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FREE RADICAL LIPID OXIDATION AND REPRODUCTIVE HEALTH OF COWS

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Abstract

Free radical lipid oxidation is currently considered as one of the dominant metabolic processes of physiological system functional activity. When it goes beyond regulated limits it is considered as an inductor of free radical pathology oxidative stress. In the conditions of a big dairy cattle breeding farm (Voronezh Province), specialized in Red-and-White breed, the functioning peculiarities of the peroxidation system lipid-oxidant defense in cows at the normal course of gestation and at gestosis, at the normal course of postpartum period and at puerperal endometritis, uterus subinvolution and ovarian dysfunction were studied. The state of lipid peroxidation processes and antioxidant system were evaluated by determining total lipid content, cholesterol, triglycerides, malonic dialdehyde (MDA), nitrogen oxides ($NO^{\bullet}$), E and C vitamins concentrations, glutathione peroxidase (GPO), glutathione reductase (GR), catalase, superoxide dismustase (SOD) activity in blood. It is demonstrated that the high activity of lipid peroxidation reactions and the system of nitrogen oxide at decrease of antioxidant defense non-enzymic link capacity is the basis of gestosis and acute postnatal complications. Thus, an increase of MDA concentration by 42.3-43.0 %, $NO^{\bullet}$ by 31.9-38.0 % was observed in cows with gestosis. At mild gestosis the glutathione peroxidase activity, catalase activity and vitamin C concentration increased by 11.0 %, 14.3 %, 38.0 %, and 24.1 %, respectively, while vitamin E concentration decreased by 11.7 % due to more consumption for neutralization of the toxic peroxidation products. At more sever pathology, the glutathione peroxidase activity and catalase activity in blood increased by 26.0 % and 17.3 %, respectively, when compared to the healthy animals, while vitamins E and C concentrations decreased by 33.3 % ($p < 0,01$) and 17.2 %, respectively. As free radical oxidation intensified, an anaerobic degradation of carbohydrates was activated to supply the tissues of developing fetus with energy under oxygen deficit occurred because of violation of the blood circulation. Postpartum inflammation in the genital organs in cows developed against the background of increase of MDA concentration by 76.0 %, GPO and GR activity by 65.8 % and 14.6 %, respectively, SOD by 46.0 %, catalase by 45.7 %, 2.9 times increase of $NO^{\bullet}$ concentration and reduction of vitamin E content by 35.5 %. Infertile animals with ovarian dysfunction were characterized by high activity of lipid peroxidation processes and by low level of nitrogen oxide generation. This is indicated by an increased concentration of MDA by 57.0 %, activity of GPO by 27.6 %, GR by 10.5 %, SOD by 31.9 %, catalase by 24.3 %, with a reduced content of $NO^{\bullet}$ and vitamin E by 56.9 % and 31.6 %, respectively, in comparison with healthy animals. A decrease in $NO^{\bullet}$ concentration in blood could result from a sharp depression of hormone synthesizing function in ovaries, and low $NO^{\bullet}$ production could disturb functions of the gonads. The high level of peroxidation is peculiar to animals with chronic uterus pathology, however, it is less expressed than in cows with acute course of the pathological process.

Keywords: cows, blood, lipid peroxidation, gestation, postpartum period, norm, pathology.

Free radical oxidation or lipid peroxidation (FRO, LPO) are currently considered as the dominating metabolic processes, which provide regulation of functional activity of any organism's physiological systems. The reactions of peroxidation have a universal character, being the source of basic energy mass necessary for vital activity and stability index of metabolic transformations in the organism.

