Comparison of Lipid Peroxidation and Several Antioxidants in Blood of Normally Calved and Dystocia Affected Cows and Their Newborn Calves

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ABSTRACT

The changes of malondialdehyde (MDA) concentrations in plasma and of enzymatic antioxidants like glutathione peroxidase (GSHPx), catalase (CAT) activities, non-enzymatic antioxidants like glutathione (GSH) concentrations in blood of normally calved and dystocia affected cows and their newborn calves were investigated. The study included 8 normally calved cows, 8 dystocic cows and their neonates. Calves of dystocic cows were delivered by traction from the birth canal. The MDA, GSH concentrations ($P<0.001$) and CAT activity ($P<0.01$) were significantly higher in dystocia affected cows compared to the normally calved cows. MDA concentrations were significantly higher in the calves of dystocia affected cows compared to the calves of normally calved cows ($P<0.001$), but CAT activity and GSH concentration were significantly lower ($P<0.01$). There were no significant differences in the GSHPx activity between the groups. In both dystocia affected cows' and normally calved cows' calves, the plasma MDA concentrations were higher than in their mothers. Moreover the CAT and GSHPx activities and GSH concentration in the normally calved cows' calves were higher than in their mothers'. Our results suggest that lipid peroxidation and antioxidant status change during dystocia, and these changes may affect the fetus by creating oxidative stress.

Keywords: Dystocia; Cows; Calves; Lipid peroxidation; Antioxidants.

INTRODUCTION

Free radicals or reactive oxygen metabolites (ROM), formed during physiological and pathological conditions in the body are extremely reactive and unstable reacting with proteins, lipids, carbohydrates and nucleic acids (1, 2). The imbalance between the production of ROM and their disposal leads to free radical accumulation, deregulation of metabolic pathways and cellular damage through oxidative chain reactions and lipid peroxidation, resulting in oxidative stress (3, 4). Under normal conditions, free radicals are neutralized by efficient antioxidant systems (3). Enzymatic antioxidants like glutathione peroxidase (GSHPx), superoxide dismutase (SOD), catalase (CAT), and non-enzymatic antioxidants like vitamins A, E, and ß-carotene and glutathione (GSH) protect living organisms against ROM (3).

Pregnancy is a state when oxidative stress can be expected due to a high energy demand and increased oxygen requirements (5). Birth due to oxidative stresses causes changes in free radical formation and in the antioxidant system of the blood and other organs (6). Difficult calving, termed as dystocia, occurs in 3% to 25% of cattle pregnancies (7). Dystocia has been a long-standing problem in both the beef and dairy industry (7). It is one of the most serious complications of

pregnancy in cattle (7). The process of parturition, though physiological, is a stressful event and abnormal parturition (dystocia) further adds to the normal stress of calving (8).

Information about the oxidant and antioxidant status in dystocia affected cows is limited in the literature (9, 10), and furthermore, there are no reports about the oxidant and antioxidant status in neonates born by dystocia-affected cows. Therefore, our aim was to investigate the changes of MDA concentrations in plasma and of antioxidant enzyme (GSHPx, CAT) activities, GSH concentrations in blood of normally calved and dystocia affected cows and their newborn calves, particularly the oxidant and antioxidant status in neonates born by dystocia-affected cows.

MATERIAL AND METHODS

Animals and Samples

The study was performed on 16 multiparous cows, 3-8 years old, brought for parturition around full term to the Clinic of Obstetrics and Gynecology of Firat University. The study was composed of 8 normally calved cows (3 Simmental, 4 Montafon, 1 Holstein), 8 dystocia affected cows (3 Simmental, 4 Montafon, 1 Holstein) and their neonates.

Normal calving was defined as a spontaneous calving of normal duration. Calves of dystocia-affected cows were delivered by traction from the birth canal after correction of presentation, position, and posture of the fetus. The blood of normally calved and dystocia affected cows and their newborn calves were taken from the jugular vein within the first half hour after birth. All blood samples were collected into sterile blood collecting heparinized tubes from all cows and newborn calves. Whole blood was separated for GSHPx and GSH assays. The remaining blood was immediately centrifuged at 1500 *g* for 5 min. The erythrocytes and plasma were collected separately for CAT and MDA assays, respectively. All samples were kept at -25°C pending analysis.

