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Redox status in the blood of ewes in the perinatal period and during lactation

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Abstract

The aim of the study was to determine the effect of the perinatal period on redox status indicators in the blood of ewes before and after lambing and during lactation. The study was performed on 12 ewes of the synthetic SCP line. Blood for testing of redox parameters was collected seven times: before pregnancy, 1.5 months and 24 h before lambing, 2 and 24 h after lambing, and in the fourth and eighth weeks of lactation. The following blood indices were determined by spectrophotometry: lipid peroxides, malondialdehyde, superoxide dismutase, catalase, plasma total antioxidant capacity, uric acid, urea, bilirubin, and creatinine. The tests showed that during the perinatal period reactions are generated which lead to oxidative stress. Oxidative stress in pregnant ewes was found to increase during the period before lambing and may persist even up to weeks 4–8 of lactation.

Keywords: ewes, lambing, lactation, redox status.

Introduction

In the normal physiological process the numerous oxidation processes taking place at the cellular and tissue level as a result of catabolism and the activity of specific forms of immune cells are counterbalanced by a complex antioxidant mechanism whose purpose is to minimise the effects of free radical species, thereby maintaining an optimal antioxidant balance (21, 22). However, due to exposure of the animal organism to numerous stress-inducing and immunosuppressive factors and due also to a high level of cellular metabolism, a very common phenomenon is destabilisation of the antioxidant balance towards excessive activity of reactive radical species, which leads to lipid peroxidation, *i.e.* the initiation of oxidative stress.

Pregnancy is a period of life in which higher levels of oxidative stress can be expected in the organism due to the mother's increased need for oxygen. Pregnancy is regarded as a physiological state of the organism, and changes in biochemical status and frequently observed complications, such as gestational diabetes, pre-eclampsia, eclampsia, abortions, premature births, or hypertension, are identified as the effect of lipid peroxidation processes which escalate during pregnancy (13, 29).

The literature provides very few data on changes in redox status indicators in the blood of sheep during the perinatal period and lactation. For this reason it was considered expedient to investigate them in this model during these periods.

Material and Methods

Animals. The study was performed on 12 ewes of the synthetic SCP line kept at the Small Ruminants Teaching and Research Station in Bezek, belonging to the University of Life Sciences in Lublin (16). The animals were of about the same age (second or third lactation) and kept under the same environmental

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conditions. They were fed fodder consisting of meadow hay, maize silage, concentrate feed (oat grain and wheat bran), and a mineral and vitamin supplement. The feeding level was in accordance with INRA standards. Prior to the experiment, the oestrus cycles were synchronised.

Laboratory analyses. Blood for testing of redox parameters was collected seven times: before pregnancy, 1.5 months and 24 h before lambing, 2 and 24 h after lambing, and in the fourth and eighth weeks of lactation. Superoxide dismutase (SOD) in the blood plasma was assayed spectrophotometrically by the adrenaline method according to Misra (in Greenwald (15)), at a wavelength of 320 nm. The method was modified in order to achieve greater selectivity of transitory reaction products at this wavelength (2). Analyses were also performed for catalase (CAT) activity according to Bartosz (2). For antioxidant system parameters, assays were performed for the ferric reducing ability of plasma (FRAP) according to Benzie and Strain (4), and vitamin C according to Omaye *et al.* (23). Samples of blood plasma were also analysed for levels of lipid peroxidation products: peroxides (LOOH) according to Gay and Gębicki (14), and malondialdehyde (MDA) as an end product of tissue lipid oxidation according to Salih *et al.* (24). Tests developed by Cormay (Poland) were used for spectrophotometric determination of plasma concentrations of uric acid (UA), urea, bilirubin (BIL), and creatinine (CREAT).

Statistical analysis. The results obtained were analysed statistically using one-way analysis of variance in the SAS statistical package (SAS, USA) (25). The significance of differences was estimated by Duncan's test.

