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Impact of Vitamin E and Selenium on the Hematological, Biochemical and Productive Parameters of Pregnant Awassi Ewes

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ABSTRACT: Twenty four pregnant ewes averaged 45.75 ± 2.7 kg were used to evaluate impact of vitamin E plus selenium (Se) injection on productive characteristics and some physiological indicators of the ewes and their kids. Randomly, The ewes divided into 2 groups (12 ewes each). The control (first group) (G1) injected with 1.0 ml of saline solution to each head, while the treated ewes (second group) (G2) were injected intramuscularly with 1.0 ml/head viteselene. The ml of viteselene contained 150 mg vitamin E acetate and 1.67 mg saodium selenite. The ewes were injected with viteselene biweekly starting at four weeks late pregnancy and after parturition (lactation) for twelve weeks. The results indicate that lambs born from treated ewes (G2) proved higher ($P<0.05$) daily gain and body weight than those born from control ewes (G1). Blood Hb concentrations significantly raised for G2 compared with G1. Lambs born from treated ewes had significantly higher values ($P<0.05$ to $P<0.01$) of Red Blood Cells (RBCs), Hemoglobin (Hb), Packed Cell Volume (PCV) and Mean Corpuscular Hemoglobin (MCH) 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 altitude ($P<0.05$) in lymphocytes and a decline ($P<0.01$) in neutrophils with insignificantly 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 $P<0.01$) high in total protein and globulin. Changes in Albumin, Total lipids and Glucose concentrations for treated ewes and their lambs were not significant, while plasma T3 and T4 levels were higher ($P<0.05$) in treated ewes (G2) compared with control ewes. Plasma level of T3 only was higher ($P<0.05$) in lambs that born from treated ewes (G2) than those born from control ewes (G1). Our results indicated that administration of vitamin E-Se to ewes at one month late pregnancy and after parturition (lactation) enhanced growth characteristics, immune responses and viability of their lambs as a consequence of favorable indicators in their physiological parameters

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INRODUCTION

Reproductive efficiency significantly contributes to overall farm productivity and economic sustainability by ensuring a stable and consistent supply of offspring for the production of meat, dairy, or fiber (1). Vitamin E plus selenium (Se) have supplementary effect, as antioxidants, to protect cells from the harmful effects of lipid peroxides and free radicals that synthesized during metabolism. At both molecular and cellular levels, the multiple functions of these nutrients, extend to protection from antioxidant, as their involvement in the feeding at levels above needs is related with variable enhancements in sheep interpretation and immune activity (2). Vitamin E plus Se in the diet improved the physiological parameters, hormonal levels and antioxidant situation of sheep, they also have the ability to enhance the toxic effects of arsenic (3). In cattle, vitamin E-selenium the supplementation may negatively recover the effect of heat stress and return the redox balance in some sheep breeds (4). Adding selenium to the animals diet might be reduced specific stress types such as heat or postpartum stress, that causes a higher selenium levels in the colostrum of ewe and goat (5). It has been emphasized that both nutrients should be supplemented, to sheep, in combination to progress their

immune competence (6). vitamin E is known as free radical scavenger due to its role in non-enzymatic protection against lipid peroxidation (7). Also helps to protect membrane integrity of cells so the health status, growth of efficiency and production should be rises up by administrated vitamin E in sheep (8).

Newborns are critical to vitamin E deficiency, so that it is necessary that colostrum provides the pregnancy with suitable amounts of vitamin E, because the amount of vitamin E passes to the fetus from uterus in a negligible manner (9). Previous studies proved that ewes treated with vitamin E and Se, during late pregnancy, had lambs with significantly higher birth weight and dropped mortality compared to lambs of non-supplemented ewes (10). It has been reported that vitamin E and Se to enhance immune activities (11). In addition, dietary Se supplementation and vitamin E injection to ewes in late pregnancy and sucking period may provide some improvement in lamb performance and livability (12). Although physiological and blood parameters are important to sings the health status of the animal and to evaluate the management practices for livestock (13), the physiological interactions of the combined effect of vitamin E and Se given to ewes on their lamb performance have not been documented fully. So that, the present study aimed to detect the mechanistic aspects through which ewe-vitamin E-Se injection, at 4 weeks late pregnancy and during lactation period, might influence the performance of their lambs via monitoring some of their growth performances and physiological features of the ewes and their lambs. Our results is executed the aim to determine the impact of vitamin E plus Se on productivity and hematological parameters.

