The Effect of Experimentally Induced Vitamin E and Selenium Deficiency on Erythrocytes Osmotic Fragility and Phagocytosis in Pregnant Awassi Ewes and Their Newborn Lambs

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Summary

Experimental induction of vitamin E and selenium deficiency using deficient diet was carried out on fourteen pregnant Awassi ewes and their newborn lambs to study the effect of the deficiency on erythrocytes osmotic fragility and phagocytosis. From the fourteen deficient ewes, seven were vaccinated with Rev I vaccine and seven ewes as control group, and their newborn lambs were used in the study. Results were showed increased in the concentration of saline solution in start and complete erythrocyte hemolysis in deficient lambs (0.52 ± 0.01 and 0.54 ± 0.01) and (0.42 ± 0.01 and 0.44 ± 0.00) respectively and in deficient ewes (0.53 ± 0.01 and 0.54 ± 0.01) and (0.43 ± 0.01 and 0.44 ± 0.00) respectively. Results showed a low phagocytic index in deficient lambs (9.40 ± 0.87 and 10.60 ± 1.16) and in deficient ewes (12.14 ± 0.85 and 12.42 ± 0.75) compared to phagocytic index in control lambs (43.85±0.91) and in control ewes (43.14 ±0.91).

Keywords: Vitamin E, Selenium, Osmotic Fragility, Phagocytosis, Awassi ewes.

Introduction

Vitamin E deficiency in sheep results in increased hemolytic susceptibility of erythrocytes, which may provide a basis for a single functional test for vitamin E deficiency in sheep (1). Selenium deficiency has been identified as the leading cause of excessive fragility of vascular and erythrocyte membranes, which leads to such condition as anemia with Heinz bodies (2).

Vitamin E is one of the major lipid soluble antioxidants. It prevents oxidation of poly unsaturated fatty acids and thus protects red blood cells from oxidative stress induced lyses (3). Supplementation of vitamin E may have an important role in maintaining red cell membrane integrity by reducing osmotic fragility of erythrocyte (4).

A significant elevation in red blood cells hemolysis in vitro as a result of lipid peroxidation was reported by (5) where they reported that red blood cells in vitro hemolysis test has long been used as a criteria for the assessment of vitamin E status and the higher red blood cells hemolysis implied that the vitamin E status might be compromised by the lipid peroxidation.

Selenium deficiency is associated with decreased intracellular killing power by bovine neutrophils, while performance of phagocytes can be improved by selenium/vitamin E injections (6).Alterations in immunity have been reported with vitamin E deficiency. Reduced lymphocyte and leukocyte killing power has been shown in humans as well as in experimental animals. Vitamin E supplementation has been reported to enhance phagocytosis in experimental and farm animals and humans (7).

Free radicals and lipid peroxidation are immunosuppressive and due to its strong lipid-soluble antioxidant, activity vitamin E is able to optimize and enhance the immune response. Supplementation with vitamin E increases lymphocyte proliferation in response to mitogens, phagocytic activity by alveolar macrophages, and causes an increased resistance against infectious agents (8). The aim of this study was to investigate the effect of vitamin E and selenium deficiency on erythrocyte osmotic fragility and phagocytosis in Pregnant Awassi ewes and their Newborn lambs.

Materials and Methods

Twenty one pregnant Awassi ewes and their newborn lambs from State Board of Agricultural Research / Ministry of

Agriculture were used. The deficient groups included 14 ewes 7 of them vaccinated with Rev I vaccine against brucellosis and the control group included 7 ewes. Ultrasound scanner was used to check the uterine health of the ewes. Estrus synchronization was scheduled. The study lasted for 9 months started from 1.3.2011, to 1.12.2011.

Induction of selenium and vitamin E deficiency was done by feeding a diet consisting of cod liver oil 3%, ground corn 0.5 kg/ animal/day, discolored bad quality hay ad lib, and water was freely offered (9). Feeding of this deficient diet lasted for three months (the last two months of gestation and one month after birth).The control group was allowed the regular feeding program adapted in the state board of agricultural research.

