Hematological, Biochemical and Hormonal Studies on Postpartum Alopecia in Ewes

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Abstract: This study was carried out on twenty ewes. Fifteen from them suffering from postpartum alopecia and other five ewes were kept as control. Two blood samples were obtained from each ewe. The first sample was collected in tube with EDTA as anticoagulant for hemogram studies. The second blood sample was collected in plain tube for serum separation for biochemical and hormonal studies. Hematological examination revealed that normocytic normochromic anemia in alopecic ewes as well as neutrophilic leukocytosis and lymphocytopenia. There were significant decrease in total protein, albumin and globulin in diseased group. Liver enzymes revealed significant increase in ALT,AST activities in alopecic ewes. Urea level also increased in alopecic group while non significant changes in ALP and creatinine. There were significant decrease in zinc, copper and calcium while non significant changes in phosphorus and magnesium levels. Hormonal studies revealed a significant increase in prolactin and cortisone hormones. It could be concluded that, nutritional deficiency as well as stress play an important role in postpartum alopecia.
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1. Introduction

Alopecia is the partial or complete lack of hairs in areas where they are normally present. It is caused by many important factors include bacteria, viruses, parasites, metabolic disorders and nutritional deficiencies. Among them, deficiencies of micro- and macro- elements are the most important **(Akgul *et al*, 2000).** The animals obtain these elements either as minerals salts or as organic compounds. The need for these elements change with age, productivity, climates as well as the levels of these elements in soil. The insufficiencies causes lesion throughout the body and specific skin diseases such as alopecia **(Underwood, 1977).** Trace elements deficiency, in particular copper, zinc and cobalt have been incriminated in the etiology of alopecia and wool eating habit in sheep **(Meyer and Lohse, 2002)**. Numerous factors may be related to alopecia and range from naturally occurring process for example seasonality, aging to various biological dysfunction including vitamin and mineral imbalances, endocrine disorders **(Melinda and Jerrold, 2009**).Zinc is a compound of almost of 300 enzymes and it is vital to the activity of a variety of hormones as well as play a key role in immunological responses **(Al-Saad *et al,* 2010)**. Zinc deficiency is associated with parakeratosis, growth retardation, wrinkled skin, wool loss **(Kendall *et al,* 2000)**. Copper is required for the activity of enzymes associated with ferrous metabolism, elastin and collagen formation, melanin production and integrity of central nervous system**(McDonald *et al.*, 1984).** Copper deficiency is associated with wool abnormalities as the fine wool becomes limp and glossy and losses its crimps **(Radostits *et al,* 2000).** Alopecia and wool abnormalities were observed in sheep suffering from copper and zinc deficiency. In addition to iron and manganese **(Abd El-Roaf and Ghanem,2006) .** Also they observed significant decrease in RBCs count and hemoglobin concentration in sheep suffering from alopecia while non significant change in total and differential leukocytic count. Serum iron, manganese, phosphorus, calcium, sodium and chloride values were not significantly different from control animals. On other hand serum zinc, copper and total protein values of diseased animals were significantly lower than the controls **(Akgul et al.2000).** The primary effect of stress on hair growth or loss mostly fully studied in mice. The hair loss is caused as result of prolonged the telogen phase and prematurely terminating the anagen phase leading to rapid development of the catagen phase (***Arck et al,2003).*** Stress may induce a local inflammatory response that lead to an inhibition of keratinocytes proliferation as well as an increase in apoptotic death of keratinocytes in the telogen – stage hair follicles***(Areck et al,2001)*** . The relation between changes in plasma prolactin level and fiber growth is well established in shedding sheep **(Pearson et al, 1996)**. Prolactin could have a role in seasonally driven follicle regression as well as recrudescence **(Choy et al; 1997)**.

The aim of this work is to evaluate the clinicopathological alterations of alopecia in ewes through selective some hematological, biochemical parameters as well as study the role of cortisone and prolactin in alopecic ewes.

2. Materials and methods:

This study was carried out on 20 ewes aged between 3 and 5 years. 5 were healthy served as control group and 15 were suffered from alopecia. The main complain was appearance of alopecia in ewes suddenly after Parturition. After clinical examination and exclude the ectoparasites as the owner administrated ivermectin injection regularly. Two blood samples were taken from each sheep. The first sample was collected in tube with EDTA as anticoagulant for study hemogram,erythrocytes, total and differential leukocytic count ,hematocrite and hemoglobin values were measured according to **(Coles1986)**. Second blood sample was collected in plain tube for serum sepration for biochemical analysis and hormonal assay. Total protein, albumin, globulin, ALT (Alanine amino transferase), AST (Aspartate amino transferase), ALP (Alkaline phosphatase), urea, creatinine,calcium, magnesium and Phosphorus were estimated with spectrophotometer (Human kits, according to the enclosed pamphlet). Zinc, cobalt, manganese and copper were estimated by using atomic absorption spectrophotometer in the laboratory of Agriculture Faculty of Suez Canal University. The hormonal assay was performed according to ELISA kits for quantitative determination of prolactin and cortisone in serum by human ELISA microplate reader (Humareader plus).

