Assessment of Antibacterial Therapy of Chlamydiosis During Pregnancy in Placenta

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The aim of the investigation was to assess morphologically an antibacterial therapy of chlamydiosis during pregnancy relying on structural and functional changes of decidual cells of placenta.

Materials and Methods. We studied 6000 decidual cells from three placental areas (central, paracentral and marginal) by means of histostereometry using standard histological techniques. The cells were taken from puerperas, who had in their past histories untreated chlamydiosis and chlamydiosis treated by antibacterial drugs during pregnancy. We measured diameters of cells and their nuclei, calculated cell number per 1 mm^2. Decidual cells were classified by cell populations, and nuclear volume variation curves were plotted.

Results. The placentas infected by *Chlamydia trachomatis* were found to have decreased cell proliferation mechanisms and diminishing total nuclear volume to placental periphery with compensatory processes being concentrated in the paracentral area. All these changes were caused mainly by the fraction of small decidual cells. Primarily, basal lamina responded to antibacterial therapy by decreased volume of cells, their nuclei and cytoplasm in the marginal area of placenta. Moreover, nuclear cytoplasmic ratio in basal lamina was near to normal, while in septa it remained rather high throughout the placenta. A therapeutic effect of antibiotics on chlamydiosis during pregnancy was shown by the decrease of total volume of cellular-nuclear material in basal lamina throughout the placenta. After therapy, K-cell count decreased and large decidual cell count grew.

Conclusion. Antibiotics have low therapeutic effect on morphological changes of placental cells in chlamydiosis treatment: cell proliferative processes are weak against the significant decrease of immunoregulatory functions.

Key words: placenta; chlamydiosis; decidual cells; nuclear cytoplasmic ratio; variation curve method.

Intrauterine infection of the fetus remains an important medical and social problem of modern obstetrics and perinatology.

Infection caused by *Chlamydia trachomatis* is the most common disease worldwide transmitted sexually, replacing gradually the causative agents of classic sexually transmitted diseases such as syphilis and gonorrhea [1–3].

The results of quite a number of studies [4, 5, and others] indicate that the presence of chlamydial infection of the genital tract in the mother can result in an increased incidence of miscarriage, stillbirth, premature labor, premature rupture of membranes, fetoplacental insufficiency, development of endometritis and salpingitis in the postpartum period, birth of children with signs of intrauterine fetal hypotrophy. Besides, the presence of chronic inflammation foci, and more than that, intrauterine infection of the fetus makes a significant imbalance in the "mother–placenta–fetus" system, causing disturbance in the mechanisms of execution of the fetal development program and timely delivery [6].

The risk of unfavorable outcomes of infection during pregnancy for both the mother and the fetus and newborn demands antibacterial therapy, which is one of the most common ones prescribed to pregnant women so far. When administering antimicrobial drugs to pregnant women it is important to ensure adequate treatment of infection avoiding unfavourable impact on the fetus, since no drug is considered to be absolutely safe during pregnancy [7].

Decidual cells (DC) are among the main "operating elements" in the placenta. Our studies [8, 9] aimed at identification of changes in the morphology and decidual cell count under the influence of various intrauterine infections prove the appropriateness of such studies.

The aim of the investigation was to assess morphologically an antibacterial therapy of chlamydiosis during pregnancy relying on structural and functional changes of decidual cells of the placenta.

Materials and Methods. The research objects were the placentas obtained from puerperas whose pregnancy was complicated by *Chlamydia trachomatis*. Group 1 consisted of women who did not receive treatment for chlamydiosis, group 2 included women who received antibacterial treatment on the standard algorithms during pregnancy. All the data were obtained from the

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prenatal records and labor and delivery records. All the puerperas participating in the experiment were informed about the study and gave consent to the processing of the obtained biomaterial and publication of the results in public media.

