VETERINARSKI ARHIV 83 (2), 153-159, 2013

The effects of Caesarean section on lipid peroxidation and some antioxidants in the blood of newborn calves

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ERISIR, M., F. M. KANDEMIR, M. YÜKSEL: The effects of Caesarean section on lipid peroxidation and some antioxidants in the blood of newborn calves. Vet. arhiv 83, 153-159, 2013.

ABSTRACT

The mode of delivery may have a considerable effect on the state and health of the newborn. Therefore, the purpose of this study was to investigate the effect of a Caesarean section on oxidant and antioxidant status in newborn calves. This study included the neonates of 8 normally calved cows and 8 cows who had undergone by Caesarean section. Malondialdehyde concentrations were significantly higher in the calves delivered by caesarean section compared to the calves of normally calved cows, but catalase activity was significantly lower ($P<0.01$). Glutathione concentrations tended to increase in the calves delivered by Caesarean section compared to the calves of normally calved cows. There were no significant differences in the glutathione peroxidase activity between the groups. Our results suggest that the malondialdehyde concentrations and catalase activities, which show lipid peroxidation and antioxidant status in newborn calves, change due to Caesarean section. In conclusion, newborn calves are exposed to higher oxidative stress in Caesarean section.

Key words: Caesarean section, newborn calves, oxidant and antioxidant status

Introduction

Reactive oxygen species (ROS) such as superoxide radical anion, hydroxyl radical, and hydrogen peroxide are produced in metabolic and physiological processes, and their harmful oxidative reactions may occur in organisms. The oxidative effects of ROS are controlled by exogenous antioxidants, such as vitamins E and C, and also by endogenous antioxidants, such as scavenger enzymes (i.e. glutathione peroxidase and superoxide

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ISSN 0372-5480
Printed in Croatia

dismutase), glutathione, and uric acid. Under some conditions, increases in oxidants and decreases in antioxidants cannot be prevented, and the oxidative/antioxidative balance shifts toward the oxidative status. Blood contains many antioxidant molecules that prevent and/or inhibit harmful reactions of free radicals that lead strongly to oxidative damage of biomolecules, such as lipids, proteins, and DNA (SAUGSTAD, 2003).

Gestation is a physiological state that increases oxygen demand and requires high energy for various body functions. These increments, in taking and using oxygen, lead to alterations in the oxidant-antioxidant balance, particularly towards the oxidant side, eventually causing oxidative stress. During parturition, this stress increases more profoundly (GITTO et al., 2002; ERISIR et al., 2009).

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 the dairy industry. It is one of the most serious complications of pregnancy in cattle (OAKES et al., 2001). The process of parturition, though physiological, is a stressful event, and abnormal parturition (dystocia) further adds to the normal stress of calving (NAKAO and GRUNET, 1990).

The process of birth potentially induces oxidative stress in the newborn (PAAMONI-KEREN et al., 2007; GITTO et al., 2009). Umbilical arterial lipids are more susceptible to peroxidation than umbilical vein lipids, indicating high oxidative stress in the fetal circulation, irrespective of the mode of delivery (FOGEL et al., 2005). The transition from the fetal to the neonatal environment exposes the newborn to a more oxidative environment (PAAMONI-KEREN et al., 2007). The mode of delivery may have a considerable effect on the state and health of the newborn (GITTO et al., 2009). Therefore, the purpose of this study was to investigate the effect of Caesarean section (CS) on oxidant and antioxidant status in newborn calves and to demonstrate whether this mode of delivery leads to more oxidative stress in neonates.

Materials and methods

Animals and samples. Sixteen parturition cows, ranging in age from 3 to 8 years old, were used in this study. The cows were admitted to the Firat University Veterinary Faculty Obstetrics and Gynaecology Department.

The study included the neonates of 8 normally calved cows (4 Simmental, 4 Montofon) and 8 cows who had undergone Caesarean section (4 Simmental, 4 Montafon). Blood of normally calved and CS affected cows' newborn calves was taken from the jugular vein in the first half hour after birth. All blood samples were collected to sterile blood collecting tubes, with heparin from all the newborn calves. Whole blood was separated for GSH-Px 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 the plasma was measured by the thiobarbituric acid reacting substance) method (TBARS) (PLACER et al., 1966), and was expressed in terms of the malondialdehyde (MDA) content, which served as the standard of 1,1,3,3-tetraethoxypropane. Values were expressed as MDA equivalents in $nmol \cdot mL^{-1}$ plasma.