The initiators of free radical oxidation are the active forms of oxygen (AFO), forming in oxidase (mitochondrial) and oxygenase (microsomal) reac-

tions of aerobic oxidation, realizing with the participation of molecular oxygen (O_2). During these reactions O_2 is subjected to sequential univalent reduction with the formation of so-called free radical compounds, possessing an unpaired electron. When the oxygen loses one electron at the beginning, we can observe formation of a superoxide anion radical ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2), the subsequent repair of which is accompanied by water (H_2O) formation and hydroxyl radical ($OH^\bullet$). The last one differs by its high reactivity and it is one of the main initiators of LPO (1-4). Under certain conditions non-enzymatic dismutation of superoxide anion can produce singlet oxygen ($^1O_2$) possessing high reactive and biological activity as well as a hydroxyl radical.

AFO undergo oxidation reaction with polyunsaturated lipids, including fatty-sour residues of phospholipids, the main structural components of biological membranes. AFO also activate the formation of a number of molecular products of LPO (peroxide radicals $RO_2^\bullet$): hydroperoxides of saturated fatty acids, aldehydes and dialdehydes, ketones, lactones, epoxides, substances like Schiff bases, etc. All of them play an important role in the processes of structural modifications of biomembranes and changes of their physicochemical properties (5). Excessive production of the active forms of oxygen and redundant in vivo accumulation of LPO products lead to the changes of physicochemical biomembrane properties, activity of many membrane-bound enzymes, penetrability disorder and then structural integrity and genotoxic oxidative DNA damages (6).

Formation, accumulation and utilization of FRO products are controlled by the system of antioxidant protection, including non-enzymatic and enzymatic links. The system of antioxidant protection (AOP) limits the processes of free radical lipid oxidation practically in all its links and maintains this class of reactions at a relatively fixed level. It controls the content of active forms of oxygen, free radicals and molecular products of LPO (5) in the organism and plays a very important role in the homeostasis maintenance.

In the enzyme link of AOP system the central part is occupied by copper-, zinc- and manganese-containing enzymes of superoxide dismutase (SOD), which catalyzes the dismutation reaction of superoxide anion radical with the formation of molecular oxygen and hydrogen peroxide, that is also capable of taking a toxic effect on the cells. The destruction of H_2O_2 molecules is done by catalase enzymes and glutathione peroxidase (8). Catalase is haematin-containing enzyme, destroying H_2O_2 without participation of oxygen acceptors. The hydrogen peroxide itself is the electron donor. Catalase continuously preserves its activity and does not require energy activation and the speed of hydrogen peroxide decomposition is only limited by the speed of substrate diffusion to the active center of the enzyme. Glutathione peroxidase is one of the components of the anti-peroxide complex, including glutathione and glutathione reductase, catalyzes the transformation of hydrogen peroxide and fatty acids of hydroxides into nontoxic compounds. The efficiency of glutathione peroxidase mechanism of hydroperoxide reduction depends on the hydrogen- glutathione content in the principal donor's organism. The support of sufficient amount of reduced glutathione, oxidizing under functioning of glutathione-dependent antiperoxide systems, is done by the enzyme of glutation reductase (GR).

In the non-enzymatic link of the AOP system the central part is occupied by tocopherols (9), among which α- tocopherol (vitamin E) possesses the highest biological activity. It enters the organism with vegetable and animal feed. It realizes its antioxidant function at the expense of forming solid membrane architecture, preventing the attack of unsaturated fatty-sour residues of membrane phospholipids by active forms of oxygen and at the expense of local damages of oxygen and lipid peroxide radicals. α-Tocopherol acts as an active

«quenching agent» of singlet oxygen and «interceptor» of free radicals, reacting directly with them at the stage of chain cut off (10, 11).

It is considered that only reduced (phenolic) form of vitamin E, possessing free hydroxyl group, can actively react with peroxide radicals. Among the substances capable of reducing oxidated kinone form into a phenolic one and by this to regenerate antiradical activity of vitamin E, the greatest importance belongs to ascorbic acid, standing as a donor of protons and synergist of vitamin E (12, 13). In addition, it itself can interact with singlet oxygen, hydroxyl radical and superoxide anion radical, and destroy hydrogen peroxide (14, 15). Reduction of the ascorbic acid is realized at the expense of reduced glutathione. Close interconnection of the ascorbic acid with tocopherol and glutathione makes it an important component of the biological non-enzymatic system of the antioxidant protection.