We studied 6000 DC (3000 from 5 placentas in group 1, 3000 from 5 placentas in group 2) from three placental areas: central (near the umbilical cord), marginal (closer to the placental edge) and paracentral (between them) — by standard histological techniques with hematoxylin and eosin staining. The main operating magnification was ×400. With the help of the ocular micrometer we measured the major and minor diameters of DC and nuclei of the basal lamina and septa, the number of DC on 1 mm^2 area of the basal lamina and septa. Using the obtained values were calculated the nuclear-cytoplasmic ratio, DC volume, their cytoplasm and nuclei volumes by the formula: $V=\pi/6 \cdot LB^2$ (where *L*: the major diameter, *B:* the minor diameter). Every studied parameter in every preparation was measured 100 times by random sampling. Based on the calculated diameters, the stromal DC of the septa and basal lamina were distributed by cell populations into K-cells, small and large DC [10]. Besides, the variation curves of the DC nuclear volume of the septa and basal lamina were made up with the pitch of 50 and 100 μm^3.

The obtained results were treated statistically and compared with the use of the Statistica 6.0 software.

Results. The findings of the morphometric study of the placenta (See the Table) indicate that in untreated chlamydiosis the DC volume significantly increases from the center to the periphery of the placenta with the maximum in the paracentral area both in the basal lamina and in the septa relative to the basal lamina. At the same time, when pathology corrected (antibiotic therapy during pregnancy), in the basal lamina this index is significantly reduced in the marginal area relative to all other areas and septa, as well as compared to the group with untreated chlamydiosis. In the septa it remains at the same level throughout the placenta, significantly diminishing in the paracentral area and rising in the marginal area relative to the basal lamina. Besides, an antibacterial effect is observed in these areas as a significant reduction of DC volumes.

The DC nuclear volume in both variants of pregnancy was significantly reduced to the placental marginal area both in the basal lamina and septa. When comparing this index in different placenta fractions, the values in the septa were found to be significantly higher than those in the basal lamina in corrected chlamydiosis, while this index was significantly higher only in the marginal area of the placenta in untreated pathology. In addition, the therapeutic effect of antibiotics manifested itself in a significant reduction in the nuclear volumes in the paracentral and marginal areas of the basal lamina, reduction in the septa in the marginal area and rise in the central area.

The volume values of DC cytoplasm varied according to changes in the nuclear volumes in inverse proportion in the septa as well as in the basal lamina.

Basal lamina and sept decidual cells properties in the placenta of pregnant women with chlamydiosis

Indices	Central area		Paracentral area		Marginal area	
	Chlamydiosis	Chlamaydiosis treated	Chlamydiosis	Chlamydiosis treated	Chlamydiosis	Chlamydiosis treated
Basal lamina						
DC volume (μm^3)	2602.46±62.89	2695.17±102.74	2940.02±72.46*	2900.24±105.15	2841.58±54.67*	2212.54±60.53*$^{\#v}$
DC nuclear volume (V_1) (μm^3)	303.82±6.78	280.59±15.59	309.29±8.37	257.65±9.24^v	262.46±5.56*$^{\#}$	206.89±5.98*$^{\#v}$
DC cytoplazm volume (V_2) (μm^3)	2298.64±61.52	2414.58±93.49	2630.73±69.41*	2642.59±100.56 (* p<0.1)	2579.12±51.94*	2005.64±58.53*$^{\#v}$
NCI (V_1/V_2)	0.170±0.010	0.120±0.004^v	0.140±0.010*	0.110±0.004*^{v}	0.110±0.002*$^{\#}$	0.110±0.004
DC count per 1 mm^2	1438.05±16.65	1032.81±22.64^v	1226.42±22.49*	928.13±14.55*^{v}	1213.75±16.92*	1021.09±22.56$^{\#v}$
Stages						
DC volume (μm^3)	2535.95±58.76	2620.84±77.72	3221.91±74.76*$^+$	2602.21±98.22^{+v}	2984.25±60.23*$^{\#}$ ($^+$ p<0.1)	2644.26±67.33^{+v}
DC nuclear volume (V_3) (μm^3)	306.29±5.47	332.62±12.59^{+v}	320.30±10.19	341.82±13.10	284.88±6.83*$^{\#+}$	308.80±8.71$^{\#+v}$
DC cytoplazm volume (V_4) (μm^3)	2229.66±57.03	2288.22±70.10	2901.62±72.26*$^+$	2260.39±89.19^{+v}	2699.37±58.32*$^{\#}$	2335.45±64.12^{+v}
NCI (V_3/V_4)	0.160±0.003$^+$	0.150±0.010$^+$	0.130±0.010*	0.160±0.004^{+v}	0.130±0.003*$^+$	0.150±0.005$^{\#+v}$
DC count per 1 mm^2	1338.15±23.48$^+$	957.81±20.73^{+v}	1156.15±16.92*$^+$	1101.56±26.04*$^+$ (^{v} p<0.1)	1121.88±14.96*$^+$	1046.09±19.79*^{v} ($^{\#}$ p<0.1)