MATERIAL AND METHODS

Animals and Management:

Twenty four pregnant ewes averaged 45.75 ± 2.7 kg, 3 years old were used in this experiment. The animals were purchased from private farms in Babylon. Before beginning the experiment, the health status of the ewes was evaluated clinically and it was proven that they were free from all parasites. Animals were fed on concentrate feed mixture and bean straw to provide their nutrient needs according to their body weight (14). The concentrate mixture included 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 levels of Se and vitamin E in the concentrate mixture fed were calculated, 0.17 ppm and 17.78 IU/kg DM, respectively. (14) 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. Ewes were feeded twice daily (8 am and 2 pm) and mineral blocks and drinking water were obtainable to the animals all times.

Blood Analysis

Blood hemoglobin, packed cell volume, red blood cells and leucocyte counts using conventional methods. Mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration were calculated. Blood smears were stained with Lieshman's stain were prepared for the differential leucocytes count. Centrifugation of blood samples at 3000 rpm for 10 minutes, was used to obtain plasma samples and stored at -20°C until assayed for biochemical analysis. Estimation of total protein was been by spectrophotometer (Spekol 11, Carl Zeiss Jena, Germany) based on instructions of the manufacturer (Diagnostic diamond, Egypt; (15)). Serum albumin levels of all lambs were estimated by spectrophotometer (Spekol 11, Carl Zeiss Jena, Germany) based on the instructions of the manufacturer (Diagnostic Biosystem, Egypt, (16)). Serum globulin levels was calculated as the difference between total protein and albumin. Plasma triiodothyronine (T3) and thyroxin (T4) levels of all lambs were estimated by spectrophotometer (Spekol 11, Carl Zeiss Jena, Germany) based on the instructions of manufacturer.

RESULTS

Animal performance:

The findings indicate that no significant differences in averages body weight (BW) between treated 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.

Table (1): Impact of vitamin E and selenium supplementation on ewes body weight.

(Body weight, kg)	G1	G2	Change(%)	SEM	Sig.
At starting	45.85	51.28	2.72	1.85	N.S
4weekspostpartum	43.53	46.80	7.69	1.93	N.S
8 weeks postpartum	48.66	51.73	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

Data in Table (2) show no significant differences in BW averages at birth between kids born from treated ewes (G2) and that born from control ewes (G1), while treated lambs recorded higher BW averages (P<0.05) than those born from control ewes. Average daily gain weights of treated lambs were more elevated (P<0.05) than those born from control group (Table, 2).

Table (2): Impact of vitamin E plus selenium administration of ewes on body weight and daily gain of lambs

Parameters	G1	G2	SEM	Sig.
Body weight BW (kg) :				
At birth	2.89	2.98	0.41	NS
4 weeks	7.55 ^{b}	8.30 ^{a}	0.85	*
8 weeks	12.26 ^{b}	13.59 ^{a}	1.01	*
12 weeks	17.47 ^{b}	19.58 ^{a}	0.97	*
Daily weight gain DWG (g/day):				
Birth- 4 weeks	153.43 ^{b}	174.77 ^{a}	3.66	*
4 - 8 weeks	165.35 ^{b}	183.5 ^{a}	2.37	*
8 - 12 weeks	167.15 ^{b}	192.71 ^{a}	3.55	**

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.

Hematological parameters

Table 3 show non-significantly changes in blood parameters (RBC, PCV, MCH, MCV and MCHC) for treated ewes, but there was a significant (P<0.05) raise in Hb of control ewes. kids born from treated ewes (G2) had a significant (P<0.05 to P<0.01) increase in 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 for treated than control ones.

Table (3): Impact of vitamin E plus Se on some hematological parameters of ewes and their lambs

Blood Parameters	Ewes		SEM	Sig.	Lambs		SEM	Sig.
	G1	G2			G1	G2		
RBC(x106/mm3)	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

Data presented in Table (4) indicate that treated ewes showed increase in total count of leucocytes,lymphocytes and eosinophils percentages (P<0.05) and no significant changes in neutrophils, basophils and monocytes compared with the control ewes. Lambs born from treated ewes showed increase in lymphocytes (P<0.05) and decrease in neutrophils (P<0.01) while there was no significant effects on eosinophils, basophils and monocytes percentages and total count of leucocytes compared to lambs born from control ewes.

Table (4): Impact of vitamin E plus Se on total and differential leucocytes counts of ewes and lambs

Parameters	Ewes		SEM	Sig.	Lambs		SEM	Sig.
	G1	G2			G1	G2		
Leucocytes (x103/mm3)	8.06 ^{b}	9.15 ^{a}	0.45	*	8.79	9.22	0.65	NS
Neutrophils (%)	30.67	27.07	1.85	NS	0.98	31.1 a	25.7 ^{b}	**
Eosinophils (%)	4.8 ^{b}	5.1 ^{a}	0.25	*	5.26	5.31	0.32	NS

Basophils (%)	0.53	0.49	0.05	NS	0.59	0.55	0.07	NS
Lymphocytes (%)	60.6 [b]	64.45 [a]	1.89	*	59.53 [b]	65.28 [a]	1.37	*
Monocytes (%)	3.65	3.43	0.38	NS	3.66	3.31	0.41	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.

