Changes in plasma malondialdehyde concentration and some erythrocyte antioxidant enzymes in cows with prolapsus uteri, caesarean section, and retained placenta

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SUMMARY

The periparturient period is especially critical for health and subsequent performance of dairy cows. The aim of the study was to detect the occurrence of an oxidative stress by the determination of the plasma malondialdehyde (MDA) concentration and the erythrocyte glutathione peroxidase (GSH-Px) and catalase (CAT) activities in cows with prolapsus uteri, caesarean section, and retained placenta. A total of 46 Holstein cows (15 cows with normal parturition, 15 cows with retained placenta, 8 cows with prolapsus uteri, 8 cows with caesarean section) of the Research and Practice Farm of Firat University were used in this study. CAT activity did not significantly vary in diseased animals (p > 0.05), while in cows with prolapsus uteri and caesarean section, plasma MDA concentrations significantly increased (p < 0.001), and GSH-Px activity was significantly lowered compared to the control group or to cows with retained placenta (p < 0.01). In retained placenta cows, plasma MDA concentrations and GSH-Px activity were not significantly altered although the enzyme activity tended to increase. These results suggest that the antioxidant systems would be impaired and peroxidation reactions accelerated in cows with prolapsus uteri and caesarean section.

Keywords : Malondialdehyde - glutathione peroxidase - catalase - cow - prolapsus uteri - retained placenta - caesarean section.

RÉSUMÉ

Variations des concentrations plasmatiques de malondialdéhyde, et des activités anti-oxydantes intra-érythrocytaires chez des vaches présentant un prolapsus utérin, une rétention placentaire ou ayant subi une césarienne. Par M. ERISIR, Y. AKAR, S. Y. GURGOZE et M. YUKSEL.

La période péri-partum est une période particulièrement critique pour la santé et les performances zootechniques des vaches laitières. Le but de cette étude était de détecter la survenue d’un stress oxydatif en déterminant les concentrations plasmatiques de malondialdéhyde (MDA) et les activités intra-érythrocytaires de la glutathion peroxydase (GSH-Px) et de la catalase (CAT) chez des vaches présentant une rétention placentaire, un prolapsus utérin ou ayant subi une césarienne. Au total, 46 vaches Hostein (15 avec un part normal, 15 ayant une rétention placentaire, 8 un prolapsus utérin, et 8 avec césarienne) issues de la ferme de l’Université Firat ont été utilisées dans cette étude. L’activité CAT n’a pas significativement varié chez les animaux malades (p > 0.05) alors que, chez les vaches ayant eu un prolapsus utérin ou une césarienne, les concentrations plasmatiques de MDA ont significativement augmenté (p < 0.001) et l’activité GSH-Px a été réduite par rapport aux contrôles ou aux animaux ayant une rétention placentaire (p < 0.01). Dans ce dernier groupe, les concentrations plasmatiques de MDA et les activités GSH-Px n’ont pas été significativement altérées, bien que l’activité enzymatique semblait accrue. Ces résultats suggèrent que les systèmes anti-oxydants pourraient être défectueux et les réactions de peroxydation accélérées chez les vaches avec un prolapsus utérin ou une césarienne.


Introduction

Reactive oxygen species (ROS) are unavoidable intermediates which are produced during metabolism. Their concentration and balance between their production and degradation are controlled by enzymatic and non-enzymatic defence mechanisms [27].

The natural defence mechanisms against free radicals consist of enzymatic antioxidant agents like glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) and non-enzymatic antioxidants like glutathione, ascorbate, urate, vitamin E and beta carotene [21].

GSH-Px (EC 1.11.1.9) is the most effective antioxidant in erythrocytes and catalyses the reduction of hydrogen peroxide and organic peroxides like lipid peroxides. CAT (EC 1.11.1.6) together with GSH-Px is responsible for the degradation of hydrogen peroxide [32].

Lipid peroxidation is a non-enzymatic chain reaction based on oxidation of mainly unsaturated fatty acids and is associated with the presence of ROS. It leads to the creation of lipid peroxides and other intermediates. These intermediates may influence the properties of cell membranes and their physiological functions [11]. The most common of these intermediates are malondialdehyde (MDA) and 4-hydroxynonenal [8].

