



IMMUNOHISTOCHEMICAL APPROACHES TO THE DIAGNOSIS OF EARLY MISCARRIAGE

Matrizaeva Gulnara Dzhumaniyazovna¹, Ikhtiyarova Gulchekhra Akmalovna²

¹ Matrizaeva G. D. - Candidate of Medical Sciences, Associate Professor, Head of the Department of Obstetrics and Gynecology, Urgench Branch of the Tashkent Medical Academy, Urgench, Uzbekistan. E-mail: gmatrizayeva@gmail.com; <https://orcid.org/0009-0001-2796-8041>

² Ikhtiyarova G. A. - Doctor of Medical Sciences, Professor, Head of the Department of Obstetrics and Gynecology, Bukhara State Medical Institute, Bukhara, Uzbekistan. E-mail: ixtiyarova7272@mail.ru; <https://orcid.org/0000-0002-2398-3711>

Introduction. The decidua is a transformed uterine mucosa that provides immune tolerance to the fetus, supports implantation processes and promotes normal pregnancy development. Women with recurrent pregnancy loss (RPL) have abnormalities in the structure and function of the decidua, which can be a key factor in pregnancy loss. Diagnosis of endometrial receptivity in women with recurrent pregnancy loss is an important step in the treatment of this condition. Endometrial receptivity is a key factor in successful embryo implantation, and its impairment can lead to recurrent pregnancy loss (RPL). Diagnosis of endometrial receptivity includes assessment of expression of hormonal receptors (estrogen, progesterone), inflammatory markers (CD20, CD3), cell proliferation (Ki-67) and vascular endothelial factor (VEGF). Modern molecular and immunological methods, including immunohistochemistry, receptivity marker tests, and molecular testing, play a key role in identifying implantation disorders and optimizing treatment. Continued research in this area will allow for the development of more accurate diagnostic algorithms and improved outcomes in women with endometrial receptivity disorders.

Key words: miscarriage, hormonal receptors, immunohistochemistry, PRP (Platelet-Rich Plasma).

Objective of the study: To characterize the immunohistochemical aspects of the endometrium, to determine the relationship between inflammatory processes and



hormonal disorders in the formation of miscarriage in women living in the Aral Sea region for the development of diagnostic methods and pre-conception preparation.

Materials and methods of research: This scientific study was conducted from 2021 to 2023 at the Department of Obstetrics and Gynecology of the Urgench branch of the Tashkent Medical Academy.

The study involved 258 women of childbearing age. All study participants were permanent residents of urban and rural areas of the Khorezm region and the Republic of Karakalpakstan.

The contingent of examined women was divided into two groups:

1. The main group - 198 women with habitual miscarriage. Women with habitual miscarriage were divided into two subgroups depending on the type of treatment.
2. The control group - 60 healthy women who applied for a medical abortion for personal reasons.

Blood biochemistry was analyzed using a Mindray-BA-88A device. Reagents from Human (Germany) were used to perform the analyses. The concentration of hormones of the hypothalamic-pituitary-ovarian system was determined on an empty stomach on the 2nd-3rd day of the menstrual cycle between 08:00 and 09:00. The studies were conducted using the enzyme immunoassay method on a Mindray MR-96A hormone analyzer. Blood tests were performed for a clinical health assessment, including biochemical (total protein, glucose, ALT, AST, lactate dehydrogenase), immunological (procalcitonin, VGF, ferritin, lupus anticoagulant, AT-TPO, antibodies to human chorionic gonadotropin) and hormonal profiles. Hormonal studies included the following parameters: tritropic hormone, T3, T4, prolactin, testosterone, estradiol, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and other reproductive hormones. Data analysis was conducted in accordance with the study objectives for each clinical group.

Methods for studying pathomorphological changes in uterine curettage in the observed women were conducted in the pathomorphology department of the Republican Institute of Obstetrics and Gynecology (RIO) and the Republican Institute of Obstetrics and Medical Technologies (RIATM). Address: Tashkent, Shaykhontohur district, Farabi street, house 383.



