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Markers of lipid metabolism and antioxidant system of organisms of cows depending on their physiological state

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Abstract. The study is aimed at determining the etiopathogenesis of reproductive ability disorder among high-producing cows. For this purpose, a group of cows amounting 37 heads was formed following the principle of analogous pairs. The cows were inseminated during the first estrum after calving, and then their blood was drawn using Monovette system taking into account sterility terms. In the drying off period, the blood was drawn 1-4 days before calving and during the first day after calving. Totally 253 blood samples were tested. Proceeding from the results, cows were divided into two groups. The first group included 20 cows impregnated after the first insemination; the second group included 17 not impregnated cows. Later the blood was drawn providing for the parturition process and postpartum period. Blood values were investigated by commonly accepted methods using certified equipment. The study showed that cows during the early gestation have lipid metabolism, lipid peroxidation and antioxidant system state significantly differing from those among non-pregnant cows. During the gestation, cows have the level and class of general lipids reducing, as well as the accumulation of lipid peroxidation products. Cattles with retained placenta have low lipid metabolism and higher level of peroxidation within interlactation period. After the calving, this difference tends to widen. The obtained data can be used for elaboration of measures preventing post-parturient complications by applying agents with antioxidant properties.

1. Introduction
Stimulation of dairy cattle breeding aims to increase the volume of milk production, which largely depends on the feeding conditions during preparation for calving and after calving. This aspect is of great importance for the industrial technology of milk production, since the preparation is required for insemination and calving of not particular heads of cattle, but of a large number of their analogues in terms of age and gestation age. Preparation for calving and calving itself demands balanced feeding. Feeding of pregnant animals greatly influences the fetal development, especially during the late gestation when the fetus grows particularly intensively. Therefore, when preparing high-producing cows for calving and subsequent lactation, it is important to establish the level of feeding that ensures lipid metabolism, both for their body and for creating a reserve of nutrients in the mother's body for the postpartum period and future lactation. Microbial digestion of fiber and other nutrients in a forestomach of ruminant animals indicates the influence nature of the formed and used metabolites of the substrate [17]. Due to the relative values of some key processes, lipogenesis among ruminant animals significantly
differs from similar processes among non-ruminant ones. Acetate is the main precursor of high molecular weight fatty acids got by ruminant animals [19]. That is why ruminants, particularly cows, are much more dependent in energy metabolism on non-glucose metabolites than nonruminant animals, although cows’ feed contains relatively small amount of lipids, approximately 5% of fat. Lipids serve as the main form of energy storage in the body. The main metabolic role of adipose tissue of all placental animals is to remove lipids and lipid precursors from the blood when there is a caloric excess and the release of fatty acids during periods of energy deficiency. This metabolic situation is peculiar to cows, especially high-producing ones – 2-3 months of lactation. Even with all the well-known nutritional balance requirements, cows lack energy and protein for milk production [6]. In this connection, there is a need to establish the boundaries of the maximum and optimal mobilization of reserve lipids in cows and to find out the factors favorable to the use of fat reserves in the life of cows. Research in this area would allow a better understanding of the effect of tissue lipids on the general body energy, lactogenesis and manifestation of the reproductive function of cows [7, 11].

Industrial technology for the production of animal products involves the permanent adaptation of animals to the effects of various adverse stress factors caused by keeping, feeding and exploitation conditions [1, 5, 9, 13]. The most significant negative effect of such factors is exerted on pregnant cows, who consumes more energy because of intensive exploitation and development of a fetus [4, 8], which leads to the intensive use of plastic tissue compounds, primarily depot fat and then structural lipids [7, 14, 15]. Mobilization of fatty acids occurs in complex oxidation reactions with carbohydrates and is often disrupted because of the relative lack of carbohydrates. In this connection, under-oxidized compounds enter the bloodstream leading to depletion of the animal organism, vitamins, and bioantioxidants arising due to high physiological load, stresses, feeding mistakes, which creates favorable conditions for the activation of non-enzymatic lipid oxidation [2, 5, 6, 10, 11, 14] which adversely affect the state of metabolism, and therefore the reproductive function of animals [12]. However, there is a lack of studies on this issue in world and domestic literature. In the available literature, there is no information about the effect of lipid metabolism and the antioxidant system of the body during gestation and the occurrence of obstetric pathology, which determined the topic of the present research.