Disturbances during the periparturient period in cows are one of the most important problems in veterinary practice, mainly because of economic losses. Retained placenta (RP) is frequently observed in Holstein cattle after parturition and may cause reduction of milk production, delay of the first
oestrus, failure to conceive, prolongation of calving intervals, and of course, expensive treatment [21]. Caesarean section (CS) which commonly occurs in cows is finally made in dystocia. It may cause various postoperative complications such as evagination, uterin adhesions, amphiysema, haemorrage unless adequate pre- and postoperative care and hygiene are taken into action [26]. Prolapus uteri (PU) is a commonly encountered disease in the cattle and its rate has been reported to vary between 0.2 and 3 %. It is also an important disease, since it causes delayed uterus involution and chronic endometritis in puerperal and following period, leading to a low fertility [24].

In order to establish a link between retained placenta, prolapsus uteri and caesarean section and complications commonly observed, the antioxidant status of peri-parturient cows was explored through the measurement of plasma MDA concentrations and of 2 erythrocyte anti-oxidant enzymes (GSH-Px, CAT).

Materials and methods

ANIMALS AND SAMPLES

Holstein cows (2.5-3.5 years old) of the Research and Practice Farm of Firat University were used in the study. Fifteen cows without any puerperal disorder and with normal parturition were considered as the control group. Fifteen cows that were unable to discharge foetal membranes totally or partially within 24 hours postpartum were considered to have retained placenta (RP). The other groups were consisted of 8 cows with prolapsus uteri (PU) and 8 cows with caesarean section (CS).

Jugular blood samples were collected into sterile blood collecting tubes with heparin from all cows between 24th and 72th hours after delivery.

BIOCHEMICAL ANALYSES

Erythrocyte GSH-Px activity was measured by the method of BEUTLER [4] in which cumene hydroperoxide was used as substrate. Oxide glutathione (GSSG) produced by the action of erythrocyte GSH-Px and cumene hydroperoxide, was reduced by glutathione reductase (GSH-az) and NADPH. The decrease of the NADPH concentration was measured at 340 nm. The enzyme activity in erythrocytes was expressed as units per gr of Hb (U/ g Hb).

Erythrocyte CAT activity were determined according to the method of AEBI [1] and expressed as k/ g Hb. The decomposition of H2O2 can be directly followed by the decrease of absorbance at 240 nm. The difference in absorbance at 240 nm per time unit allows determining the CAT activity.

The end-product of polyunsaturated fatty acid peroxidation, MDA, which reacts with thiobarbituric acid in serum samples, was determined by the methods of SATOH [25] and YAGI [35]. Reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) has been applied widely to assess lipid peroxidation in biological material. The reaction yields to a red MDA-TBA adduct, the product of 2 mol of TBA plus 1 mol of MDA. The coloured complex can be quantified spectrophotometrically from its visible absorbance (max 532 nm) and is readily extractable into organic solvents such as butanol. The values of MDA reactive material was expressed as MDA quantities for plasma volume (nmol / ml plasma).

STATISTICAL ANALYSIS

Results were expressed as mean ± SEM. Analysis of variance (ANOVA) followed by Duncan test were used to determine whether there were significant differences among the groups. Differences were considered as significant when p values were less than 0.05.

Results

No statistically significant difference was found for CAT activity in all the groups (P>0.05). In cows with prolapsus uteri and caesarean section, plasma MDA concentrations were significantly higher than in the control group (P<0.001), whereas they were not altered in the RP group. The GSH-Px activities were significantly depressed in the CS group compared to the control and RP groups (P<0.01). This parameter was also reduced in cows with PU (retained placenta cows vs. prolapsus uteri cows: p < 0.01) but the difference with the healthy cows was not significant. On the contrary, GSH-Px activity was slightly increased but not significantly in the RP group in comparison to controls (Table I).

![Table I. — Plasma MDA concentrations and erythrocyte GSH-Px and CAT activities in healthy cows and in cows with retained placenta (RP), prolapsus uteri (PU) and caesarean section (CS) between 24th and 72th hours after delivery. Results are expressed as means ± standard errors.](image)
Discussion

Oxidant and antioxidant status in fetal and maternal parts of bovine retained and not-retained placenta has already been investigated [13, 15, 34], but only few reports [6, 21] have studied the blood oxidant and antioxidant status of cows with retained placenta during the last 6 weeks of gestation. There is no study in which antioxidant enzyme activities were assessed during prolapsus uteri and caesarean section, although these 2 situations frequently induced an exacerbated inflammatory reaction leading to delayed uterus involution and chronic endometritis in puerperal and following period [24, 26].

