

# Chronic endometritis and the state of the receptor apparatus in women with reproductive losses

## Abstract

The purpose of this study was to determine the expression of lymphocytes and content of estrogen receptors (ER) and progesterone (PR) in the stroma and epithelium of the endometrium in women with histologically confirmed endometritis. Surveyed 56 women with infertility and miscarriage, in which the immune histological method was used to determine ER and PR in stroma and epithelium of the endometrium and expression of lymphocytes. It is revealed that the inflammatory reaction in the endometrium was expressed mainly in the lymphocytic infiltration and was predominantly CD3, CD4, CD8 lymphocytes. The affected endometrial receptivity has been broken: the middle phase of proliferation in the stroma and in the endometrium was dominated by progesterone receptors. The ratio of ER and PR was  $<1$ , indicating a lack of maturity and readiness of the endometrium to its secretory transformation and the adoption of a blastocyst. Despite the low expression of ER and focal hyperplasia of the basal layer occurred 53, 10% of women. The incidence of hyperplasia correlated with the expression of CD3 and CD68 cells.

**Keywords:** infertility, habitual miscarriage, chronic endometritis, receptors to estrogens, progesterone receptors, immunohistochemistry, lymphocytes CD3, CD4, CD8

Volume 4 Issue 2 - 2018

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**Received:** February 25, 2018 | **Published:** April 26, 2018

## Introduction

Chronic endometritis (CE) is a clinical and morphological syndrome, in which as a result of persistent damage to the endometrium by an infectious agent, multiple secondary morphofunctional changes occur that violate the cyclic transformation and receptivity of the uterine mucosa.<sup>1,2</sup>

According to the decision of the XVIII world Congress of gynecologists and obstetricians (FIGO, 2006),<sup>3,4</sup> the diagnosis of chronic endometritis should put all women who have had non-developing pregnancy, not performing more tests. In addition to CE, clinicians often face this contingent of patients with the situation of "escape" of the endometrium from under normal hormonal influences and desynchronization of ovarian and endometrial cycles, insufficient growth of endometrium, especially in ovarian stimulation programs in patients with infertility.<sup>5,6</sup> The receptivity of the endometrium is a complex of structural and functional characteristics of the endometrium with clear time constants that determine the ability of the endometrium to provide embryo implantation. Currently, the most widely used in clinical practice to determine the receptor apparatus is immunohistochemical verification of estrogen receptor (ER) and progesterone receptor (PR) in endometrial tissue.<sup>7,8</sup> The amount of estrogen and progesterone receptors varies throughout the normal menstrual cycle.<sup>9</sup> Depending on the stage and phase of the menstrual cycle, these or those receptors prevail in the epithelium of the glands or stromal component.

## Objective

To study the expression of lymphocytes and estrogen and progesterone receptors in stroma and endometrial epithelium in women with CE. A comprehensive survey of 56 women with

reproductive problems: 36 women had a history of habitual pregnancy loss and 20 had infertility.

Ultrasoundography (USG) of the pelvic organs was performed on 20-22 day of the menstrual cycle. The indicators of endometrium thickness and structure were evaluated. The uniform structure and its correspondence to the day of menstrual cycle, the thickness of endometrium were considered as normal parameters not less than 8mm.

In all examined in histological examination of the endometrium, CE was diagnosed. For histological examination, the endometrium was obtained by a pipel biopsy, which was performed according to the standard method on the 7-11 day of the menstrual cycle.

Immunohistochemical (IHC) examination of endometrial tissue it was carried out using the method of peroxidase technique on paraffin sections 3-4 microns thick. The system has been used for the detection UltraVision Quanto (DAKO, Denmark) and monoclonal mouse antibodies firm "DAKO": estrogen receptor (clone 1D5), progesterone (clone PgR 636), for receptors of lymphocytes - CD3 (clone SP-7); CD4 (clone 4B12); CD8 (clone), CD138 (clone).

To determine the expression level of estrogen and progesterone receptors, a semi-quantitative system "Allred Scoring Guideline" was used to estimate the percentage of colored cells (proportion score, PS) and the intensity of cell coloring (intensity score, IS), which were then summed up: TS (total score) = PS + is. The percentage of stained cells was assessed in points: 0-0% cells, 1-0,1-1% of cells, 2-10% of cells, 3-11-33% of cells, 4-34-66% of cells, 5-67-100% of cells. The intensity of staining was assessed in points: 0-no staining, 1-weak staining, 2-moderate, 3-marked coloring. Thus, the result of Allred Score ranged from 0 to 8 points.

Viewing and photographing of micro-products was carried out on the microscope AxioLab ("Carl Zeiss Jena", Germany) using a digital camera ("Carl Zeiss Jena", Germany). Quantitative analysis of the results of immunohistochemical reactions was carried out under the lens magnification of 20×, 40×. Statistical data analysis was carried out on a personal computer using the software package "Statistica" version 7. Used non-parametric methods of statistical analysis. When comparing the performance of different groups used the student's t-test, Mann-Whitney, Wald-Wolfowitz. Significant differences between groups were considered at the significance level of  $p<0.05$ . Research result.

In patients with ultrasound, the echo structure of the endometrium was changed in 69% of cases. In the structure of the ultrasonic signs of CE prevailed: heterogeneous echo - structure of the endometrium, its uneven contour and increased echogenicity. Less frequently observed hyperechogenic inclusions in the basal layer of the endometrium, gaseous bubbles in the uterine cavity and its extension.

