EFFECTS OF DYSTOCIA ON LIPID PEROXIDATION AND ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANTS IN CROSSBRED DAIRY COWS

HAMİT YİLDİZ, HALİL ŞİMŞEK¹, NEVZAT SAAT, AND MURAT YÜKSEL

Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, University of Firat, 23119 Elazig, Turkey

¹ Sanitary Services Vocational School of Higher Education, University of Bingöl, 12000 Bingöl, Turkey

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Abstract

The aim of the study was to evaluate oxidative stress in cows undergoing normal parturition and cows suffering from dystocia. The erythrocytic glutathione peroxidase (GSH-Px) activity, plasma vitamin A and ß-carotene concentrations, and paraoxonase (PON1) activity were lowered (P<0.001, P<0.01, 0.05, and 0.05, respectively) in cows with dystocia compared to normal calving cows. The erythrocytic malondialdehyde (MDA) concentration was markedly increased in the dystocia group compared to normal calving group. However, erythrocytic glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) activities, and plasma vitamin E and MDA concentrations were not significantly changed in the dystocia-affected cows in comparison to eutocia cows. In the difficult calving cows, there were significant correlations between the activities of GSH-Px and SOD (r=-0.41, P<0.01), plasma ß-carotene levels and paraoxonase activity (r=0.34, P<0.05), body condition score (BCS) and plasma MDA (r=0.46, P<0.05). Similarly in the eutocia cows there were significant correlations between CAT activity and MDA concentration (r=-0.76, P<0.01), levels of plasma ß-carotene and PON1 (r=0.58, P<0.01), BCS and MDA concentrations (r=0.50, P<0.05), and BCS and vitamin E (r=0.53, P<0.05) concentrations. These results suggests that evaluation of plasma vitamin A and ß-carotene concentrations, PON1 and GSH-Px activities, and MDA concentration seems to be useful in the assessment of dystocia in cows.

Key words: cows, dystocia, lipid peroxidation products, antioxidants.

Difficult calving, termed as dystocia, occurs in 3% to 25% of cattle pregnancies. Dystocia has been a long-standing problem in both the beef and dairy industry. It is one of the most serious complications of pregnancy in cattle, which is believed to be influenced by numerous factors such as pelvic area, calf’s birth weight, age of dam, twin pregnancy, presentation, disposition, hormonal control, and nutrition of dam (17).

Reactive oxygen species (ROS) or free radicals, formed during physiological and pathological conditions in the body, are extremely reactive and react with proteins, lipids, carbohydrates, and nucleic acids. Uncontrolled increase in free radicals may cause damage of cells and tissues through oxidative chain reactions and lipid peroxidation, resulting in oxidative stress (16). Enzymatic antioxidants like GSH-Px, SOD, together with CAT, and non-enzymatic antioxidants like vitamins A, E, and ß-carotene and GSH protect living organisms against ROS (16).

Recently, some researchers reported that serum paraoxonase - PON1 was reduced in dry cows and early postpartum dairy cows (30), and in late pregnancy (29). Oxidative stress leads to various disorders in the prepartum and postpartum cattle (18). Therefore, there is a possibility that PON1 activity is a useful indicator for diagnosis of diseases in pregnant cattle. There is little information in the literature on biochemical profile in cattle with dystocia. Therefore, the present study was to determine the lipid peroxidation products and some enzymatic and non-enzymatic antioxidants in cows with or without difficulties during parturition.

Material and Methods

The study was performed on 55 crossbreds (Brown Swiss x Simmental) dairy cows, 3-8 years old, brought for parturition around full term to the Clinic of Obstetrics and Gynaecology between September 2007 to October 2008. Body condition scores (BCS) of the cows before parturition in dystocia and normal birth groups were determined as 2.87 ±0.53 and 2.90 ±0.38, respectively. The assessments of the BCS were made by the same individual throughout the experimental period.
as described by Edmonson et al. (8). The scores were assigned using a five-point scale (0-very thin to 5-grossly fat). It was based on the palpation of the traverse processes of the loin vertebrae, cranial coccyeal vertebrae, and tuber ischii. Out of these animals, 20 gave normal birth to calves, while 35 cows were admitted with dystocia for required assistance at calving. Normal calving may be defined as a spontaneous calving of normal duration. All cows were pluripara.

