared to lambs born from control ewes.

Table 1: Averages of body weight (± SEM) of ewes as affected by vitamin E and Se administration.

(Body weight, kg)	G1	G2	Change (%)	SEM	Sig.
At starting	49.85	51.28	2.87	1.85	N.S
4 weeks postpartum	42.53	45.80	7.69	1.93	N.S
8 weeks postpartum	47.66	50.73	6.44	1.08	N.S
12 weeks postpartum	51.00	53.65	5.20	2.42	N.S

G1 = Control, G2 = Vitamin E and Se, NS = Not significant

Table 2: Averages of body weight and daily gain (± SEM) of lambs as affected by ewe vitamin E and Se administration.

Parameters	G1	G2	SEM	Sig.
Body weight (kg) :				
At birth	2.85	2.96	0.42	NS
4 weeks	7.45 ^{b}	8.20 ^{a}	0.86	*
8 weeks	12.36 ^{b}	13.69 ^{a}	1.05	*
12 weeks	17.37 ^{b}	19.48 ^{a}	0.95	*
Daily weight gain (g/day)				
Birth- 4 weeks	153.33 ^{b}	174.67 ^{a}	3.64	*
4 - 8 weeks	165.30 ^{b}	183.00 ^{a}	2.35	*
8 - 12 weeks	167.00 ^{b}	192.67 ^{a}	3.53	**

a, b: Means in the same row having different superscripts are significantly different (* P<0.05, ** P<0.01), NS = Not significant. G1 = Control G2 = Vitamin E plus Se

Table 3: Combined effect of vitamin E plus Se on some hematological parameters of ewes and their suckling lambs (Means ± SEM).

Blood Parameters	Ewes		SEM	Sig.	Lambs of		SEM	Sig.
	G1	G2			G1	G2		
RBC (x10^6/mm^3)	9.28	9.65	0.18	NS	9. 60 ^{b}	10.65 ^{a}	0.22	*
Hb (g/dl)	10.40 ^{b}	11.28 ^{a}	0.32	*	10.95 ^{b}	12.88 ^{a}	0.25	**
PCV (%)	29.85	31.22	2.45	NS	31.80 ^{b}	34.55 ^{a}	2.85	*
MCH (µ/µg)	11.2	11.7	0.35	NS	11.5	12.1	0.55	NS
MCV (Cu/µ)	32.2	32.4	1.85	NS	33.1	32.6	2.00	NS
MCHC (%)	34.8	36.1	2.35	NS	34.4 ^{b}	37.20 ^{a}	1.66	*

a, b: Means in the same row having different superscripts are significantly different (* P<0.05 and ** P<0.01), NS= Not significant. G1 = Control G2 = Vitamin E plus Se RBC=Red blood cells, Hb=Hemoglobin, PCV=Packed cell volume, MCH= Mean corpuscular hemoglobin, MCV= Mean corpuscular volume, MCHC= Mean corpuscular hemoglobin concentration

Table (4): Combined effect of vitamin E plus Se on total and differential leucocytes counts of ewes and their suckling lambs (Mean ± SEM).

Parameters	Ewes		SEM	Sig.	Lambs		SEM	Sig.
	G1	G2			G1	G2		
Leucocytes (x10^3/mm^3)	8.05 ^{b}	9.14 ^{a}	0.45	*	8.78	9.20	0.63	NS
Neutrophils (%)	30.65	27.05	1.84	NS	31.0 ^{a}	25.6 ^{b}	0.97	**
Eosinophils (%)	4.7 ^{b}	5.0 ^{a}	0.22	*	5.24	5.30	0.30	NS
Basophils (%)	0.51	0.48	0.04	NS	0.59	0.53	0.05	NS
Lymphocytes (%)	60.5 ^{b}	64.44 ^{a}	1.88	*	59.52 ^{b}	65.27 ^{a}	1.36	*
Monocytes (%)	3.64	3.42	0.38	NS	3.65	3.30	0.40	NS

a, b: Means in the same row having different superscripts are significantly different (* P<0.05 and ** P<0.01). G1= Control G2= Vitamin E plus Se.

Table 5: Combined effect of vitamin E plus Se on plasma metabolites and thyroid hormones concentrations of ewes and their suckling lambs (Means ± SEM).