Results

The data illustrating the changes in the redox status of the blood plasma of ewes in the perinatal period and during lactation are presented in Table 1. During pregnancy the highest ($P \leq 0.01$) LOOH level (5.72 μmol L^{-1}) was noted 1.5 months before lambing. The level was similar to that noted in the sheep before pregnancy. A significant decrease in the level of this index (on average by 56%), persisting until the fourth week of lactation, was noted 24 h before lambing. Before and during pregnancy, up to 2 h before lambing, the MDA level remained at a similar level (0.68–0.77 μmol L^{-1}). A statistically-significant ($P \leq 0.01$) increase in the MDA level, persisting until the eighth week of lactation, was noted from 24 h after lambing. The highest ($P \leq 0.01$) SOD activity (90.93 U mL^{-1}) was noted 1.5 months before lambing. The SOD activity 1.5 months before lambing was similar to the value recorded in the sheep before pregnancy. A not inconsequential decrease in SOD activity, by about 36%, was noted 2–24 h after lambing. SOD activity continued to be reduced during lactation by 50% in the fourth week of lactation and 68% in the eighth week. The lowest ($P \leq 0.05$) catalase activity (3.31 U mL^{-1}) was noted 1.5 months before lambing. Catalase activity in the ewes before pregnancy and 1.5 months before lambing was at a similar level. A significant ($P \leq 0.05$) increase (about twofold) in CAT activity was noted 24 h before lambing and 2 h after lambing. CAT activity remained elevated during the lactation period. The highest significant ($P \leq 0.01$) FRAP level was also noted in the ewes before pregnancy and 1.5 months before lambing. The greatest significant ($P \leq 0.01$) decrease in the FRAP level was noted 24 h before and 2 h after lambing. The reduced ($P \leq 0.01$) FRAP level persisted up to the eighth week of lactation. Vitamin C content in the ewes in the perinatal period (before and after lambing) remained at a similar level. A decrease in the content of vitamin C was noted during lactation.

The results of the analyses of low-molecular-weight antioxidants in the blood plasma of the sheep are presented in Table 2. UA content was within the range of reference values (0–110 μmol L^{-1}) presented by Winnicka (28). It should be emphasised, however, that UA content was the highest ($P \leq 0.05$) during the prenatal period (24 h before lambing and 2 h after lambing). A substantial increase in UA content was noted in the eighth week of lactation. Urea content, both in the perinatal period and during lactation, was not consistent with the reference values (4.15–7.47 mmol L^{-1}) given by Winnicka (28). From 24 h before lambing, urea content was nearly 20 times higher than before pregnancy and 1.5 months before lambing. Urea concentration higher than the reference values for this index persisted after lambing and during lactation. The CREAT content was within the range of reference values (79.6–160.9 μmol L^{-1}) (28). It should be emphasised, however, that the CREAT values noted in weeks 4–8 of lactation were lower than before pregnancy and during the perinatal period. The BIL level was higher than the reference values (1.7–6.0 μmol L^{-1}) (28) and its highest values were noted during 4–8 weeks of lactation.

Discussion

A measure of the antioxidant status of an organism is the balance between oxidative agents, *i.e.* lipid peroxidation products (radicals, lipid peroxides, or aldehydes) and endogenous and exogenous substances suppressing radicals, the reactive oxygen species (ROS). These are special groups of enzymes including superoxide dismutase, glutathione peroxidase, catalase, and numerous low-molecular-weight antioxidants, such as bilirubin, creatinine, uric acid, urea, glutathione, active forms of vitamins (E and C), and dissociated metal cations (Cu, Zn, Mn, Fe, and Se), constituting the total antioxidant capacity of plasma (FRAP) (18).

