The dairy cows post-partum period dynamics during the ovaries ovulatory function recovery and depression

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Abstract. A significant problem in the dairy farming industry is the decrease in the livestock reproductive capacity due to the ovaries physiological functions violation. This study examines the lipids, proteins and carbohydrates metabolism state, depending on the sexual cyclicity recovery in animals degree, while observing adequate feeding. For the experiment, 16 new-calving Holstein-Friesian cows with an average annual milk yield of 9.5 thousand kg were selected, divided into 2 equal groups, depending on the ovulatory function resumption. All animals received a balanced diet. Blood was taken on days 6, 12, 19, 40 and 68 after calving. Blood serum was separated and the total protein and individual fractions content, as well as the creatinine, urea, total lipids, cholesterol, triglycerides, glucose amount, were determined. To determine the hormonal status, the content of estradiol and progesterone was examined. The reproductive organs condition was assessed using transrectal palpation and using an ultrasound apparatus. We found that cows with ovarian dysfunction are characterized by protein and glucose globulin fraction decreased concentrations in the blood, increased creatinine content and hypothalamus-pituitary-gonad system hormone-producing function inhibition. With an accuracy of 87.5%, the glucose threshold level in the blood serum was revealed, at which ovarian dysfunction can be predicted: a decrease in fertility in cows is observed at a rate of <2.2 mmol/l. Taking into account the previous studies results, further research expediency on finding the hormonal and metabolic status relationship with ethology typological features and the central nervous system state is substantiated.

1. Introduction
The decline in the highly productive dairy cows reproductive capacity and fertility is one of the modern dairy cattle breeding significant problems [1, 2]. It is associated, as a rule, with the ovaries functional disorders that develop in cows after giving birth during the lactation dominant formation. This pathology is manifested by ovo-folliculogenesis depression, sexual cyclicity delayed renewal, the interbody interval lengthening and is recorded in 17 ... 35% of new-calf animals [3, 4, 5, 6]. It is believed that the main reason for its manifestation lies in unfavourable shifts in metabolic, immune and cows endocrine homeostasis caused by a negative energy balance formed in the prenatal and early postnatal periods, which is not fully compensated for by the feed intake [1, 7, 8, 9]. It is called a critical stage in the lipids and carbohydrates metabolism, aimed at meeting the mammary gland metabolic requirements [10]. The
highly productive lactating ruminants organism functions in metabolic processes and metabolic needs special regime chronic stress necessary for the high milk productivity genetic potential realization [11, 12].

2. Materials and methods

The research objects are Holstein-Friesian cows with average annual milk production of 9.5 thousand kg, belonging to Verbilovskoye LLC, the Lipetsk region, in complete feeding conditions in basic nutrients terms. The diet included hay, haylage, corn silage, beet pulp, a concentrated feed and dietary supplements mixture. There were 16 animals under observation with physiologically proceeding childbirth and the post-partum period. On days 6, 12, 19, 40 and 68 after calving, the total protein content in the venous blood serum was determined using an R2-1 refractometer (Poland), its fractions by electrophoresis in agarose gel, urea, creatinine and cholesterol using a biochemical analyzer "Hitachi - 902" (Japan) using reagents from Randox (Great Britain) and Vital (Russia), triglycerides and glucose by the enzymatic colourimetric method using reagents from Vital and a KFK-3M spectrometer (Uniko SIS, Russia), total lipids by the photometric method with phosphovaniline using the reagents Total - Lipids - DAC. Lg "(Moldova). The ovaries' ultrasound scanning was carried out using the Draminski iScan apparatus (Poland). The data obtained were analyzed for the cows' group with the ovaries ovulatory function restored (n=8) and the group with depression (n=8). Digital data mathematical processing was carried out under the descriptive statistics methods using the application program Statistica 8.0 (Statsoft Inc, USA). The research results were expressed as the arithmetic mean and standard deviation (M±SEM). Differences were considered statistically significant at P<0.05, which were determined using the paired Wilcoxon W test.

3. The results

In the lactation first days, total protein content in blood serum indicators in all cows was at the reference values lower border (table 1). By the lactation second weekend, its concentration increased by 9.7%, and after 10 weeks - by 19.4 ... 21.3%. No pronounced differences were found between animals groups.

The albumin and globulins content in the cows' blood during the ovarian function restoration for lactation six weeks was at a stable level. Two months after calving, the albumin amount in the blood decreased by 13.7%, with an increase in α-globulins by 15.0%, β-globulins by 9.6 and γ-globulins by 3.5%. The protein index changed from 0.58 to 0.46 (20.7%).