Parameters	Ewes		SEM	Sig.	Lambs		SEM	Sig.
	G1	G2			G1	G2		
Total protein (g/dl)	6.75 [b]	7.64 [a]	0.14	*	6.54	7.75	0.19	*
Albumin (g/dl)	3.29	3.30	0.09	NS	3.32	3.38	0.12	NS
Globulin (g/dl)	3.46[b]	4.34 [a]	0.08	*	3.22 [b]	4.37 [a]	0.16	**
Total lipids (mg/dl)	147.5	152.7	6.50	NS	124.5	130.0	4.48	NS
Glucose (mg/dl)	50.80	53.75	3.13	NS	57.50	61.21	4.36	NS
T_3 (ng/ml)	1.95 [b]	2.25 [a]	0.10	*	1.60 [b]	2.05 [a]	0.05	*
T_4 (ng/ml)	41.0 [b]	46.5 [a]	2.30	*	39.8	41.3	3.99	NS

a, b: Means in the same row having different superscripts are significantly different (* P<0.05 and ** P<0.01), NS= Not significant. G1 = Control G2 = Vitamin E plus Se

Plasma metabolites and thyroid hormones

The results presented in Table (5) indicate that treated ewes (G2) exhibited higher (P<0.05 to P<0.01) concentrations of plasma total protein and globulin amounted by 13.2 and 18.5 %, respectivelythan those in the control ewes. Also, lambs born from treated ewes recorded significant (P<0.05) increases in plasma total protein and globulin by 25.4 and 35.7 %, respectively compared to those born from control ewes. No significant changes were observed in plasma albumin, total lipids and glucose either for treated ewes or for their lambs. The data showed that plasma T_3 and T_4 levels were higher (P<0.05) by 15.4 and 13.4 % for treated ewes compared to control ones. Lambs born from treated ewes revealed a significant (P<0.05) increases in plasma levels of T_3 by 28.2 %, with no significant change in T_4 levels compared to lambs born from control ewes.

DISCUSSION

In the current study, injection of vitamin E plus Se to ewes at 4 weeks late gestation and during suckling period for 12 weeks significantly improved averages BW and DWG of offspring from birth up to weaning with insignificant increase in birth weight and average body weights for treated ewes. Such improvement may indicate a high efficiency of feed utilization for treated-ewes and viability of their lambs. Feed efficiency and DWG could also be improved by vitamin E supplementation to coarse-wool lambs (Shetaewi *et al*., 1992). Weekly injection of 900 IU of vitamin E to ewes in late gestation did not affect lambs birth weight, but increased the pre-weaning weight and DWG of lambs while supplement of Se at 90 ppm to ewes in late gestation and during lactation insignificantly increased birth weight (3.68 % only) (Ali *et al.,* 2004). In addition, injection of ewes with vitamin E Se before mating and lambing seasons had significant increase in DWG from birth up to 60 days and mean lambs' BW at 60 days (Koyuncu and Yerlikaya, 2007). Administration of Se alone improved reproductive performance of ewes and ADG of offspring (Gabryszuk and Klewiec, 2002). However, when ewes received Se alone in organic and inorganic forms during pregnancy and lactation, Se had no significant effect on either birth weight or growth of their lambs (Rodinova *et al.,* 2008). Recently, supplementation with 50 mg vitamin E plus 0.3 mg of Se /kg diet to Baladi ewes starting at 2 weeks before mating and extended through pregnancy till lambing improved significantly their reproductive performance and growth performance of their lambs born from birth up to weaning compared to lambs from ewes supplemented with vitamin E or Se alone (El-Shahat and Abdel Monem, 2011). Since vitamin E and Se improve immune competence in sheep (Hamam and Abou-Zeina, 2007) and the health status and subsequently the efficiency of growth and production (Ali *et al.,* 2004; El-Shahat and Abdel Monem, 2011). The presented results are agreeable with some ruminant studies. For

instance, buffaloes injected with 30 ml of viteselen at late gestation led to calves of heavier BW, daily growth rate and better vitality (Amer and Hashem, 2008). This improvement in productive performance of buffaloes was accompanied with higher ($P<0.05$) levels of IgG in both dam colostrum and newborn serum after administration of viteselen. Another recent study on buffaloes showed that injection of 10 ml of vitamin E-Se (containing 50 mg of vitamin E and 0.5 mg of sodium selenite/ml) during late gestation increased colostrum production and birth weight and weight at 4 weeks of age in calves (Qureshi *et al*., 2010). Therefore, the beneficial effects of supplementation with vitamin E plus Se on animal's immunity and health may be reflected through positive responses on productive performance.

