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THE CONCENTRATION OF VITAMIN A AND ITS PROVITAMIN – BETA CAROTENE IN BOVINE RETAINED AND NOT RETAINED PLACENTA

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The relationship between oxidative stress that may accompany the periparturient period and retention of fetal membranes (RFM) in cows still rises questions not only about the biochemical mechanisms but on prevention, as well.

The aim of the present study was to describe the relationship between placental vitamin A concentrations and its provitamin and placental release with respect to different time and mode of delivery.

Pregnant (n=62), healthy cows were divided into six groups as follows: A – caesarian section before term without RFM (n=10), B – caesarian section at term with RFM (n=10), C – caesarian section before term at RFM (n=12), D – caesarian section at term with RFM (n=12), E – spontaneous delivery at term without RFM (n=10), F – spontaneous delivery at term with RFM (n=8). The concentrations of beta carotene and vitamin A in homogenates of maternal and fetal parts of the placenta were determined spectrophotometrically at 325 nm and 453 nm and expressed in μg/g protein (mean±SEM).

Values of beta carotene were significantly (p<0.05) lower in the maternal part of the placenta. The opposite relationship was shown by vitamin A concentrations. The comparison of mode of delivery at term in healthy cows showed, opposite to vitamin A, significantly (p<0.05) higher values of beta carotene in vaginal delivery compared to the surgical. In RFM affected cows the same significant (p<0.05) relationship was observed, moreover values of beta carotene were significantly (p<0.05) higher, and lower with regard to vitamin A, as compared to healthy animals. In preterm placenta beta carotene concentration was significantly (p<0.05) higher in RFM cows. Values of vitamin A showed the opposite relationship.

Vitamin A and its provitamin may influence the process of fetal membrane retention.

Key words: beta carotene, vitamin A, retained placenta

INTRODUCTION

Reactive oxygen species (ROS) are unavoidable intermediates which in low concentrations exert positive effects, but any imbalance between their production and neutralization may lead to serious metabolic alterations (Sies, 1993). Such alterations, called oxidative stress, were already detected in many human and animal diseases (Halliwell and Gutteridge, 1985). Among others the retention of fetal membranes (RFM) in cows is supposed to be connected to oxidative stress (Miller *et al*., 1993; Kankofer, 2001a, b, c). Clinically important symptoms are defined as the disturbances in steroid hormones, as well as prostaglandin $F_{2\alpha}$ concentration (Leidl *et al*., 1980; Heuwieser and Grunert, 1987). Biochemically relevant changes cover, among others, the disturbances in the antioxidative defence systems and the increase in the intensity of peroxidative processes (Castillo *et al.,* 2005).

Parturition itself due to changes in oxygen use and pressure may induce the increase in ROS production.

Antioxidative functions in biological systems are more complicated than the simple free radical scavenging process. That is why all components of the antioxidative defence should be examined prior to a clear statement that oxidative stress is implemented in particular pathological processes.

Carotenoids – ROS quenchers – are lipid soluble antioxidants. They have a typical conjugated polyene structure and trap singlet oxygen most efficiently (Pryor, 2000).

β-carotene may react with peroxyl radical to form a carbon-centered radical, which can be stabilized as a result of its high delocalization possibilities. The antioxidative activity of carotenoids seems to change according to oxygen pressure, from an efficient antioxidant at low pressure to a pro-oxidant at high pressure (Burton and Ingold, 1984).

There is evidence that vitamin A insufficiency may appear during RFM (Kleczkowski, 1987; LeBlanc *et al*., 2004). Moreover, the supplementation with vitamin E may decreases the risk for RFM incidence (Gwazdauskas *et al*., 1979) but no data concerning placental vitamin A and its provitamin concentration in this syndrome is available.

The objective of this study was to describe the relationship between placental concentrations of vitamin A and its provitamin and disturbed placental release with respect to different time and mode of delivery.

MATERIAL AND METHODS

A total of 62 pregnant cows (Holstain-Friesian) included in this study were clinically healthy and from 2 to 6 years of age. Days of gestation were calculated using dates of insemination. Caesarian sections were indicated because of fetus oversize.

Placentomes were collected from the pregnant horn (one per cow) immediately after spontaneous delivery of a calf at term (282-288 days of pregnancy) and after extraction of a calf during caesarian section before term

(272-277 days of pregnancy) and at term as well. The remaining fetal membranes were left *in situ* until they were released spontaneously within 8 hours after parturition or removed by a veterinarian after 8 hours and defined as retained placenta (Grunert, 1983). Cows were divided into six groups according to the time of partuition and the mode of delivery as follows:

A – caesarian section before term without RFM (n=10),
B – caesarian section before term with RFM (n=10),
C – caesarian section at term without RFM (n=12),
D – caesarian section at term with RFM (n=12),
E – spontaneous delivery at term without RFM (n=10),
F – spontaneous delivery at term with RFM (n=8),

Placental tissue was divided into the maternal and fetal part of the 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 x g. The whole procedure was performed at 4°C.