Recently $NO^{\bullet}$ was reported also to be involved into the oxidative stress and antioxidation defense mechanisms (16-22). Its protective effect presumably is due to capability to enhance activity of antioxidative enzymes (23, 24), interact with superoxide anion radical and provide detoxication of potentially harmful reactive oxygen species (5, 17, 22).

Under initial insufficiency of the AOP system, its capacity decrease, because of extreme external factors or due to the internal physiological causes, LPO processes outlet over the regulated limits, redundant accumulation of its toxic products, development of oxidative stress and free radical pathology (1, 25, 27) with the structural-metabolic changes in the reproductive organs and occurrence of such diseases as fetoplacental insufficiency, late gestosis, placentitis, antenatal fetal hypoxia, retained placenta, postnatal subinvolution of uterus and endometritis (28-32), chronic pathologies of uterus and ovaries accompanied by infertility (33-35) are marked.

That is why modern scientific literature actively discusses the role of LPO in the molecular mechanisms of adaptive reactions and in the genesis of many diseases of reproductive animals.

The aim of this work was to study the functioning peculiarities of lipid peroxidation—antioxidant protection system and nitric oxide in highly productive dairy cows under physiological and pathological course of pregnancy and puerperal period.

Technique. The experiment has been run during winter keeping cattle stalled period in 2009 at a pedigree farm «Druzhba» (Pavlovskiy region, Voronezh Province). A total of 96 cows of Red Motley breed with average annual productivity of 6.5 thousand kg in the herd under tethered-pasture keeping participated in the experiment. Their ration included corn silage, meadow hay, spring corn straw, concentrated food, treacle and table salt. Common nutritiousness of the ration was 100 % with provision of 98 % digestible protein, 98 % sugar, 73 % calcium, 72 % phosphorus, 92 % carotene; sugar-protein ratio was 1:1 and calcium-phosphorus ratio was 1.8:1. There were seven groups of animals formed as follows: I (n = 9) with a normal gestation course, II (n = 9) with the signs of light gestosis, III (n = 9) with the signs of severe gestosis, IV (n = 17) with a normal course of puerperal period, V (n = 28) with an acute postnatal endometritis, VI (n = 12) with a chronic uterus subinvolution and VII (n = 12) with ovarian hypofunction. Functional state of the reproductive organs was examined by transrectal palpation. The gestosis was diagnosed on the basis of visual detection of pathological subcutaneous edema in the area of hind limbs, abdominal wall and dewlap, measurement of blood pressure using clinical thermometer, detection of protein concentration in urine using test strips AlbuPHAN (Lachema, Czechia).

Blood was obtained from the jugular vein during morning hours. Heparin was as an anticoagulant. The amount of malonic dialdehyde (MDA), activity of glutathione peroxidase (GPO), glutathione reductase (GR), catalase, superoxide dismutase (SOD) (36), the sum of nitric oxide ($NO^{\bullet}$) stable metabolites (37) were evaluated in the blood. E and C vitamins content was determined in blood serum using spectrophotometric method (38), common lipids, cholesterol and triglyceride were determined using Vital Diagnostica (Russia) and Lachema (Czechia) sets, blood lactic acid was determined according to the reaction with paroxy diphenyl (36).

Statistical treatment was done using program Statistica v. 6.0. The validity of the differences was evaluated by paired comparison method using Student's *t*-criterion.

Results. Gestation pathology, clinically revealing as gestosis symptom complex in cows, developed against the background of lipid peroxidation activation under simultaneously increased activity of antioxidant protection system as a compensatory reaction to damaging action of LPO products (Table 1).