Samples of uterine curettage were collected for the study from 40 women with miscarriage (2 or more spontaneous miscarriages or fetal arrest) and from 20 women who had a medical abortion during a normally developing pregnancy at their own request. The histological features of tissues obtained from curettage of the uterus of women belonging to patients who applied to the gynecology department of the Perinatal Center of the Khorezm region, as well as the obstetrics and gynecology complex of the Urgench City Medical Association and the private clinic "Dilorom Medical" were assessed. Tissue sections obtained by curettage or aspiration of the uterus were made on the same day. The materials were processed using unified methods of histological examination of biopsy and surgical material using a Thermo Fisher Scientific histoprocessor for 16 hours in accordance with the instructions.

In this study, we were able to identify the causes of early pregnancy loss at the cellular level using immunohistochemical analysis. We studied the correspondence between the number of estrogen, progesterone and hCG receptors, the degree of cell proliferation, signs of inflammation and the state of molecular adhesion in the vessels.

Inclusion criteria - study participants must be between 18 and 45 years old, have a history of two or more cases of pregnancy loss, and also be currently pregnant at the time of the study.

Exclusion criteria - age outside the established range, male factor infertility, alloimmune abortion, uterine pathologies, high body mass index and other conditions are excluded from the study.

After a full examination and determination of existing disorders

(3 menstrual cycles):

1. From the 5th to the 21st day of the menstrual cycle, 17- β -ethinyl estradiol preparations are prescribed (TTC-25, TTC-100, Divigel, Progynova, etc.). The route of administration of the drug is determined individually
2. From the 16th to the 25th day of the menstrual cycle - progesterone preparations (progesterone, Utrozhestan)



3. For ovulation - human chorionic gonadotropin 5000-10000 U once
4. Intrauterine plasma lifting (every week once for 3 months)
5. Vaginal ozone therapy (5 days every month from the 9th day of the menstrual cycle for 3 months)

Results of the study:

When examining the general blood test, it was revealed that patients with miscarriage have a significantly lower hemoglobin level of 90.4 g / l (CI 95% - 88.68; 92.1) compared to the control group 112.88 g / l; (CI 95% -109.4; 114.9), ($\chi^2 = 4.47$; $p < 0.05$). Our attention was drawn to another important point, the total number of leukocytes was increased by 1.5 times in the main group compared to the control, respectively $9.1181 \pm 0.181 * 10^9 / l$ and $5.923 \pm 0.1167 * 10^9 / l$. But did not exceed the reference values. High level of band neutrophils in women of the main group ($77.782 \pm 0.0,547\%$) compared to the control group ($62.085 \pm 0.468\%$) indicated the presence of an inflammatory process ($\chi^2=3.96$; $p<0.05$). Although during the examination, women did not have symptoms indicating the presence of acute infectious processes. A decrease in the level of lymphocytes ($20.151 \pm 0.6077\%$) in women with miscarriage compared to the control group ($31.712 \pm 0.51\%$) may indicate suppression of the immune system ($\chi^2=4.21$; $p<0.05$;). The incidence of increased ESR in patients of the main group (16.112 ± 0.429 mm/h) had a significant difference from the control group (9.883 ± 0.6336 mm/h) ($\chi^2=3.92$; $p<0.05$).

Antiphospholipid antibodies of IgM class are significantly higher in the main group (1.325 ± 0.05581 vs. 0.3281 ± 0.0564 U/ml), the difference between the groups did not reach statistically significant difference ($\chi^2=3.028$; $p>0.05$), but the indicator has clinical significance. Antiphospholipid antibodies of IgG class are also significantly higher in the main group (4.19638 ± 0.0783 U/ml; 95% CI- 3.94; 4.42 vs. 1.6532 ± 0.0798 U/ml; 95% CI-1.39; 1.64). This indicates the presence of an autoimmune process in women with miscarriage, and has a statistically significant difference ($\chi^2=3.91$; $p<0.05$). Antiphospholipid antibodies IgG can interact with the membranes of vascular wall cells, leading to thrombus formation and impaired blood circulation in the placenta. Antibodies to hCG class IgM in the main group are significantly higher compared to the control



