

Problem of chronic endometritis in cows and ways to solve it

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Abstract. Chronic endometritis is diagnosed on average in 18.1 % of infertile cows. With chronic endometritis, the uterus has a pronounced cavity of 18.4±0.9 mm (11–35 mm), while the wall thickness of the uterus 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 compared with clinically healthy animals showed an increase in leukocyte content by 12.2 %, 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 the bactericidal and lysozyme activity of blood serum, respectively by 10.7 and 12.9 % and phagocytic activity of leukocytes by 12.4 %. The effectiveness of the application of the developed method for the treatment of chronic endometritis, involving the use of aminoseleton five times with a 72-hour interval at a dose of 30, 35, 40, 45 and 50 ml, magestrofan on the 1st and 13th days at a dose of 2 ml, uterotone on 3rd, 4th and 5th days at a dose of 10 ml and intrauterine administration of primalact on 1st-3rd days at a dose of 20 ml, is 88.0 %. Recovery of animals after the 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.

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, 2].

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 [3, 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 broad-spectrum antibiotics into veterinary practice, which led to noticeable disturbances in the environmental relationships between a macroorganism and its microflora [5, 6].

The tactics of therapeutic effects in chronic endometritis in cows is aimed, first of all, at suppressing the vital functions of microorganisms in the uterine cavity and completely eliminating pathological changes in the endometrium. However, the nonspecific use of chemotherapeutic agents in the treatment of acute postpartum endometritis leads to an increase in the drug resistance of microorganisms, weakening of protective mechanisms, spontaneous mutations, causing the appearance of genetically resistant populations, which complicates the treatment, and chemotherapy becomes a

link of etiopathogenesis of chronic endometritis of cows [7, 8].

Untimely and ineffective treatment of chronic endometritis in cows leads to impaired reproductive function, decreased milk productivity and premature culling [9, 10].

In this regard, the study of the clinical and pathogenetic features of the development of chronic endometritis in dairy cows and the development of methods for its pharmacotherapy are high on the agenda and require detailed study.

2 Materials 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.

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Morphometric studies of histological material were performed according to G.G. Avtandilov [11].

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 [12]. 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 [13].

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

The effectiveness of the developed 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 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 (Stat-Soft Inc., USA).

3 Results and discussion

Studies on the prevalence of chronic endometritis were carried out on 736 infertile cows in 5 farms of the Voronezh 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.

In chronic endometritis, in 36.6 % of cases, functionally active cyclic yellow bodies with a size of 13.9 ± 0.9 mm are diagnosed in the ovaries. In most cases (48.7 %), the course of chronic endometritis occurs against the background of ovarian hypofunction, in 11.0 % luteal cysts with a diameter of 29.1 ± 1.3 mm are diagnosed.

The degree of microbial contamination of cervical-vaginal mucus in chronic endometritis was 3913.7 ± 204.8 CFU/ml, which is 19.9 % ($P < 0.05$) less compared with the pyometra and 3.37–3.98 times more than with pathologies of the uterus of a functional nature (chronic uterine subinvolution, glandular cystic hyperplasia of the endometrium). With the development of chronic endometritis, exudate in 66.6 % is contaminated with enterobacteria. 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. The content of neutrophilic leukocytes in the cervical-vaginal mucus of cows with chronic endometritis is 14.1 ± 0.91 %, which is 1.38–2.2 times higher ($P < 0.01$) than in functional disorders, confirming the presence of an inflammatory process in the uterus.