Table 1. Levels of redox status indices in the blood plasma of ewes

Parameter		Before pregnancy	Before lambing		After lambing		Lactation	
			1.5 months	24 h	2 h	24 h	week 4	week 8
		n = 12	n = 12	n = 12	n = 12	n = 12	n = 12	n = 12
LOOH μmol L^{-1}	$\bar{x}$	5.62^{Aa}	5.72^{Aa}	2.10^{Bc}	2.29^{Bbc}	2.99^{Bbc}	2.57^{Bbc}	3.87^{ABb}
	SD	0.78	0.84	0.40	0.48	0.57	0.42	0.61
MDA μmol L^{-1}	$\bar{x}$	0.74^{C}	0.77^{C}	0.69^{C}	0.68^{C}	1.36^{BC}	2.18^{B}	3.84^{A}
	SD	0.92	0.88	0.89	0.49	1.09	1.70	0.91
SOD U mL^{-1}	$\bar{x}$	101.2^{A}	90.93^{A}	79.15^{AB}	58.31^{BC}	58.22^{BC}	45.54^{CD}	28.61^{D}
	SD	24.41	22.28	31.16	21.59	20.00	23.53	0.63
CAT U mL^{-1}	$\bar{x}$	3.75^{b}	3.31^{b}	6.93^{a}	7.44^{a}	4.70^{ab}	6.14^{ab}	6.48^{a}
	SD	0.93	1.49	3.97	4.66	2.69	3.33	3.61
FRAP μmol L^{-1}	$\bar{x}$	1308.5^{A}	1269.12^{A}	46.81^{B}	63.08^{B}	78.08^{B}	69.23^{B}	70.20^{B}
	SD	43.5	73.68	22.87	12.36	45.05	19.37	14.17
VIT C mg L^{-1}	$\bar{x}$	0.46^{Aa}	0.21^{ABbc}	0.33^{ABab}	0.45^{Aa}	0.30^{ABabc}	0.12^{Bc}	0.12^{Bc}
	SD	0.06	0.06	0.15	0.16	0.08	0.03	0.03

a, b – mean values within a row with no common superscript differ significantly at $P \leq 0.05$
A, B – mean values within a row with no common superscript differ significantly at $P \leq 0.01$
LOOH – peroxides; MDA – malondialdehyde; SOD – superoxide dismutase; CAT – catalase; FRAP – ferric reducing ability of plasma; VIT C – vitamin C

Table 2. Level of low-molecular-weight antioxidants in the blood plasma of ewes

Parameter		Before pregnancy	Before lambing		After lambing		Lactation	
			1.5 months	24 h	2 h	24 h	week 4	week 8
		n = 12	n = 12	n = 12	n = 12	n = 12	n = 12	n = 12
UA μmol L^{-1}	$\bar{x}$	37.98^{BCcd}	31.83^{Cd}	54.20^{ABCab}	46.95^{ABCabc}	38.17^{BCcd}	43.13^{ABCbcd}	56.63^{Aa}
	SD	6.47	17.63	12.93	17.59	9.43	10.60	13.47
Urea mmol L^{-1}	$\bar{x}$	5.30^{CDb}	1.28^{Dc}	20.96^{ABa}	24.17^{Aa}	12.25^{BCb}	8.52^{CDb}	12.87^{BCb}
	SD	2.12	0.50	10.12	9.96	6.12	1.39	2.03
CREAT μmol L^{-1}	$\bar{x}$	104.94^{A}	107.81^{Aa}	93.75^{ABCab}	85.78^{ABCab}	101.72^{BCa}	70.64^{Cb}	74.35^{BCb}
	SD	13.28	17.27	26.53	33.68	38.03	17.43	9.37
BIL μmol L^{-1}	$\bar{x}$	7.13^{b}	10.17^{ab}	8.74^{ab}	7.03^{b}	12.52^{ab}	16.34^{a}	13.09^{ab}
	SD	4.77	5.23	7.30	6.40	9.03	11.27	10.95

a, b – mean values within the row with no common superscript differ significantly at $P \leq 0.05$
A, B – mean values within a row with no common superscript differ significantly at $P \leq 0.01$
UA – uric acid; CREAT – creatinine; BIL – bilirubin

The results of our study showed that the redox status indicators in the pregnant ewes up to 1.5 months before lambing were similar to the values noted in these animals before pregnancy. These observations suggest that up to this point the organism of the sheep was free of oxidative stress, and thus there was homeostasis in the redox status of the organism. Erisir *et al.* (13) showed a predisposition to oxidative stress in pregnant sheep as early as the second to third months of pregnancy. The results of the study also show that the greatest changes in redox status indicators indicating increasing oxidative stress (reductions in FRAP, LOOH, and SOD, and increases in MDA and CAT) were noted 24 h before lambing. These tendencies persisted up to 2 or 24 h after lambing. When reactive oxygen species such as superoxide anion radical are generated in the organism, the antioxidant enzyme superoxide dismutase transforms the radical into hydrogen peroxide in a dismutation reaction. Hence the decrease noted in the concentration of lipid peroxides is probably linked to a reduction in superoxide dismutase activity. The