Whole blood Glutathione Peroxidase (GSH-Px; EC 1.11.1.9) activity was assayed by the method of LAWRENCE and BURK (1976) and expressed as unit per g of Hb ($U.g^{-1}$ Hb). GSH-Px activity was determined in the presence of GSH and cumene hydroperoxide substrates, using an end-point direct assay. The activity was expressed as loss of reduced GSH/min.

Erythrocyte Catalase (CAT; EC 1.11.1.6) activity were determined according to the method of AEBI (1987) and expressed as $kat \cdot g^{-1}$ Hb. The principle of the assay is based on the determination of the constant rate or the H_2O_2 decomposition rate at 240 nm. Results were expressed as k (rate constant) / g Hb protein.

The concentration of reduced glutathione (GSH) was assayed by the method of BEUTLER et al. (1963) and expressed as $\mu mol \cdot g^{-1}$ Hb. This method is based on the capacity of sulphhydryl groups present in whole blood to react with 5, 5'-dithiobis-(2-nitrobenzoic acid) (Ellmann's reagent) and form a yellow dye, with maximum absorbance at 412 nm.

Haemoglobin (Hb) concentration was determined according to the cyanmethaemoglobin method (FAIRBANKS and KLEE, 1986).

Statistical analysis. Results were expressed as mean ± SEM. 2- Independent samples followed by the Mann-Whitney U test was used to determine whether there were significant differences among the groups. Differences were considered significant when P values were less than 0.05.

Results

MDA concentrations were significantly higher in the calves of CS affected cows compared to the calves of normally calved cows, but CAT activity were significantly lower ($P<0.01$). GSH concentrations tended to increase in the calves of CS affected cows, compared to the calves of normally calved cows. There were no significant differences in the GSH-Px activity between the groups.

Table 1. Whole blood glutathione peroxidase (GSH-Px), erythrocyte catalase (CAT) activities and plasma malondialdehyde (MDA), erythrocyte glutathione (GSH) levels in normally calved (n = 8) and newborn calves delivered by Caesarean section (n = 8)

	Normally calved newborn calves			Caesarean section newborn calves			
	Mean ± SEM	Min	Max	Mean SEM	Min	Max	P
MDA (nmol/mL)	8.92 ± 0.37	7.44	9.97	10.54 ± 0.21	9.72	11.18	**
CAT (k/g Hb)	47.01 ± 3.91	31.47	61.43	31.25 ± 3.20	20.55	42.42	**
GSH-Px (U/g Hb)	14.14 ± 0.71	12.83	18.28	13.42 ± 0.72	11.40	16.33	NS
GSH (μmol/g Hb)	4.45 ± 0.67	2.02	6.59	5.80 ± 0.70	2.70	7.48	NS

Values with different superscripts within the same line were significant (P<0.05); NS: Non significant; ** P<0.01

Discussion

Newborns are especially prone to oxidative stress. The reasons for this are several. Infants very often (a) are exposed to high oxygen concentrations, (b) have infections or inflammation, (c) have reduced antioxidant defense, and (d) have high levels of free iron which enhances the Fenton reaction, leading to the production of highly toxic radicals (GITTO et al., 2009).

The mode of delivery may also have a considerable effect on the state and health of the newborn. In humans, controversial information has been available on the effects of CS on oxidant status in newborns. Some studies (VAKILIAN et al., 2009; SRIDHAR et al., 2007; INANC et al., 2005) have found a significant increase in TBARS in the cord blood of newborns following vaginal delivery (VD) compared to newborns by elective CS. In contrast, in various studies (HRACSKO et al., 2007; MUTLU et al., 2011; BUONOCORE et al., 1998) have reported that lipid peroxidation and total oxidant status in the neonatal cord blood and newborns were significantly higher in babies delivered by CS as compared to VD. In the present study, we also found that MDA concentrations were significantly higher in the calves delivered by CS compared to calves normally calved. This was probably due to indications of higher oxidative stress in calves born by CS. Likewise, a significant increase of plasma MDA concentrations was observed in cows after CS (ERISIR et al., 2006). High levels of oxidative stress product and low antioxidant in newborns could also be a consequence of such products in the mother's blood (TURK et al., 2008).

