Selective Serum Oxidant, Antioxidant and Trace Elements Profile in Ossimi Sheep Affected with Pregnancy Toxemia

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Abstract: Pregnancy toxemia is a metabolic disorder affecting mainly sheep usually during last period of gestation specially those bearing twines and reflected by nervous signs in affected animals. A totally (265) examined sheep, about (8) cases was tentatively diagnosed as pregnancy toxemia according to the previous case history, present clinical signs and serum biochemical analysis. Our serum chemistry parameters revealed a significant elevation in creatine kinase (CK) activity, β-Hydroxybuteric acid (βHBA), creatinine and urea levels with a significant reduction in glucose value in pregnancy toxemia comparing to control pregnant. Oxidative stress and antioxidant biomarkers in serum from pregnancy toxemic sheep show a significant increase in hydrogen peroxide (H_2O_2) level more than control pregnant ones. Also, a significant decrease of glutathione peroxidase (GPx) activity, total antioxidant capacity (TAC) and vitamin C (Vit C) in pregnancy toxemia group compared to control pregnant group. The trace elements study revealed a significant increase in the level of copper (Cu) and iron (Fe) while a marked decrease of zinc (Zn) value in pregnancy toxemia cases than control pregnant group. In conclusion, the oxidative stress markers and antioxidants with trace elements analysis could provide a great overview for their role in pathogenesis of pregnancy toxemia in Ossimi sheep.
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1. Introduction

There are no 'blueprint' approaches in the health management of pregnant ewes. Actually, there are differences in the health management of pregnant ewes between various production systems, which are related to the production priorities in the various systems, as well as between flocks within the same production system **(Fthenakis *et al.,* 2012)**. Pregnancy toxemia is a metabolic disorder in sheep and goats which usually develops during late gestation and which is always associated with hyperketonaemia and hypoglycaemia. Ewes of certain breeds, mainly when bearing two or three lambs, are more susceptible than ewes carrying only one fetus **(Harmeyer and Schlumbohm, 2006)**.

The origin of oxidative stress could be from a variety of mechanisms, such as excessive oxygen radical production from the autooxidation of glucose. Increased glucose oxidation leads to increased levels of glucose metabolites such as NADPH, which stimulates the cytochrome P450-like activity of hemoglobin in erythrocytes or of microsomes in various tissues, resulting in increased production of oxygen radicals and leading to cellular lipid peroxidation. Thus, glucose can react with proteins in vivo to form stable covalent adducts (glycation) **(Jain *et al.,* 2006)**. Antioxidants also ensure defenses against ROS-induced damage to lipids, proteins and DNA. ROS and antioxidants have been implicated in the regulation of reproductive processes in both animal and human, such as cyclic luteal and endometrial changes, follicular development, ovulation, fertilization, embryogenesis, embryonic implantation, and placental differentiation and growth **(Al-Gubory *et al.,* 2010)**.

Glutathione peroxidases (GPXs) are a family of enzymes divided to two groups, selenium (Se)-independent and Se-dependent enzymes, present in the cytoplasm and the mitochondria, and catalase which found within peroxisomes, both catalyze the conversion of H_2O_2 to H_2O **(Al-Gubory *et al.,* 2004; Garrel *et al.,* 2010).** Vitamin C has a variety of roles, one of which is the regeneration of vitamin E. Vitamin C is found at higher than plasma levels in a variety of tissues, including the brain (there is a greater than 10-fold gradient between the concentration of ascorbic acid in brain and serum). It is believed to be a critical cofactor of dopamine β-hydroxylase, and to be

involved in catecholamine biosynthesis. It also inhibits peroxidation of membrane phospholipids, and acts as a scavenger of free radicals**.** Human and other primates cannot synthesize this vitamin endogenously in the liver. **(Path, 1990; Gilgun-Sherki *et al.,* 2001).**

Copper and iron are a key structural component of many proteins and act as co-factors for the activity of many enzymes that are critical for brain function including enzymes involved in antioxidant defense and catecholamine synthesis involved in multiple biological processes required for growth, development, and maintenance of the nervous system **(Gaetke and Chow, 2003; Frederickson *et al.,* 2005).** Zinc deficiency is therefore accompanied by consequences as dramatic as erosion of the gastrointestinal tract, or skin lesions, or cardiac failure, or malformations of brain and the male reproductive system **(Frazzini *et al.,* 2006).** The dual aspects of cellular zinc are dealt by distributing the metal in highly regulated gradients across the plasma membrane and among intracellular compartments. In the same way, an adequate supply of zinc must be available for insertion into proteins, while preventing its accumulation and the potentially devastating effects it can readily initiate **(Sekler *et al.,* 2007)**.