The supernatants were subjected to biochemical analysis in accordance to the methods described below.

The protein content of supernatants was determined using Lowry's method and bovine serum albumin as standard (Lowry *et al*., 1951).

Vitamin A and β-carotene determination (Suzuki and Katoh, 1990)

Supernatant was mixed 1:1 with ethanol. The mixture was extracted by 3 mL hexane, shaked for 30 min and centrifuged at 800 x g for 10 min. The absorbance of the hexane layer was measured against hexane alone at 325 nm for a total amount of retinol plus β carotene and at 453 nm for β carotene alone.

The concentration of retinol was calculated by subtraction of measured values at 325 and 453 nm. The results were recalculated by a standard curve prepared with different dilutions of retinol and β carotene and expressed as μg/g protein (mean±SEM).

Statistical analysis

Paired observations were averaged and subjected to statistical analysis of significance of differences between groups as well as correlation analysis with the aid of program Statistica 5.0.

RESULTS

Values of beta carotene were significantly ($p<0.05$) lower in the maternal part of the placenta. The comparison of mode of delivery at term in healthy cows showed significantly ($p<0.05$) higher values in vaginal delivery (group E – maternal 2.32±0.1 μg/g prot, fetal 3.71±0.24) than in the surgicaly treated ones (group C – maternal 1.89±0.076, fetal 2.76±0.1). In RFM affected cows the same relationship ($p<0.05$) was observed, moreover values were significantly ($p<0.05$)

higher as compared to healthy animals (group F – maternal 3.23±0.11, fetal 4.38±0.18 vs group D – maternal 2.45±0.14, fetal 3.48±0.23).

In preterm placenta beta carotene concentration was significantly ($p<0.05$) higher in RFM cows than in healthy animals (group B – maternal 1.83±0.076, fetal 2.44±0.23 vs group A – maternal 0.73±0.027, fetal 2.07±0.061). Results are presented in Figure 1.

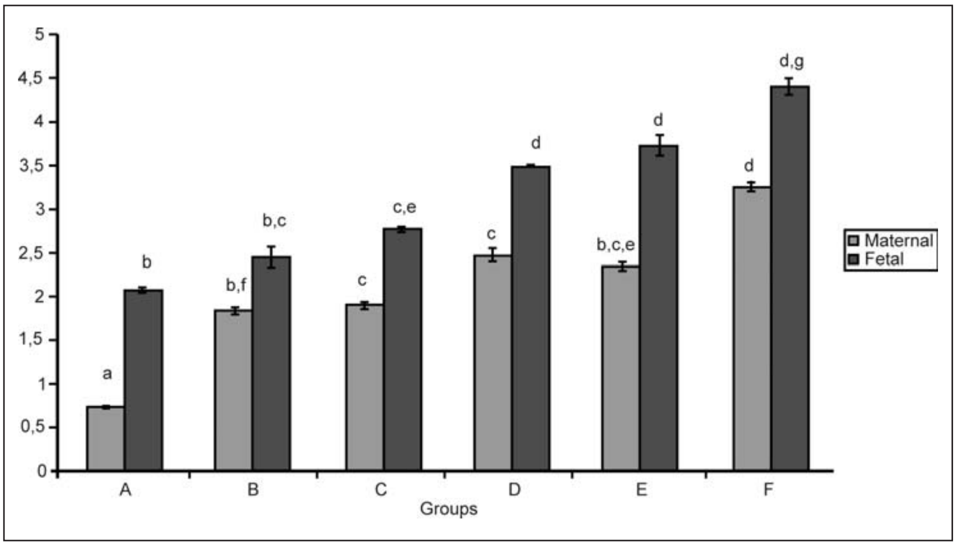

Figure 1. Beta carotene concentrations in maternal and fetal bovine placenta
Groups: A – caesarian section before term without RFM, B – caesarian section at term with RFM, C – caesarian section at term without RFM, D – caesarian section before term with RFM, E – spontaneous delivery at term without RFM, F – spontaneous delivery at term with RFM. Different superscripts denote significant differences at $p<0.05$

The concentration of vitamin A was significantly ($p<0.05$) higher in fetal placenta in all examined groups (except group B). The comparison of mode of delivery at term in healthy cows showed, opposite to the above, significantly ($p<0.05$) lower values in the vaginal delivery group (group E – maternal 112.3±4.4 μg/g prot, fetal 143.6±3.9) than in the surgicaly treated (group C – maternal 149.0±3.54, fetal 172.6±3.4). In RFM affected cows the same significant ($p<0.05$) relationship was observed, moreover values were significantly lower compared to healthy animals (group F – maternal 81.2±4.2, fetal 113.5±5.66 vs group D – maternal 131.5±4.65, fetal 155.2±3.9).