Statistical Analysis:

The significance of variation of sheep with alopecia and the control group with regard to their certain blood parameters,biochemical,mineral and trace element as well as hormonal assay values were determined by the independent student t-test **(Petrie and Waston 1999)**.

3. Results and discussion:

Hematological examination **(Table1)**, our results revealed that there were significant decrease in RBCs,Hb and PCV in diseased group when compared with control one. These result agree with **Abd-El Raof and Ghanem (2006)** who reported anemia in cases of alopecia in sheep. This anemia may be due to disturbance in iron metabolism as result of copper deficiency which decrease the absorption of iron, releasing of iron from body stores and utilization in hemoglobin synthesis **(Church and Pond, 1988).** Also agree with **Al-Saad et al.,(2010)** who reported normocytic normochromic anemia in sheep suffering zinc deficiency.

Regarding to leukocytic examination, there were significant leukocytosis in diseased group as well as neutrophilia and lymphocytopenia. These results were disagree with **Abd-El-Raof and Ghanem (2006)** and Al-**Saad et al;(2010)** who reported there were non-significant changes in total leukocytic and differential count in both diseased and control one. This difference may be due to ewes in our study were lactating and so exposed to high stress. Stress is considered one cause of leukocytosis, neutrophilia and lymphocytopenia **(Coles, 1986)**. In addition to among the various role of zinc in immunity are gene expression, mitosis and apoptosis of lymphoid cells because DNA polymerase the major ezyme regulating DNA replication is zinc dependent , proliferative response of macrophage T-cells and B-cells may have use as early indicators of zinc status**(Shanker and Prasad,1998)**. Zinc deficiency lead to impairment of cell replication and protein synthesis and thus generation of blood cells **(Payne, 1989)**.

Biochemical examination **(Table 2)** revealed that there were significant decrease in total protein, albumin and globulin in diseased ewes when compared with control one. These result was agree with **Abd-El-Raof and Ghanem (2006)** and **Al-Saad et al; (2010)**. This may be due to loss of appetites and loss of albumin as result of increased capillary permeability in copper deficient in alopecic ewes **(Rucker and Tinker, 1977)**. Also these result agree with **Nelson et al.(1984)** who reported there were significant decrease in total protein and globulin values in sheep with alopecia. Also agree with **(Fouda et al., 2011)** who reported reduction in the level of total protein, albumin and globulins in case of diseased sheep. This reduction is attributed to the increased level of blood cortisol in diseased animals. Increased cortisol cause catabolism of protein leading to negative nitrogen balance and increased urinary elimination of nitrogen **(McDonald 1980)**. it was recently suggested be due to increase basal cortisol concentration **(Cerundolo et al;2001) .**Micronutrient play an important role in specific and non specific immune mechanisms so deficiency of zinc, copper and selenium can influence several components of innate immunity **(Erickson, et al.2000)**. Zinc is major elements affecting the immune status in sheep and this element deficiency adversely affects the cellular and humeral immune status in sheep **(Fouda,et al,2011**)

Liver enzymes examination **(Table 2)** revealed that there were significant increase in alanine aminotransferase, aspartate aminotransferase while non significant changes in alkaline phosphatase activities in diseased sheep compared with control ones. These result partially agree with **Abd-El-Raof and Ghanem (2006)** who reported significant increase in ALT and AST in alopecic sheep as well as with **Al-Saad et al;(2010) who** reported significant increase in ALT and AST in zinc deficient sheep while significant decrease in ALP. Also **Akgul et al;(2000)** reported there were significant increase in

ALT in diseased sheep while there was non significant differences in AST . The divergent results obtained in the present study and those reported by others are due to different etiological agents and age of sheep. The higher level of the enzyme occur in disorders or conditions that involve the hepatocyte or muscles damage **(Stockham and Scott, 2002).** Urea examination revealed that there was significant increase in urea level in diseased group while non significant change in creatinine level when compared with controls one. These results agree with **Abd-El-Raof and Ghanem (2006)** who reported increase urea level in alopecic sheep which may be due to trace elements deficiency particularly cupper. Increased cortisone cause catabolism of protein leading to negative nitrogen balance and increased urinary elimination of nitrogen **(McDonald 1980)**.