The present work was aimed to investigate the role of some selective oxidant, antioxidant and trace elements parameters in pathogenesis of pregnant toxemia affected ewes.

2. Material and Methods
2.1. Animal Description

A total of (265) Ossimi sheep from different sheep flocks, different localities, and different flock size and different ages were investigated for presence of obvious nervous signs and apparently healthy sheep were collected during the period of investigation and considered as control group for this study (n=8). Based on competent case history, clinical findings the affected sheep and complete final diagnosis, affected sheep were categorized into two main groups; control pregnant group (n=8) and pregnancy toxemia group (n=8).

2.3. Serum Sampling:

Blood samples were collected in control pregnant group and diseased group from jugular vein. The blood samples were taken in plain centrifuge tube and in an inclined position for 20 minutes at room temperature, then, centrifuged at 3000 rpm for 10 minutes and the clear serum was separated carefully and was stored in Epindorf tubes at -20 °C until estimation of oxidant, antioxidant and trace elements parameters **(Coles, 1986)**.

2.4. Serum Chemistry Analysis.

Kinetic determination of CK activity was determined spectrophotometerically at 340 nm using a ready made kits provided by ELITech company according to **Henderson and Donald (2001)**. β-hydroxybutyrate was determined using ready made kits provided by Biochemical Enterprise according to **Young (2000).** Glucose was determined by GOD–PAP method without deproteinization, by using ready made kits provided by Spinreact according to **Young (2001)**. Determination of creatinine by photometric colorimetric test for kinetic measurement, method without deproteinization , using ready made kits provided by Human , Germany, according to **Henry *et al.* (1974)**. Urea was determined by enzymatic colorimetric method by using ready made kits provided by Diamond according to **Numann *et al.* (1977)**.

2.5. Serum Oxidant and Antioxidant Analysis.

Glutathione peroxidase, total antioxidant capacity, ascorbic acid and hydrogen peroxide is determined by colorimetric determination method by ready made kits provided by Bio-diagnostic, Egypt according to the instruction of the enclosed pamphlet.

2.5. Serum Trace Element Analysis.

Serum samples were defrosted and homogenized. The procedure consisted in placing 0.2 ml of serum/CSF samples in a flask, together with 1.0 ml of HNO3/HClO4 (4:l) mixture and heating to 120°C for 60 min in a mineralization block. The digest obtained was cooled and diluted to 1.5 ml with ultrapure water **(Terres-Martos *et al.,* 1997)**. The amount of copper, zinc and iron was determined by the air/acetylene atomic absorption spectrophotometer (Perkin Elmer 2380 AAS) **(L'vov, 2005)**.

2.7. Statistical Analysis:

Data were subjected to statistical analysis using statistical software program (SPSS for Windows, version 15, USA). Means and standard error for each variable were estimated. Differences between means of different groups were carried out using t-test.

3. Results
3.1. Clinical Findings:

Sheep with pregnancy toxemia were isolate themselves from the flock, poor body condition, mild to moderate depression, decreased appetite or anorexia standing apart from the flock, decreased flight response to approach, and decreased eye preservation and auditory reflexes. Lastly, not responding ketotic ewes were sternly recumbent but able to rise if enforced, absence of eye preservation/auditory reflex.

3.2. Serum Chemistry analysis.

Serum biochemical parameters are summarized in **Table (1)**. Serum CK activity, βHBA, creatinine and urea levels show a significant elevation in pregnancy toxemia affected sheep than control pregnant group. Glucose level show a significant decrease in pregnancy toxemia affected sheep than control pregnant group.

3.3. Serum Oxidant and Antioxidant.

Oxidative stress and antioxidant markers results are shown in **Table (2)**. There is a significant decrease in GPx activity, TAC and ascorbic acid levels in pregnancy toxemia group than control pregnant group. On the contrary, a significant increase in H_2O_2 value is seen in pregnancy toxemia group than control pregnant group.

3.4. Serum Trace Elements.

Trace elements are categorized in **Table (2)**. Pregnancy toxemia affected sheep show a significant increase in copper and iron values while a significant decrease in zinc level is observed in pregnancy toxemia group than control pregnant group.

Table (1): Serum Biochemical Analysis (mean values ± SE) in Clinically Healthy Pregnant Sheep and Those with Pregnancy Toxemia:

Groups	CK U/L	βHBA mg/dl	Glucose mg/dl	Creatinine mg/dl	Urea mg/dl
Control Pregnant (n=8)	143.31	10.18	59.13	0.96	169.50
	± 3.28	± 0.20	± 1.99	± 0.06	± 2.47
Preg. Toxemia (n=8)	827.12 *	17.76 *	41.63 *	1.30 *	184.54 *
	± 26.04	± 0.88	± 1.74	± 0.05	± 2.62

CK= creatine kinase, βHBA= β-Hydroxybuteric acid.
* Significant variance (*P* value ≤ 0.05).