In preterm placenta vitamin A values were significantly ($p<0.05$) lower in RFM cows (group B – maternal 114.4±4.7, fetal 120.1±8.8 vs group A – maternal 165.1±7.0, fetal 220.2±9.88). Results are presented graphically in Figure 2.

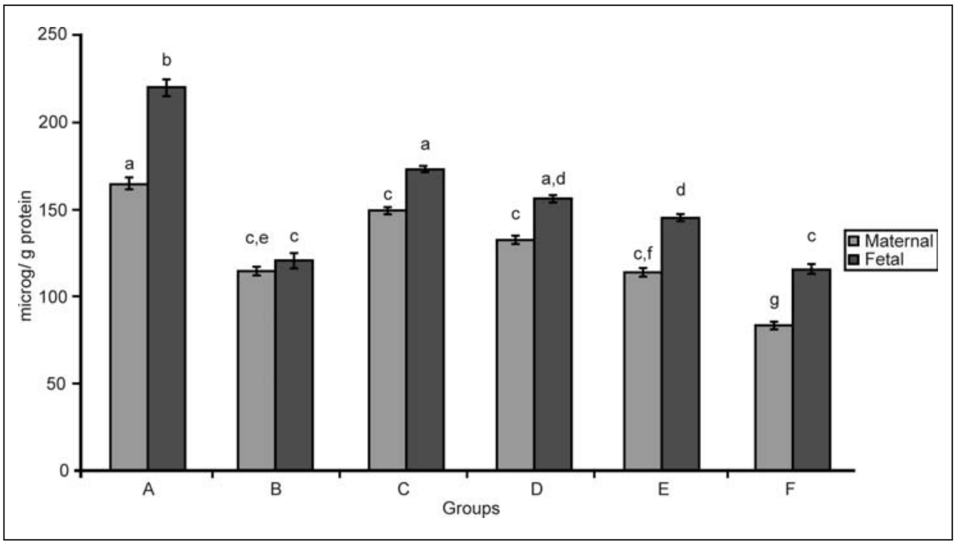

Figure 2. Vitamin A concentrations in maternal and fetal bovine placenta
Groups: A – caesarian section before term without RFM, B – caesarian section before term with RFM, C – caesarian section at term without RFM, D – caesarian section at term with RFM, E – spontaneous delivery at term without RFM, F – spontaneous delivery at term with RFM. Different superscripts denote significant differences at $p<0.05$

A negative correlation was found between beta carotene in the maternal placenta between groups A and B (r = - 0.65). A similar correlation was observed between vitamin A level in the maternal placenta in groups C and D (r = - 0.74). A negative correlation was found as well between beta carotene and vitamin A concentration in fetal placenta of group B and D (r = - 0.73). Positive correlation between two examined parameters was noticed in fetal part of group F (r = 0.93).

DISCUSSION

Beta-carotene is the main dietary precursor of vitamin A in dairy cattle. Apart from its role as provitamin A, it can exert antioxidative effects as well (Chew 1993). Synergistic action to vitamin E is shown by beta carotene which may neutralize a singlet oxygen and interrupt the peroxidative chain (Sies, 1993).

Present results confirm that the retention of fetal membranes can be related to oxidative stress as well as the mode of delivery may influence the antioxidative/oxidative status of cow. Beta-carotene and its provitamin show an opposite relationship with regard to maternal – fetal tissue content.

Dimenstein *et al*. (1996) suggested that beta carotene may be a precursor of retinol in human placenta but this conversion may depend on the nutritional status of the mother, being particularly effective in a more depleted state.

Dietary vitamin A usually appears as retinyl esters or beta carotene. They are transported by retinol binding protein to the liver and later on to extra-hepatic tissues such as eye, placenta or mammary gland (Sundaram *et al*., 1998). In cows, maternal plasma retinol and beta carotene concentrations decrease at the end of gestation. They reach the lowest values at parturition and increase during lactation (Goff *et al*., 2002). A sharp decrease in plasma β carotene and vitamin A during prepartum period was already reported in the late 40-ties by Sutton *et al*. Authors suggested that it might be due to, at least in part, to a rapid transfer to colostrum. A similar decrease was reported in mammectomized cows that gave birth to a premature calf (Wise *et al*., 1947). Due to limited placental transfer, newborns rely on colostral vitamin A supply or external supplementation.