**Blood collection and preparation of blood samples.** Blood samples were taken after a gynaecological examination- before the obstetrical manipulation to dystocia. Similarly, normally calving cows were sampled before delivery. Blood was taken from jugular venapuncture by means of vacuum tubes (Vacutainer®), 5 ml with added EDTA, and then blood was separated into plasma and RBC by centrifugation at 1,800 × g for 10 min.

**Biochemical assay.** Biochemical examinations were performed in the Central Research Laboratory of Bingöl University. Lipid peroxidation levels in plasma and haemolysed RBC were measured using method described by Placer (20), based on thiobarbituric-acid reaction. The quantification of thiobarbituric-acid reactive substances was determined by comparing the absorption to the standard curve of malondialdehyde equivalents generated by acid catalysed hydrolysis of 1,1,3,3-tetramethoxypropane. The values of MDA were expressed as nmol/mL for plasma and RBC. Every sample was assayed in duplicate, and the assay coefficients of variation for MDA were less than 3%.

The methods of Goth (11) were used for the determination of catalase activity in haemolysed RBC. The GSH content in RBC was measured at 412 nm using the method of Siedak and Lindsay (23). GSH-Px activities in RBC were measured at 37°C and 412 nm according to Lawrence and Burk (14). Total (Cu–Zn and Mn) SOD activity was determined according to the method of Sun et al. (25). The protein content in the plasma and haemolysed RBC was measured using Lowry’s et al. method (15).

Vitamins A and E were determined in frozen plasma samples by a modification of the method described by Desia (7). The levels of β-carotene in plasma samples were determined according to method of Suzuki and Katoh (26). PON1 activity was measured using diethyl-p-nitrophenylphosphate as a substrate, as previously described by Furlong et al. (10).

**Statistical analysis.** All data were expressed as mean values with standard deviation (±SD). The t-test of independent-samples was performed in order to compare the normal and dystocia groups for each evaluated parameter. Pearson’s correlation coefficients were tested for significance using t-test. Analyses were performed using the Statistical Package for Social Sciences software (SPSS12. 0, Chicago, 2003). P<0.05 was considered statistically significant.

**Results**

This study did not include animals subjected to foetotomy or caesarean section. Epidural anaesthesia was not used for the cows, which needed mechanical extraction. The duration of the obstetrical procedures was recorded as 25 to 30 min for dystocia affected cows. All the animals were able to stand, and no complication was seen after their delivery. No important complications after dystocia were observed in the selected animals. The majority of dystocia cases were caused by weight of calf, head and limb deviations, and carpal and hock flexion. Some cases were due to maternal causes (uterus inertia, inadequate of birth canal). Calves of dystocia-affected cows were delivered by traction from birth canal after correction of the presentation, position, and posture of foetus.

The GSH-Px activity was significantly lower in the dystocia group compared to the normal labour group (P<0.001), whereas MDA concentration was significantly higher in the dystocia group compared to the normal labour group (P<0.001). There were no significant difference in GSH, CAT, and SOD activities between dystocia and normal delivery groups (Table 1).

The results of plasma MDA concentration, PON1 activity, and vitamins A, E, and β-carotene in normally calving and dystocia cows were presented in Table 2. In cows with dystocia, vitamin A and β-carotene concentrations and PON1 activity were significantly lower than those in the normal delivery group (P<0.01, 0.05 and 0.05, respectively), whereas there were no significant differences in vitamin E and MDA levels between the groups (Table 2).