The animals in the deficient group and the control group were watched at a regular daily basis. Erythrocyte osmotic fragility test was carried out according to (10) and the saline concentration was recorded for beginning of hemolysis and complete hemolysis. Phagocytic index was carried out according to (11).

Selenium in serum was estimated according to (12) by using flameless atomic absorption, and vitamin E in serum was estimated according to (13) by using spectrophotometer. Statistical analysis was conducted using ready – made statistical design statistical package for Windows Integrated Student Version (SPSS)(14).

Results and Discussion

Clinical signs of the deficiency appeared after three months of feeding deficient diet to ewes and were mainly loss of body weight, decreased milk production, loss of wool, weakness, dullness and recumbency. The levels of selenium and vitamin E in serum of deficient ewes were (0.02 ppm, 0.61mg/L respectively) compared with that in the control ewes (0.45 ppm and 2.72 mg/L respectively).While the clinical signs of the deficiency in lambs appeared within three days of life and when the serum selenium and vitamin E levels in deficient lambs reached (0.01 ppm and 0.34 mg/L respectively) compared with that in the control lambs which were (0.45 ppm and 2.45 mg/L respectively).

The results showed an increased in the concentration of saline solution in start and complete erythrocyte hemolysis in lambs of groups (1 and 2) (0.54 ± 0.01 and 0.52 ± 0.01) and (0.44 ± 0.00 and 0.42 ± 0.01 respectively) compared to that of the control group (0.42 ± 0.01) and (0.34 ± 0.01 respectively) with a significant difference (P<0.05) between groups (1 and 2) and the control group (Table, 1).

Table, 1: Start and complete erythrocyte osmotic fragility in newborn lambs (control and vit.E and selenium deficient.

Groups	Osmotic fragility (mean±S.E) (Start hemolysis)	(Complete hemolysis)
Group 1* Lambs born to deficient and vaccinated ewes	0.54 ± 0.01 A	0.44 ± 0.00 A
Group 2* Lambs born to deficient ewes	0.52 ± 0.01 A	0.42 ± 0.01 A
Control group lambs born to control ewes	0.42 ± 0.01 B	0.34 ± 0.01 B

***Two lambs died, Different letters mean significant (P<0.05) results between different groups**

The results showed an increased start and complete erythrocyte hemolysis in ewes of groups (1 and 2) (0.53 ± 0.01 and 0.54 ± 0.01) and (0.43 ± 0.00 and 0.44 ± 0.01 respectively) compared to that of the control group (0.43 ± 0.01) and (0.33 ± 0.01 respectively) with a significant difference (P<0.05) between groups (1, 2) and the control group (Table, 2).

Table, 2: Start and complete erythrocyte osmotic fragility in ewes(control and vit.E and selenium deficient

Groups	Start hemolysis	Complete hemolysis
Group 1 Deficient and vaccinated ewes	0.53 ± 0.00 A	0.43 ± 0.00 A
Group 2 Deficient ewes	0.54 ± 0.01 A	0.44 ± 0.01 A
Control group	0.43 ± 0.00 B	0.33 ± 0.01 B

n=7 Different letters mean significant (P<0.05) results between different group

The results showed a lower phagocytic index in lambs of groups (1 and 2) than that of lambs of the control group with a significant difference in phagocytic index (P<0.05) between groups 1 and 2 with control group (Table, 3).

Table, 3: The mean percentage of phagocytic activity in newborn lambs (control and Vit. E and selenium deficient.

Groups	Phagocytic index percentage mean ±S.E
Group 1* Lambs born to deficient and vaccinated ewes	9.40 ± 0.87 A
Group 2* Lambs born to deficient ewes	10.60 ± 1.16 A
Control group Lambs born to control ewes	43.85 ± 0.34 C

n=7

***Two lambs died , Different letters mean significant (P<0.05) results between different group**

The results also showed lower phagocytic index in ewes of groups 1 and 2 than that in control group with a significant difference (P<0.05) between groups 1, 2 with control group (Table, 4).

