Oxidative stress and its significance in the pregnancy pathogenesis in dairy cows

V Safonov, I Ventsova and G Pelevina
Voronezh State Agrarian University named after Emperor Peter the Great, 1, Michurina street, Voronezh, 394087, Russia

Abstract. The lipid peroxidation and the antioxidant defence system indices in red-and-white dairy cows with the pregnancy pathology development - gestosis and with its physiological course - were investigated. The study objects were the Druzhba breeding plant (Voronezh region) animals with average annual milk production of 6.5 – 6.7 thousand kg. For the experiment, two groups were formed, the first (n=18) consisted of animals with gestosis clinical manifestations, the second (n=12) - animals with a pregnancy physiological course. The animals' clinical condition was assessed using conventional methods. An increase in the malondialdehyde content in the animals' blood with pathology, an increase in the catalase and glutathione peroxidase activity were found. The antioxidant defence non-enzymatic link components content - vitamin E and vitamin C - was reduced. With clinical gestosis, a decrease in the content of progesterone, testosterone, estradiol-17 β and cortisol in the blood plasma was noted, which indicates a fetoplacental complex functional insufficiency. Thus, oxidative stress arising against the imbalance background in the system "lipid peroxidation - antioxidant protection" is one of the factors determining the gestosis pathogenesis. The study results enhance the processes understanding occurring during the pregnancy pathological conditions development and will be useful in choosing prevention and therapy effective methods.

1. Introduction
To date, it has been established that a living organism universal reaction to any stress factors effect is the oxidative stress development with a decrease in the enzymes and other substances' activity responsible for antioxidant protection [1, 2].

Oxidative stress (OS) occurs due to the excess free radicals' formation in the body - reactive oxygen species (ROS). Free radical oxidation (FRO) is recognized as one of the dominant metabolic processes that spontaneously occur at the cellular level, which underlie plastic and energy metabolism. FRO directly influences adaptive changes in the body due to its participation in the oxygen, proteins, lipids and carbohydrates conversion regulation.

Free radical reactions in the body occur as a result of the reactive forms and oxygen radicals formation (‘O₂, O₂⁻, ‘OH) in the molecular form O₂ and in the oxidation substrates presence, which are polyunsaturated fatty acids, and variable valence metal ions (Meⁿ⁺). During the oxygen molecular form reduction, activated forms are formed, which in turn activate the lipid peroxidation (LPO) reactions ROS represented by hydroperoxide radical (HO₂⁻), superoxide anion radical (O₂⁻), hydrogen peroxide (H₂O₂) and singlet oxygen (‘O₂) react with polyunsaturated lipids, some of which serve as cell membranes structural components. The result is the LPO primary, secondary and final products - peroxide radicals.
(RO$_2^\cdot$). Free radicals are involved in electron transfer, cell membrane conductivity modification, cell proliferation and apoptosis, ovulation and egg fertilization processes also occur with the LPO products participation. It is generally accepted that the free radicals' inclusion in these processes arose as a living organisms' adaptation result to oxygen respiration.

Free radicals excessive synthesis and accumulation due to their increased reproduction by macrophages, monocytes and granulocytes, with a utilization reduced possibility, leads to oxidative phosphorylation uncoupling in mitochondria, the biological membranes fluidity and integrity disruption, increases the permeability to various substances, changes the ion channels, receptors and membrane proteins physiological functions [2, 3, 4]. The chromatin structural organization can also change, errors occur in reading genetic information. Thus, the free radical oxidation affects all levels of adverse effects, starting with the molecular one. A shift in the balance between the FRO processes and the antioxidant defence system (AOD) triggers pathological reactions in the body that lead to the diseases' development [1, 2, 5, 6, 7, 8].

One of the lipid peroxidation key products is malondialdehyde (MDA), which is highly reactive. With an increase in its content in the body, structural and functional disorders in living systems are associated. MDA modifies the nucleic acids' structure, increases the calcium channels permeability, inhibits mitochondrial enzymes, and stimulates the cytochrome C release from mitochondria. MDA is utilized in a successive reactions' series with the reduced glutathione and inert D-lactate formation [4].

Antioxidant protection is carried out by a multicomponent system, which includes enzymatic and non-enzymatic links. Superoxide dismutase (SOD), catalase, glutathione peroxidase (GPO) and other components that are part of the defence enzymatic link together with vitamins E and C, carotene, albumin, ceruloplasmin (non-enzymatic link) in the normal physiological state of a living organism keep the lipid peroxidation processes under control within the framework ensuring the homeostasis maintenance.

