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THE IMPACT OF IMMUNE STATUS CHARACTERISTICS ON THE RESULTS OF IN VITRO FERTILIZATION IN PATIENTS WITH INFERTILITY

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The purpose of our study was to assess the levels of immunological indicators in infertile patients with positive and negative results of in vitro fertilization procedure. The study of indicators of immune status was conducted in 54 married couples. The concentration of immunoglobulins A, M and G were tested by the radial immunodiffusion method. The main lymphocyte populations—T-lymphocytes, T-helpers, cytolytic T-cells, natural killers, B-lymphocytes against monoclonal antibodies, respectively, CD3, CD4, CD8, CD16, CD19, CD25, HLA-DR, CD4/CD8 were studied. Indicators of immune imbalance in both partners were found in 7 % of married couples (in men were determined in 9 % of cases, in women—in 15 % of cases). In infertile women the number of T-helpers significantly exceeds the indicators of men in both the main and control groups ($p < 0.001$), a decrease in the number of T-suppressors was determined in infertile women compared to the indicators in men and the indicators in the control group. The level of IgM was significantly higher in women (3.7 g/l) than in men (2.4 g/l) of infertile couples and the control group (1.7 g/l), ($p < 0.05$). There were no significant changes in the amount of IgA and IgG in patients in the observation groups.

Key words: T-lymphocytes, natural killers, immunoglobulins, infertility

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ВПЛИВ ПОКАЗНИКІВ ІМУННОГО СТАТУСУ НА РЕЗУЛЬТАТИ ЕКСТРАКОРПОРАЛЬНОГО ЗАПЛІДНЕННЯ У ПАЦІЄНТІВ З БЕЗПЛІДДЯМ

Метою нашого дослідження була оцінка рівня імунологічних показників у безплідних пар із позитивними та негативними результатами процедури екстракорпорального запліднення. Дослідження показників імуного статусу було проведено у 54 подружніх пар. Концентрацію імуноглобулінів А, М та G перевіряли методом радіальної імунодифузії. Основні популяції лімфоцитів: Т-лімфоцити, Т-хелпери, цитолітичні Т-клітини, природні кілери, В-лімфоцити (антитіла) проти моноклональних антитіл, відповідно, CD3, CD4, CD8, CD16, CD19, CD25, HLA-DR, CD4/ CD8 були вивчені. Показники імуного дисбалансу в обох партнерів виявлено у 7 % подружніх пар (у чоловіків визначалися у 9 % випадків, у жінок – у 15 % випадків). У безплідних жінок кількість Т-хелперів достовірно перевищує показники чоловіків як в основній, так і в контрольній групах ($p < 0,001$), у безплідних жінок встановлено зниження кількості Т-супресорів порівняно з показниками у чоловіків та контрольної групи. Рівень IgM був достовірно вищим у жінок (3,7 г/л), ніж у чоловіків (2,4 г/л) безплідних пар та групи контролю (1,7 г/л), ($p < 0,05$). Достовірних змін кількості IgA та IgG у пацієнтів груп спостереження не виявлено.

Ключові слова: Т-лімфоцити, натуральні кілери, імуноглобуліни, безпліддя.

The problem of infertility remains one of the important issues of modern medical science. Medical aspects of infertility have changed depending on the progress made in the last decade, leading to the formation of a new direction such as immunology of reproduction. The goal of reproductive immunology is to study the role of immune factors and the influence of immunological mechanisms on the reproductive process. The close interaction between the immune system and the endocrine system at various stages of reproductive function is known from the literature [4, 11].

Modern advances in reproductive medicine have made it possible to closely study the microenvironment of follicles and demonstrated the important role of immunocompetent cells (IMCs) and the cytokines produced by them in the maturation of oocytes, on the degree of which the occurrence of pregnancy depends for a long time [5, 13]. Pregnancy is an example of a unique interaction between the mother's body and the fetus. During pregnancy, the main function of the immune system is aimed at ensuring special interactions between the pregnant woman and the fetus and developing resistance. Also, adaptation to the development of the fetus takes place and the safety of the mother's body is ensured. An important aspect of the transformation of the mother's immune response is the formation of immune tolerance, in the development of which macrophages, T-lymphocytes and NK-cells play an important role [3, 6, 7].

Some authors emphasize that the successful outcome of pregnancy largely depends on mandatory monitoring and full examination of both men and women (couples) [9].

There are two possible reasons for the disruption of the mother's immune system during miscarriages: the first – defects at the level of recognition of fetal antigens; the second – increased immune reactivity against fetal antigens.

In cases of miscarriages due to the first cause, the normal state of tolerance of the mother's immune system against fetal antigens is disturbed. Most researchers emphasize the fact that the immune system serves as a unique "body screen" reflecting the state and work of all major organs and systems, including the reproductive system [8, 10]. One out of every 6 women with a history of miscarriage has a wide range of non-pregnancy-specific autoantibodies –antiphospholipid, anticardiolipin, antinuclear antibodies [12]. Acquired immune deficiency is distinguished among the possible autoimmune causes of pregnancy pathologies. At this time, under the influence of stress, infections, pregnancy, the antigenic specificity of own tissues changes, which stimulates the formation of autoantibodies that can damage normal cells [13]. The role of immunological reasons in the formation of infertility remains poorly understood.