N o t e: * statistically significant differences in the values compared to central area (p<0.05); $^{\#}$ paracentral area; $^+$ compared to basal lamina with the septa within the same area; ^{v} compared to the group with untreated chlamydiosis. DC: decidual cells; NCI: nuclear-cytoplasmic index.

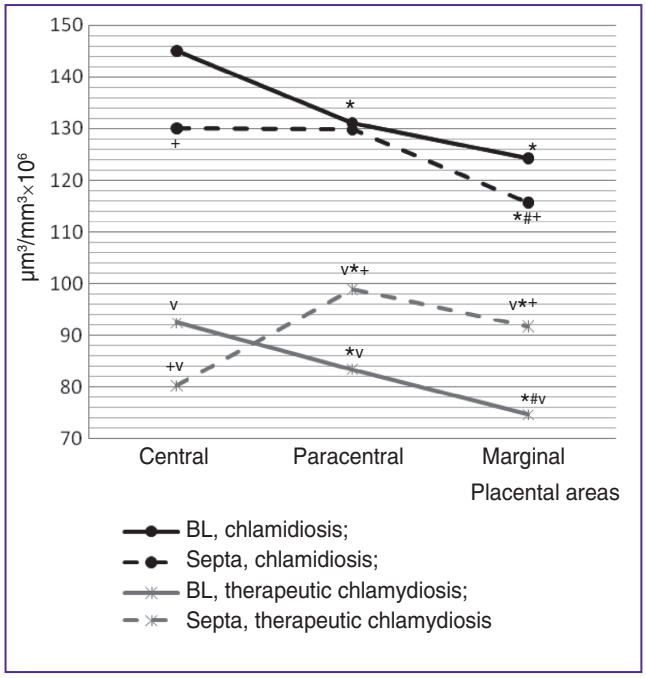

Figure 1. The total volume of decidual cells in the basal lamina (BL) and septa per 1 mm³. Here: * statistically significant differences in the values ($p<0.05$) compared to central area; # paracentral area; + basal lamina; v chlamidiosis group

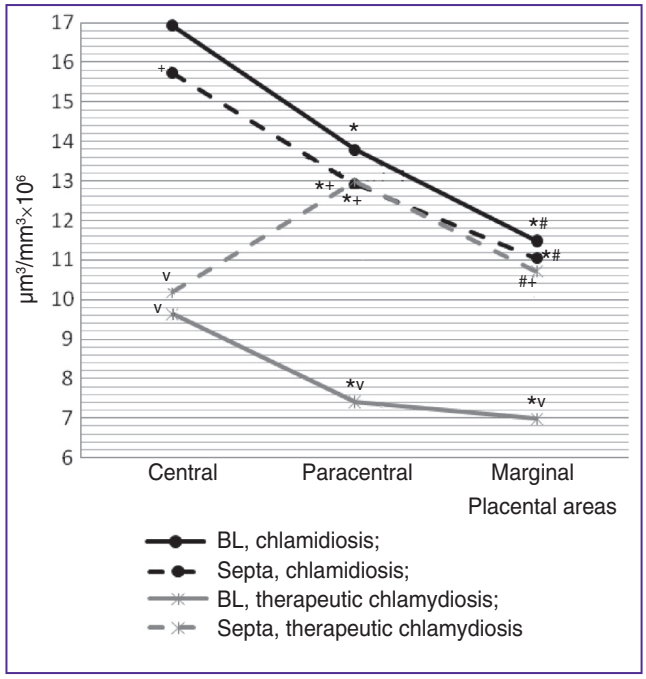

Figure 2. The total volume of the decidual cell nuclei in the basal lamina (BL) and septa per 1 mm³. Here: * statistically significant differences in the values ($p<0.05$) compared to central area; # paracentral area; + basal lamina; v chlamidiosis group

The indices discussed above are reflected in the values of nuclear-cytoplasmic ratio. In uncorrected pathology they significantly diminished to the periphery of the placenta in the basal lamina, and in the septa, while in treated chlamydiosis they remained within the same values in all areas of the placenta, though in the septa these values were significantly higher than those in the basal lamina. A therapeutic effect on chlamydiosis during pregnancy was reflected in the reduction in the cell tension in the basal lamina, though these changes had no effect on the placenta septa.

