Short Paper

Some acute phase proteins, oxidative stress biomarkers and antioxidant enzyme activities in ewes with pregnancy toxemia

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Summary

The aim of this study is to investigate antioxidant enzymes, oxidative stress biomarkers and the acute phase proteins levels for subclinical and clinical pregnancy toxemia and to determine the effect of early diagnosis on the success of curing. According to the results of clinical and biochemical parameters, from 39 ewes, 10 were healthy ewes (control group), 13 ewes had subclinical pregnancy toxemia (subclinic group) and 16 had clinical pregnancy toxemia (clinic group). Glucose level and glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) activity in clinic group were found statistically lower than in the other groups ($P<0.05$); as for BHBA, cortisol, MDA, SAA and Hp were found higher than in the other groups ($P<0.05$). In subclinic group BHBA, SAA and Hp were statistically higher than in control group ($P<0.05$). Conclusively, the parameters of oxidative stress, antioxidant enzymes activity and acute phase proteins (AAPs) can be used for the diagnosis of pregnancy toxemia in pregnant ewes.

Key words: Pregnancy toxemia, Acute phase protein, Oxidative stress, Antioxidant, Ewe

Introduction

Pregnancy toxemia is a metabolic disease commonly occurring in the last 6 weeks of gestation that causes significant economic losses with a high mortality rate in pregnant ewes (Caldeira *et al*., 2007).

Markers of oxidative stress increased in cows with subclinical ketosis (Sahoo *et al*., 2009). Normal cells have the capacity to detoxify superoxide radicals using antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase, and catalase (CAT), which help maintain the intracellular concentration of reduced glutathione and NADPH necessary for the optimal function of the antioxidant defense system (Al-Qudah, 2011).

Previous studies in ruminants suggested a relationship between selected APPs and lipid mobilization. Some results revealed significant ($P\leq0.05$) increase in the levels of Hp, SAA, Fb in ewes with pregnancy toxemia when compared to healthy pregnant ewes (EL-Dee, 2012). It has been postulated to be related to negative energy balance, since cows with high milk Hp also showed high non estrified free fatty acids (NEFA) concentration in serum (Hiss *et al*., 2009). A significant correlation between Hp and BHB in lactating goats has been also reported by (Trevisi *et al*., 2005).

The aim of this study is to investigate some clinical and biochemical parameters for subclinical and clinical pregnancy toxemia and to determine the effect of early diagnosis on the success of curing.

Materials and Methods

The disease occurred in flock consisting of 82 Ivesi sheep (fat tail) in the province of Elazig in Turkey during September 2013. All animals in the flock were checked for the periods of pregnancy and the number of fetus by ultrasonography in the first month of estimated pregnancy. In addition, the ewes were examined by systemic and clinical examination. The ewes in the study were at 17th week of gestation and aged between 2 and 6 years and weighing 45-56 kg. The mean body condition score (BCS) was 3.5 ± 1.6 measured on a 0-5 point scale (Russel, 1991).

According to the results of clinical and biochemical parameters, it has been determined that from 39 ewes, 10 were healthy ewes (control group), 13 ewes had subclinical pregnancy toxemia (subclinic group) and 16 ewes had clinical pregnancy toxemia (clinic group). Clinical pregnancy toxemia in 16 ewes was diagnosed according to declared symptoms by Scott (1993) and demonstration of plasma BHBA concentrations greater than 3.0 mmol/L (Marteniuk and Herdt, 1988). Subclinical pregnancy toxemia developed in 13 ewes (plasma BHBA concentration >0.86 mmol/L without any clinical signs of disease) (Lacetera, 2001).

In the study, plasma MDA, which is the last product of lipid peroxidation, was determined spectrophotometrically (Placer *et al*., 1966). The serum GSH-Px activity was determined according to the method of Lawrence and Burk (1976). The serum CAT activity was

measured as previously described by Goth (1991). The serum SOD activity was determined according to the method of Sun *et al*. (1988). All measurements were performed using spectrophotometer (Schimadzu UV-1208 UV-VIS, Japan) according to the manufacturer's recommendations. Blood samples were analysed twice (during one assay) and an arithmetic mean was calculated. Serum BHBA (Randox Laboratories Ltd., UK, Cat # RB 1008) concentration was determined spectrophotometrically. Serum glucose concentration was measured by spectrophotometry using a commercially available kit (Glucose-RTU®, BioMérieux, Lyon, France) following the manufacturer's instructions. Serum cortisol concentration was measured using a commercially available ELISA kit (Biocheck, Foster City, CA, USA). The plates were read at 450 nm on a computerized automated micro plate ELISA reader (BioTek, ELx808, USA).

Serum Hp was measured based on prevention of the peroxidase activity of hemoglobin, which is directly proportional to the amount of Hp. The analytical sensitivity of this test in serum has been determined as 0.0156 mg/ml for Hp by the manufacturer (Tridelta Development Plc., Wicklow, Ireland). Serum SAA was measured by a solid phase sandwich-ELISA. The analytical sensitivity of this test in serum has been determined as 0.3 µg/ml for SAA by the manufacturer (Tridelta Development Plc., Wicklow, Ireland).

All results were expressed as mean ± SD. SPSS/PC software one-way repeated measure analysis of variance (ANOVA) was used to determine statistical differences between mean values of the studied parameters among the groups. Differences were considered as significant at $P<0.05$.

Results

Urine analysis was unremarkable, except for ketonuria (urine ketone bodies in subclinic group ++, in clinic group +++ with Bayer Multistix 10 SG®).