2. Study purposes
The study is aimed at determination of etiopathogenesis of reproductive dysfunction of high producing cows. To achieve this goal, the following tasks were solved:

- to study the lipid peroxidation and the state of the ascorbant and thiol-disulfide redox system and the content of vitamins A and E depending on the physiological state;
- to determine the effect of the lipid spectrum of blood serum of dry cows on calving and postpartum period.

3. Materials and methods
The studies were performed with black-and-white cows aged 3-5 lactations with a live weight of 480-520 kg. For these purposes, a group of cows was formed in the amount of 37 heads of cattle inseminated after calving of the first estrum, with the further regular blood drawing using Monovette system in the following gestation periods: 4-7; 14-17; 24-34; 35-50; 88-95; 195-210 days; during the drying off period; 1-4 days before calving and on the first day after calving. Totally 253 blood samples were examined. Then depending on the result of insemination, animals were divided into two groups. The first group included 20 cows fruitfully inseminated. The second group included 17 cows that were not impregnated after the first insemination. During the research, the first group of animals was divided into two groups according to the results of the parturition and postpartum period. The first group included animals with a normal physiological course of parturition and the postpartum period (15 animals). The second group included cows with parturition pathology (retained placenta) and postpartum complications (postpartum endometritis) (5 animals).
The content of serum lipids was investigated using a unified method with application of equipment LAKHEMA made in Czech Republic, lipid extraction was studied according to Folch method followed by thin-layer chromatography on FIXION-50x8 plates and densitometry. Lipid peroxidation products (malondialdehyde, diene conjugates, triene conjugates) were studied by the method of Kitabchi and Bulge. The state of ascorbate and thiol disulfide redox systems was studied by the method of V. Sokolovsky (1974). Determination of vitamin A and E was performed spectrophotometrically using Bier method.

The obtained data of biochemical studies were interpreted by the method of retrospective analysis. All obtained digital materials were processed biometrically using Microsoft Excel, the difference criterion in percent was determined.

4. Results
As a result of the studies, the biochemical parameters of cows’ blood had significant differences in a number of indicators depending on the productivity of insemination (Table 1).

The content of total serum lipids of pregnant animals was 15.8% higher compared to unfertilized animals. In the class composition of lipids, the most significant difference was found in the content of unesterified fatty acids (UEFA), triglycerides and cholesterol, both free and ether-bound. These differences are explained by high energy consumption among pregnant animals, which is necessary for the formation of an embryo, development and growth of a fetus, as well as for maintaining homeostasis of mother’s body. The decrease in free cholesterol (by 28.65%), especially its ester-bound fraction (14.91%), observed among non-pregnant animals, indicates a lower synthetic function of the liver, as evidenced by the low serum levels of vitamins A and E (a difference of 11.3 and 23.65%).

Table 1. Biochemical blood values of pregnant and unfertilized cows.