Regarded to heavy metals result the cobalt and manganese were non detected in serum of ewes. On other hand our result **(Table 3)** revealed that there were significant decrease in calcium, copper and zinc while there were none significant changes in phosphorus and magnesium between diseased ewes and control ones. These results are agree with result obtained by **Hasan et.al;(2008)** and **Abd-El-Raof and Ghanem (2006)** who reported significant decrease in serum zinc and copper and non significant changes in phosphorus levels of diseased ewes. While disagree with them in calcium result as they reported none significant changes in calcium level these difference in our study may be due to ewes were lactating. Zinc and copper deficiency cause loss of hair and unthriftiness **(Abd-El-Raof and Ghanem, 2006).**

Our result revealed that there were significant increase in both prolactin and cortisone in diseased ewes when compared with control ones. Prolactin could have a role in seasonally driven follicle regression as well as recrudescence **(Choy, et.al; 1997)**. The relationship between changes in plasma prolactin level and fibre growth is well established in shedding sheep **(Pearson et.al; 1996)**. Change in hormone level have long been known to play a role in hair loss, Prolactin acts in an autocrine and / or paracrine manner on locally expressed high affinity receptors and functions as a catagen promoting signal in hair follicles in mice. One of classic signs of hyperadrenocorticism in dogs is a symmetrical pattern of hair loss that affects nearly the entire body except for the head and lower extremities. Also stress induce hair pulling behavior **(Foitzik et al.,2006)**.Wool breaking off at very short length and coming out in handfuls may be caused by stressful occasion . Hair loss has been associated with pregnancy and postpartum period in both humans and animals.**(Foitzik et al.,2009)**

It could be concluded that, we should examine appropriate hematological as well as biochemical studies and stress factors to diagnose alopecia in sheep. Cortisone and prolactin in addition to zinc and copper as well as other nutritional deficiency play an important roles in alopecia in ewes. Sheep breeder must pay attention to reduce stress and give balanced ration to prevent alopecia.

Table (1) Hematological results in diseased and control ewes (Mean ±SE)

Parameters	Control group	Diseased group
RBC×10^6/µl	9.096±0.20	6.892±0.37*
Hb g/dl	11.32±0.17	9.18±0.48*
Hct %	39.28±1.86	24.82±4.4*
MCV fl	35.48±6.03	39.86±1.35
MCH pg	15.88±3.2	13.32±0.17
MCHC %	29.12±1.17	33.6±1.23*
WBC×10^3/µl	5.67±0.71	8.0±o.91*
Lymphocytes×10^3/µl	2.85±0.53	1.95±0.22*
Neutrophils×10^3/µl	2.48 ±1.3	5.55±2.6*
Monocytes×10^3/µl	0.255±0.5	0.353±0.47
Eosinophils ×10^3/µl	0.088 ± 0.28	0.085±0.17

Symbol * denotes significant different from control at $P \leq 0.05$

Table (2) Some biochemical parameters in diseased and control ewes (Mean ±SE)

Parameters	Control group	Diseased group
Total protein(gm/dl)	8.84±0.28	6.87±0.37*
Albumin (gm/dl)	3.47±0.10	2.45±0.26*
Globulin (gm/dl)	5.36±0.30	4.42±0.21*
Alanine aminotransferase µ/l	15±0.63	22.6±0.67*
Aspartate aminotransferase µ/l	17.4±1.5	23±0.54*
ALP µ/l	34.0±1.8	38±3.7
Urea mg/dl	21± 1.3	33±1.24*
Creatinine mg/dl	1.4±1.6	1.55±1.0

Symbol * denotes significant different from control at $P \leq 0.05$

Table (3): Minerals, heavy metals and hormonal assay in diseased and control ewes (Mean ±SE)

Parameters	Control group	Diseased group
Calcium (mg/dl)	8.92±0.19	4.8±0.38*
Phosphorus (mg/dl)	6.04±0.18	5.98±0.48
Magnesium(mg/dl)	2.57±0.12	2.12±0.22
Cupper(µg/dl)	1.7±0.23	0.99±.07*
Zinc (µg/dl)	1.81±0.22	0.95±0.02*
Cortisone µg/dl	22±2.0	34±2.6*
Prolactin ng/ml	19.66±2.0	33.3±4.4*

Symbol * denotes significant different from control at $P \leq 0.05$

Ewe suffering from postpartum alopecia at back area

Ewe suffering from postpartum alopecia at back area

Ewe suffering from postpartum alopecia at lateral aspect of abdominal and chest area

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