In the present study, some hematological parameters were positively changed up as a result of vitamin E plus Se treatment, which may point out the active metabolism and biological oxidation effects on the cellular base that might lead to availability of metabolites required for tissue growth. The significant increase in RBCs, blood Hb and PCV (%) due to injection of vitamin E plus Se agrees with the results reported on growing Ossimi lambs (Soliman *et al.,* 2001) and on dairy calves (Mohri *et al*., 2005). Likewise, these findings are in accordance with the study of Qureshi *et al.* (2001) which found significant higher Hb, RBC count and PCV values, while MCV, MCH and MCHC remained unchanged for adult buffaloes supplemented with vitamin E and Se during late gestation. The lambs born from vitamin E plus Se-injected ewes, had significant increase in blood Hb and MCHC and showed tendency to increase MCH values. In contrary to the above results, supplementation of Se and vitamin E in the diet of buffalo calves had no significant effect on hematological parameters (Hb, PCV, RBCs and WBCs counts) as reported by Shinde *et al*. (2009). The hematological responses obtained in the current study could not be discussed away from the recent report of Jilani and Iqbal (2011). They stated that vitamin E supplementation led to increase the number of colony forming units of erythroid precursors, preventing the oxidation of polyunsaturated fatty acids in RBCs membrane, thus inhibiting the premature erythrocyte lysis, enhancing erythropoiesis and decreasing the premature erythrocyte hemolysis by reducing the fragility of erythrocytes. Thus, vitamin E may improve the post-supplemental blood Hb and PCV levels. As far as supplementation of vitamin E alone is concerned, Shetaewi *et al*. (1992) supplemented vitamin E at rate 100 mg/head/weekly found increased RBCs, HB and PCV in coarse-wool lambs. While in case of supplementation of Se alone to lambs, total RBCs count and osmotic resistance of RBCs were higher (Faixova *et al.,* 2007). However, El-Ayouty *et al*. (1996) found that Se alone had no marked effect on RBCs count, Hb and hematocrit in buffalo calves. Likewise, supplementation of different forms of Se did not markedly influence the dynamics of blood parameters in non-pregnant, pregnant and lactating ewes if the intake of vitamins and other essential microelements was adequate (Pisek *et al*., 2008). In the current study it is likely that the positive effect of vitamin E plus Se injection on hematological parameters, might be attributed to the effect of vitamin E on keeping efficient metabolic activity, reflecting the increase in blood parameters observed in born lambs. It could also be noticed that changes in blood hematological parameters, in the present study, were within the normal physiological values of sheep as previously documented (Blunt, 1975).

Moreover, the present work showed significant changes in leucocytes count and some of its differential cell percentages in the treated ewes and their lambs. These findings confirm that administration of vitamin E plus Se could improve immune function in sheep. At this point, Hamam and Abou-Zeina, (2007) suggested that injection of both vitamin E plus Se, in Baladi ewes, significantly increase the concentrations of natural antioxidants (α-tocopherol and glutathione peroxidase) in blood of sheep, hence ensures that they mount adequate immune globulins, concluding that both nutrients should be administered to sheep in order to improve their immune competence. The effect of vitamin E plus Se on increasing