group, respectively $0.98431 \pm 0.01361 \mu\text{l}$; 95% CI-0.42; 0.5 and $0.168 \pm 0.0054 \mu\text{l}$ 95% CI-0.16; 0.27. This indicator has high statistical significance ($\chi^2=3.966$; $p<0.05$). High levels of antibodies to human chorionic gonadotropin (hCG) of the IgG class in the main group ($1.706331 \pm 0.0617 \mu\text{l}$ versus 0.4175 ± 0.0518) were 4 times higher than in the control group, indicating impaired hormonal support of pregnancy. Statistically significant differences ($\chi^2=3.97$; $p<0.05$) confirm the clinical importance of this indicator. The highest level of Anti hCG IgG was $4.66 \mu\text{l}$, which was 11.7 times higher than the normal values. These antibodies can block the action of hCG, which will lead to impaired implantation and fetal development.

Increased levels of lupus anticoagulant in women with miscarriage $44.7557 \pm 0.1965 \text{ sec}$; 95% CI-37.6; 46.8 compared to the control group $33.23 \pm 0.2856 \text{ sec}$; 95% CI-32.7; 33.7 ($\chi^2=3.99$; $p<0.05$) indicate an increased risk of thrombus formation, which can block normal blood flow in the placenta. The level of antibodies to thyroid peroxidase is significantly higher in women with miscarriage ($141.506 \pm 11.671 \text{ IU/ml}$ versus $22.22 \pm 2.3956 \text{ IU/ml}$). Statistical significance $\chi^2=640.3$; $p<0.001$ indicates a high risk of complications associated with thyroid dysfunction and thereby disrupting hormonal support of pregnancy.

Immunohistochemical markers in the decidua, which help to assess the expression level of various hormones and proteins associated with cell proliferation and vascular development in tissues, which is important for successful pregnancy. Pronounced hypoexpression of estrogen receptors in the decidua is 2 times lower in the main group compared to the control (7.525 ± 0.2259 vs. 15.15 ± 0.538 , $\chi^2=3.83$; $p<0.05$). Progesterone receptors are also significantly lower in the main group (10.025 ± 0.172 vs. 18.8 ± 0.1785 , $\chi^2=4.2$; $p<0.05$). The results of the study showed no significant differences in the levels of hCG expression between the main (16.6 ± 0.1265) and (18.8 ± 0.1785) control groups $\chi^2=0.25$; $p>0.05$. The expression of CD34, an angiogenesis marker, was 2 times lower in the main group compared to the control group, 8.525 ± 0.1625 and 15.5 ± 0.349 , respectively, $\chi^2=3.85$; $p<0.05$. CD34 is an indicator of vascularization and growth of new blood vessels, which is critical for the development and maintenance of pregnancy. A decrease in the level of this marker in the main group may indicate insufficient tissue blood supply and lead to placental or



vascularization disorders. Ki-67, a marker of cell proliferation, showed increased activity in the control group (6.2 ± 0.3703) compared to the main group (10.275 ± 0.1421), indicating increased cell division in the decidua during normal pregnancy, but this indicator was not statistically significant ($\chi^2=2.16$; $P>0.05$). A significantly increased expression of CD20, a marker of B-lymphocytes, was revealed, which was 2 times higher in the main group (20.675 ± 0.169 versus 9.9 ± 0.5257), indicating increased immune activity in the decidua in women with miscarriage ($\chi^2=11.7$; $p<0.001$).

The daytime body temperature of women with miscarriage was higher (37.168 ± 0.035 ; 95% CI-37-37.3) than that of healthy women (36.65 ± 0.0376 ; 95% CI-36.5-36.7), although the difference was not statistically significant $\chi^2=0.007$; $p>0.05$. As in the daytime, in the evening the temperature of women with miscarriage was higher (37.32 ± 0.0496 ; 95% CI-37.1-37.5) than that of women in the control group (36.46 ± 0.044 ; 95% CI- 36.4-36.5), although not significantly ($\chi^2=0.02$; $p>0.05$). When comparing the described criterion between the groups, it turned out that its maximum median was registered in women with early miscarriages (38.7). When analyzing the data in this table, it is necessary to take into account that women did not have acute inflammatory diseases at the moment, we excluded them from the study. This means that an increase in body temperature may be associated with increased activity of the immune system or other reactions of the body to the stresses of pregnancy.