There is evidence that age and parity of the cow may influence vitamin A concentration. In primiparous cows these levels are higher than in multiparous. This probably results from greater body storage and lack of secretion in colostrum and milk (Kumagai *et al*., 2001).

Previous experiments in our laboratory concerning antioxidative enzymes, as well as parameters of peroxidative damage to macromolecules during the retention of fetal membranes in cows, revealed significant alterations with regard to placental release disturbances (Kankofer, 2001a,b,c, 2002a,b). But antioxidant contribution *in vivo* goes far beyond scavenging free radicals. Moreover, a single antioxidant is usually not present alone in biological systems but acts in combination with other antioxidants. It was measured by total antioxidant capacity in placental homogenates and confirmed existing data (Kankofer, 2005). Moreover, the influence of the mode of delivery on antioxidative status was described as well.

Biochemical consequences of ROS excess mainly concern fatty acid peroxidative damage which in turn influences the properties of cell membranes. Cytotoxic effects of peroxidative end products are known (Marnett, 1999). The alterations in fatty acid concentrations in retained and released placental tissues were detected (Kankofer *et al*., 1996). The decrease in SH groups content and the shift in GSH:GSSG ratio appear and it may influence the activity of enzymes involved in metabolic pathways. Finally the concentration of NADPH decreases and in consequence the level of ATP as well. This in turn interferes energetic metabolism. Antioxidants with their different chemical structure and mode of action are able to interrupt the chain reaction and neutralize ROS excess.

More recent papers indicate that supplementation with β carotene and vitamin A reduces the incidence of retained placenta and other postpartum disorders (Gwazdauskas *et al*., 1979; Kleczkowski, 1987; Michal *et al*., 1994; Leblanc *et al*., 2004). A possible relationship may rely on the importance of vitamin A for maintenance of epithelial integrity. The differences in the histological picture of properly released and retained bovine placenta with regard to epithelial cell lysis were previously described (Schoon, 1989).

Clinically relevant are the data stating that vitamin E and Se may decrease RFM incidence (Miller *et al*., 1993) but whether interaction and relationship between vitamin A and E exists in the placenta remains to clarify. Several studies report decreased bioavailability of vitamin E when large amounts of vitamin A are provided simultaneously (Ametaj *et al*., 2000).

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KONCENTRACIJA VITAMINA A I NJEGOVOG PROVITAMINA BETA KAROTINA U SLUČAJEVIMA RETENCIJE PLACENTE KRAVA

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SADRŽAJ

Cilj ovog rada je bio da se odnos vitamina A i njegovog provitamina β karotina i retencije placente krava u zavisnosti od termina i načina porođaja. U ogled su bile uključene zdrave krave (62 grla) koje su bile podeljene u 6 grupa na sledeći način: A – krave oteljene carskim rezom pre termina i bez retencije (10), B – krave oteljene carskim rezom pre termina sa retencijom (10), C – krave oteljene carskim

rezom u terminu bez retencije (12), D – krave oteljene carskim rezom u terminu sa retencijom (12), E – spontani porođaji u terminu bez retencije (10), F – spontani porođaji u terminu sa retencijom (8). Koncentracija β karotina i vitamina A u homogenatima placentalnog tkiva poreklom od majke i ploda je određivana spektrofotometrijski na 325 nm i 453 nm i izražavana je u μg/g proteina (srednja vrednost $\pm$ standardna devijacija).

Koncentracija β karotina je bila značajno niža ($p<0.05$) u majčinim nego u fetalnim delovima placente, što je bilo u suprotnosti sa koncentracijom vitamina A. Kod zdravih krava su registrovane statistički značajno veće vrednosti ($p<0.05$) koncentracije β karotina pri vaginalnom porođaju u odnosu na carski rez. Kod krava sa retencijom su takođe uočene statistički značajno veće vrednosti ($p<0.05$) koncentracije b karotina, a niže A vitamina u poređenju sa zdravim jedinkama. U nezrelom placentalnom tkivu koncentracija b karotina je bila značajno veća ($p<0.05$) u slučajevima retencije, što je bilo u suprotnosti sa koncentracijom A vitamina.

Autori zaključuju da vitamin A i njegov provitamin utiču na proces odbacivanja placente kod krava.