One of the main antioxidant defence enzymes is superoxide dismutase, an enzyme that catalyzes the superoxide radicals’ dismutation with the hydrogen peroxide and molecular oxygen release and protects intracellular structures.

The hydrogen peroxide's optimal amount by its destruction is maintained by catalase and peroxidases. The extremely significant enzyme catalase destroys H$_2$O$_2$ without the oxygen acceptors participation, retains its activity for a long time and does not require activation energy. The rate of inactivation of H$_2$O$_2$ is limited only by the substrate diffusion intensity to the enzyme active centre.

In addition to SOD, the superoxide radical dismutation reaction is carried out by ceruloplasmin (CP), a metalloprotein containing copper, which is included in the blood serum α-globulin fraction. Due to the compound high resistance to the free radicals toxic effects, CP maintains its activity for a long time during ROS intensive production. CP protects lipid structures by trapping free radicals circulating in the bloodstream [9].

The enzyme glutathione peroxidase catalyzes the hydrogen peroxide and fatty acid hydroperoxides' conversion hydrogen peroxide and fatty acid hydroperoxides to hydro compounds that can be utilized by the cellular system's components and prevents the hydroperoxides further formation. Selenium-dependent SOD is included in the anti-peroxide complex, which also includes glutathione and glutathione reductase (GR). Glutathione reductase maintains the glutathione reduced form required level, which in turn acts as a hydrogen donor for the glutathione peroxidase mechanism for the hydroperoxides' reduction [10].

Ascorbic acid, which is part of the non-enzymatic link in antioxidant defence, has a wide range of antioxidant properties, including the ability to reduce the α-tocopherol oxidized form [4]. Vitamin E, or α-tocopherol, has the non-enzymatic link sulfur components the highest biological activity as a singlet oxygen form “quencher”, an anion-radical acceptor and free radicals “interceptor”, with which it reacts at the chain termination stage [11, 12, 13, 14].

Β-carotene and other carotenoids exhibit antioxidant activity. It also possesses nitric oxide NO$^-$ in cases where its excessive accumulation does not occur. It participates in the superoxide radical anion utilization process and increases the EL enzymatic link activity [15].
LPO rates rise in cows towards the lactation final stage, usually coinciding with the pregnancy period. It has been proved that the lipid peroxidation processes in deep-wounded animals are more intensive than in non-fertile ones. The pregnant cows' antioxidant defence system is under increased stress, and oxidative stress is aggravated by the iron-deficiency anaemia presence in animals [16, 17, 18, 19, 20]. Often, when the LPO-AOD system components balance is disturbed, the reactive oxygen species formation goes out of control, leading to cellular respiration and homeostasis violation in general, affecting the reproductive system organs functioning [21, 22, 23].

In assessing the pregnancy pathologies, the LPO-AOD system is of high diagnostic value. Traditionally used metabolic parameters show less diagnostic specificity and sensitivity. Among the LPO-AOD system indicators, the most significant are the superoxide dismutase activity, the ketodienes and conjugated trienes intermediate products' concentration in the pregnant animals' blood, and the malonic aldehyde content. The above indicators' highest values are observed with the gestosis development against the subclinical ketosis background (gestosis-ketosis syndrome) [24].

This study aim was to determine the free radical oxidation features and the antioxidant defence system functioning in pregnant dairy cows with normal pregnancy and pathology, taking into account hormonal levels.

2. The materials and methods
The study was carried out on the Agricultural Academy of the Druzhba breeding plant basis, located in the Voronezh region, Pokrovskoe village. For the experiment, the red-and-white breed cows with average annual milk production of 6.5-6.7 kg were selected. The selected animals were formed into groups: gestosis clinical signs (n=18) and with the pregnancy physiological course (n=12).

The animals' clinical condition was assessed using conventional methods.
LPO processes and the AOD system state were assessed by the malondialdehyde (MDA) determination, glutathione peroxidase (GPO), catalase, the stable nitric oxide metabolites amount (NO'), ceruloplasmin (CP), the vitamins E and C content in the venous blood.
The animals' hormonal status was assessed by estradiol, progesterone, testosterone, cortisol. The hormones content in blood plasma was determined by EIA. Blood was taken from the animals in the morning from the jugular vein under the aseptic and antiseptic conditions.
The obtained measurements were processed using the Statistica 5.0 computer program in compliance with the mathematical statistics accepted methods used in medicine and biology. The differences significance between indicators was assessed by Student's t-test. Differences were considered statistically significant at P<0.05.