Statistical analysis of associations demonstrated highly reliable direct correlation changes between anti-hCG and anti-TPO; there is a positive correlation (correlation coefficient = 0.0886), which indicates a relationship between these two indicators in women with miscarriage. The χ^2 value = 640.3757784 indicates strong statistical significance of these data, which is confirmed by $p < 0.05$. The correlation coefficient = 0.0534 indicates a positive correlation between anti-hCG and LA antigens. The χ^2 value = 3.997645516 indicates statistical significance, confirmed by $p = 0.0456$. The relationship between anti-hCG and antiphospholipid antibodies shows a positive correlation ($r = 0.0199$). The significance level is $p = 0.028$; $\chi^2 = 4.82518528$ indicates the presence of a statistically significant relationship between these indicators. Correlation relationships between antiphospholipid antibodies and anti-hCG, anti-TPO and LA in women with miscarriage indicate complex mechanisms of interaction between



the immune system and hormonal regulation. After the treatment, the level of expression of estradiol receptors in the stroma and glands in both groups significantly increased, but there was an intergroup difference. In women who did not receive pregravid preparation, there was still a low positive reaction in the endometrium in 40% of cases. In group 1a, estrogen expression with a high positive reaction was detected in 75% of women, which had statistical significance $p < 0.05$.

After treatment, the level of expression of progesterone receptors in the complex treatment group was significantly higher compared to the standard therapy group, $p < 0.05$. Targeted correction of the level of inflammatory factors led to a sharp decrease in the expression of SD 20 in the 1a group, where a negative reaction was noted in 60% and a low-positive reaction in 40%, $p < 0.05$.

Conclusion: Thus, the study of the expression of estrogens and progesterone in the endometrial structures of the study groups revealed significant differences in the form of a reliable decrease in indicators in women suffering from habitual miscarriage, in contrast to patients with uncomplicated pregnancy. Anti-hCG are antibodies that can interfere with the normal function of hCG, which disrupts hormonal support of pregnancy and can lead to its termination. An increase in body temperature can be accompanied by activation of the immune system, which can lead to an increase in the level of anti-hCG. This may be a marker of autoimmune processes that affect the development of pregnancy. Pregravid preparation has a significant effect on reducing the frequency of complications during pregnancy, such as retrochorial hematoma, non-developing pregnancy and spontaneous abortion. The threat of miscarriage and vomiting of pregnant women are also less common in women who have undergone preparation. Preparing the body for pregnancy, including hormonal correction, improving health, can be a key factor in successful gestation.

References:

1. ESHRE Guideline Group on RPL; Bender Atik R., Christiansen O.B. et al. ESHRE guideline: recurrent pregnancy loss: an update in 2022. Hum Reprod Open. 2023;2023(1):hoad002. DOI: 10.1093/hropen/hoad002.



2. Marini, M.G., et al. Effects of Platelet-Rich Plasma in a Model of Bovine Endometrial Inflammation In Vitro. *Reprod Biol Endocrinol*, 2016. doi:10.1186/s12958-016-0195-4 (<https://doi.org/10.1186/s12958-016-0195-4>)
3. Matrizayeva G.D., Ikhtiyarova G.A. Immunohistochemical features of the endometrium in miscarriage// *World Bulletin of Public Health Vol.17* (2022): WBPH
4. Van den Boogaard E., Cohn D.M., Korevaar J.C. et al. Number and sequence of preceding miscarriages and maternal age for the prediction of antiphospholipid syndrome in women with recurrent miscarriage. *Fertil Steril*. 2013;99(1):188–192. DOI: 10.1016/j.fertnstert.2012.09.002.
5. Friedler, S., et al. Ultrasonography in Endometrial Receptivity Evaluation Post-ART. *Hum Reprod Update*, 1996. doi:10.1093/humupd/2.4.323 (<https://doi.org/10.1093/humupd/2.4.323>)