Aetiology of RP is not yet understood: the biochemical mechanisms involved in proper release or retention of fetal membranes in cows still require clarification. In last years, RP in cows is supposed to be connected with an imbalance between production and neutralisation of reactive oxygen species in placenta [13, 15, 16, 34]. There are reports indicating some changes in antioxidative defence systems of placenta in cases of foetal membranes retention [13, 14, 34] as well as alterations in lipid peroxidation products [15].

WISCHRAL et al. [34] reported that, i) in RP cows, the SOD activity in the foetal placenta decreased in comparison to controls (no RP) whereas CAT activity was not modified, ii) in the maternal placenta, an increase of CAT activity was observed while SOD activity did not vary and iii) the GSH-Px activity and the TBARS contents were not modified in the 2 placentas. Differently, KANKOFER [15] evidenced an increase of lipid peroxidation metabolites in placenta of RP group compared to no RP group. Moreover, this author also demonstrated [13] that the GSH-Px activity in the placenta was significantly increased in cows with RP compared to no RP cows in peri-partum period, while CAT activity was more variable. Besides, by achieving caesarean sections before the term or at term and by comparing placenta GSH-Px and CAT activities, he has shown that the enzyme activities in placenta enhanced at the end of pregnancy and at term, suggesting that eventual imbalance between production and neutralization of ROS would appear closely to, or at parturition. In blood, the antioxidant status of cows with RP also changed [6, 21]. Before parturition, the GSH-Px activity in red blood cells was significantly lowered in diseased animals compared to controls (no RP) on 2 and 0 weeks before expected calving. However, TBARS in red blood cells did not differ in cows with or without RP [6]. MILLER et al. [21] reported that the total antioxidant activity of plasma did not differ between RP and no RP cows 6 and 4 weeks before parturition. However, 2 weeks before and at calving, antioxidant activity decreased in RP animals. By contrast, in the present study, GSH-Px activity tended to increase, but not significantly compared to controls and CAT activity and MDA concentrations remained unchanged in RP group between 24th and 72th hours after delivery. Elevated GSH-Px activity in erythrocytes of RP group may indicate the free radical formation and the beginning of an oxidative stress.

There are accumulated evidence that free radicals are involved in the physiology of the reproductive system [3, 5, 18, 22, 23, 31], and many studies showed the effects of free radicals in the inflammatory diseases in different species. While some studies have reported a reduction of the GSH-Px activity in the inflammatory diseases [2, 30, 33], some others have indicated either no significant change [7] or an increase [19, 20]. The reports on the MDA concentrations during inflammation are also controversial: some investigators [2, 7, 12, 20, 30] showed increases, while some others [9, 33] reported no any significant change during inflammatory diseases. In the present study, a significant increase (p<0.001) of plasma MDA concentrations was observed in cows with prolapsus uteri, while CAT activity remained unchanged, and GSH-Px activity decreased. In the same way, plasma MDA concentrations in cows with caesarean section was significantly higher than in the control group (P<0.001), and GSH-Px activity was significantly depressed (P<0.01). Our results were in agreement with other investigations [17, 28, 29] that reported an increase of plasma MDA concentration and a decrease of antioxidant enzyme activity at the beginning of postoperative period after abdominal operations. Consequently, these results suggest that prolapsus uteri or caesarean section induce an inflammatory reaction 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.

Oxidative stress is a secondary factor in most diseases. The resultant antioxidative defence mechanisms against ROS, although activated, might be not enough efficient and clinical symptoms of illness may occur [10]. Deficiencies of natural protective substances or excess exposure to substances that can stimulate ROS production may impair health and performance. Therefore, cows with prolapsus uteri and caesarean section would require supplement of non-enzymatic antioxidants because of inadequate antioxidant system against ROS. The assessment of ROS generation intensity process may help in understanding of molecular processes observed in the intracellular environment after operations. The evaluation of antioxidant level may give an answer about the efficiency of antioxidative defence mechanisms [28].

Our findings give support that radical scavenging enzymatic system, GSH-Px, is consumed by the increased lipid peroxidation in prolapsus uteri and caesarean section. Results suggest that the antioxidant system is impaired and peroxidation reactions are accelerated in patients with prolapsus uteri and caesarean section.

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