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NEW BIOTECHNOLOGICAL WAY OF SOLVING SUCH PROBLEM AS CHRONIC ENDOMETRITIS IN COWS

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1 Introduction. At the present stage of development of dairy animal husbandry, one of the constraining factors is the gynecological diseases of cows, which include chronic endometritis registered in 15.0-67.0 % of infertile animals [1]. In most cows with chronic endometritis, the uterine cavity is populated by a variety of microflora, which for a long time can support the inflammatory process and prevents the fertilization of animals [2-4].

In recent years, highly productive cows have increased the frequency of inflammatory processes caused by opportunistic microorganisms. First of all, this is due to the introduction of biopharmaceutical broad-spectrum antibiotics into veterinary practice, which led to noticeable disturbances in the environmental relationships between a macroorganism and its microflora [5, 6].

Untimely and ineffective biotechnological treatment of chronic endometritis in cows leads to impaired reproductive function, decreased milk productivity and premature culling [7]. In this regard, the study of the clinical and pathogenetic features of the development of chronic endometritis in dairy cows and the development of biotechnological methods for its pharmacotherapy are high on the agenda and require detailed study.

2 Material and methods. 2.1 Objects of study. The objects of the study are clinically healthy cows and those diseased by chronic endometritis.

2.2 The study of clinical, ultrasound and morphometric diagnostic criteria. The criteria for the diagnosis of chronic endometritis in infertile cows were studied using an Easi-Scan ultrasound scanner equipped with a linear sensor with a frequency of 7.5 MHz. According to the results of the studies, animals were divided into two groups: clinically healthy and those with chronic endometritis.

2.3 Morphological, biochemical, immunological studies of blood of cows.

Blood samples were taken from cows with chronic endometritis and clinically healthy animals for morphological, biochemical and immunological studies. A hemomorphological blood test was performed on an ABX Micros 60 hematology analyzer, and biochemical studies were carried out in accordance with the Methodological Guidelines for the Diagnosis, Treatment, and Prevention of Metabolic Disorders in Productive Animals. Immunological indicators: serum bactericidal activity (SBA), serum lysozyme activity (SLA), total immunoglobulins, circulating immune complexes, leukocyte phagocytic rate (LPR) were determined using standard and unified methods in accordance with the Methodological Recommendations for the Assessment and Correction of the Immune Status of Animals.

2.4 Determining the effectiveness of a comprehensive method for the treatment of chronic endometritis in cows.

The effectiveness of the developed biotechnological method for the treatment of chronic endometritis was studied in 46 sick cows. The animals of the first group (n = 25) on the 1st, 4th, 7th, 10th and 13th days were injected subcutaneously with aminoseleton in increasing doses: 30, 35, 40, 45 and 50 ml and starting from the third day of treatment. Daily during 2-3 days an antimicrobial biotechnological preparation primalact in a dose of 20 ml was administered in the uterine cavity. In the second group (n = 21) in the 1st, 5th and 9th treatment days the animals were subcutaneously injected denatured emulsified placenta at a dose of 25 ml, while intrauterine injection involved metricur according to the instructions for its use. In addition, on the 1st and 13th day of treatment, the animals of both groups were injected intramuscularly with magestro-fan at a dose of 2 ml and tetrahydrovit at a dose of 6 ml, while at 3, 4, 5 days, uterotone was injected intramuscularly at a dose of 10 ml. Assessment of therapeutic efficacy was carried out according to the number of recovered animals, the number of intrauterine administrations of antimicrobial drugs, the percentage of fertilized cows, the rate of fertilization and the period from the start of treatment to fertilization. From 5 animals from each group, blood samples were taken for laboratory tests before and after treatment. The experimental data were processed using the Statistica 8.0 applied statistical program.

3 Results and discussion. Studies on the prevalence of chronic endometritis were carried out on 736 infertile cows in 5 farms of the Kharkiv region. It was found that chronic endometritis was diagnosed in 256 infertile animals, or in 18.1 % of those examined.

Clinical-echographic studies have shown that chronic endometritis is characterized by an increase in the size of the uterus by 1.5-2.0 times, a weakly expressed response to massage, and an elastic consistency. The horns of the uterus in chronic endometritis hang over the edge of the pubic symphysis into the abdominal cavity. With chronic endometritis, catarrhal-purulent, purulent-catarrhal exudate, echographically represented as single echopositive inclusions, is noted. The uterus has a pronounced cavity: 18.4±0.9 mm (11-35 mm), the thickness of the uterine wall in chronic endometritis is on average 8.7±0.37 mm.

The species composition of microflora isolated from the uterine content of

patients with chronic endometritis of cows is represented by *E. coli* (66.7 % of cases), *Staph. aureus* (25.0 %), *Ent. faecalis* (25.0 %), *Ent. faecium* (25.0 %), *Staph. epidermidis* (8.3 %), yeast-like fungi (41.7 %), *Asp. fumigatus* (33.3 %). At the same time, microflora was isolated in the form of monoculture in 41.7 % of cases and in the form of associations in 58.3 % of cases. In the case of chronic endometritis, the fraction of functionally active elements of the endometrium decreases to 12.17 %; a decrease in the volume of epithelial cells of the integumentary epithelium and uterine glands is noted, which indicates the development of dystrophic processes.