3. Results and discussion
The substances content characterizing the LPO-AOZ system state is presented in table 1. Compared with the pregnancy physiological course with clinical preeclampsia, a significant increase of 42.3% in the MDA level in the blood was established, the stable nitrogen metabolites level was increased by 31.9% in gestosis. The AOZ system enzymes increased activity indicates a body compensatory reaction development in response to oxidative stress. The GPO and catalase activity is higher in animals with pathology: The GPO exceeds by 26.0%, catalase - by 17.3% in comparison with the healthy animals' indicators.

In relation to the protection non-enzymatic link, deviations from the norm towards a decrease in concentrations are observed. The vitamin E content is reduced by 44.5% with the gestosis and vitamin C development - by 20.8%. In animals with pathology, a slight decrease in the ceruloplasmin activity was noted - by 5.6%. These indicators are probably due to the non-enzymatic link substances increased consumption for the LPO products neutralization.

If there is a significant difference in the LPO-AOZ system indicators in animals' two groups, it can be concluded that the pregnancy pathology in dairy cows considered in the study develops against the FRO processes background.
Table 1. The LPO-AOZ system state indicators content in cows during the pregnancy physiological course and during the gestosis development.

| Indicator                | Gestosis       | Physiological course (norm) |
|--------------------------|---------------|----------------------------|
| MDA, μM/l                | 1.48 ± 0.14   | 1.04 ± 0.14                |
| GPO, mMG-SH/l min        | 18.4 ± 2.58   | 14.6 ± 1.54                |
| Catalase, mM H2O2/l min  | 35.3 ± 2.44   | 30.1 ± 1.26                |
| NO⁺, μM/l                | 79.3 ± 8.19   | 60.1 ± 8.02                |
| Vitamin E, μM/l          | 7.7 ± 0.93    | 11.2 ± 0.89                |
| Vitamin C, mM/l          | 12.0 ± 1.69   | 14.5 ± 5.73                |
| CP, μM benzoquinone/l min| 268.5 ± 9.68  | 284.3 ± 11.08              |

The animals' hormonal status with abnormal pregnancy was also adversely affected by oxidative stress caused by the free radicals' accumulation in the body. The EIA tests results are presented in table 2.

Table 2. Sex and corticosteroid hormones concentration in cows during the pregnancy physiological course and during the gestosis development.

| Indicator             | Gestosis       | Physiological course (norm) |
|-----------------------|---------------|----------------------------|
| Estradiol-17β, pg/ml  | 215.2 ± 17.90 | 273.4 ± 38.40              |
| Progesterone, ng/ml   | 10.5 ± 2.09   | 24.7 ± 4.62                |
| Testosterone, ng/ml   | 0.7 ± 0.09    | 1.3 ± 0.22                 |
| Cortisol, ng/ml       | 24.4 ± 3.01   | 32.7 ± 5.79                |

The sex and corticosteroid hormones concentration is reduced in the animals' blood with gestosis clinical manifestations. The progesterone level is 42.5%, testosterone - 53.8%, estradiol-17 β - 78.8%, cortisol - 74.6% of the hormones corresponding level in the animals' clinically healthy group. Such indicators reflect the fetoplacental complex functional insufficiency, which leads to negative consequences for the reproductive system functioning and affects the offspring viability. The oxidative stress damaging effects negatively affect the body endocrine system, in this case, the ovaries and adrenal glands.

4. Conclusion
The study found that the gestosis development in dairy cows occurs against the imbalance background in the LPO-AOZ system. A significant increase in the nitric oxide stable metabolites' concentration, MDA, GPO activation with a decrease in the body non-enzymatic defence link indicators has been proved.

An oxidative stress negative consequence in sick animals was a physiological hormonal status violation, which is confirmed by a decrease in the sex hormones and cortisol concentration and indicates fetoplacental complex inhibition.

The study results enhance the processes' understanding occurring during the pregnancy pathological conditions development and will be useful in choosing prevention and therapy effective methods.

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