total count of leucocytes agree with similar response observed on Friesian heifers (Suwanpanya *et al.,* 2007); adult buffaloes during late gestation (Qureshi *et al.,* 2001); growing Ossimi lambs (Soliman *et al.,* 2001) and dairy calves (Mohri *et al*., 2005). However, Shinde *et al.,* (2009) found that total count of leucocytes was not affected by Se alone or vitamin E plus Se supplementation to buffalo calves. The observed increase in eosinophils that amounted by 8.5 % for vitamin E plus Se-injected ewes is compatible with the finding of increasing eosinophils percentage in adult buffaloes received intramuscular administration of 500 mg vitamin E and 15 mg Se during late gestation (Qureshi *et al.,* 2001). However, the exact mechanism by which eosinophils number is increased by immunomodulators is still unclear and needs to be clarified. In the present study, neutrophil percentages did not significantly changed due to administration of vitamin E plus Se to ewes, but they showed a significant decrease in their lambs. The neutrophil response to supplement of vitamin E and/or Se was focused in some studies. Hogan *et al.* (1992) reported that neither phagocytic index nor percentage of neutrophils differed between vitamin E-injected and non-injected cows. However, El-Ayouty *et al.* (1996) found that supplement of Se alone to suckling buffalo calves had no effect on neutrophil percentages. But, Suwanpanya *et al.* (2007) illustrated that neutrophil phagocytosis was improved in vitamin E plus Se supplemented-heifers. Such differences in neutrophil responses could be attributed to the different levels of vitamin E and/or Se used, route of administration, physiological states and species of the experimental animals. The present study showed a significant increase in lymphocytes percentages either for vitamin E plus Se-injected ewes or their male lambs. The enhanced effect of injectable vitamin E and Se to increase lymphocytes (%) was previously reported on growing lambs (Soliman *et al.,* 2001) and adult buffaloes (Qureshi *et al.,* 2001). It has been noticed that lymphocyte proliferation was declined following prolonged exposure of lambs to a diet deficient in both vitamin E and Se, while their supplementation restored lymphocyte function within a week **(**Turner and Finch**,** 1990). The enhanced lymphocyte function and its proliferative responses due to the interacted effect of vitamin E and Se were also reported with cattle (Pollok *et al*., 1994). Exposure of peripheral blood lymphocytes to vitamin E and Se treatment in *in vitro* study enhanced lymphocyte proliferation in cattle. These results may optimize resistance to diseases by enhancing the lymphocyte populations (Ndiweni and Finch, 1995). Generally, the usefulness of Se to the immune status appears to be increased by giving vitamin E. In buffalo calves, supplementation of Se improved the humoral immune response, whereas vitamin E showed a tendency towards improvement in cell mediated immune response (Shinde *et al*., 2007). A marginal vitamin E deficiency impaired the immune response, while supplementation with higher than recommended dietary levels of vitamin E enhanced humoral and cell-mediated immunity (Beharka *et al*., 1997). Among the circulating leucocytes, the lymphocytes are responsible for humoral and cellular immune responses, and an increase in their number in blood may be a good indicator of an immunomodulatory response (Qureshi *et al.,* 2001). Thus, the increase or decrease in the numbers of the different types of leucocytes can help identify infection or to monitor the animal body's response to diseases treatment.

The responses of ewes and their male lambs' leucocytes and its differential cell percentages, in the present study, to vitamin E plus Se injection may be considered a useful response to improve the immune function, disease resistance, general health and enhancing their adaptability against adverse environmental conditions which reflected on the productive performance. Both vitamin E and Se act synergistically and protect the tissues against oxidative damage which improve immune responses (Shinde *et al.,* 2007). Numerous studies support the view that vitamin E and Se together have an important beneficial effect on immunity than administration of Se alone (Pollok *et al*., 1994; Ramos *et al*., 1998; Hamam and Abou-Zeina, 2007). It has been also suggested that Se can protect immune cells

for a long period, whereas vitamin E has an immediate effect (Pollok *et al*., 1994). The effects of vitamin E and Se on immune system are most probably attributed to their antioxidant properties (Hamam and Abou-Zeina, 2007). The total leucocytes count and its differential cell percentages, in the current study, were within the normal hematologic reference ranges of sheep as documented by Duncan and Prasse (1986).

