The relationship between lipid peroxidation intensity and total antioxidant capacity in cases of spontaneously released and retained bovine placenta.

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SUMMARY

The retention of foetal membranes (RFM) is supposed to be related to oxidative stress which can be evaluated through indirect and direct parameters, among others, total antioxidant capacity (TAC) and lipid peroxidation intensity (LPI).

Placental samples (maternal and foetal parts) were collected from Holstein-Friesian healthy cows immediately after caesarean section (n = 13, group A) at time or spontaneous delivery (n = 10, group C) and from RFM affected cows after caesarean section (n = 15, group B) or spontaneous delivery (n = 8, group D), and TAC and LPI were measured spectrophotometrically in corresponding supernatants obtained from placenta parts. The TAC values were lowered in the healthy cows (without RFM) surgically delivered (group A) compared to the group C (spontaneous delivery) (p < 0.05) while the LPI was similar whatever the mode of delivery. The TAC and LPI values were markedly increased in both maternal and foetal placenta of RFM affected cows (groups B and D) compared to the respective controls (groups A and C) (p < 0.05). The LPI was more intense in the foetal part than in the maternal placenta (p < 0.05) whatever the mode of delivery or the occurrence of RFM whereas the TAC was higher in the maternal part except in cases of spontaneous delivery with RFM (group D).

The results confirm the occurrence of oxidative stress during RFM particularly in the foetal part but the relationship between oxidative/antioxidative processes in bovine placenta still needs clarification.

Key words : cow, retained placenta, caesarean section, oxidative stress, total antioxidant capacity, lipid peroxidation intensity.

Introduction

The balance between reactive oxygen species (ROS) production and neutralisation is crucial for proper metabolic pathways and biochemical processes of living organisms. The proper balance is maintained by concert action of enzymatic and non-enzymatic antioxidant defence systems [19]. Any alteration in this balance leads to cellular dysfunction and cell membrane damage and in consequence to oxidative stress and clinical symptoms of disease [5, 16].

The retention of foetal membranes (RFM) is one of the most important problems of peri-parturient period in cows. It is supposed to be connected with oxidative stress conditions which in result may influence hormonal balance and the process of proper separation of foetal membranes. Alterations appear not only in metabolic pathways but also in function of cell membranes. Disturbances in the steroid hormone and prostaglandin concentrations were described during RFM not only in general circulation but also in...
placental tissues [4, 14, 20]. RFM affects not only a cow but also newborn and causes serious economic losses.

The control of proper antioxidant/oxidative balance can be performed by the estimation of intensity of peroxidative processes. The determination of intermediates or end products as well as indirect methods can be used. Activity of antioxidant systems can be considered in terms of the determination of single antioxidant or by measurement of total antioxidant capacity (TAC). Both ways have advantages and disadvantages but TAC seems to be of clinical meaning due to easy, not time consuming and not expensive method.

The aim of the present study was to compare the method for determination of lipid peroxidation based on the use of diethyl-p-phenylenediamine (DEPPD) with the total capacity of antioxidant systems measured by the reaction with 2,4,6-tri-pyridyl-s-triazine with respect to spontaneously released and retained bovine placenta as well as different modes of delivery.

Material and methods

ANIMALS AND PROTOCOL DESIGN:

A total of 46 Holstein-Friesian, 2-6 years old, pregnant cows were included in this study. Days of gestation were calculated using dates of insemination and caesarean sections for all the cows included in this study. Days of gestation were calculated using dates of insemination and caesarean sections (groups A, B, C, D). The cows were divided into four groups according to the time of expulsion and the mode of delivery which were averaged and subjected to statistical analysis. Significance of differences between examined groups were determined by t-Student test and analyses of correlations were calculated by use of Statgraph 5.0 and Statistica 5.0 programme. A p value < 0.05 was considered as significant.

Material and methods

Results

The results of the total anti-oxidant capacity (TAC) determinations are graphically presented in figure 1. The TAC values were significantly lower in foetal part than in maternal parts of bovine placenta except for the group D (spontaneous delivery with RFM, maternal parts : 45.85 ± 6.39 μmol/g proteins). Cases with RFM (spontaneous delivery (group D) or caesarean section (group B) showed significantly higher values compared to healthy animals (without RFM, groups C and A) (p < 0.05) and healthy animals which delivered spontaneously (group C) had significantly higher TAC values than the healthy cows undergoing caesarean section (group A).
(p < 0.05) whatever the placenta origin (maternal or foetal parts). Nevertheless, the TAC values of placenta foetal parts obtained in cows spontaneously delivered with RFM (group D) were increased compared to cows submitted to caesarean section with RFM (group B) (p < 0.05) while maternal parts showed similar values.

The lipid peroxidation intensity (LPI) was significantly higher in foetal than in maternal parts of placenta in all examined groups (p < 0.05) (figure 2). Control animals delivered spontaneously (group C) or by caesarean section (group A) showed similar hydroperoxide concentrations in placenta maternal or in foetal parts. In cows suffering from RFM, the LPI measured in maternal and foetal parts were similar in animals submitted to caesarean section (group B) and in those spontaneously delivered (group D). Moreover, values in maternal and foetal parts evidenced in cases with RFM (groups B and D) were higher than those of the respective controls (groups A and C) (group B vs. group A and group D vs. group C : p < 0.05).