In the structure of the endometrium of cows with chronic endometritis, the fraction of integumentary epithelium is 2.12 ± 0.21 %, uterine glands 5.21 ± 0.33 %, blood vessels 4.84 ± 0.29 %, and endometrial stroma 87.83 ± 6.1 %, while in the case of uterine pathologies of functional nature the figures are 3.21–5.71 %, 6.72–28.64 %, 7.45–12.31 % and 53.34–79.24 %, respectively. Thus, in the case of chronic endometritis, the fraction of functionally active elements of the endometrium decreases to 12.17 %. The height of the integumentary epithelium cells in the case of chronic endometritis is 17.9 ± 1.48 μm , which is 13.5–20.1 % ($P < 0.05$) less in comparison with chronic pathologies of the uterus of an inflammatory nature, the thickness of the endometrium is 297.8 ± 21.6 μm , and the height of the epithelial cells is of the uterine glands is 11.8 ± 0.88 μm , which is less by 13.9–34.9 % ($P < 0.05$ – 0.01) and 15.5–30.2 % ($P < 0.05$), respectively. The volume of epithelial epithelium in the case of chronic endometritis is 472.9 ± 33.8 μm^3 , and the

volume of their nuclei is $156.8 \pm 12.4 \mu\text{m}^3$, which is respectively less by 36.6–47.2 ($P < 0.001$) and 20.7–34.1 % ($P < 0.01$) compared with chronic diseases of the uterus of a functional nature, and the volume of uterine gland epithelial cells and their nuclei is $357.8 \pm 29.6 \mu\text{m}^3$ and $119.5 \pm 9.1 \mu\text{m}^3$, which is respectively less by 23.7–46.4 ($P < 0.01$ – 0.001) and 19.6–36.8 % ($P < 0.01$). Thus, in chronic endometritis, a decrease in the volume of epithelial cells of the integumentary epithelium and uterine glands is noted, which indicates the development of dystrophic processes.

The results of morphometric studies are additional markers for the diagnosis of chronic endometritis in dairy cows, which also include indicators of morphological and biochemical status (Table 1).

Table 1. Morphological and biochemical blood parameters of cows with chronic endometritis

Indicators	Clinically healthy, n = 5	Chronic endometritis, n = 5
Leukocytes [$10^9/l$]	8.2 ± 0.37	$9.2 \pm 0.19^*$
Eosinophils [%]	2.8 ± 0.21	3.0 ± 0.14
Neutrophils [%]		
stab	2.8 ± 0.13	$4.7 \pm 0.14^{***}$
segmented	43.4 ± 3.0	40.7 ± 3.1
Monocytes [%]	2.8 ± 0.45	$7.0 \pm 0.34^{***}$
Lymphocytes [%]	48.5 ± 2.4	44.6 ± 3.3
Total protein [g/l]	78.1 ± 4.2	77.4 ± 4.7
Albumins [%]	40.7 ± 2.9	$35.5 \pm 2.1^*$
α -globulins [%]	11.6 ± 0.6	11.9 ± 0.4
β -globulins [%]	21.3 ± 0.6	$23.7 \pm 0.8^*$
γ -globulins [%]	26.4 ± 1.6	28.9 ± 1.8
Creatinine [$\mu\text{M/L}$]	67.4 ± 4.1	$87.4 \pm 5.7^*$
AST [units/l]	62.7 ± 4.1	$90.7 \pm 4.8^{***}$
ALT [units/l]	15.4 ± 1.1	$22.7 \pm 1.2^{**}$
ALP [units/l]	55.4 ± 3.1	$70.4 \pm 5.1^{**}$
GTP [units/l]	13.7 ± 1.1	$21.7 \pm 1.4^{**}$
MMP [a.u.]	0.7 ± 0.01	$1.0 \pm 0.02^{***}$
MDA [$\mu\text{M/L}$]	2.21 ± 0.19	$2.54 \pm 0.11^*$
Catalase $\mu\text{M}_{\text{H}_2\text{O}_2}/\mu\text{min}$	52.8 ± 3.4	$40.8 \pm 2.4^*$
GPx [$\mu\text{M/l} \cdot \text{min}$]	16.8 ± 0.8	$13.4 \pm 0.8^*$
IEI	20.5 ± 0.5	$25.7 \pm 1.2^{**}$
NO_x [$\mu\text{M/l}$]	36.5 ± 1.2	40.1 ± 2.8
Vitamin A [$\mu\text{M/l}$]	1.51 ± 0.07	$1.22 \pm 0.05^{**}$
Vitamin E [$\mu\text{M/l}$]	18.8 ± 1.4	15.6 ± 0.9
Vitamin C [$\mu\text{M/l}$]	20.6 ± 1.7	18.4 ± 1.2
Carotene [$\mu\text{M/L}$]	15.4 ± 1.3	12.7 ± 1.0
Selenium [$\mu\text{M/L}$]	1.51 ± 0.08	$1.22 \pm 0.07^*$
Total immunoglobulins [g/l]	22.9 ± 2.2	19.4 ± 1.3
CIC [g/l]	0.13 ± 0.02	0.18 ± 0.01
SBA [%]	81.6 ± 2.4	72.9 ± 3.3
SLA [$\mu\text{g/ml}$]	2.09 ± 0.17	1.82 ± 0.11
LPR [%]	82.8 ± 3.7	72.5 ± 3.9
Estradiol [pg/ml]	44.7 ± 3.4	$22.3 \pm 1.7^{***}$
Progesterone [nmole/l]	1.54 ± 0.07	$18.3 \pm 1.2^{***}$
Cortisol [nmole/l]	132.7 ± 10.5	$90.4 \pm 6.1^{**}$
IFN γ [pg/ml]	176.4 ± 15.7	$775.4 \pm 55.7^{***}$
IL-2 [pg/ml]	33.1 ± 1.6	$79.6 \pm 4.8^{**}$
TNF α [pg/ml]	248.4 ± 19.7	$489.7 \pm 28.4^*$