**Table 1**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal cows (n=20)</th>
<th>Dystocia cows (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (µmol/mL)</td>
<td>1.77 ±0.07</td>
<td>1.78 ±0.07</td>
</tr>
<tr>
<td>GSH-Px (U/g protein)</td>
<td>164.93 ±11.38</td>
<td>140.62 ±21.23*</td>
</tr>
<tr>
<td>CAT (k/g Hb)</td>
<td>80.61 ±12.51</td>
<td>81.59 ±14.29</td>
</tr>
<tr>
<td>MDA (nmol/mL)</td>
<td>18.11 ±1.91</td>
<td>22.35 ±1.35*</td>
</tr>
<tr>
<td>SOD (U/g Hb)</td>
<td>309.03 ±38.14</td>
<td>314.59 ±35.05</td>
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</tbody>
</table>

*P<0.001.
Plasma concentrations of vitamins A and E, β-carotene, and MDA, and paraoxonase activity in normal and dystocia cows

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal cows (n=20)</th>
<th>Dystocia cows (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E (nmol/L)</td>
<td>6.09 ±1.39</td>
<td>6.30 ±1.38*</td>
</tr>
<tr>
<td>Vitamin A (µmol/L)</td>
<td>2.99 ±1.17</td>
<td>2.51 ±2.38**</td>
</tr>
<tr>
<td>β-carotene (µmol/L)</td>
<td>0.84 ±0.45</td>
<td>0.59 ±0.26**</td>
</tr>
<tr>
<td>MDA (nmol/mL)</td>
<td>1.28 ±0.23</td>
<td>1.34 ±0.17</td>
</tr>
<tr>
<td>Paraoxonase (IU/L)</td>
<td>47.46±12.33</td>
<td>42.64±12.99**</td>
</tr>
</tbody>
</table>

* - P<0.01; ** - P<0.05.

In the dystocia cows, there were significant correlations between the activities of GSH-Px and SOD (r=-0.41, P<0.01), plasma β-carotene levels and PON 1 activity (r=0.34, P<0.05), and BCS and plasma MDA (r=0.46, P<0.05). Similarly as in the eutocia cows, there were significant correlations between the CAT activity and MDA concentration (r=-0.76, P<0.01), levels of β-carotene and PONI (r=0.58, P<0.01), BCS and MDA concentrations (r=0.50, P<0.05), BCS and vitamin E (r=0.53, P<0.05) concentrations.

**Discussion**

Body condition scoring was used as a management tool to assess the energy reserves and nutritional status of dairy cattle. BCS must be optimal to ensure an easy calving. Overconditioned cows have been demonstrated to have a higher risk of dystocia and metabolic disorders (22). In this study, BCS status in cows with dystocia and normal parturition was similar in both groups and was determined as 2.87 and 2.90, respectively. This finding was consistent with earlier observations of Berry et al. (4) who reported that there was no significant effect of BCS on dystocia in the perinatal period.

Vitamins E and A, and β-carotene are known to be effective antioxidants in living organisms. Sathya et al. (21) showed that antioxidant vitamins are effective in reducing oxidative stress in dystocia-affected cows. A decrease in plasma concentrations of vitamins E, A, and β-carotene during parturition in cows were reported (31). A significant increase in plasma vitamin A level was observed in cows with dystocia, while β-carotene levels remained unchanged (6). It has been reported that there was no difference in the levels of serum vitamin E between cows with retained and non-retained placenta (5). Trevisi et al. (28) found that plasma vitamin A concentration was significantly lower in cows having retained placenta as compared to healthy animals. In our study, plasma vitamin A and β-carotene concentrations were lower in dystocia group compared to normal parturition group. However, there was no statistical difference in vitamin E level between these groups. These results are in accordance with the observations of other researchers. (5, 28, 31). A decrease in vitamin A and β-carotene levels in dystocia cows may be an indicator for an impaired oxidant and antioxidant balance.

The erythrocyte SOD activity was increased in dystocia-affected buffaloes in comparison with normally calved buffaloes, though it was not statistically significant. This increase could be due to higher degree of inflammation in the reproductive tract in the immediate postpartum period and higher degree of stress due to dystocia (21). CAT activity was not significantly different among Holstein cows with prolapsed uteri, caesarean section, and with or without retained placenta (9). In the latter study, buffalo cows with retained placenta showed decreased GSH, SOD, and CAT values, indicating that these animals were under oxidative stress (1). However, there were no differences in GSH levels in a study with retained placenta as opposed to those without the disorder at 2 weeks before calving, with a tendency to increase towards delivery (5). In our study, no significant difference was noted in SOD and CAT activities and GSH concentration in cows suffering from dystocia, as compared to cows calving normally. Similar results were obtained by others (5, 9, 21).