Table 1. Metabolism proteins and end products contained in the cows’ blood serum in the post-partum period and lactation dynamics.

| Indicator     | 6     | 12    | 19    | 40    | 68    |
|---------------|-------|-------|-------|-------|-------|
| Total protein, g/l | 72.9±0.47 | 80.0±1.13 | 82.6±0.56 | 84.4±1.48 | 88.4±2.14 |
| Albumins, %    | 36.6±2.73 | 35.6±1.60 | 36.0±2.46 | 31.6±3.28 |
| α-globulins, % | 12.0±0.58 | 11.6±0.55 | 12.3±0.56 | 13.8±0.49 |
| β-globulins, % | 11.0±0.83 | 10.9±0.97 | 11.2±0.68 | 11.8±0.49 |
| γ-globulins, % | 27.8±1.09 | 27.4±1.05 | 27.4±1.03 | 25.3±0.99 |
| Protein index, A/g | 0.58 | 0.55 | 0.56 | 0.46 |
| Urea, mM/l    | 4.07±0.49 | 4.49±0.31 | 4.78±0.52 | 5.50±0.55 | 5.46±0.41 |
| Creatinine, μM/l | 87.1±4.5 | 83.0±3.4 | 78.8±4.8 | 79.1±8.0 | 77.1±5.6 |

*Note: The upper number - cows indicators with the ovaries restored ovulatory function, the lower - with the ovaries ovulatory function depression.
For animals with the ovaries ovulatory function depression, an albumin higher content in the blood is characteristic: by 8.7 ... 15.7% in the lactation first six weeks and by 29.7% after two months, and an α-globulins lower content - by 6.4 ... 11.9%, β-globulins - by 3.2 ... 14.1%, γ-globulins - by 2.5 ... 16.6%, reflecting their reduced immunological reactivity state. At the same time, the protein index in these animals exceeded the cows' first group indicators after three weeks by 21.8% and ten - by 50.0%. Based on these data, it can be concluded that the ovaries folliculogenesis and ovulatory function depression in cows in the post-partum period is accompanied by a body reduced immune competence.

In cows with the ovaries restored ovulatory function, a gradual decrease in the protein metabolism, creatinine end product blood content, was noted. Its amount during the active follicular growth period (day 19) decreased by 10.4%, and after 10 weeks - by 20.6%. In cows with ovarian depression, this metabolite content in the research different periods was higher by 5.1 ... 23.6%. It can be assumed that an increased creatinine level in the blood, reflecting the liver, kidneys and endogenous toxicosis functional state, has a negative effect both on the ovarian function regulation central axis (at the hypothalamus-pituitary gland level) and directly on the ovaries' tissue structures.

In the urea content terms in the different cows' groups blood, no pronounced differences were recorded. They were within the reference values. In the post-partum period, the urea concentration in the first group cows' blood gradually increased by 34.2, the second - by 50.1%.

The studies have shown that the early lactation period in cows is associated with main energy substrates low blood content - lipids and glucose, which ensure normal lactogenesis and lactopoiesis, the endocrine system functioning and intracellular signalling pathways. So, with the lactation onset, the total lipids content in the cows' blood serum was only 1.76 ± 0.68 ... 1.80 ± 0.08 g/l and cholesterol 1.60 ± 0.01 ... 1.57 ± 0.21 mM/l, which is 1.97 ... 1.94 and 2.94 ... 2.99 times lower than the reference values (table 2). This reflects the lactational stress and peroxidation reactions manifestation high level. Cholesterol low levels in the blood in the lactation first week should also be associated with high activity of its hydroxylation processes perinatal period, aimed at the steroid hormones and labour synthesis.

The total lipids and cholesterol content normative indicators in the cows' blood were registered only by the 40th day of the postpartum period, which may indicate the new-born animals' adaptation reactions completion to lactation. During follicles, ovulation active growth and a functionally active corpus luteum formation in the first group cows, in comparison with animals with depression of ovarian function, the total lipids content in the blood was 6.3 ... 14.8 lower and cholesterol by 3.2 ... 12.0%.

Consequently, the ovarian cyclicity restoration in cows after childbirth occurs against lipid compounds increased consumption background and the activity of oxidative processes aimed at increasing the steroid hormones biosynthesis. This is confirmed by the fact that the concentration level in their blood of the lipid peroxidation - malondialdehyde intermediate product during this period was 1.78 ... 2 times higher than in the second group animals (1.57 ± 0.23 ... 1.59 ± 0.19 μM/l versus 0.88 ± 0.11 ... 0.78 ± 0.07 μM/l), estradiol - 17β - 1.77 ... 1.33 times (0.39 ± 0.003 ... 0.32 ± 0.003 nM/l versus 0.22 ± 0.02 ... 0.24 ± 0.02 nM/l), progesterone - 9.0 ... 27.4 times (8.18 ± 0.09 ... 23.11 ± 1.87 nM/l versus 0.91 ± 0.02 ... 0.84 ± 0.01 nM/l). This leads to the logical assumption that the cow's body in the early lactation period, combined with the ovarian and sexual cyclicity resumption, functions in an oxidation-reduction processes more active and intense mode in comparison with animals with reproductive function depression.