The results of this study showed some positive appreciable changes in the plasma metabolites and thyroid hormones (T_3 and T_4) with vitamin E plus Se-injected ewes and their lambs. The results revealed that metabolic activity processes might be enhanced in these animals. The present study revealed that ewes received injection of vitamin E plus Se at late gestation and during suckling period had significant increases in plasma total protein and globulin with no significant change in plasma albumin when compared to untreated ewes. These results are in agreement with the study of El-Shahat and Abd El-Monem (2011), who reported that Baladi ewes supplemented with 50 mg vitamin E plus 0.3 mg of Se /kg diet, at 2 weeks before mating and extended through pregnancy till lambing resulted in a significant ($P<0.05$) increase in total serum protein and globulin. They also noticed that the higher level of serum albumin was found in vitamin E alone supplemented-ewes compared to vitamin E plus Se or Se alone supplemented ewes. However, ewes received Se alone had significantly lower total serum protein, albumin and globulin. These observations may signify a lack of an effect of supplemental Se alone on serum albumin in sheep as previously observed by Hamam and Abou-Zeina (2007). They found that in Baladi ewes, vitamin E plus Se-supplemented group had significantly higher ($P<0.05$) total serum globulins, specifically γ-globulins, than did the control or Se-supplement alone. The mode of action of vitamin E is closely associated with Se metabolism, and it can make up for Se deficiency to a certain degree (Makimura *et al.*, 1993). In this regard, vitamin E supplementation increased antioxidant recycling and improved synergistic antioxidant effect (Ramos *et al*., 1998). In other ruminants, similar response of elevated serum total protein and globulin were obtained in buffaloes supplemented with vitamin E and Se during the last stage of pregnancy (Helal *et al.*, 2009). However, Shinde *et al*., (2009) found no effect on serum total protein and globulin for supplementation of Se and vitamin E in the diet of buffalo calves. In the present study, changes in plasma total lipids and glucose concentrations were not significant either for ewes or for their lambs due to injection of vitamin E plus Se when compared to respective control. According to Njeru *et al.* (1994), dietary supplement with vitamin E in sheep had no effect on serum α-tocopherol, total cholesterol, triglyceride concentrations or the sum of the two lipid fractions. Vitamin E and Se could modify the lipid metabolism via elevating both high density lipoprotein and triglycerides in cows at early stage of lactation (Falkowska *et al*. 2000). In the present study, no significant changes in plasma glucose concentrations in ewes or their lambs due to injection of vitamin E plus Se. Also, Shinde *et al.* (2009) indicated that supplementation of buffalo calves with Se alone or vitamin E plus Se had no effect on serum glucose levels as compared to control. Contrary to the above results, Singh *et al*. (2002) observed low blood glucose concentration in buffalo calves fed wheat straw containing high Se. It should be also considered that in ruminants, glucose is constantly synthesized from volatile fatty acids, the main energy source, in the liver since the amount of change in circulating glucose is small (Udum *et al.,* 2008). In any case, plasma glucose levels, in the current study, were maintained within the normal physiological ranges of sheep (Blunt, 1975).

The present study also showed that thyroid activity and secretion of its metabolic hormones could be modified in ewes and their male lambs injected with vitamin E plus Se. Ewes received injection of vitamin E plus Se had higher levels of plasma T_3 and T_4 than the untreated ewes. Similar T_4 response was reported by El-Shahat and Abd El-Monem (2011) with Baladi ewes , but they found insignificant increase in plasma T_3 levels. In dairy cows, parental administration of vitamin E

and Se, at 4 weeks prior parturition, significantly ($P< 0.05$) increased T_3 concentration (Pavlata *et al.*, 2004). They reported that such significant increase in T_3 levels could probably explained by an increased activity of iodothyronine deiodinase, which involves in the conversion of T_4 into active T_3 as its activity influenced by Se (Arthur *et al*., 1992). Similar results confirming the positive effect of Se on T_3 concentrations were presented by Wichtel *et al.* (1994) and Awadeh *et al.* (1998), whom noticed increased T_3 concentrations in cows and calves after higher Se intake. In this regard, it has been documented that Se is essential for normal thyroid hormone metabolism, and selenoperoxidases protect the thyroid gland from peroxides produced during the synthesis of hormones, a matter play an important role in regulation of thyroid status in animal tissues in various physiological and pathological situations (Arthur *et al*., 1992). Se supplementation in goats resulted in a significant increase in T_3 concomitant with decrease in T_4 and increased T_3/T_4 ratio (El-Sisy *et al*., 2008). Donald *et al*. (1994) reported no change in T_4 levels and T_4/T_3 ratio in ewes supplemented with different levels of Se alone (0-8 mg). The result could be explained by the fact that type I iodothyronine-5´-deiodinase is a Se dependent enzyme, since it is responsible for the deiodination of T_4 to T_3. In the present study, lambs born from ewes received injection of vitamin E plus Se exhibited higher concentration of plasma T_3 with no significant change in their T_4 levels. In agreement, supplementation of buffalo calves with Se alone or vitamin E plus Se significantly increased serum level of T_3, but did not affect T_4 levels and T_4/T_3 ratio (Shinde *et al.*, 2009). However, the present results disagree with the study of Nazifi *et al.* (2008) who revealed no significant correlation between selenium and thyroid hormones in the serum of Iranian fat-tailed sheep. The beneficial effects of vitamin E plus Se supplementation on blood T3 and T4 levels indicate their favourable metabolic role and so efficient thyroid activity that reflected on higher growth performance of lambs born from treated ewes.