MDA is one of the final decomposition of lipid peroxidation and it is also formed as a product of the cyclooxygenase reaction in prostaglandin metabolism. While some studies have reported an increase in the MDA concentrations in buffaloes (1) and cows with retained placenta (12), some others have reported no significant changes in erythrocytic MDA concentrations of the dystocia-affected buffaloes (21) and cows with retained placenta (5, 9). In the presented study, a significant increase in the MDA levels was observed in cows with dystocia, while plasma MDA concentrations remained unchanged. These findings were consistent with earlier observations of Ahmed et al. (1) and Gupta et al. (12). Higher levels of MDA in cows with dystocia may be explained by higher levels of glucocorticoids and eicosanoids, and adrenaline-induced pathways of aerobic energy production associated with parturition, which generate reactive oxygen metabolites and lipid peroxidation.

GSH-Px activity in erythrocytes before parturition was significantly lowered in the cows with retained placenta, compared to cows with no retained placenta at 2 and 0 weeks before expected calving (5). Sathya et al. (21) reported that there was no significant difference in erythrocyte GSH-Px activity between
dystocia-affected buffaloes and euctocia. GSH-Px activity tended to increase in animals with retained placenta, but the change was not significant (9). Moreover, these authors also demonstrated that GSH-Px activity was significantly decreased in cows with caesarean section compare to cows with or without retained placenta. In addition, Kankofer et al. (13) found that cows with retained placenta had lower GSH-Px activity in maternal and placental tissues compared with the cows without retained placenta. In our study, a significant decrease in GSH-Px activity was observed in cows with dystocia. These results were consistent with findings of some researchers (5, 9, 13). In the dystocia cows, a decreased GSH-Px activity and increased MDA concentrations may indicate the free radical formation and the beginning of an oxidative stress.

Bernabucci et al. (3) reported that cows with higher BCS before calving and with more pronounced BCS losses during the transition period showed higher plasma reactive oxygen metabolites (ROM) and thiobarbituric acid reactive substances (TBARS), and these cows are more sensitive to oxidative stress. In the patients with β-thalassaemia, serum total antioxidant capacity was significantly correlated with serum paraoxonase activity (24). Erythrocyte GSH-Px activity in equine were positively correlated with erythrocyte SOD activity (r=0.50, P<0.05) (19). However, there were negative correlations between plasma MDA levels and CAT activity in the preeclamptic rats (r=-0.22, P<0.01) (27). Similarly in the present study, there was negative correlations between GSH-Px and SOD activity (r=-0.41, P<0.01) in cows with dystocia, and CAT activity and MDA concentration (r=-0.76, P<0.01) in euctocia animals, and positive correlations between plasma β-carotene and PON1 activity (r=0.34, P<0.05; r=0.58, P<0.01, respectively), and BCS and plasma reactive oxygen metabolites (ROM) and CAT activity in the preeclamptic rats (r=-0.22, P<0.01) (19). However, there

PON 1 activity can vary depending on different physiological conditions or pathological states (24). Turk et al. (29, 30) suggested that a decreased serum PON1 activity in dairy cows during late pregnancy and early lactation may probably be a consequence of an antioxidant imbalance influenced by reproductive stress. Bademkiran et al. (2) reported that there has been a tendency to a decrease in PON 1 activity in cows with dystocia and twin parturition. The authors speculated that the tendency to decreased PON1 activity is probably due to the increased free radical production resulting from a negative energy balance caused by increased requirements. In the present study, plasma PON1 activity in dystocia-affected cows was significantly lower than that in cows with normal parturition. Dystocia reduced the PON1 activity and this decrease may be associated with increased oxidative stress, resulting in raised lipid peroxidant products, which impair the balance between oxidant and antioxidant systems.

In conclusion, the obtained findings suggest that dystocia appears to be associated with the occurrence of a systemic oxidative stress evidenced by the elevation of the MDA concentration and the reduction of erythrocyte GSH-Px activity, plasma β-carotene, vitamin A, and PON1 activity.

References