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Total antioxidant capacity of bovine spontaneously released and retained placenta

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Abstract

Exposure of living organisms to a constant generation of reactive oxygen species (ROS) resulted in the development of antioxidative defence systems which protect cells and tissues against their harmful effects.

The retention of fetal membranes (RFM) in cows is hypothesized to be connected with the imbalance between production and neutralization of ROS.

The efficiency of enzymatic and non-enzymatic antioxidative systems can be detected by the determination of single components of this system or by so-called total antioxidant capacity (TAC). In the present study, total antioxidant capacity was compared with previously measured parameters of antioxidative defence mechanisms in placental tissues of cows with respect to time of fetal membranes expulsion and mode of delivery. Placental samples were divided into: (A) caesarian section before term (272–277 days of pregnancy) without RFM ($n = 9$), (B) caesarian section before term with RFM ($n = 14$), (C) caesarian section at term (282–288 days of pregnancy) without RFM ($n = 12$), (D) caesarian section at term with RFM ($n = 16$), (E) spontaneous delivery at term without RFM ($n = 8$), (F) spontaneous delivery at term with RFM ($n = 8$).

TAC was measured spectrophotometrically at 593 nm by use of 2,4,6-tri-pyridyl-*s*-triazine in homogenates of maternal and fetal part of placenta and expressed as μmol/g protein (mean ± S.E.M.).

The values of TAC were significantly higher ($P \leq 0.05$) in the fetal than in maternal part in preterm samples (A – maternal: 27.24 ± 4.17 μmol/g prot, fetal: 63.67 ± 18.16, B – maternal: 49.80 ± 5.11, fetal: 70.96 ± 13.23). The opposite relationship was noticed in term samples. Significantly higher values were observed in retained than in not retained placental tissues (C – maternal: 32.40 ± 6.12, fetal: 16.29 ± 3.97, D – maternal: 48.17 ± 6.91, fetal: 27.92 ± 4.72, E – maternal: 40.55 ± 2.66, fetal: 27.90 ± 1.23, F – maternal: 45.85 ± 6.40, fetal: 43.50 ± 4.61).

Values of TAC are comparable with previously determined single parameters of antioxidative defence mechanisms in placental tissues and may be of clinical importance. Whether they reflect plasma values as well requires further evaluation.
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Keywords: Total antioxidant activity; Bovine placenta; Retention of fetal membranes

1. Introduction

Aerobic metabolism is connected with incessant generation of reactive oxygen species (ROS). Although certain amounts of ROS are indispensable, their excess might be dangerous for cells and tissues. Thus effective mechanisms protecting cells from the imbalance between production and generation of ROS are important [1].

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Living organisms are endowed with non-enzymatic, water and lipid soluble components as well as enzymatic defence mechanisms against ROS. Only the concert action of both systems based on current challenge of adequate tissue can guarantee the proper response. When ROS formation exceeds the capacity of antioxidative mechanisms to neutralize them, various pathologies may appear [2,3].

One of such pathologies, where ROS might be involved is the retention of fetal membranes in cows (RFM). It affects cows by endometritis as well as causes a delay to next ovarian cycle and pregnancy and causes serious economic losses. There is evidence that some parameters of oxidative stress are altered in cases of RFM as compared to properly released placenta. Non-enzymatic and enzymatic components of antioxidative defence mechanisms against ROS showed different patterns in maternal and fetal part of retained placenta as compared to control animals [4,5]. Processes of peroxidative damage to biomolecules such as lipids, proteins and DNA occurred and showed an increase in their intensity in retained membranes as compared to not retained tissues [6–9].

There is evidence that selenium and Vitamin E supplementation before parturition can improve the ratio of affected cows confirming possible participation of ROS in RFM [10].

The alteration in prostaglandin metabolism, detected in RFM cows, may provide another piece of evidence for ROS involvement in the ethiopathology of RFM [11,12].

Antioxidative status of tissues can be described by the analysis of single components in defence systems against ROS as well as by the determination of total antioxidant capacity. Both ways show advantages and disadvantages. The components of these defence systems act synergistically and their activities should not be considered separately. The methods for total antioxidant capacity, which use a variety of detection systems, do not cover the whole spectrum of chemical properties of antioxidants [13]. However, these methods are easy, not time consuming and, once correlate with other parameters of oxidative stress, may have clinical meaning.

The question was stated whether the determination of total antioxidant capacity of placental maternal and fetal homogenates will reflect previously measured single components of antioxidative defence mechanisms.

The aim of present study was to describe total antioxidant capacity of placental tissue directed against different possible oxidants and to compare this capacity between tissues obtained after spontaneous delivery, caesarian section as well as retained and properly released placenta. The relationship between total antioxidant capacity and components of antioxidative defence mechanisms was considered.

2. Material and methods

Pregnant cows ($n = 67$) included in this study were clinically healthy and of Holstein–Friesian breed, 2–6 years old. Days of gestation were calculated using dates of insemination. Preterm caesarian sections were indicated for teaching purposes, term sections because of fetus oversize.

Placentomes were collected from pregnant horn (one per cow) immediately after spontaneous delivery of a calf at term (282–288 days of pregnancy), after extraction of a calf during caesarian section before term (272–277 days of pregnancy), and during caesarian section at term. The remainder of fetal membranes were left in situ until they were released spontaneously within 8 h after parturition or removed by a veterinarian after 8 h and defined as retained placenta [14]. Cows were divided into six groups according to the time of expulsion and the mode of delivery as follows:

(A) caesarian section before term without retained placenta ($n = 9$),
(B) caesarian section before term with retained placenta ($n = 14$),
(C) caesarian section at term without retained placenta ($n = 12$),
(D) caesarian section at term with retained placenta ($n = 16$),
(E) spontaneous delivery at term without retained placenta ($n = 8$),
(F) spontaneous delivery at term with retained placenta ($n = 8$).

Placental tissues were divided into maternal and fetal part of placenta, washed in cold 0.9% NaCl, and stored frozen at −80 °C until analysed.

Placental maternal and fetal tissues were homogenised in phosphate buffer (0.05 mol/l, pH 7.0) using an Ultra Turrax T 25 (Ikawerk Janke and Kunkel Inc., Staufen, Germany) for 5 min and centrifuged for 20 min at 3000 × *g*. The whole procedure was performed at 4 °C.

The supernatants were subjected to the determination of total antioxidant capacity.

The protein content of supernatants was determined using Lowry's method and bovine serum albumin as standard [15].

The determination of total antioxidant capacity by ferric reducing ability of supernatants ([16] with modifications).

Working reagent was prepared immediately before use by mixing 300 mmol/l acetate buffer (pH 3.6), 10 mmol/l 2,4,6-tri-pyridyl-*s*-triazine (TPTZ, Sigma, Poznan, Poland) in 40 mmol/l HCl and 20 mmol/l $FeCl_3 \times 6\ H_2O$ in the ratio of 10:1:1.

Working reagent (2250 μl) was mixed with 25 μl of supernatant and absorbance was measured at 593 nm against the working reagent alone. After exactly 10 min of incubation at room temperature, the absorbance was measured again. The difference in absorbance at zero and 10 min time was compared with standard curve prepared with different dilutions of Fe(II) – 0–1000 μmol/l. The results were recalculated per protein content of supernatants and expressed as μmol/g protein (means ± S.E.M.).

The change in absorbance was directly related to the "total" reducing power of the electron donating antioxidants present in samples.

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