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## IMMUNO-ENDOCRINE MECHANISMS OF REPRODUCTIVE FUNCTION DISORDERS IN WOMEN WITH AUTOIMMUNE THYROIDITIS AND APPROACHES TO THE THERAPY

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**IMMUNO-ENDOCRINE MECHANISMS OF REPRODUCTIVE  
FUNCTION DISORDERS IN WOMEN WITH AUTOIMMUNE  
THYROIDITIS AND APPROACHES TO THE THERAPY**

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**ABSTRACT**

**Objective:** to study the features of the immune and neuroendocrine links of the reproductive function disorder pathogenesis in women with autoimmune thyroiditis and on this basis to substantiate fertility reproduction.

**Material and Methods:** The research presents the analysis of the dynamic monitoring results and the evidences of clinical, immunologic and hormonal examinations of 325 women with reproductive disorders and autoimmune thyroiditis. The control group included 30 healthy women of maternal age.

**Results and conclusions:** clarified and expanded conceptions on the immunological and neuroendocrine links of the reproductive disorders formation pathogenesis in women with AIT; presented data of immunoregulatory and effector role cytokines in the progression of immune system dysregulation in various clinical events of AIT in women with reproductive failure; the relationship between immune and endocrine changes was investigated for the first time and progression of reproductive disorders was shown in association with a number of unidirectional changes in the immune system: imbalance of cytokines, a decrease in the number of activation markers of early activation lymphocytes, increase of late activation lymphocytes, increase in the number of the B-lymphocytes quantity, antibodies to thyroperoxidase and antibodies to thyroglobulin; it is established that

the pathogenesis of menstrual cycle and reproductive disorders in women with AIT is caused by a combination of endocrine changes and immunological abnormalities, the nature and severity of immunological and endocrine disorders depends on the stage of AIT progress; the features of the formation of ovarian insufficiency syndrome in patients with AIT were studied taking into account the stage of development of the autoimmune process.

**Key words:** autoimmune thyroiditis, antithyroid antibodies, immune system, cytokines, hypothalamic-pituitary-ovarian system, reproductive disorders.

### **Introduction**

Diseases of the thyroid gland occupy the first place among the causes of reproductive disorders. The prevalence of thyroid pathology in the world is 10-15%, of which hypothyroidism accounts for 7 to 14%. The most common cause of hypothyroidism is autoimmune thyroiditis (AIT). According to the WHO AIT occurs in 5-26% of women of reproductive age and in 13-20% of pregnant women.

In women with autoimmune thyroiditis, reproductive disorders are manifested by early termination of pregnancy and the development of infertility, which are a significant medical and social problem. Various exogenous and endogenous factors influence on the course of AIT, including hereditary factors, endocrine diseases, adverse environmental and other factors. The relevance of AIT is due to the lack of study of etiological factors, the lack of clear understanding pathogenic mechanisms of disease development. The priority directions of modern scientific researches are the study of cellular and humoral immunity, changes in cytokine status, early detection of the disease, the use of modern methods of treatment and improvement of preventive measures.

Currently, there is an accumulation of sometimes quite contradictory clinical and experimental data on the participation of various cytokines in the pathogenesis of AIT. The authors provide contradictory data on the participation of cellular and humoral factors of the immune system in the occurrence and progressing of the autoimmune process, which may be the result of insufficient consideration of the stage of the disease (Gataulina R. G., 2001; Andreeva A.V. et al., 2011). This

makes it necessary to study the features of humoral and cellular immunity depending on the stage of the disease (Diez J. J. et al., 2003; Yunusov A. A., 2014). The study of the dynamics of cytokine production, immunocompetent blood cells and their relationship will complement the current information on the pathogenesis of AIT, and will contribute to the development of treatment protocols for reproductive disorders.

The pathogenic mechanisms of the relationship between AIT and reproductive disorders have not been fully studied. Analysis of the results of clinical studies shows that underestimation of the mechanisms of development of menstrual cycle disorders, ovulation in patients with AIT, the lack of clear protocols for restoring fertility, leads to an increase in the frequency of miscarriage and infertility (Jones R. L. et al., 2004; Fang Y, 2008; Yunusov A. A., 2014).