Note: * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$

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.

With the progression of chronic endometritis, a reduced content of vitamin A was found to be 19.2 % ($P < 0.01$) than in clinically healthy animals, vitamin E decreased by 17.0 %, vitamin C by 10.7 %, carotene by 17.5 %, selenium by 19.2 % ($P < 0.05$), indicating a more intense accumulation of lipid peroxidation products against the background of a decrease in the functioning of the non-enzymatic antioxidant defense link and selenium level. Cows with chronic endometritis showed a decrease in the level of total immunoglobulins by 15.3 %, in comparison with clinically healthy animals, bactericidal activity of blood serum by 10.7 %, lysozyme activity of blood serum by 12.9 %, and phagocytic activity of leukocytes by 12.4 %, with an increase in the content of circulating immune complexes in them by 38.5 %.

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 ($P < 0.01$), TNF α 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 therapy method (Table 2).

It has been established that the method providing for the use of aminoseletonone 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, 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 agent.

Table 2. Therapeutic efficacy of a comprehensive 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 the treatment, the indicators of the immune-biochemical status of the cows normalize, while in animals of the first group, the changes are more pronounced (Table 3).

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 ($P<0.001$), γ -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 agent and primalact as an antimicrobial is accompanied by an increase in the indicators of the general nonspecific resistance of the organism: 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 body of the cows of the first group decreases, which is manifested by a decrease in the activity of gamma-glutamyltransferase by 26.3 %, the content of medium-molecular 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 14.34.5, 14.4 %, 7.5 and 6.2 %, correspondingly. The recovery of cows of the first group 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 3. Indicators of immune-biochemical status of cows before and after chronic endometritis treatment

Indicators	Before treatment, n = 14	After treatment	
		First group, n = 7	Second group, n = 7
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 [μ g/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/phagocyte]	5.8±0.24	7.5±0.31***	6.6±0.36
PN [cells/active phagocyte]	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 [μ M/l]	2.04±0.18	2.38±0.11*	2.18±0.16
MMC [a.u.] 254 nm	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 [μ M/L]	2.39±0.19	1.75±0.13***	2.09±0.19
Index of intoxication	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 Central Federal District of the Russian Federation. 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.

4 Conclusion

The effectiveness of the developed method for the treatment of chronic endometritis, involving the use of aminoselestone as a general stimulating agent and primalact as an etiotropic agent is 88.0 %. Recovery of animals after the 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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