Table (2): Serum Oxidative Stress Markers, Antioxidant and Trace Elements (mean values ± SE) in Clinically Healthy Pregnant Sheep and Those with Pregnancy Toxemia:

Groups	GPx mU/mL	H_2O_2 mM/L	Vit C mg/L	TAC mM/L	Cu ppm	Zn ppm	Fe ppm
Control Pregnant (n=8)	29.22	2.00	143.13	0.86	0.25	0.49	5.32
	± 0.62	± 0.33	± 0.52	± 0.03	± 0.01	± 0.02	± 0.19
Preg. Toxemia (n=8)	21.19 *	8.88 *	99.38 *	0.31 *	0.34 *	0.28 *	9.70 *
	± 0.90	± 0.72	± 0.89	± 0.04	± 0.01	± 0.02	± 0.31

GPx= glutathione peroxidase, H_2O_2= hydrogen peroxide, Vit C= vitamin C, TAC= total antioxidant capacity, Cu= copper, Zn= zinc, Fe= iron.
* Significant variance (*P* value ≤ 0.05).

4. Discussion.

Pregnancy toxemia is a metabolic disorder in sheep and goats which usually develops during late gestation and which is always associated with hyperketonaemia and in most cases also with hypoglycaemia. Ewes of certain breeds, mainly when bearing two or three lambs, are more susceptible than ewes carrying only one foetus (**Harmeyer and Schlumbohm, 2006**). Our observation over pregnancy toxemia affected sheep are isolation from the flock, poor body condition, mild to moderate depression, decreased appetite or anorexia standing apart from the flock, decreased flight response to approach, and decreased eye preservation and auditory reflexes. (**Andrew *et al.*, 1996; Sargison, 2007**).

Our serum CK activity in pregnancy toxemia affected group show a significant elevation than control pregnant group agreeing with **Andrew *et al.* (1996)** who explain this elevation due to cardiac or skeletal degeneration, and CK levels generally rose in ewes before death. Serum βHBA show a significant elevation in pregnancy toxemia affected sheep than control pregnant group. In contrary, hypoglycemia is observed in pregnancy toxemia affected sheep than control pregnant group indicating the high demand of energy for dam and their fetuses, also the reduced ability of the late gestating ewe to utilize BHBA (**Sargison *et al.,* 1994; Rook, 2000; Ramin *et al.,* 2005; Harmeyer and Schlumbohm, 2006**). Renal malfunction and protein catabolism can cause an increase in urea levels. Fatty infiltration and degeneration of the kidneys in pregnancy toxemia may impair renal function (**Andrew *et al.,* 1996**). Our serum creatinine and urea results show a significant elevation in pregnancy toxemia affected sheep than control pregnant group.

ROS and antioxidants have been implicated in the regulation of reproductive processes in both animal and human, such as cyclic luteal and endometrial changes, follicular development, ovulation, fertilization, embryogenesis, embryonic implantation, and placental differentiation and growth. In contrast, imbalances between ROS production and antioxidant systems induce oxidative stress that negatively impacts reproductive processes (**Al-**

Gubory *et al.*, 2010). Our results show a significant increase in H_2O_2 value is observed in pregnancy toxemia groups than control pregnant group. On the contrary, there is a significant decrease in GPx activity, TAC and ascorbic acid levels in pregnancy toxemia group than control pregnant group. The decrease in antioxidants is probably due to a compensatory nature responding to the increased lipid peroxide load in pregnancy toxemia **(Al-Gubory *et al.*, 2004; Patil *et al.*, 2009; Al-Gubory *et al.*, 2010; Garrel *et al.*, 2010; Al-Qudah, 2011)**.

Copper and iron are a key structural component of many proteins and act as co-factors for the activity of many enzymes that are critical for brain function including enzymes involved in antioxidant defense and catecholamine synthesis involved in multiple biological processes required for growth, development, and maintenance of the nervous system **(Gaetke and Chow, 2003; Frederickson *et al.*, 2005)**. Our trace elements results in pregnancy toxemia affected sheep show a significant increase in copper and iron values. Moreover, zinc level shows a significant decrease in pregnancy toxemia group than control pregnant group which agree with **Connor *et al.* (2001), Potts *et al.* (2006) and Maia *et al.* (2007)**. **Ilhan *et al.* (2002)** suggests a relationship between increased MDA, Cu levels and decreased SOD, Zn levels in pregnancy and preeclampsia.

In conclusion, the results of the present study revealed that serum analysis for, oxidative stress markers and antioxidants with trace elements could provide a great overview for the degree of tissue damage during pregnancy toxemia and their role in pathogenesis of pregnancy toxemia in Ossimi sheep.

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