In conclusion, the present results declare that parental administration of vitamin E plus Se to ewes at the 4 weeks of late gestation and during suckling period improved growth performance, some immune responses and viability of the produced male lambs. This treatment was accompanied with favourable signs on physiological reactions, blood hematological profile, plasma metabolites and thyroid activity.

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الملخص العربى

التأثير المشترك لفيتامين ه والسلينيوم على بعض الإستجابات الفسيولوجية للنعاج وحملانها الذكور أثناء فترة الرضاعة

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أجريت هذه الدراسة على عدد ٢٤ من النعاج (1/2 أوسيمىx 1/2كيوس) بمتوسط وزن ٤٩.٧٥ ± ٢.٦٥ كجم وذلك لتقييم تأثير معاملة هذه النعاج بفيتامين ه والسلينيوم على النمو وبعض الإستجابات الفسيولوجية لهذه النعاج وحملانها الرضيعة من الذكور. قسمت النعاج عشوائيا إلى مجموعتين متساويتين الأولى للمقارنه (الكنترول) وتم حقنها بمحلول فسيولوجى (٠.٩ % كلوريد صوديوم) بمعدل ١.٠ مل/رأس، بينما تم حقن المجموعة الثانية بالفيتسيلين بمعدل ١.٠ مل/رأس، وقد تم الحقن بمعدل مرة كل أسبوعين خلال الأربعة أسابيع الأخيره من فترة الحمل وأثناء فترة الرضاعة لمدة ١٢ أسبوع. وقد أظهرت النتائج أن معاملة النعاج بفيتامين ه والسلينيوم لم يكن له تأثير معنوى على متوسطات وزن الجسم ، بينما سجلت الحملان المولودة للنعاج المعاملة قيم أعلى معنويا فى كل من متوسطات وزن الجسم ومعدلات الزيادة اليومية فى الوزن. أظهرت النعاج المعاملة زيادة معنوية فى تركيز هيموجلوبين الدم وكذلك فى العدد الكلى لكريات الدم البيضاء متضمناً زيادة معنوية فى نسبة الكريات حمضية الصبغ وكذلك الكريات الليمفاوية وتغيرات غير معنوية فى نسبة الكريات المتعادلة، الكريات قاعدية الصبغ والكريات الأحادية مقارنة بالنعاج الكنترول. وأيضاً أظهرت الحملان المولودة للنعاج المعاملة انخفاض معنوى فى نسبة الكريات المتعادلة مع زيادة معنوية فى نسبة الكريات الليمفاوية مصحوبا بزيادة غير معنوية فى العدد الكلى لكرات الدم البيضاء وتغيرات غير معنوية فى نسبة الكريات قاعدية الصبغ والكريات الأحادية مقارنة بالحملان المولودة للنعاج الكنترول. أظهرت النعاج المعاملة زيادة معنوية فى محتوى البلازما من البروتينات الكلية والجلوبيولين وكذلك هرمونات الدرقية مقارنة بالنعاج الكنترول. سجلت الحملان المولودة للنعاج المعاملة قيم أعلى معنويا فى كل من عدد كريات الدم الحمراء، تركيز هيموجلوبين الدم، النسبة المئوية للمكونات الخلوية، متوسط هيموجلوبين الخلايا، متوسط تركيز هيموجلوبين الخلايا وكذلك محتوى البلازما من البروتينات الكلية والجلوبيولين والهرمون ثلاثى اليود مقارنة بالحملان المولودة للنعاج الكنترول. لم يتأثر معنوياً محتوى البلازما من الليبيدات الكلية والجلوكوز سواء للنعاج المعاملة أو حملانها بالمقارنة بالكنترول لكل منها. لم يتأثر معنوياً محتوى البلازما من هرمون الثيروكسين للحملان المولودة للنعاج المعاملة

نستنتج من هذه الدراسة أن معاملة النعاج بالحقن بفيتامين ه والسلينيوم بدءاً من الأربعة أسابيع الأخيره من فترة الحمل وأثناء فترة الرضاعة أدى إلى تحسين أداء النمو وبعض خصائص القدرة المناعية لحملانها الذكور كنتيجة لإستجاباتها الفسيولوجية الإيجابية.