The purpose of the study was to assess the levels of immunological indices in infertile couples with positive and negative results of in vitro fertilization procedure.

Material and methods. The study of immune status indices was carried out in 54 married couples. Immunological tests were performed on the day of blood sampling from both partners at the same time. Immune indicators were analyzed according to the norms of healthy people of similar age and nationality living permanently in the territory of the Republic of Azerbaijan.

The condition of the immune system was evaluated based on the concentration of immunoglobulins A, M and G, which were tested by the radial immunodiffusion method according to Mancini. Phenotypic characteristics of lymphocytes in peripheral blood were studied by flow cytofluorometry in the "Bio Rad" (USA) device. At the same time, using "Becton Dickenson" monoclonal antibodies and based of the differentiation antigens of the main lymphocyte populations – T-lymphocytes, T-helpers, cytolytic T-cells, natural killers, B-lymphocytes against monoclonal antibodies, respectively, CD3, CD4, CD8, CD16, CD19, CD25, HLA–DR were studied. CD4/CD8 immunoregulatory index was also determined.

The obtained data of clinical and laboratory examinations were processed by variation statistics methods in statistical analysis system STATISTICA 10 (STATISTICA Software Package, USA, version 10 for Windows 8). In all samples, the normality distribution characteristics were determined according to the Kolmogorov-Smirnov, Shapiro-Wilkie and Levene criteria. If there is a normal distribution or the number of research objects is very large (more than 100), Student's parametric criteria were used for comparative analysis. a Mann-Whitney U test was used for comparisons between two groups of non-normally distributed quantitative variables. Statistical significance was accepted as $p < 0.05$.

Results of the study and their discussion. It was determined that in 69 % of cases, no disorders were detected in the immune system of both partners. Indicators of immune imbalance in both partners were found in 7 % of married couples, but immune disorders in men were determined in 9 % of cases and in women only in 15 % of cases. Thus, in the absence of other unfavorable factors for fertilization, various immune disorders are found in 1/3 of couples of reproductive age with primary infertility.

The analysis of the hemogram indicators of the examined patients made it possible to detect leukocytosis, lymphocytosis or leukopenia, which are direct precursors of possible infections or disturbances in the immune system. It was determined that the absolute indicators of leukocytes were within normal limits in 90 % of the examined persons.

Absolute and relative lymphocytosis was noted in 18 % of patients, and in women 1.5 times more often than in men.

Analysis of lymphocyte subpopulation composition was performed in married couples. Immune phenotyping of lymphocytes in the peripheral blood of consanguineous married couples made it possible to detect certain disorders in the immunogram. It was determined that during the examination of 50 infertile married couples (100 persons), 83 of them had normal T-lymphocytes (CD3+) indicators. Also, indicators of T-helpers (CD4+) in 75 patients and T-suppressors (CD8+) in 79 patients and values of in index CD4/CD8 in 72 examined persons were 1.3-2.0 conditional units. it corresponded to the normative values within.

In 21 of patients examined, the number of CD4+ lymphocytes was above normal, which indicates the strengthening of the effector mechanisms of the immune response, and in 14 patients, the number of CD8+ lymphocytes was reduced.

The changes in the relative number of T-helpers and T-suppressors were accompanied by an increase in index (CD4/CD8), the values of which were significantly higher in this category of couples with infertility or a history of spontaneous abortion than in those with physiological pregnancy.

A comparative analysis of the number of immunocompetent T-lymphocytes of women and men between the examined patients showed that maximum indicators of the absolute number of T-lymphocytes and T-helpers, as well as a decrease in the number of T-suppressors compared to control indicators ($p < 0.01$) were noted in infertile women, which indicates an imbalance in the immune system (Fig. 1).

The percentage of total T-lymphocytes of women and men in the examined couples practically did not differ from the control indicators (72–80 %). The same tendency was observed for the number of CD4+ (T–helpers) and CD8+ (T–suppressors) lymphocytes. In the examined men, their indicators corresponded to normal values. A completely different picture is observed in infertile women, where the number of T–helpers significantly exceeds the indices of men in both the main and control groups ($p < 0.001$). At the same time, a decrease in the number of T–suppressors was determined in infertile women compared to the indices in men and the indices in the control group.

During the study of subpopulations of natural killer cells of the examined contingent of patients, it was determined that the number of cells with the CD16/CD56+ phenotype in 74 % of infertile women exceeded the analogous indices of their partners and control values. Only 26 % of the examined individuals had normal numbers of natural killer cells

During the normal functioning of the immune system, along with cellular immunity, specific humoral mechanisms represented by classes of immunoglobulins are also of great importance. A particularly important role is played by classes of serum immunoglobulins IgG, IgA, IgM, which protect the body against infection and form a normal immune response to antigens.