Moreover, in untreated chlamydiosis significant reduction in the DC count was observed to the placenta marginal area in the basal lamina and septa, where these indices were also significantly diminished relative to the basal lamina, too. Under the therapeutic action the DC count increased significantly to the placenta periphery only in the septa, whereas in the basal lamina it remained approximately the same except for the paracentral area, where this index was significantly diminished relative to the other areas as well as the sept fraction. Estimating the DC count after therapy, it should be said to decrease significantly in all areas and fractions of the placenta.

The above changes had an effect on the total volumes of DC and their nuclei: in the group with untreated chlamydiosis (Figure 1) as a significant reduction in the both indices to the placenta periphery in the basal lamina and septa; in the group with corrected pathology (Figure 2) in the basal lamina — as a statistically significant reduction to the placenta periphery relative to all the areas and sept fraction as well as the group with untreated chlamydiosis; in the septa — as a significant increase in these indices in the paracentral area of the placenta relative to the other areas. At the same time, the total nuclear volumes of the paracentral and marginal areas were noted to have virtually the same values in both variants of pregnancy.

When evaluating the DC distribution on the population, large DC were found to appear under the therapeutic effect on chlamydiosis (Figure 3 (a)) due to the reduction of the remaining fractions, but mainly K-cell fraction. In turn, in the group with untreated pathology (Figure 3 (b)) the highest K-cell count is characteristic of the basal lamina in central placenta and septa, and small DC — for the paracentral area. At the same time in the group with treated pathology the small DC count remains approximately the same in all the areas and fractions of the placenta, and the ratio of K-cells and large DC becomes inversely directed.

While making up the variation curves of the DC nuclei with the pitch of 50 and 100 μm³ it was found that the major modes are formed within the nuclei volumes with the values of 1–100, 201–300, 501–600 μm³. Furthermore, when distributing nuclear volumes by