The bacteriological study of aspirates from the maternal cavity showed no microflora growth in 81% of infertile women and 79% of the usual pregnancy loss ( $p>0.05$ ). Structure of detected pathogens *Staphylococcus epidermidis*; *Enterococcus faecalis*; *Staphylococcus sapphyticus*; *Staphylococcus aureus*.

Morphological criteria for the diagnosis of "chronic endometritis" were: inflammatory infiltrates, consisting mainly of lymphocytes, neutrophilic leukocytes, located more often around the glands and

blood vessels, less diffuse; the presence of plasma cells;<sup>10</sup> endometrial stroma alterations, including fibrosis; sclerotic changes in spiral arteries;<sup>11</sup> deformation of the glands; focal hyperplasia of the basal layer.<sup>12,13</sup>

In immunohistochemical study of plasma cells, CD138, 4,5% of the examined patients were identified. The presence of lymphohistiocytic only infiltration in the stroma of endometrium without plasma cells was evaluated, the picture is incomplete as CE. Focal endometritis occurred in 13.2%, and diffuse -in 86,8% of women.

In women with chronic diffuse endometritis in the middle of the proliferation phase, the expression of ER in the stroma made up 181,11±50,0, in the epithelium –198,89±28,72, while expression of PR was higher in the stroma and in the epithelium, accounting for 220,0±40,0 or 237,78±28,72 respectively. To ensure the maturity of the endometrium and its readiness for secretory transformation and blastocyst administration, it is important not only the actual number of steroid receptors, but also their ratio. Determining the ratio of ER to PR, the following results were obtained: 0.89 in stromal and 0.86 in glandular components of the endometrium. Lymphocytic reaction in diffuse and focal endometritis did not differ in the qualitative and quantitative composition of lymphocytes and was represented by CD2–35.4%, CD3–81.8%, CD4–54.5%, CD8–77.3%, CD68–40.9%. Despite the decreased expression of RE focal hyperplasia, there were 53.10% of women. The incidence of hyperplasia correlated with the expression of CD3 and CD68 cells (Tables 1–3).

**Table 1** The incidence of focal hyperplasia in the expression of different types of lymphocytes (T-tests)

	Mean	Mean	t-value	p	Std.dev.	Std.dev.	F-ratio	p
CD2	0,61535	0,55555	0,14835	0,88355	1,04,390	0,72648	2,06,478	0,30964
CD3	1,76,923	0,88888	2,32,903	0,03045*	1,01,273	0,60092	2,84,024	0,14626
CD4	1,23,076	0,66666	1,23,176	0,23232	1,16,575	0,86602	1,81,197	0,40579
CD8	1,69,230	1,11,111	1,28,798	0,21245	1,18,213	0,78173	2,28,671	0,24684
CD68	1,30,769	0,11111	2,93,674	0,00815*	1,18,213	0,33333	1,25,769	0,00131*
CD138	0,07692	0,000000	0,82572	0,41870	0,27735	0,00000	0,00000	1,00,000

\*, Indicators are statistically significant at  $p < 0.05$

**Table 2** The incidence of focal hyperplasia in the expression of different types of lymphocytes (Mann-Whitney U Test)

	Rank Sum	Rank Sum	U	Z	p-level	Z	p-level	2*1sided
CD2	14,60,000	10,70,000	55,00,000	0,200334	0,841219	0,233646	0,815260	0,844582
CD3	18,10,000	7,20,000	27,00,000	20,70,120	0,038442	21,66,455	0,030277*	0,036416
CD4	16,55,000	8,75,000	42,50,000	10,35,060	0,300642	11,02,840	0,270098	0,292051
CD8	16,80,000	8,50,000	40,00,000	12,02,005	0,229362	12,45,299	0,213023	0,234852
CD68	18,25,000	7,05,000	25,50,000	21,70,287	0,029986	24,54,152	0,014122*	0,025202
CD138	15,40,000	9,90,000	54,00,000	0,267112	0,789383	0,739600	0,459543	0,793760

\*, Indicators are statistically significant at  $p < 0.05$

**Table 3** The incidence of focal hyperplasia in the expression of different types of lymphocytes (Wald-Wolfowitz Runs Test)

	Mean	Mean	Z	p-level	Z adjstd	p-level
CD2	0,615385	0,555556	1,52,253	0,127878	12,96,207	0,194905
CD3	17,69,231	0,888889	-1,19,333	0,232740	0,967012	0,333539
CD4	12,30,769	0,666667	-0,74069	0,458882	0,514368	0,606995
CD8	16,92,308	11,11,111	-0,28805	0,773312	0,061724	0,950783
CD68	13,07,692	0,111111	-2,55,126	0,010734*	23,24,943	0,020076*
CD138	0,076923	0,000000	0,61724	0,537076	0,390920	0,695857

\*. Indicators are statistically significant at p <0.05

Thus, the inflammatory reaction in endometrium in women with reproductive problems was mainly expressed in lymphocytic infiltration and was mainly represented by CD3, CD4, CD8 lymphocytes. The affected endometrial receptivity has been broken: the middle phase of proliferation in the stroma and in the endometrium was dominated by progesterone receptors. The ratio of ER and PR was <1, which testified to the lack of maturity of the endometrium and its readiness for secretory transformation and blastocyst acceptance. Despite the decreased ER expression, focal hyperplasia of the basal layer occurred in 53.10% of women. The incidence of hyperplasia correlated with the expression of CD3 and CD68 cells.

## Acknowledgements

None.

## Conflict of interest

The authors declare there is no conflict of interest.

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