1. Indices of lipid peroxidation- antioxidant protection system in Red motley cows under physiological and pathological gestation course (*M*±*m*; pedigree farm «Druzhba», Pavlovskii region, Voronezh Province; winter keeping cattle stalled period 2009)

Index	Clinically healthy (n = 9)	Light gestosis (n = 9)	Severe gestosis (n = 9)
MDA, μmol/l	1.04±0.140	1.49±0.120	1.48±0.140
GPO, mmol GSH/(l · min)	14.6±1.54	17.2±2.11	18.4±2.58
Catalase, mmpl H_2O_2/(л · min)	30.1±1.26	34.4±0.93	35.3±2.44
Vitamin E, μmol/l	11.2±0.89	9.9±1.20	7.7±0.93
Vitamin C, mmol/l	14.5±5.73	18.1±4.02	12.0±1.69
$NO^{\bullet}$, μmol/l	60.1±8.02	83.0±7.87	79.3±8.19

C o m m e n t s. MDA — malonic dialdehyde, GPO — glutathione peroxidasea, $NO^{\bullet}$ — sum of nitric oxide stable metabolites.

In terms of malonic dialdehyde blood concentration increase, the POL product level in animals with slight gestosis increased by 43.0 % in comparison with that in healthy cows ($p < 0.05$). Along with it, the activity of GPO and catalase, concentration of stable $NO^{\bullet}$ metabolites and vitamin C increased by 11.0 %, 14.3 %, 38.0 % and 24.1 %, respectively. Meanwhile, the amount of vitamin E, which is not synthesized in the organism, decreased by 11.7 %, that was connected with the increase of its expenditure on the neutralization of LPO toxic products.

The intensification of the pathological process severity caused the subsequent increase of enzymatic link of AOP system and decrease of non-enzymatic one. Compared to clinically healthy animals, activity of GPO and catalase increased by 26.0 % and 17.3 %, respectively, and the amount of vitamin E and C decreased by 33.3 % ($p < 0.01$) and 17.2 %, respectively.

Against the FRO intensity increase the decrease of total lipid concentration in blood from 4.1±0.35 to 3.1±0.18 g/l or by 24.4 % ($p < 0,05$), and increase of the level of triglycerides from 0.5±0.02 to 1.1±0.04 mmol/l or 2.1 times ($p < 0.001$) and lactic acid from 1.8±0.07 to 4.5±0.13 mmol/l or 2.5 times ($p < 0,001$) were shown. The last one confirms the enhancement of anaerobic decay of carbons for developing fetus's tissue provision with the energy in case of oxygen scarcity, which arises in connection with uteroplacental blood circulation disorder.

Sufficiently active LPO was found under the development of inflammatory processes in reproductive organs after parturition (Table 2). This was proved by a high MDA blood concentration, exceeding analogous index in clinically healthy animals by 76.0 %. Such a tendency was determined by an abrupt in-

crease of neutrophilic and macrophage production of active forms of oxygen, observed under an inflammatory process. At the same time such animals have compensatory inclusion of the enzymatic link of the antioxidant protection. GPO blood activity in sick animals appeared to be higher by 65.8 % ($p < 0.001$), GR by 14.6 % ($p < 0.05$), SOD by 46.0 % ($p < 0.001$), catalase by 45.7 % ($p < 0.001$). However, not high increase of GR activity compared GPO can confirm insufficiency of the functional potential of glutathione link of the AOP system and impossibility of the adequate replenishment of the reduced glutathione pool (39).