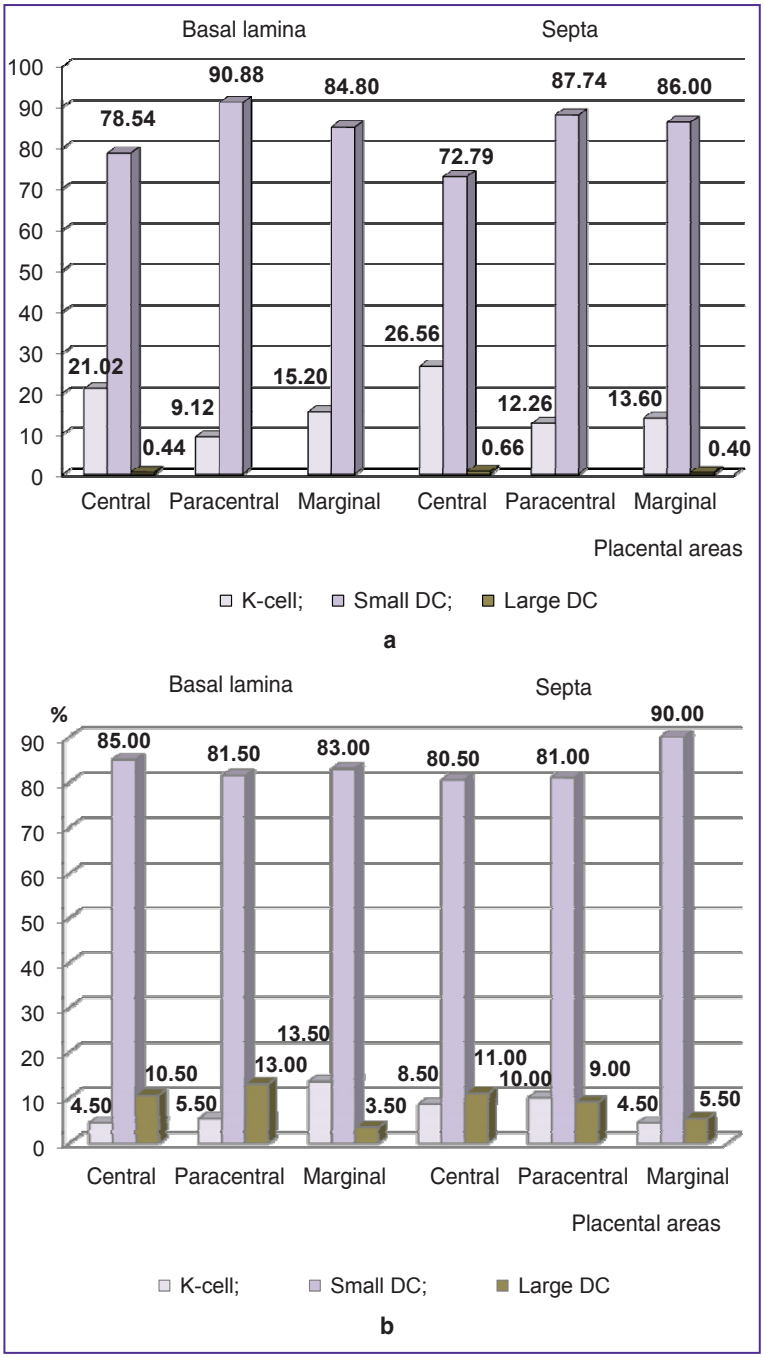

Figure 3. Distribution of stromal decidual cells by cell populations in untreated (a) and treated (b) chlamydiosis during pregnancy

frequency with the interval of 50 μm³ several additional smaller peaks are traced — a "submode" in the classes with the intervals of 351–400, 651–700 and 801–850 μm³, the last two of which are of a "damped" character.

Discussion. When there is an intrauterine infection of the placenta with *Chlamydia trachomatis,* the cellular constituents get stressed with a decrease in cell proliferative mechanisms, nuclear material volume and, consequently, protein-synthetic function throughout the placenta from the center to the periphery with the concentration of compensatory processes in the paracentral area. An increase in all the studied cell compartments, particularly nuclei is observed in it, indicating their intensity in the form of functional swelling. All these changes occur mainly at the expense of the small DC fraction, responsible for intercellular substance synthesis. The DC immune regulatory function is reduced. Based on the DC morphological analysis, the latter in the basal lamina and septa should be assumed to be on the same level of the structural and functional status.

With the introduction of antibacterial drugs in the therapeutic measures aimed at arresting chlamydiosis, predominantly the basal lamina responds by a decrease in the volumes of the cells, their nuclei and cytoplasm in the paracentral and marginal areas of the placenta. This is accompanied by a similar decrease in the immune regulatory K-cells. The formation and increase in the large diploid DC count suggests activation of extra- and intracellular regeneration processes. Similar processes occur in the septa, but without significant changes in the DC morphology throughout the placenta. Besides, in the septa of the marginal and particularly paracentral areas the total DC volume and their nuclei volume is significantly increased by functional swelling and increasing ploidy, which is reflected primarily in increasing the proliferative activity of this fraction nuclei. And nucleocytoplasmic ratio in the basal lamina is close to normal values, while it remains high in the septa throughout the placenta.

Assessing a therapeutic effect on chlamydiosis during pregnancy we can state the reduction of the total volume of the cellular nuclear material in the basal lamina throughout the placenta, and in the septa — only in the central area. In the septa of the paracentral and marginal areas the amount of nuclear material is at the level of the same parameters in untreated chlamydiosis. After treatment the K-cell count decreases and the large DC count increases which indicates a reduction in the immune-response modulating processes and an increase in cell regenerative processes with the production of placental proteins and hormones. This is also confirmed by the nuclear volume and nuclear-cytoplasmic ratio, which is higher in the septa throughout the placenta in the group with therapeutic chlamydiosis.

Conclusion. Antibiotics have low therapeutic effect on morphological changes of the cell apparatus of the

placenta in chlamydiosis treatment, since we observe weak cell proliferative processes against a significant decrease in immunoregulatory functions with their use.

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Conflict of Interest. The authors do not have any conflicts of interests.

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