Plasma metabolites and thyroid hormones

The results presented in Table (5) indicate that levels of plasma total protein and globulin were higher in treated ewes (G2) than the control ewes. Also, lambs that born from treated ewes recorded high (P<0.05) in plasma total protein and globulin than those born from control ewes. No significant changes were noticed in plasma albumin, total lipids and glucose either for treated ewes or for their lambs. The data showed that plasma T3 and T4 levels for treated ewes were higher (P<0.05) compared to control ones. Lambs born from treated ewes had a significant (P<0.05) increases in plasma levels of T3, with non-significantly change in T4 levels compared to lambs born from control ewes. The results of this study indicated some significant positive changes in the blood metabolites and thyroid hormones (T3 and T4) with vitamin E plus Se-injected ewes and their lambs. Our results showed that metabolic activity processes could be improved. The present study revealed that there was significantly high in globulin and total protein in ewes injected with vitamin E and Se at late pregnancy and sucking period, and non-significantly effect in albumin compared to control ewes.

Table (5): Impact of vitamin E plus Se on plasma metabolites and thyroid hormones concentrations of ewes and their suckling lambs (Mean ± 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
T3 (ng/ml)	1.95 [b]	2.25 [a]	0.10	*	1.60 [b]	2.05 [a]	0.05	*
T4 (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

DISCUSION

The results revealed an increase in body weight between treated ewes and controls ewes. Such improvement may show a high feed efficiency utilization for treated ewes and lambs viability. Feed efficiency and DWG might also be improved by vitamin E supplementation to coarse-wool kids (17). This supports earlier research emphasize that injected ewes by a mixture of selenium and vitamin E was higher in body weight in All stages of the experiment (18). Our findings are in agree with the study of (19) which found that BW at birth of lambs has non-significant affect by E plus Se administration compared with control. injected Ewes with 900 IU of vitamin E by weekly in late pregnancy appeared no effect in birth weight of lambs, but raised in the weight and DWG of lambs in pre-weaning period while administration of Selenium at 90 ppm to pregnant ewes in pregnancy and through sucking period non-significantly increased BW (3.6 %) (20). In addition, the earlier studies found that ewes which injected with vitamin E-Se before mating and parturition had significant increase in DWG from birth to two months and mean BW at two months (21). However, when ewes obtained only Se in both organic and inorganic forms during gestation and sucking period, Se had non significantly effect on either BW or growth of their lambs (22). Recently, supplementation with vitamin E (50 mg) and Se (0.3 mg) for each kg diet to ewes beginning within 2 weeks before mating to lambing enhanced significantly the reproductive parameters and productive characteristics of their kids born from the birth to be weaned compared to kids from ewes treated with vitamin E or/and Se (23). Since vitamin E-Se improve immune competence in ewes and the health situation and subsequently the production efficiency of (24). Therefore, the positive effects of supplementation with vitamin E plus Se on animal's immunity and health may be reflected through positive responses on productive performance.

In this study, many hematological characteristics were modified because of vitamin E plus Se administration, that can indicate to the active metabolism roles and biological oxidation on the cellular base that could lead to availability of the necessary metabolites for tissue development. The significantly high in RBCs, blood Hb and PCV resulting to injection of vitamin E-Se agrees with the

earlier study on lambs in developing period (25) and on dairy calves (22). In a similar way, these findings are compatible with the study of (26) which found significant increase in Hb, RBC count and PCV values, while MCV, MCH and MCHC were still without changes for buffaloes treated with vitamin E plus Se in late pregnancy. Lambs born from treated ewes, were significantly higher Hb and MCHC and indicate to rise MCH levels. In another hand, administration of Se plus vitamin E in the feeding of buffalo calves had non significantly effect on physiological traits (Hb, PCV, WBCs and RBCs counts) as proved by (27). In addition, The earlier study indicated that vitamin E supplementation caused to raise the colony number that forming erythroid precursors units, which benefit in prevent the polyunsaturated fatty acids oxidation of in RBCs cell membrane, resulting reduce of the premature decomposition of erythrocyte, improvement of erythropoiesis and reducing the premature erythrocyte lysis by decreasing the erythrocytes fragility. So that, vitamin E can enhance the Hb and PCV levels.(28) Supplementation of various forms of Se did not significantly affect hemodynamic traits in ewes in diestrus, gestation and sucking periods if the intake of vitamins and other necessary microelements was enough (29). In the present study it is possible that the high impact of vitamin E plus Se injection on hematological parameters, might be due to the role of vitamin E on keeping adequate metabolic efficiency, reflecting the high in blood parameters reported in born lambs. It could also be observed that changes in blood hematological parameters, in the our study, were within the normal physiological values of sheep as previously documented (30).