Changes of the biochemical parameters in the plasma and serum and significant differences of data between control, subclinic and clinic groups are presented in Table 2.

Glucose, GSH-Px, SOD and CAT levels were found statistically lower ($P<0.05$) than the other two groups in clinic group; as for BHBA, cortisol, MDA, SAA and Hp were found higher ($P<0.05$) than the other groups. BHBA, SAA and Hp were found higher ($P<0.05$) than the control group in subclinic group.

Table 1: Avaragedailyintake of metabolizable energy (MJ) and digestible crude protein (nitrogen × 6.25 g) by the ewes

	Pregnant ewes (d pregnant)	
	64-119	119-147
Metabolizable energy	12.75	18.71
Digestible crude protein	95	115

Discussion

Oxidative stress has been reported in several studies of healthy pregnancy in animals (Castillo *et al*., 2005). This is caused by increased free radical production resulting from increased metabolic activity during pregnancy, negative energy balance and ketone body formation, reduction of antioxidant reserve during pregnancy, and physiologic adaptation of pregnant animals to lactation (Sahoo *et al*., 2009). In this study, MDA levels were found to be higher than normal physiological levels, whereas GSH-Px, SOD and CAT levels decreased in all groups. However, MDA levels increased significantly in clinic group compared to other groups. Also, GSH-Px, SOD and CAT levels were found to be significantly lower in clinic group compared to other groups. The decrease in SOD and GSH-Px enzyme activity in this investigation can be explained by the serious damage that occurred in the erythrocyte membrane and other cellular structures depending on inability to fully detoxify oxygen free radicals. Also, an association between hyperketonemia and lipid peroxidation was noted, suggesting that ketonemia is a risk factor for lipid peroxidation and oxidative stress in ewes affected with pregnancy toxemia (Al-Qudah, 2011).

The acute phase response has been studied in ruminant species such as cow, sheep and goats, and Hp and SAA are considered the most important and useful indicators of inflammatory process (Murata *et al*., 2004). Besides serum, SAA and Hp levels in the sera of aborted

Table 2: The mean levels and ±SD of BHBA, glucose, cortisol, MDA, GSH-Px, SOD, CAT, Hp and SAA in clinic, subclinic and control groups

Parameters	Clinic group	Subclinic group	Control group
BHBA (mmol/L)	5.05 ± 0.12^{a}	1.18 ± 0.08^{b}	0.45 ± 0.01^{c}
Glucose (mmol/L)	1.11 ± 0.03^{a}	2.19 ± 0.09^{b}	4.72 ± 0.04^{b}
Cortisol (mmol/L)	2.02 ± 0.05^{a}	0.33 ± 0.03^{b}	0.19 ± 0.01^{b}
MDA (mmol/L)	5.46 ± 0.24^{a}	3.93 ± 0.18^{b}	3.21 ± 0.21^{b}
GSH-Px (U/g Hb)	44 ± 3.86^{a}	63 ± 3.50^{b}	77 ± 2.98^{b}
SOD (U/g Hb)	963 ± 27.85^{a}	1175 ± 32.16^{b}	1416 ± 26.82^{b}
CAT (U/g Hb)	99 ± 6.24^{a}	110 ± 7.52^{b}	112 ± 5.63^{b}
SAA (µg/ml)	65.24 ± 6.20^{a}	24.63 ± 4.36^{b}	5.62 ± 0.84^{c}
Hp (mg/ml)	0.835 ± 0.072^{a}	0.527 ± 0.070^{b}	0.048 ± 0.008^{c}
Urine multistix	+++	++	-

[a, b, c] Different superscript letters within same row indicate significant differences among groups ($P<0.05$)

goats were lower than the non-aborted goats (Balikci *et al*., 2013). In addition to inflammatory conditions, the acute phase proteins are also released in normal physiological conditions such as pregnancy (Georgieva *et al*., 2011). Nazifi *et al*. (2008) also reported that the Hp concentrations (0.300 ± 0.090 g/L) in dry cows near parturition were slightly higher than in dairy cows (0.120 ± 0.050 g/L) and pregnant cows (0.220 ± 0.030 g/L) and that the APP concentrations measured during pregnancy or lactation were markedly higher than in non-pregnant females (0.080 ± 0.060 g/L). In the present study, SAA and Hp levels were found significantly higher when compared to others in clinic group. In addition, SAA and Hp levels were found significantly higher than the control group in subclinic group. Increased Hp and SAA levels due to inflammation in placenta are thought to be the reason of fetal losses. Moreover, these increases are probably because of up-regulation of its expression by cortisol and non-steroid fatty acids. Concerning the acute phase response, there was significant ($P \leq 0.05$) increase in the levels of Hp, SAA (1.2 mg/L, 29.4 mg/L) in ewes with pregnancy toxemia when compared to healthy ewes (EL-Dee, 2012). Some authors have reported increases in Hp without increases in other APP such as alpha 1-acid glycoprotein after starvation of cows (Yoshino *et al*., 1993). In addition, ketosis did not produce any change in serum transferrin (an APP) in cows (Moser *et al*., 1994). Similarly, increased concentrations of BHBA and normal Hp concentrations were reported in cows with subclinic ketosis (Skinner *et al*., 1991).

In conclusion, the parameters related with oxidative stress, antioxidants and acute phase proteins in the present study may be considered in the diagnosis and prognosis of pregnancy toxemia in ewes.

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