2. Indices of lipid peroxidation-antioxidant protection system in Red motley cows under physiological and pathological course of puerperal period (*M*±*m*; pedigree farm «Druzhba», Pavlovskii region, Voronezh Province; winter keeping cattle stalled period 2009)

Показатель	Physiological course of the puerperal period (n = 17)	Acute endometritis (n = 28)	Chronic subinvolution (n = 12)	Ovarian hypofunction (n = 12)
MDA, μmol/l	1.00±0.050	1.76±0.400	1.45±0.030	1.57±0.060
GPO, mmol GSH/(l · min)	9.4±0.32	15.8±0.44	13.7±0.45	12.0±0.64
GR, μmol G-SS-G/(l · min)	293.1±10.88	336.2±9.06	299.0±7.11	324.0±8.24
SOD, standard unit/mg of haemoglobin	0.72±0.030	1.05±0.030	0.97±0.040	0.95±0.050
Каталаза, mmol H_2O_2/(l · min)	25.9±0.57	37.6±0.63	33.0±1.28	32.2±0.84
Витамин E, μmol/l	23.7±3.48	15.3±0.93	28.3±2.79	16.2±2.78
$NO^{\bullet}$, μmol/l	47.8±0.29	138.7±7.14	–	20.6±2.21

Comments. MDA — malonic dialdehyde, GPO — glutathione peroxidasea, GR — glutathione reductase, SOD — superoxide mutase, $NO^{\bullet}$ — of nitric oxide stable metabolites. Dash indicates the absence of data.

The decrease of AOP non-enzymatic link activity was simultaneously detected in the animals, which have become sick. Vitamin E content in their blood appeared to be lower by 35.5 % ($p < 0.01$). The imbalance in AOP system didn't allow to support LPO processes at a relatively fixed level, which could be a background of endometrium cell structures damage by means of FRO toxic products accumulation and postnatal pathology development. At the same time production of nitric oxide in the sick cows' increased 2.9 times ($p < 0.01$). Immunocompetent cells, the macrophages and neutrophils, were the source of its generation (16). Possessing antioxidant and muscle relaxing effect, $NO^{\bullet}$, on the one hand, restricted the intensity of the peroxide reactions, and on the other hand, it depressed uterine activity and caused the breakdown in postnatal involutional processes of the reproductive organs.

Lipid peroxidation in cows with uterus inflammatory diseases was accompanied by the decrease of total lipid concentration in blood by 17.9 % (2.71±0,04 versus 3.30±0.18 g/l, $p < 0.001$), cholesterol by 44.0 % (2.68±0.12 versus 4.78±0.33 mmol/l, $p < 0.001$).

The high activity of LPO reaction remained in cows under the development of chronic reproductive organs pathology (see Table 2). So, the animals with chronic uterus subinvolution had higher MDA blood concentration compared to healthy animals by 45.0 %, GPO activity by 45.7 %, SOD by 34.7 % and catalase by 27,4 % ($p < 0.01$-0.001). In cows with ovarian dysfunction the difference according to the same indices was 57.0, 27.6, 31.9 and 24.3 % ($p < 0.001$), respectively. There were no evident differences in GR activity and vitamin E content under chronic uterus pathology. The activity of this enzyme in animals with sexual glands hypofunction exceeded the index in healthy animals by 10.5 %, but vitamin E concentration was lower by 31.6 %, and the cows were characterized by a lower generation of nitric oxide. The concentration of its stable blood metabolites was 56.9 % lower ($p < 0.001$).

Therefore, $NO^{\bullet}$ formation in the organism was connected with the activity of sex steroids biosynthesis (40, 41), and the decrease of its concentration

must be referred to an abrupt reduction of hormone-synthesizing ovarian function. At the same time low NO• production under a mentioned pathology may be the basis for gonads generative function disorder, because this compound is included into the control of hypothalamus secretion of gonadotropin-releasing hormone and hypophysis secretion of luteinizing hormone responsible for the gonad ovulatory function (41-44).

Thus, the activation of free radical oxidation, development of oxidation stress and free radical pathology against the background of imbalanced changes in nitric oxide generation and glutathione link of antioxidant protection (AOP) should be referred to the main mechanisms, leading to reproductive function disorders in highly productive cows. The regularities ascertained in connection with reproductive health of animals, functions of AOP-lipid peroxidation and nitric oxide may be used in developing treatment-prophylactic measures.

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