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, in Baladi ewes, (31) suggested that ,by significantly, injection of vitamin E and Se increased the levels of antioxidants in blood, it then confirm that they synthesize sufficient immune globulins, concluding that it is necessary to administer nutrients together to improve immune efficiency of sheep. In earlier study, They also indicated that there was on increasing total count of leucocytes in vitamin E plus Se treated Friesian heifers (32) and dairy calves (33). In the our study, management of vitamin E-Se to ewes revealed insignificantly effects on neutrophil percentages, but they showed a significant decrease in their lambs. Neutrophil response to supplement of vitamin E and/or Se was focused in some studies. The present study showed a significant high in the percentages of lymphocytes in both ewes injected with vitamin E plus Se and male lambs. Previously, The researcher reported that the vitamin E and Se injection enhanced lymphocytes concentration on growing lambs (17). Among the circulating leucocytes, a high number of the lymphocytes in blood and their responsible for cellular and humoral immune responses can be a good sign of an immune response (34). 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. Vitamin E and Se effect synergistically to protect the tissues from oxidative effects so that enhance immune responses (35).

Our results are agree with the study of (23), that revealed Baladi ewes treated with vitamin E and Se at 2 weeks before mating also in all gestation period till lambing had a high ($P<0.05$) in globulin and total protein. In addition, indicated that the higher level of albumin was noticed in ewes treated with only vitamin E than ewes treated in vitamin E and Se or only Se. However, ewes that treated with Se alone had a significant reduction in total protein, albumin and globulin. These observations can indicate that there was no impact of Se alone on albumin in sheep as previously amplified by (31). In other ruminants (buffaloes), a same effect of vitamin E and Se during the late gestation had been proven in elevated total protein and globulin (36). In the present study, changes in plasma total lipids and glucose levels were not significant either for ewes or for their lambs because of injection of vitamin E plus Se when compared to respective control. According to (37), dietary supplement with vitamin E in sheep had no act on serum α-tocopherol, cholesterol, triglyceride concentrations or the sum of the two lipid fractions. Vitamin E and Se could change the lipid metabolism via elevating high density lipoprotein and triglycerides in cows at early stage of lactation. In our study, non-significantly changes in plasma glucose concentrations in ewes or their kids because of injection of vitamin E and Se. Also, the study indicated that thyroid efficiency and its metabolic hormones secretion could be changed in ewes and their male lambs administered with vitamin E plus Se. Ewes treated with injection of vitamin E plus Se showed increased levels of plasma T3 and T4 compared with the untreated ewes. Similar T4 response was reported by (23) with Baladi ewes, but they found a non-significantly high in plasma T3 levels. In cows, administrated parents with vitamin E and Se, at 4 weeks before calving, significantly ($P<0.05$) raised T3 levels (29). Similar results were reported that the positive effect of Se on T3 levels were presented by (38),who noticed a high levels of T3 in cows and calves after higher Se administration. In this regard, it has been documented that Se is necessary for normal thyroid hormone metabolism, and seleno peroxidases save the thyroid gland from peroxides produced during the hormones production, so that Se plays a necessary role in thyroid regulation of animal tissues in both physiological and pathological situations. In posterior study on goats, Se supplementation showed a significant high in T3 levels with a low in T4 and increased T3/T4 ratio (39). The result could be explained by the fact that responsible for the deiodination of T4 to T3 due to type I iodothyronine-5´-deiodinase is a Se dependent enzyme. In the present study, lambs that born from ewes treated with injection of vitamin E- Se showed that there was a higher level of plasma T3 with no significant change in their T4 levels. In agreement, supplementation of buffalo calves with Se

alone or vitamin E plus Se significantly increased level of T3 in serum, but did not affect T4 levels and T4/T3 ratio (40). The beneficial impacts of vitamin E plus Se supplementation on blood T3 and T4 levels indicate their favorable metabolic role and so efficient thyroid activity that reflected on higher growth performance of lambs that born from treated ewes.

CONCLUSION

From this study, it was found that, parental administration of vitamin E and Se to ewes at the one month of late pregnancy and through lactation improved growth characteristics, some responses of immune and viability of the produced male lambs. This treatment was accompanied with favorable indicators on physiological characteristics, hematological parameters, plasma metabolites and thyroid activity.

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INTEREST CONFLICT

The author has declared, in this manuscript, no interest conflict.

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