Changes in the morphological status of blood of cows with chronic endometritis are characterized by an increase in leukocyte content, in comparison with clinically healthy animals, by 12.2 % ($P<0.05$), eosinophils by 7.1 %, stab neutrophils by 1.68 times ($P < 0.001$), monocytes by 2.5 times ($P < 0.001$), with a decrease in the level of segmented neutrophils by 6.2 % and lymphocytes by 8.0 %. This indicates the depletion of the granulocyte system, pronounced monocytosis and eosinophilia.

In the progression of chronic endometritis, a decrease in the alpha globulin fraction of the protein by 12.8 % was found in the blood of cows, with an increase in the gamma globulin fraction by 9.5 %, creatinine levels by 29.7 % ($P < 0.05$), and midmolecular peptides (MMP) by 25.0 % ($P < 0.001$), malondialdehyde (MDA) by 14.5 % and index of endogenous intoxication (IEI) by 25.4 % ($P < 0.01$), which characterizes the intensification of the processes of endogenous intoxication of the body.

The development of chronic endometritis occurs against the background of increased blood progesterone, the level of which is 11.9 times higher ($P < 0.001$) than in clinically healthy animals, with a decrease in estradiol by 2.0 times ($P < 0.001$) and cortisol by 31.9 % ($P < 0.01$). In the case of chronic endometritis, there is a sharp increase in the level of pro-inflammatory cytokines: IL-2 by 2.4 times ($P < 0.01$), TNFa by 1.9 times ($P < 0.05$) and interferon gamma by 4.4 times ($P < 0.001$). Thus, chronic endometritis is characterized by the presence in the ovaries of functionally active yellow bodies and luteal cysts, as well as a sharp increase in the level of pro-inflammatory cytokines in the blood.

Thus, the established changes in the hematologic and biochemical status of cows with chronic endometritis indicate the presence of inflammatory phenomena, endogenous intoxication, intense functioning of the excretory system against the background of a decrease in the indicators of the general nonspecific resistance of the body and serves as the basis for the development of a complex biotechnological therapy method (Table 1).

It has been established that the method providing for the use of aminoseletone as a general stimulating agent and primalact as an etiotropic agent has the greatest therapeutic effectiveness of 88.0 %, which is 7.0 % higher compared to the use of metricur as an antimicrobial agent.

To achieve the therapeutic effect, 1.71 intrauterine administration of primalact was required, which is 0.43 ($P < 0.05$) less in comparison with metricur. After the treatment via biotechnological method, 80.0 % of the animals included in the

experiment were fertilized, with a decrease in the period from the start of treatment on fertilization by 14.2 days ($P < 0.01$) and decrease in the fertilization rate by 0.48 ($P < 0.05$) compared to metricur usage as etiotropic biotechnological agent.

Table 1. –Therapeutic efficacy of a comprehensive biotechnological method for the treatment of chronic endometritis in cows

Indicators	First group	Second group
Number of animals	25	21
Number of intrauterine preparation administrations	1.71±0.12*	2.14±0.16
Cured [cows]	22	17
Therapeutic efficacy [%]	88.0	80.9
Fertilized, from among experimental animals [%]	80.0	66.7
Fertilization coefficient	1.81±0.14*	2.29±0.18
Time from treatment start to fertilization [days]	34.7±2.81**	48.9±3.19

Note: * - P<0.05; ** - P<0.01; *** - P<0.001

In the process of recovery after treatment via biotechnological method, indicators of immune-biochemical status of cows normalize, while in animals of the first group, the changes are more pronounced (Table 2).

Indeed, in cows of the first group, recovery is accompanied by a decrease in the leukocyte content by 15.2 %, including eosinophils by 32.2 % ($P < 0.001$), stab neutrophils by 1.65 times ($P < 0.001$), monocytes by 2.24 times ($P < 0.001$), y-globulin fraction of the protein by 30.1 %, with a simultaneous increase in segmented neutrophils and lymphocytes by 10.5 and 7.9 %, respectively, which indicates a decrease in the inflammatory response.

In addition, complex treatment with the use of aminosletone as a general stimulating biotechnological agent and primalact as an antimicrobial is accompanied by an increase in indicators of general nonspecific organism resistance: bactericidal and lysozyme activity of blood serum by 34.9 ($P < 0.01$) and 50.0 % ($P < 0.02$), respectively, the phagocytic activity of leukocytes by 13.4 % ($P < 0.05$), and in animals of the second group by 13.8, 26.9 and 7.2 %, correspondingly. In the process of healing, the endogenous intoxication of the cow's body for the first group decreases, which is manifested by a decrease in the activity of γ-glutamyltransferase by 26.3 %, the content of medium molecules by 18.8 %, malondialdehyde by 36.6 % ($P < 0.001$) and the index of intoxication by 24.6 %, with an increase in the actioxidative activity of blood serum by 16.8 %, and in animals of the second group by 14.35, 14.4 %, 7.5 and 6.2 %, correspondingly. The recovery of the first group cows is accompanied by a decrease in the functional load on the liver, as evidenced by a decrease in the activity

of alkaline phosphatase by 31.6 % ($P < 0.05$) and aspartate aminotransferase by 62.8 % ($P < 0.01$), and in cows of the second group, respectively by 17.1 % and 23.7 %.