| Values                          | Group of cows                      | Difference, % |
|--------------------------------|------------------------------------|---------------|
|                                | first (pregnant)                   | second (non-pregnant) |               |
| Amount of heads                | 20                                 | 17            |               |
| Lipids, mg%                    | 426.1±23.4                         | 358.6±21.9    | -15.84        |
| Phospholipids, mg%             | 267.5±18.3                         | 259.0±18.7    | -3.18         |
| Free cholesterol, mg%          | 34.2±2.8                           | 24.4±3.2      | -28.65        |
| Unesterified fatty acids, mg%  | 44.7±3.0                           | 17.0±3.1      | -61.91        |
| Triglycerides, mg%             | 21.6±2.7                           | 3.7±1.9       | -99.61        |
| Esters of cholesterol, mg%     | 58.11±4.7                          | 49.44±4.2     | -14.91        |
| Lipid hydroperoxides (MDA), umole/l  | 4.89±0.45                         | 5.03±0.62     | +2.85         |
| Diene conjugates (rel. absorbance unit per 1 ml of plasma) | 1.48±0.21                         | 2.11±0.26     | +42.57        |
| Tocopherol, mg%                | 0.712±0.092                        |               | -25.65        |
| Vitamin A, ug%                 | 42.3±2.9                           | 37.5±3.8      | -11.3         |
| Ascorbate system: (oxide forms), mg% | 0.38±0.06                        | 0.41±0.06     | +7.89         |
| (deoxide forms), mg%           | 0.39±0.05                          | 0.28±0.04     | -26.3         |
| Thiol Disulfide system: (oxide forms), mole/l  | 22.0±1.8                          | 29.3±3.1      | +33.18        |
| (deoxide forms), mole/l        | 32.8±2.3                           | 28.4±2.5      | -13.42        |

In addition, a higher level of malondialdehyde (MDA) and diene conjugates of fatty acids (products of lipid peroxidation) characterizes the increased degree of free radical oxidation. A shift towards the oxidized forms of the components of both the ascorbate and thiol disulfide antioxidant systems, as well as the lower tocopherol content, indicate inadequate tension of the antiradical chains and a decrease in their buffer capacity, which negatively affects the ovarian function [18].

With an increase in the gestation period, some changes were detected in the lipid spectrum of blood serum of pregnant animals. For instance, a decrease in the level of lipids, unsterilized fatty acids and
triglycerides was observed, which is quite typical for this physiological period of a gradual decrease in lactation performance and yet insignificant growth of the fetus. In the redox ascorbate and thiol disulfide systems, a slight increase in oxidized products comparing with reduced products and a slight increase in lipid peroxidation products were recorded.

The drying off period of animals was a significant stress for organisms of pregnant cows that affected the biochemical pattern of blood, which was manifested as the significant increase in lipid peroxidation products: malondialdehyde by 12-17%, diene and triene conjugates by 10-15 and 8-15%. 1-4 days before calving, animals that further would have had the pathological course of calving, in particular retention of the placenta (5 goals), were characterized by an increased plasma content of lipid oxidation products. Thus, the difference in the number of dienes, trienes, and malondialdehyde products was 14.0, 12.0, and 23.5%, compared with animals without obstetric pathology. During the first days after calving, this difference increased more. Cows with retention of placenta had a significantly higher level of lipid peroxidation products and were characterized by a predominance of oxidized products over the antiradical thiol disulfide and ascorbate systems restored in the antioxidant balance. The content of vitamins A and E in blood of these animals both during the drying off period (a few days before calving), and after calving, was significantly lower (averagely by12-18%).

Changes in the lipid spectrum of blood serum of pregnant cows during the drying off period, shortly before calving and immediately after calving, are presented in table 2.

As follows from the data presented, animals with retention of the placenta had a lower serum lipid content and, accordingly, all their classes. Moreover, during the drying off period, the amount of total lipids in blood of cows with obstetric pathology amounted to 90.3% of the level of normally calving cows, before calving – 85.4%, after calving – 87.0% respectively. A similar pattern was observed for the class of phospholipids.

Table 2. Biochemical markers of lipid metabolism depending on the manifestation of parturition and postpartum pathology.