Among the infertile couples examined by us, the levels of IgG, IgA, IgM were determined in the blood serum of both partners. More pronounced trends were determined in IgM, the level of which was significantly higher in women (3.7 g/l) than in men (2.4 g/l) and the control group (1.7 g/l) ($p < 0.05$). Thus, the indicators of IgM in women were 2 times higher than the control values and 1.5 times higher than the indices in men.

A moderate decrease in the level of IgG was found in the examined women, but this decrease did not have significant differences compared to other groups of examined persons ($p > 0.05$).

There were no significant changes in the amount of IgA in patients in all three observation group (Fig. 2).

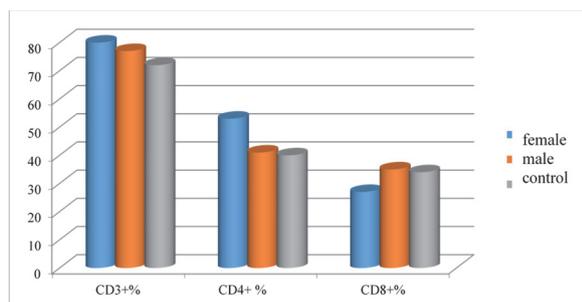


Fig. 1. Characteristics of subpopulations of T-lymphocytes in infertile couples

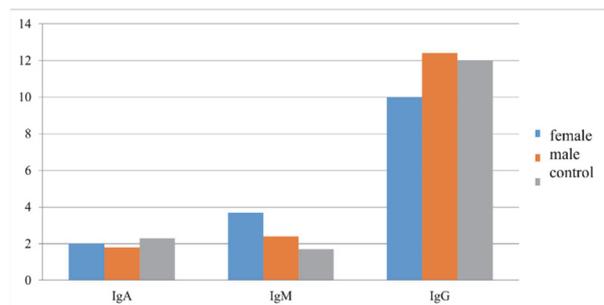


Fig. 2 Serum immunoglobulin levels in examined infertile married couples

Analyzing data obtained we revealed various changes in some immunological indices in infertile couples related to success of IVF procedure. So, we determined a decrease in the number of T–suppressors in infertile women compared to the indices in men and the indices in the control group.

In his work Dons'koi BV (2015) showed that NK-CD8 expression being increased ($> 60\%$) was predictive for IVF failure (OR 3.523, $p = 0.0193$, $n = 23$) while being decreased ($< 40\%$) was significantly predictive for subsequent pregnancy failure (OR 4.571, $p = 0.0418$, $n = 37$). The author noted that balanced “conditionally normal” NKCD8 expression (40–60 %, $n = 93$) was very significantly predictive for whole reproductive success after IVF (OR 3.972, $p = 0.0021$) [6].

Russell et al. (2013) noted focal perivascular aggregation of CD8 T cells in the endometrium of most women with recurrent reproductive failure [10].

According to Chernyshov et al. (2019), no changes in CD8 T lymphocytes were found between endometrial and peripheral blood. All the discrepancies cannot be attributed to the lack of a standardized CD4 measurement location [3].

Our results showed that the number of cells with the CD16/CD56+ phenotype in 74 % of infertile women exceeded the analogous indicators of their partners and control values. In contrast, Ho YK et al. (2020) used the determination of the percentage of peripheral CD16/CD56+ natural killers (NK cells) and concluded that a low percentage of peripheral CD16/CD56+ NK cells ($\leq 10.6\%$) in the early follicular phase is a potential index of reduced pregnancy rates and implantation success in patients with recurrent implantation failure [7]. This difference can be explained by the time of detection.

There are a few studies on the role of T–cell and NK cell marker changes in women with primary infertility. In some studies, the immunologic profile showed a significant difference in the percentage of

CD 8–T cells and CD 56 NK cells of females with primary infertility without a successful pregnancy and those who had [1, 11].

The level of CD56 NK cells in women with primary infertility was considerably lower than in controls, confirming that NK cells play a key role in human reproductive performance. According to a recent meta-analysis of studies that looked at peripheral NK cell levels, the percentage of NK cells in the blood is much higher in women who have recurrent abortions than in those who don't [11].

According to results of our study a significant imbalance was determined in the immune system of infertile women, which is related to the composition of the main subpopulations of immunocompetent cells. Apparently, the weakening of the suppressor function of the immune system can play a role in reducing the tolerance of the mother's immune system to the fetus, preventing fertilization and the development of a normal pregnancy.

Thus, our assesment showed that immune disorders can participate in the pathogenesis of infertility and cause embryo implantation disorders. However, more in-depth scientific research is required to reveal the causes of the development of immunological infertility forms in women.

Conclusions

1. Indices of immune imbalance in both partners were found in 7 % of married couples, but immune disorders in men were determined in 9 % of cases and in women only in 15 % of cases.

2. In infertile women the number of T-helpers significantly exceeds the indices of men in both the main and control groups ($p < 0.001$), a decrease in the number of T-suppressors was determined in infertile women compared to the indices in men and the indices in the control group

3. The level of IgM was significantly higher in women (3.7 g/l) than in men (2.4 g/l) of infertile couples and the control group (1.7 g/l), ($p < 0.05$). There were no significant changes in the amount of IgA and IgG in patients in the observation groups.

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