enzyme glutathione peroxidase, which was not a subject of this study, is responsible for the breakdown of hydrogen peroxide. However, as demonstrated in a study by Celi *et al.* (10) on pregnant goats, the activity of the antioxidant enzymes SOD and GSH-Px during the perinatal period decreases and remains at a low level even up to four weeks after lambing, which means that oxidative stress persists for a fairly long time in the organism of the mother, including the lactation period. Moreover, the enzyme catalase, whose activity increases when there is a high concentration of lipid peroxides generated during the dismutase reaction, is also responsible for breaking down hydrogen peroxide into water. An increase in catalase is also observed in inflammatory states, during which a phagocytotic process generating large quantities of hydrogen peroxide is activated as a defence mechanism of the organism (12). A high level of H_2O_2 (10^{-8} mol/L) increases catalase activity (2). The increase in MDA as an end product of lipid peroxidation is confirmation that the perinatal period is a powerful stressor for the mother.

An increase in MDA concentration in the plasma of women in the third trimester of pregnancy with respect to non-pregnant women has been noted by Arikan *et al.* (1), Djordjevic *et al.* (11) and Nakai *et al*. (20). Moreover, Celi *et al.* (10), analysing the blood of goats in the perinatal period, confirm an increase in ROS in the blood of the mothers. An increase in lipid peroxidation products during the perinatal period in dairy cows was also noted by Bernabucci *et al.* (5, 6) and Bouwstra *et al.* (7). On the other hand, Castillo *et al.* (8, 9) found no significant changes in the concentration of malondialdehyde in the plasma of pregnant dairy cows or in their subsequent lactation. Oxidative stress during pregnancy is linked to physiological changes taking place in the organism that lead to a shift in metabolic processes to adapt conditions to those ensuring normal development of the foetus. These mainly affect hormone and carbohydrate-lipid metabolism. There is an increase in lipogenesis (elevated concentration of cholesterol and triglycerides) and in oxidation of free fatty acids in the liver, which additionally favours the generation of ROS (17, 30).

During pregnancy a marked decrease in the content of low-molecular-weight antioxidants is also observed. This increase was indicated in the present study by a decrease in total antioxidant capacity (FRAP). Celi *et al.* (10) also observed a decrease in antioxidant capacity in pregnant goats. According to Venditti and Meo (26), a decrease in non-enzymatic antioxidants in the organism is considered to be a consequence of oxidative stress. The main component of FRAP is uric acid (constituting as much as 60%), which in small quantities, like bilirubin or creatinine, serves as a low-molecular-weight antioxidant in the blood (3). An elevated level of uric acid and urea and a reduced level of creatinine during the perinatal period may be indicative of kidney dysfunction, which is often observed during pregnancy. In early pregnancy the plasma concentration of uric acid generally decreases due to increased renal clearance of this compound. Uric acid, which is an end product of purine metabolism, is reabsorbed and secreted in the proximal tubule. Later, in more advanced pregnancy, clearance of uric acid in the renal tubules decreases. However, the concentration of this compound in the blood does not increase, probably due to an increase in the total volume of circulating blood. Elevated concentrations of uric acid in the blood in advanced pregnancy may indicate the development of pre-eclampsia (27). Increased glomerular filtration in pregnancy results in a reduction in the plasma concentration of creatinine. Plasma concentrations of creatinine considered normal in non-pregnant women may indicate impairment of kidney function in pregnant women (19).

The study showed that during the perinatal period reactions leading to oxidative stress are generated in the sheep organism. Oxidative stress in the organism of pregnant ewes was found to increase during the period before lambing and may persist even up to weeks 4–8 of lactation.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Animal Rights Statement: The authors declare that the experiments on animals were conducted in accordance with local Ethical Committee laws and regulations as regards care and use of laboratory animals (2nd Local Ethical Committee for Animal Experiments in Lublin, Resolution No 2/2015 of 22nd January, 2015).

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