| Markers          | Parturition and postpartum period | Physiological periods                              | 1-4 days before calving | 1-2 days after calving |
|------------------|-----------------------------------|----------------------------------------------------|-------------------------|------------------------|
| Lipids, g/l      | Without pathology                 | interlactation                                     | 3.42±0.22               | 3.38±0.25              |
|                  | With pathology                    | 3.09±0.27                                          | 2.99±0.11               | 2.63±0.08              |
| Difference, %    | Without pathology                 | -9.65                                              | -16.54                  | -12.92                 |
| Phospholipids, mg%| With pathology                    | 233.97±20.19                                       | 227.87±17.87            | 208.49±18.96           |
|                  | Difference, %                     | 207.23±13.47                                       | 201.05±17.18            | 178.45±5.47            |
|                  | Without pathology                 | 22.29±1.62                                         | 22.30±3.61              | 18.58±1.71             |
| Free cholesterol, mg% | With pathology               | 19.21±2.34                                         | 17.65±1.57              | 15.81±1.25             |
|                  | Difference, %                     | -13.82                                             | -28.60                  | -14.91                 |
|                  | Without pathology                 | 19.36±2.36                                         | 24.75±2.02              | 19.02±2.65             |
| Unesterified fatty acids, mg% | With pathology               | 20.71±1.85                                         | 22.64±1.88              | 20.58±1.49             |
|                  | Difference, %                     | +6.97                                              | -8.86                   | +8.20                  |
|                  | Without pathology                 | 10.42±1.23                                         | 8.96±1.13               | 9.37±1.05              |
| Triglycerides, mg%| With pathology                    | 9.16±0.99                                          | 10.76±2.01              | 8.32±1.03              |
|                  | Difference, %                     | -12.10                                             | +20.08                  | -11.21                 |
|                  | Without pathology                 | 48.93±2.23                                         | 51.79±6.57              | 43.75±3.69             |
| Cholesterol ester, mg% | With pathology               | 46.67±5.01                                         | 47.37±4.14              | 40.23±2.39             |
|                  | Difference, %                     | -4.62                                              | -8.54                   | -8.05                  |

Note: without pathology - blood markers of cows with normal calving: 15 heads; with pathology - retention of the placenta and endometritis (5 heads).

Among cows with the retention of placenta, the amount of phospholipids after calving had a greater difference (85.5%, if compared with animals without obstetric pathologies). The fraction of cholesterol,
both free and ether-bound, during all of the above periods among normally calving cows excelled this fraction among cows with the retention of placenta. The most notable difference was recorded in the class of unbound cholesterol 1-4 days before calving. The level of this metabolite within this period among animals with obstetric pathology amounted 79% of the level among healthy animals. Parturition of animals with normal calving featured by more intensive preparatory period, as indicated by a significant mobilization of energy reserves from the fat depot 1-4 days before calving. It implied the decrease in triglycerides and increase in unesterified fatty acids. Animals with the placenta retention energetically prepared for calving less intensively.

5. Discussion
Since the problem is multifactorial, the authors have studied the influence of the physiological state of cows on indicators of lipid metabolism and antioxidant system of the body, which are one of the main parameters determining the reproductive ability of animals [8, 10, 13].