Table 2. – Indicators of immune-biochemical status of cows before and after chronic endometritis biotechnological treatment

Indicators	Before treatment, n =	After treatment	
		First group,	Second
Leukocytes [10^9/l]	9.2±0.55	7.8±0.33	8.5±0.51
Eosinophils [%]	8.7±0.44	5.9±0.26**	8.1±0.33
Neutrophils [%]			
stab	3.3±0.12	2.0±0.11**	3.5±0.14
segmented	25.7±2.1	28.4±1.5	26.1±1.7
Monocytes [%]	5.6±0.22	2.5±0.17**	3.5±0.22**
Lymphocytes [%]	56.7±4.4	61.2±3.8	58.8±4.3
Total protein [g/l]	85.3±4.5	83.1±5.7	82.9±5.0
Albumins [%]	45.4±2.5	51.6±3.8	43.1±3.0
γ-globulins [%]	9.3±0.64	10.8±0.6	10.0±0.8
β-globulins [%]	19.8±1.3	18.0±1.3	20.7±1.9
γ-globulins [%]	25.5±1.7	19.6±1.2	26.2±2.3
GTP [units/l]	19.2±1.7	15.2±1.1	16.8±1.5
ALP [units/l]	112.8±7.2	85.7±4.1*	96.3±8.8
AST [units/l]	81.9±7.8	50.3±4.2**	66.2±5.2
SBA [%]	58.6±4.5	79.1±2.9**	66.7±4.6
SLA [μg/ml]	0.26±0.02	0.39±0.02*	0.33±0.02
LPR [%]	73.3±3.9	83.1±3,6*	78.6±5.4
PI [cells/	5.8±0.24	7.5±0.31**	6.6±0.36
PN [cells/ active	7.9±0.44	9.0±0.41*	8.4±0.51
Carotene [mg %]	0.53±0.03	0.65±0.03	0.55±0.02
Vitamin A [μM/l]	2.04±0.18	2.38±0.11*	2.18±0.16
MMC [a.u.] 254	0.27±0.01	0.23±0.02	0.26±0.03
AOA [%]	45.3±3.3	52.9±3.7	48.1±3.4
MDA [μM/L]	2.39±0.19	1.75±0.13*	2.09±0.19
Index of	17.2±1.2	13.8±0,8	16.0±0.9

Note: * - P<0.05; ** - P<0.01; *** - P<0.001

After the treatment, the degree of microbial contamination of the cervical-vaginal mucus decreased in the cows of the first group by 3.6 times (from 417.2±36.4 to 115.2±10.5 CFU/ml), and in animals the second only by 1.8 times (from 389.9±23.8 to 212.2±16.4 CFU/ml), which indicates incomplete rehabilitation of the uterine cavity.

Thus, chronic endometritis is diagnosed on average in 18.1 % of infertile cows of the Kharkiv region. The course of chronic endometritis in 48.7 % of cases occurs against the background of ovarian hypofunction, in 11.0 % of cases luteal cysts are diagnosed. With chronic endometritis, catarrhal-purulent, purulent-catarrhal exudate, echographically represented as single echopositive inclusions, is noted. The uterus has a pronounced cavity: 18.4 ± 0.9 mm (11-35 mm), the thickness of the uterine wall in chronic endometritis is on average 8.7 ± 0.37 mm. Morphometrically chronic endometritis is characterized by a decrease in the fraction of functionally active elements of the endometrium (integument epithelium, uterine glands, blood vessels) to 12.17 %. In the development of chronic endometritis, cows showed an increase in leukocyte content by 12.2 % compared with clinically healthy animals, monocytes by 2.5 times, creatinine by 29.7 %, average molecular peptides by 25.0 %, and endogenous intoxication index by 25.4 %, circulating immune complexes by 38.5 %, with a decrease in bactericidal and lysozyme activity of blood serum by 10.7 and 12.9 %, respectively, and phagocytic activity of leukocytes by 12.4 %, indicating the presence of inflammatory phenomena, endogenous intoxication, tense functioning of excretory system against the background of decreasing indicators of general nonspecific resistance of the organism.

Conclusion. The effectiveness of the developed biotechnological method for the treatment of chronic endometritis, involving the use of aminoseletone as a general stimulating biotechnological agent and primalact as an etiotropic agent is 88.0 %. Recovery of animals after biotechnological treatment is accompanied by a decrease in the inflammatory response, endogenous intoxication, normalization of liver and kidneys, an increase in overall non-specific resistance and a decrease in uterine microbial contamination.

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