The higher MDA elevation in neonates due to the CS may be the cause of some neonatal diseases. Free radicals have been reported to play an important role in the pathogenesis of several pathological conditions, such as haemolytic disease of the newborn, bronchopulmonary dysplasia, and retinopathy of prematurity. Indeed, neonates born by CS have an increased incidence of these conditions (HALLIWELL and GUTTERIDGE, 1999).

The body has a defense system to counteract damage produced by free radicals. The cellular, extracellular, and membranous antioxidant substances included in the antioxidant system react very rapidly with radicals in order to prevent the progression of autooxidation/peroxidation. In various studies fetal oxidative stress in the cord blood of fetuses born by spontaneous vaginal delivery (VD) was compared to that in fetuses born by elective cesarean delivery (CD) in humans, by measuring the umbilical cord venous blood antioxidants. Some studies reported a reduction of CAT and GSH-Px activities (GEORGESON et al., 2002), whereas others reported an increase in superoxide dismutase (SOD) and CAT activities (INANC et al., 2005), or non-significant changes of CAT, GSH-Px, SOD (HRACSKO et al., 2007) activities in the elective CD babies compared to VD. Likewise, in various studies low total antioxidant capacity has been found in both cord blood (MUTLU et al., 2011) and newborns (VAKILIAN et al., 2009; MUTLU et al., 2011) in elective CS cases in humans. Newborns of diabetic mothers delivered by CS also showed lower total antioxidant capacity than those delivered vaginally (RAJDL et al., 2005). In the present study, CAT activity was significantly higher in the normally calved calves compared to the CS calves. This may indicate insufficiency and/or depletion of antioxidant response due to high oxidative stress in the calves born by CS. In normal births, the formation and inactivation of free oxygen radicals is balanced. After the changes in the antioxidants' defense enzyme activities, the balance in calves born by CS may be unstable.

Reduced glutathione is an important major antioxidant in red blood cells (PAAMONI-KEREN et al., 2007). GSH levels in reports in humans are also variable (PAAMONI-KEREN et al., 2007; VAKILIAN et al., 2009; RAIJMAKERS et al., 2003). In the present study, GSH concentrations tended to increase in the calves born by CS compared to the normally calved calves. PAAMONI-KEREN et al. (2007) suggest that GSH levels may be indicative of the fetal oxidative stress status. We may consider that each antioxidant may be affected differently in calves born by CS.

In conclusion, our results showed that systemic oxidative stress exists, which is evidenced by the evaluation of the MDA concentration and the reduction of CAT activity in calves born by Caesarean section. Calves born by Caesarean section are at risk of free radical damage and are susceptible to the deleterious effects of free radicals, as evidenced by the evaluation of MDA concentrations. Antioxidant supplementation for calves born by Caesarean section may be useful to prevent oxidative stress related problems.

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Received: 23 March 2012
Accepted: 5 December 2012

ERISIR, M., F. M. KANDEMIR, M. YÜKSEL: Učinci carskog reza na peroksidaciju lipida i neke antioksidanse u krvi netom oteljene teladi. Vet. arhiv 83, 153-159, 2013.

SAŽETAK

Način porođaja može znatno utjecati na stanje i zdravlje novorođenčeta. Stoga je svrha ovoga rada istražiti učinak carskoga reza na oksidacijsko i antioksidacijsko stanje u netom oteljene teladi. Istraživanje je provedeno na osam normalno oteljene teladi i na osam teladi oteljene carskim rezom. Koncentracije malondialdehida bile su značajno veće u teladi oteljene carskim rezom nego li u prirodne oteljene teladi, ali je aktivnost katalaze bila značajno slabija ($P<0,01$). Koncentracije glutationa bile su povećane u teladi oteljene carskim rezom u odnosu na prirodno oteljenu telad. Nisu bile ustanovljene značajne razlike u aktivnosti glutation peroksidaze među dvjema skupinama. Rezultati upućuju na zaključak da su koncentracije malondialdehida i aktivnost katalaze kao pokazatelji lipidne peroksidacije i antioksidacijskog stanja u novorođene teladi bile promijenjene zbog carskog reza. To govori da je netom oteljena telad zbog carskog reza izložena jačem oksidacijskom stresu.

Ključne riječi: carski rez, telad, oksidacijsko i antioksidacijsko stanje