Table 4: The mean percentage of phagocytic activity in ewes (control and vit.E and selenium deficient.

Groups	Phagocytic index percentage mean ±S.E
Group 1 Deficient and vaccinated ewes	12.14 ± 0.85 A
Group 2 Deficient ewes	12.42 ± 0.75 A
Control group	43.14 ± 0.91 B

n=7

Different letters mean significant (P<0.05) results between different group.

The vaccine against brucellosis with Rev I vaccine had no effect on the parameters studied in this study. The results of this study indicated that in deficient animals the erythrocyte osmotic fragility was high, this agrees with (1) who reported that vitamin E deficiency in sheep results in increased hemolytic susceptibility of erythrocytes, which may provide a base for a single functional test for vitamin E deficiency in sheep. Furthermore (2) reported that selenium deficiency has been identified as the leading cause of excessive fragility of vascular and erythrocyte membranes.

The fact that vitamin E was protects the red cell membrane from oxidative destruction is concert with (4) who mentioned that supplementation of vitamin E may have an important role in maintaining red cell membrane integrity by reducing osmotic fragility of erythrocyte. In addition (3) mentioned that vitamin E prevents oxidation of polyunsaturated fatty acids and thus protects red blood cells from oxidative stress induced lyses.

The results showed a lower phagocytic index in deficient animals this agrees with (6) who reported that selenium deficiency was associated with decreased intracellular kill by bovine neutrophils, while performance of phagocytes can be improved by selenium/vitamin E injections. The results in this study also agrees with (7) they mentioned that alterations in immunity have beenrecordedand reduced lymphocyte and leukocyte killing power has been shown in humans as well as in experimental animalsin vitamin E deficiency.

The ability of peripheral blood polymorph nuclear leukocytes to engulf yeast cells, in vitro, was impaired by both vitamin E and selenium deficiencies and was impaired sooner by the combined vitamin E and selenium deficiencies than by individual deficiencies of vitamin E or selenium (15).

Immune cells such as the neutrophil, macrophage and other cells are prone to be affected by oxidative stress which can be prevented by vitamin E. The protection of cell membranes and other cellular components of immune cells against lipid peroxidation is probably the most important mechanism of vitamin E in the immune response (16).

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دراسة تأثيرنقص فيتامين هـ والسلينيوم المستحدث تجريبآ على فحص هشاشة كريات الدم الحمر وعملية البلعمة في النعاج العواسية الحوامل ومواليدها

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الخلاصة
تم أستحداث نقص السلنيوم وفيتامين E باستعمال عليقةغذائية لاستحداث النقص الغذائي في النعاج العواسية الحوامل ومواليدها لغرض دراسة تأثير النقص على فحص هشاشة كريات الدم الحمر وعملية البلعمة في النعاج العواسية ومواليدها.تم أستعمال أربعة عشرنعجة استحدث فيها النقص وسبعة منها لقحت بلقاح Rev I وسبعة نعاج في مجموعة السيطرة وتم استخدام مواليدها في الدراسة. أظهر فحص هشاشة كريات الدم الحمرزيادة في تركيز المحلول الملح الفسلجي في بداية التحلل واكتماله حيث كانت عالية في الحملان التي أظهرت النقص وكانت أيضا عالية في النعاج التي أظهرت النقص . وأظهرت النتائج أنخفاض في عملية البلعمة في الحملان التي أظهرت النقصوكذلك في النعاج االتي أظهرت النقص بالسلينيوم وفيتامين Eمقارنة بحملان مجموعة السيطرة ونعاج مجموعة السيطرة .

الكلمات المفتاحية: فيتامين هـ , السلينيوم , فحص الهشاشة , كريات الدم الحمر , عملية البلعمة ,النعاج العواسية.