Biochemical assays

Lipid peroxidation in plasma was measured by the thiobarbituric acid reacting substance (TBARS) method (11) and was expressed in terms of the MDA content, which served as the standard of 1,1,3,3-tetraethoxypropane. The formed MDA created a pink complex with thiobarbituric acid (TBA) and the absorbance was read at 532 nm. Values were expressed as MDA equivalents in ηmol/ml plasma.

Whole blood GSHPx activity was assayed by the method of Matkovics *et al.* (12) and expressed as unit per g of Hb (U/g Hb). GSHPx activity was determined by using cumene hydroperoxide and reduced glutathione (GSH) as co-substrates and the loss of GSH following enzymic reaction at 37°C was measured spectrophotometrically with Ellman's reagent at 412 nm (12).

Erythrocyte CAT activity were determined according to the method of Aebi (13) and expressed as kat/g Hb. The decomposition of H_2O_2 was directly followed by the decrease of absorbance at 240 nm. The difference in absorbance at 240 nm per time unit allowed determining the CAT activity.

The concentration of reduced glutathione was assayed by the method of Beutler *et al.* (14) and expressed as μmol/g Hb. The method was based on the capacity of sulfhydryl groups present in whole blood to react with 5, 5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) and form a yellow dye with maximum absorbance at 412 nm.

Haemoglobin (Hb) concentration was determined according to cyanmethaemoglobin method (15) and expressed as g/ml for GSHPx and CAT activities, as g/dl for GSH concentration. An aliquot of whole blood was mixed with a solution of potassium cyanide and potassium ferricyanide. All forms of haemoglobin except sulphaemoglobin were converted to cyanmethemoglobin. The absorbance was measured in a colorimeter at a wavelength of 540 nm.

Statistical analysis

All data were and analyzed by means of the SPSS 15.0 software (SPSS 15.0, SPSS, Inc., Chicago, IL, USA). Results were expressed as mean ± SEM (Standard Error of the Mean). The independent t-test was used to determine statistically significant variations between groups and differences which were considered as significant for P values less than 0.05.

RESULTS

The MDA, GSH concentrations ($P<0.001$) and CAT activity ($P<0.01$) were significantly higher in dystocia affected cows compared to the cows undergoing normal parturition (Table 1). MDA concentrations were significantly higher in the calves of dystocia affected cows compared to the calves of normally calved cows ($P<0.001$), but CAT activity and GSH concentration were significantly lower ($P<0.01$) (Table 2). There were no significant differences in the GSHPx activity

Table 1: GSHPx, CAT activities and MDA, GSH levels in normally calved cows (n=8) and dystocia affected cows (n=8)

	MDA (ηmol/ml)	CAT (kat/g Hb)	GSHPx (U/g Hb)	GSH (µmol/g Hb)
Normal calved cows	7.38 ± 0.37	21.95 ± 2.20	13.49 ± 0.43	1.98 ± 0.31
Dystocia affected cows	10.25 ± 0.76	34.93 ± 2.77	13.48 ± 0.47	3.60 ± 0.21
P	***	**	NS	***

Table 2: GSHPx, CAT activities and MDA, GSH levels in normally calved cows' newborn calves (n=8) and dystocia affected cows' newborn calves (n=8)

	MDA (ηmol/ml)	CAT (kat/g Hb)	GSHPx (U/g Hb)	GSH (µmol/g Hb)
Normal calved cows' newborn calves	8.88 ± 0.32	51.79 ± 5.85	14.18 ± 0.84	4.52 ± 0.79
Dystocia affected cows' newborn calves	11.07 ± 0.23	32.07 ± 3.43	12.79 ± 0.28	3.59 ± 0.55
P	***	**	NS	**

** $P<0.01$, *** $P<0.001$, NS: No significant difference

between the groups (Table 1, 2). In both dystocia affected cows' and normally calved cows' calves, the plasma MDA concentrations were higher than in their mothers. Moreover the CAT and GSHPx activities and GSH concentration in the normally calved cows' calves were higher compared to their mothers' values (Table 1, 2).

DISCUSSION

The process of parturition, though physiological, is a stressful event and abnormal parturition (dystocia) further adds to the normal stress of calving (8). Controversial information has been available on the oxidative status in dystocia (9,10). While some studies have reported an increase in the erythrocytic MDA concentrations of the dystocia-affected cows (9), others have reported no significant changes in erythrocytic (10) and plasma (9) MDA concentrations. However in the present study, a significant increase in the plasma MDA levels was observed in cows with dystocia. Higher levels of MDA in cows with dystocia may be explained by higher levels of glucocorticoids, eicosanoids, and adrenaline-induced pathways of aerobic energy production associated with parturition, which generate reactive oxygen metabolites and lipid peroxidation (9, 10).