Currently, there is no single point of view on the role of antithyroid antibodies to the thyroid gland in the pathogenesis of infertility and miscarriage. Many authors agree that the risk of spontaneous termination of pregnancy at an early stage in women with an increased titer of antithyroid antibodies exceeds that of women without them by 2-4 times, so carriers of antibodies to thyroperoxidase (ATP) they are a group of risk for early reproductive loss, which requires special monitoring of this category of women by obstetricians and gynecologists at the stage of pregnancy planning (Yukhnovets A. A., 2004; Abalovich M. et al., 2007). According to other researchers it makes no sense to determine the level of thyroid antibodies in order to assess the dynamics of treatment and predict AIT (Fadeev V. V., 2010).

Available data in the literature suggest that changes in the hormone-producing function of the thyroid gland lead to a pronounced violation of the production of gonadotropins, which may result in various disorders in the functioning of the female reproductive system (Tatarchuk T. F. et al., 2003; Potin V. V., 2008; Petunina N. A., 2011). Literature data on the dynamics of gonadotropins depending on the stage of AIT are contradictory. However, knowledge of the content of gonadotropins, hormones of the peripheral glands,

could shed light on the pathological mechanisms of anovulation and lutein phase insufficiency in AIT. This problem is particularly relevant in women with AIT and reproductive dysfunction, when the doctor faces the issue of restoring fertility and he limits to time parameters.

### **Purpose of the study**

To study the features of the immune and neuroendocrine components of the pathogenesis of reproductive functional disorders in women with autoimmune thyroiditis and on this basis to justify the principles of the fertility restoration.

### **Material and methods**

Modern methods of instrumental diagnosis, hormonal and immunological research methods were used. The analysis of the structure of causes of reproductive disorders in 465 patients with infertility and miscarriage was carried out. Because of the screening study, 325 women with AIT were identified and groups were formed depending on the functional state of the thyroid gland, the control group consisted of 30 healthy women of fertile age. The diagnosis of AIT was formed on the basis of characteristic complaints, data from Doppler studies of the thyroid gland, and the detection of elevated ATPO and ATG titers in the blood serum. All patients were divided into groups depending on TG activity and phase of AIT at the time of the examination: 65 patients with AIT in a stage of hyperthyroidism (group 1), 106 patients with AIT in a stage of subclinical hypothyroidism (group 2), 80 women with AIT in a stage of manifest hypothyroidism (group 3) and 74 women with AIT in a stage of euthyroidism (group 4).

To assess the state of the immune system there was used determination of the level of antithyroid antibodies of class G: ATPO and ATG were identified using standard sets of the company "Insep" (Russia). The study of cytokine levels: IL-1 $\beta$ , IL-6, IL-18 and TNF- $\alpha$  was performed on a StatFax-2100 device using standard kits from firm Vector-best (Russia). The relative and absolute number of B-lymphocytes (CD20+), markers of lymphocyte activation (CD25+, CD95+) were determined using monoclonal antibodies produced by Sorbent LLC (Russia) with method of indirect rosette formation using stabilized erythrocytes.

Hormonal studies included determining the level of gonadotropins (FSH, LH), prolactin, TSH, ovarian steroid hormones (progesterone, estradiol, testosterone), thyroid hormones (T3C, T4C) in peripheral blood serum was performed on the 3rd-5th days of the menstrual cycle, and progesterone levels were evaluated on the 20th-22nd days of the cycle. We used standard kits for enzyme immunoassay of hormones of the German company "Human".

Ultrasound examination showed the position of the uterus, evaluated the nature of its contours and internal structure. The median uterine echo (m-echo), correspondence to the day and phase of the menstrual cycle, and cyclic changes were studied. Ultrasound echometry of the uterus and ovaries was performed on the device "MindrayDC-3". Mode of operation: image in module B, ultrasound head 5.0 MHz, electronic convex sensor, 384 elements, radius 40 mm, secretory angle 60°. Ultrasound examination of the thyroid gland was performed using a 7.5 MHz multi-frequency linear sensor using various scanning modes. The examination of patients included gray-scale echography (the thyroid gland linear sizes determination, the tissue volume calculation, the echostructure and echogeneity evaluation). Such criteria as diffuse heterogeneity of the echostructure and reduced echogeneity of the parenchyma were used as the main ultrasound signs allowing establishment of the autoimmune nature of the thyroid gland lesion in gray-scale echography.