It is known that the main source of energy in the diet of ruminants is carbohydrates, since rumen microorganisms convert up to 50% of feed carbohydrates into acetic, propionic, butyric and other acids, which are absorbed into the walls of forestomachs, enter the bloodstream and are actively used in metabolic activity, providing animal’s organism with 70% of energy. Lack of energy negatively affects the success of insemination [6, 18]. The drying off period is a significant stress for a cow’s body, which naturally affects the biochemical markers of blood. The negative effect manifested 1-4 days before calving as a significant increase in products of lipid peroxidation (malondialdehyde – by 12-17%, dionic and triene conjugates, respectively, by 10-15 and 8-15%), which is confirmed by blood markers with an increased content of lipid peroxidation products in plasma among animals with retention of placenta. They were also characterized by the predominance of oxidized products over reducing ones in the antioxidant balance of the antiradical thiol disulfide and ascorbate systems. In addition, a higher level of malondialdehyde (MDA) and dionic fatty acid conjugates, products of lipid peroxidation, characterize the increased degree of free radical oxidation, which negatively affects the function of bovine ovary [18]. Animals with retention of the placenta had lower serum lipids and, accordingly, all their classes. Moreover, during the drying off period, the amount of lipids among cows with obstetric pathology was 90.3% of the level of normally calving cows before calving - 85.4%, and after calving, respectively - 77.0%. The indicators of lipid metabolism and antioxidant system [6, 18] of cows that were not pregnant after the first insemination are also significantly less than of successfully inseminated ones. During the gestation development, there is a decrease in lipids by 15.84%, uncertified fatty acids - by 61.91%, triglycerides - by 59.61% and there is a tendency to the accumulation of lipid peroxidation, which is apparently associated with preparation for calving and high productivity of cows in the first 80-100 days after calving. If a deficiency of carbohydrates occurs and an insufficient amount of acetic and propionic acids is formed in the rumen, it develops into the reduction of the glucose synthesis in the liver, meanwhile the lack of energy is compensated by the use of adipose tissue of the body. The data of present research coincide with the opinion of a number of authors [4, 8, 14] who notes that in the first three weeks after calving, energy is necessary primarily for the normal functioning of the uterus and the activation of cow’s ovaries. At the same time the level of energy consumption at the beginning of lactation is up to 60%, which higher than of non-lactating cows - 30-40%. Within the gestation development, some dynamics of a decrease in the level of lipids, non-certified fatty acids and triglycerides is observed among pregnant high producing cows, which is quite typical for this physiological period when there is a gradual decrease in milk productivity and a slight intensity of fetal growth in the embryonic period. The markers of lipid metabolism and antioxidant system of the organism of non-pregnant cows are significantly less than of successfully inseminated cows. Along with the gestation development, the level of lipids of cows decreases by 15.84%, uncertified fatty acids - by 61.91%, triglycerides - by 59.61%, and there is a tendency to the accumulation of lipid peroxidation products, which is apparently caused by preparation for calving and high productivity of cows in the first 80-100 days after calving. During this period, due to a deficiency of carbohydrates in the rumen, an insufficient amount of acetic and propionic acids is formed, as a result of which the synthesis of
glucose in the liver is reduced, and the lack of energy is compensated by the use of adipose tissue of the body. According to a number of authors [4, 8, 14], in the first 2-3 weeks after birth, energy is necessary, first of all, for the normal functioning of the uterus, the activation of the ovaries. The level of energy costs in lactating animals at the beginning of lactation is 30-60% higher than in non-lactating animals [9]. Lack of energy in the diet of newborn cows leads to a violation of carbohydrate-fat metabolism, which is expressed by a low energy level of lipid metabolism and a higher level of lipid peroxidation in animals with postpartum pathology [1, 2, 10]. The indicators of lipids, phospholipids, free cholesterol, non-verified fatty acids during the interlactation period, 1-4 days before calving, 1-2 days after calving, were higher among cows that passed through parturition and postpartum period without pathology.

The firstly obtained data on indicators of lipid metabolism and antioxidant system of cows, that depend on the physiological condition of cows and the manifestation of parturition and postpartum pathology, can be used for timely correction of metabolic disorders among cows, as well as serve as an indicator of prevention of reproductive disorder of high producing cows with the use of antioxidant agent.

6. Conclusions
Based on the studies, the following facts were ascertained:
- at the stage of early gestation, cows have significant differences in lipid metabolism, lipid peroxidation and in the state of antioxidant systems, compared with unfertilized animals, which is manifested as a sufficiently high content of lipids of various classes, low level of lipid peroxidation and significant capacity of antioxidant systems;
- during the development of cows’ gestation, there is a decrease in the level of lipids, their classes, as well as a tendency to the accumulation of lipid peroxidation products;
- animals with the placenta retention within interlactation period are characterized by a lower energy level of metabolism, in particular lipid metabolism, and a higher level of lipid peroxidation.
- cows with placenta retention have the amount of phospholipids that is significantly less compared to animals without obstetric pathology. The cholesterol fraction, both free and ether-bound, of normally calving cows exceeds the one of cows with placenta retention during all the periods;
- the obtained data can be used for timely correction of lipid metabolism and antioxidant system disorders among cows, also serving as the indicator of reproductive disorder of high producing cows.

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