The process of parturition potentially can induce oxidative stress to the newborn (16). The transition from fetal to neonatal environment, exposes the newborn to a more oxidative environment (16). Arguelles *et al.* (17) found higher oxidative stress of the newborn based on measurements in umbilical cord blood compared to mother's blood at the moment of the birth. Gaal *et al.* (6) reported that in newborn calves, the concentration of free radicals in blood was 30% higher than their mother's samples at calving. We also found that in both dystocia affected cows and normal parturition cows' calves, the plasma MDA concentrations were higher than in their mothers' samples indicating that neonates in both normal birth and dystocia are under an increased oxidative stress when compared to their mothers.

In the present study it was found that MDA concentrations were significantly higher in the calves of dystocic cows compared to the calves of normally calved cows. Hracsko *et al.* (18) reported that in humans, lipid peroxidation in the neonatal cord blood was significantly higher in caesarean section as compared to vaginal delivery. This is probably an indication of higher fetal oxidative stress in dystocia. The greater the MDA elevation in neonates due to dystocia may be the cause of some neonatal diseases. Free radicals were reported to play an important role in the pathogenesis of several pathological conditions such as hemolytic disease of the newborn, broncho-pulmonary dysplasia, and retinopathy of prematurity. Indeed, neonates born by caesarean section have an increased incidence of these conditions (19).

Glutathione and glutathione-related enzymes, are one of the major detoxification and free-radical scavenging systems may play a role in controlling diseases (20). In our study,

no significant differences were found in GSHPx activity in dystocic cows as compared to cows calving normally, but significantly higher CAT activity and GSH levels in cows suffering from dystocia were detected. Similarly, the enzymatic and non-enzymatic antioxidant levels in the reports were variable (9, 10, 21). We suggest that an increase in the GSH concentration and CAT activity in the dystocia-affected cows may be due to high concentrations of lipid peroxidation. Hermes-Lima *et al.* (22) proposed that the activation of antioxidant defenses, in which the actual production of oxyradicals should decrease, is a preparative mechanism against oxidative stress caused by stress situations.

In various studies fetal oxidative stress in cord blood of fetuses born in elective cesarean delivery (CD) was compared to fetuses born in spontaneous vaginal delivery (VD) in humans, by measuring umbilical cord venous blood antioxidants (16,18,23-25). Differences between elective CD and VD groups were non-significant for SOD, CAT, GSHPx enzyme activities, except for the GSH concentrations (18). Some studies have reported an increase of SOD and CAT activities (23) in the elective CD compared to the VD, yet others have indicated a reduction of CAT and GSHPx activities (24). GSH concentrations were significantly lower in the elective CD than in VD (16, 18, 25).

An important point in the present study is the CAT activity and GSH concentrations in the calves of dystocia affected cows compared to the calves of normally calved cows which were lower. These results indicate that the antioxidant system may be impaired in the calves of dystocia affected cows. Paamoni-Keren *et al.* (16) suggested that neonates who were delivered by CD have a lower GSH level than delivered by VD in human. Therefore, they appeared to be exposed to the higher oxidative stress when compared to neonates who were delivered by VD.

In present study it was found that the CAT, GSHPx activities and GSH concentration in the normally delivered cows' calves were higher than in their mothers' samples. Gaal *et al.* (6) have suggested that both healthy cows and their newborn calves are well prepared for the event of birth, and that calves are able to counter effectively the oxidative stress inevitably present in neonates after the first inhalation of atmospheric oxygen.

In conclusion, the obtained results suggest that in their newborn calves and their mothers in conditions of dystocia appear to be associated with the occurrence of a systemic oxidative stress evidenced by the changes of the MDA concentration, CAT activity and GSH level. Moreover the results of this study indicate that dystocia exposes the fetus to a higher oxidative stress than uncomplicated parturition as measured by MDA, GSH concentrations and CAT activities.

Based on our measurements we conclude that the neonates born under dystocic conditions might be predisposed to pathological conditions in which the reactive oxygen species may play a pathogenic role, due to deficient antioxidant defenses. Thus, we suggest that providing antioxidant supplementation to newborn calves and cows following dystocia may be helpful in reducing the oxidative damage and improving post-parturtion convalescence.

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