At the first stage of reproductive function restoration we performed correction of AIT by two known methods: levothyroxine monotherapy and combined therapy of levothyroxine with thiamazole. Against the background of AIT correction there was carried out the comparative assessment of thyroid status, levels of gonadotropins, prolactin and ovarian steroid hormones as well as the functional state of the ovaries in 6 months after treatment. All women who, in the process of correcting AIT alone (6 months) did not have a pregnancy, in order to restore reproductive function and induce ovulation, a preparation was prescribed, containing ethinylestradiol to 30 µg/drospirenone 3 mg from day 5 of the

menstrual cycle 21 days for 3 months. Patients with preserved ovulation received clomiphene citrate 50 mg from 5 to 9 days of the cycle, maximum 3 courses.

Statistical processing of the obtained results carried out on a personal computer using standard packages of applied statistical analysis programs (Statistical Package for Social Science-22, Microsoft Excel). Correlation analysis was performed by Pearson.

### **Results and discussion**

The menstrual disorders and infertility were monitored in 465 women suffering from menstrual cycle disorders and infertility. After a primary screening examination of the total number of patients 103 (22.2%) were diagnosed with early pregnancy failure (PF), 362 (77.8%) had infertility. Of the women with infertility 261 (72.1%) had primary and 101 (27.9%) had secondary infertility. Of 103 patients with PF up to 12 weeks, 30 (29.1%) had one spontaneous miscarriage, 44 (42.7%) had two miscarriages, 29 (28.2%) had three or more. Of 103 patients with PF, spontaneous interruption at 4-6 weeks was noted in 17 (16.5%), at 6-8 weeks in 45 (43.7%), 8-10 weeks in 28 (27.2%) and at 10-12 weeks in 13 (12.6%).

In order to study the functional state of the hypothalamic-pituitary-ovarian system and clarify the pathogenic mechanisms of formation of menstrual cycle disorders and ovulation processes we studied the concentration of gonadotropins, ovarian steroid hormones and thyroid status in 465 patients with impaired reproductive function. The disturbance of reproductive function in the examined patients in 62.2% of cases was associated with a hypogonadotropic state, caused in 25.2% by hyperprolactinemia, in 54.0% by thyroid dysfunction, in 29.0% by hyperandrogenia and in 4.7% by hypergonadotropic ovarian insufficiency. Elevated ATPO and ATG titers were found in 67.5% and 55.5% of women with impaired reproductive function, respectively. A comparative study of the results of clinical examination, the nature of menstrual cycle disorders and hormone levels led to the conclusion that disturbances of the gonadotropic function of the pituitary gland can be caused by insufficient function of the pituitary gonadotrophs as well as by hyperprolactinemia, hyperandrogenism and thyroid gland dysfunction.



Comparison the results of studying the thyroid status allowed us to form 4 groups of patients with AIT depending on the functional state of the thyroid gland:

Group 1 - 65 patients with complaints of palpitations, lack of air, sweating, sleep disorders, irritability, tremor, puffiness of the face and hands. In this group of patients, the presence of an increased serum titer of ATPO (93.8%), ATG (73.8%), and characteristic changes in the hormonal status in the form of low TSH values in combination with an increased or normal content of the free thyroxine fraction (T4C) was noted. Based on the data obtained, the diagnosis of AIT at the stage of thyrotoxicosis was made.

The criteria for inclusion of patients in group 2 (106 patients) were complaints of dry skin, hair loss, drowsiness, fatigue, irritability, periodic heartbeat, a feeling of "a lump in the throat". Changes in the hormonal status manifested in isolated increase in the level of TSH, with normal parameters of T4C. ATG values were increased in 76 (71.7%) and ATPO in 99 (93.4%) patients. These parameters corresponded to AIT at the stage of subclinical hypothyroidism.

Group 3 included 80 patients with complaints of dry skin, weight gain, headaches, irritability, drowsiness, rapid fatigue, lack of air, constipation. Along with an increase in the level of TSH, a decrease in the value of T4C observed. APO values increased in 100% (80) of women, and APG-in 88.8% (71). This group included patients with AIT at the stage of manifested hypothyroidism.

Among patients with impaired reproductive function and normal values of TSH and thyroid hormones, 74 (34.6%) showed an increase in the level of ATPO and 62 (29.0%) an increase in the titer of ATG. We included these patients in group 4 (n=74). These parameters corresponded to AIT at the stage of euthyroidism.

It seemed appropriate to perform clinical and laboratory comparisons depending on the phase of development of the disease and the nature of reproductive dysfunction in women with AIT. In patients with AIT at the stage of thyrotoxicosis, the level of FSH was 1.2 times lower, and the level of LH was increased 1.3 times, the level of estradiol was 1.1 times higher, and the level of

progesterone was 2.9 times lower compared to similar parameters obtained in the control group and in these patients NLF 30 (46.2%) was more often observed, anovulation was noted in 26 (40.0%), SLNF in 9 (13.8%).