**Table 2.** Lipids and glucose content in cows blood serum in the post-partum period dynamics (M±SEM).

| Indicator      | 6     | 12    | 19    | 40    | 68    |
|---------------|-------|-------|-------|-------|-------|
| Total lipids, g/l | 1.76±0.08 a | 2.12±0.09 | 2.57±0.19 | 3.51±0.23 | 4.34±0.28 |
|               | 1.80±0.08 | 2.32±0.13 | 2.95±0.20 | 3.73±0.27 | 4.31±0.24 |
| Cholesterol, mM/l | 1.60±0.10 | 2.67±0.22 | 3.39±0.25 | 5.34±0.53 | 6.58±0.59 |
|               | 1.57±0.21 | 2.45±0.27 | 3.50±0.39 | 5.98±0.65 | 7.33±0.86 |
| Triglycerides, mM/l | 0.13±0.02 | 0.13±0.02 | 0.13±0.01 | 0.15±0.02 | 0.14±0.02 |
|               | 0.14±0.01 | 0.12±0.02 | 0.15±0.02 | 0.15±0.01 | 0.13±0.02 |
Changes in triglyceride content are more correlated with lactation than with reproductive health. Their concentration low level in all animals in the first two to three weeks of lactation reflects their increased accumulation by the liver during an energy imbalance and the mammary gland increased need for fat synthesis [7]. When the animal organism leaves the energy imbalance, their concentration increases by 15.4 ... 25.0%.

Evaluating the glucose content indicators in the cows' blood, as one of the metabolic substrates in ensuring the energy balance, it can be stated that in cows with restored ovulatory function, its concentration was within the reference values during the research all periods (table 2). This may be due to hepatic gluconeogenesis active processes and a decrease in its use activity as an energy material due to increased lipid catabolism. At the same time, in cows with the ovaries ovulatory function depression, glucose in the blood serum in the study all periods was less than the normative indicators, and lower than in the first group cows 6 days after calving by 18.7%, 12 - 35, 19 - 33, 4 - 40 - 16.1 and on the 68th - by 12.5%. The differences in the lactation first three weeks were statistically significant (p<0.05-0.001). The glucose concentration threshold value in blood serum on days 6 and 12 after calving, at which ovarian dysfunction and decreased fertility of ruminants should be predicted, is below 2.2 mMol/l. The forecast accuracy was 87.5%.

Consequently, the energy costs replenishment for lactation in animals is due to both lipids and glucose - the most labile energy source. The total lipids and glucose blood concentration indices ratio in cows with the ovaries restored ovulatory function during the postpartum period was at the level of 3.6 ... 9.0, and in animals with gonadal function depression 4.5 ... 10.3. If in the lactation first week and after six weeks the difference in the ratio index between the groups is 25.0 ... 27.4%, then during the period when the energy balances the lowest level (12th day after calving) the these metabolites' ratio index in the second group cows exceeded the cows' same indicator in the first group by 65.8%, and on the 19th day of the postpartum period - by 48.1%.

Analyzing the data obtained on the proteins, carbohydrates and lipids metabolism state in cows in the early postpartum period, it can be concluded that their provision with energy resources during the transit period is carried out by lipoid compounds, glucose and, partially, blood proteins active inclusion in the oxidation reactions. Cows with the ovaries restored ovulatory function are characterized by a lipid use more active regime and a glucose use protective regime. Also, these animal populations have higher immunocompetence. The glucose and immunological reactivity compensatory deficiency in cows with ovarian depression is accompanied by a hypothalamus-pituitary-gonad hormone-producing function system certain inhibition (one of the folliculogenesis and ovulation depression causes).

It is necessary to agree with the opinion of ATM Van Knegsel et al [13] that cows balancing the diets in the lactation in glucogenic and lipogenic compounds terms early period is one of the key problems in metabolism in highly productive animals. At the same time, the question remains open why, same breed and equal productivity animals feeding and exploitation under equal conditions, their metabolic needs implementation in the lactation and ovulation formation differs. We have previously shown [14] that the highly productive dairy cows hormonal-metabolic, immune and reproductive statuses state is largely associated with the animal behaviour typological characteristics, determined by the higher nervous activity types. It is also impossible to exclude the certain lines of animals genetic predisposition to the folliculogenesis in the ovaries abnormal course in the early postpartum period. Further research in these areas will make it possible to confirm the relationship between the animals’ metabolic status and the central nervous system and genetic homeostasis functional state, as well as to propose new methods for controlling their fertility.
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