The TAC values positively correlated between maternal and foetal parts from placenta of healthy cows spontaneously delivered (group C, r = 0.92, p < 0.01) and of RFM affected cows delivered with caesarean section (group B, r = 0.99, p < 0.01) (figure 3), whereas no correlation was evidenced in the group A (healthy cows with caesarean section), or in the group D (RFM affected cows with spontaneous delivery). Furthermore, TAC values did not correlate between foetal and maternal placenta neither in the overall cows without RFM (group A + C), with RFM (group B + D), with spontaneous delivery (group C + D) nor with surgical delivery (group A + B).

For LPI, no correlation between values measured in foetal parts and those measured in maternal parts was evidenced whatever the considered group (groups A, B, C and D ; groups [A + C], [B + D], [C + D] and [A + B]).

Finally, LPI and TAC values significantly correlated in the maternal placenta from cows surgically delivered (group A + B), r = 0.54, p < 0.05, whereas no correlation was obtained between TAC and LPI values measured in foetal parts in any group.

Revue Méd. Vét., 2006, 157, 8-9, 405-409
Discussion

RFM is a complex syndrome appearing after parturition where many biochemical disturbances can be detected: changes in the concentrations of steroid hormones as well as prostaglandins are, among others, clinically detected symptoms of RFM [4, 14, 20].

Phospholipids which serve as a source of fatty acids can come from processes of lyses of epithelial cells of maternal crypts that occur before parturition. Histological and histochemical studies have shown lower amounts of lipid droplets in trophoblast cells and a lack of caruncular epithelial cell lysis in cows affected with RFM [18]. Besides, chromatographic determinations of placental fatty acids demonstrated significantly lower concentrations of arachidonic acid in RFM compared to control animals. The amount of this fatty acid was higher in maternal than in foetal part of placenta. On the other hand, linoleic acid concentration increased in RFM cows in maternal as well as in foetal part of placenta compared to healthy animals [7]. As unsaturated fatty acids are highly susceptible to peroxidative processes, the exacerbation of lipid peroxidation in placenta evidenced in RFM animals in the present study may be responsible for these disturbances. Unsaturated fatty acids, mainly arachidonic acid, serve as the precursors for prostaglandins (“arachidonic acid cascade”). Acids with peroxidation damage are not able to initiate this biochemical pathway. Moreover, although certain amounts of ROS are necessary for this cascade, their excess may inactivate some of cascade enzymes. All together it may directly or indirectly influence the concentrations of prostaglandins.

The intensity of lipid peroxidation previously measured by the use of different markers and methods, i.e. thiobarbituric acid reactive substances, showed similar results, confirming that imbalance between production and neutralisation of ROS appears during RFM [8]. The question, however, is still open whether alterations in fatty acid concentrations precede ROS imbalance or whether ROS imbalance leads to changes in fatty acid concentrations. The consequences of ROS imbalance are related not only to direct toxic effects of peroxidation products such as malondialdehyde, 4-hydroxyenal or hydroperoxides but also to alterations of some metabolic pathways and disturbances in cell membrane functions. Except from fatty acids, other macromolecules such as proteins or nucleic acids are susceptible to peroxidative damage as well. The determination of markers of protein and nucleic acid peroxidative damage has shown alterations in their intensity between retained and properly released bovine foetal membranes [9, 12].

Antioxidant defence against ROS relies on compounds using different mode of action in order to neutralize ROS but the cooperation between these compounds is indispensable. The estimation of the efficiency of the antioxidant defence systems can be analysed by the determination of single components of each system or by the total antioxidant capacity, called TAC. Due to the heterogeneity of compounds implicated in defence systems, different methods based on different chemical properties of these compounds were developed [6]. One of them based on TPTZ (Tripyridyl Triazine) reactivity was used in this study in order to find correlation between lipid peroxidation intensity and total antioxidant capacity. Although significant differences in TAC were evidenced according to the mode of delivery (spontaneous delivery and caesarean section) and between RFM and control animals, correlations between the 2 examined parameters were not found except in the maternal placenta from cows undergoing caesarean section. In the same way, the differences in superoxide anion release between women delivering spontaneously and by caesarean section were detected [3]. The disadvantage of the method based on TPTZ is the low pH of the incubation mixture which does not allow a proper estimation of the contributing thiol groups. In result, the whole spectrum of antioxidant system could be under-estimated. It could be the reason why no other correlation was detected. On the other hand, the LPI and TAC differences between examined groups were more important.

Previous determinations of single antioxidant enzymes as well as non-enzymatic antioxidants gave additional evidence for imbalance between production and neutralisation of ROS in bovine retained and released placenta [10, 11]. Experiments of other authors based on dietary supplementation of pregnant cows with antioxidants showed the decrease of percentage of RFM-affected cows, confirming in this way the presence of oxidative stress conditions [13, 17].

In conclusion, further experiments are necessary for investigating the relationships between ROS production/lipid peroxidation and ROS neutralization/antioxidant capacities in RFM affected cows. Furthermore, the biochemical alterations responsible for ROS imbalance and those leading to clinical symptoms of RFM should be elucidated.

References


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