In patients with AIT at the stage of subclinical hypothyroidism, the level of FSH was 1.5 times lower, estradiol was 1.5 times lower, progesterone was 3.6 times lower, and the level of LH was 1.2 times higher relatively to similar parameters obtained in the control group and anovulation was observed with a higher frequency (56.6%), less often NLF – 25 (23.6%) and SLNF – 21 (19.8%).

In the group of patients with AIT at the stage of manifest hypothyroidism, the level of FSH was lower by 2.4 times, estradiol was reduced by 2.1 times and progesterone was reduced by 4.5 times compared to similar values obtained in the control group and this group of patients was mainly characterized by anovulation - 59 (73.7%), NLF was noted in 12 (15.0%) and SLNF in 9 (11.3%) ones.

In patients with AIT at the stage of euthyroidism, the level of FSH was 1.3 times lower, estradiol was reduced by 1.2 times and progesterone was reduced by 2.9 times compared to the similar values obtained in the control group, with a higher frequency there was found SLNF 36 (48.6%), anovulation was noted in 23 (31.1%), NLF in 15 (20.3%) patients..

At the next stage of research, we studied the features of cellular and humoral immunity in women with impaired reproductive function and AIT, depending on the stage of the disease.

It was necessary to determine the features of cytokine-mediated mechanisms of dysregulation in the immune system and to characterize changes in the subpopulation composition of lymphocytes in patients with AIT and impaired reproductive function.

To achieve this purpose, 80 women were selected among all patients with impaired reproductive function and AIT (465), whose levels of ATPO and ATG were more than 5 times higher than the standard parameters specified in the test set: 20 patients with AIT at the stage of thyrotoxicosis, 20 patients with AIT at the

stage of subclinical hypothyroidism, 20 patients with AIT at the stage of manifested hypothyroidism and 20 patients with AIT at the stage of euthyroidism.

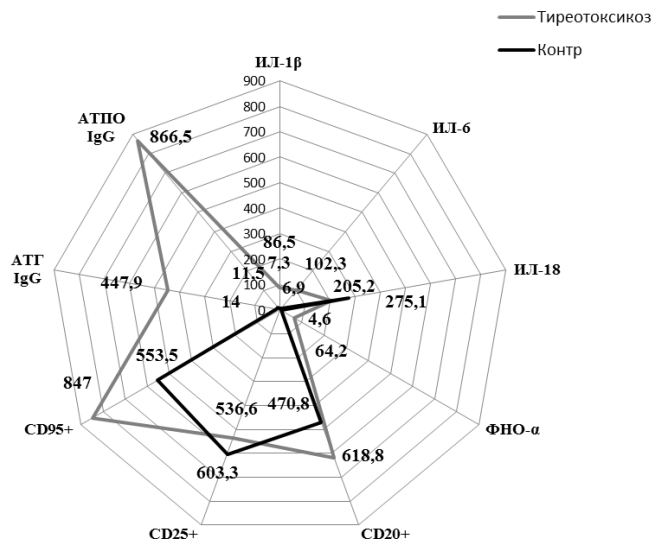


Fig.1. Peculiarities of the cellular and humoral immunity in the patients with AIT at the stage of thyrotoxicosis.

The control group consisted of 10 women without thyroid pathology.

The highest values of IL-1β were found in patients with thyrotoxicosis ( $83.2 \pm 44.2$  pg / ml) and euthyroidism ( $81.1 \pm 49.6$  pg/ml). A less pronounced reaction was found in manifest hypothyroidism and the concentration of IL-1β was  $63.1 \pm 47.7$  pg / ml. A different picture was observed in the IL-6 level. The highest values were found in subclinical hypothyroidism ( $133.9 \pm 66.1$  pg/ml), and relatively low values were found in thyrotoxicosis ( $88.9 \pm 62.5$  pg/ml). All patients with AIT had significantly high levels of TNF-α, the highest values were obtained in the group of women with euthyroidism ( $79.9 \pm 62.0$  pg / ml) and thyrotoxicosis ( $61.4 \pm 39.6$  pg/ml). IL-18 values remained within the standard parameters in all patients with AIT, but were significantly low relative to control.

When analyzing the content of lymphocytes in the blood of patients expressing activation antigens, a significant increase in the relative and absolute number of CD20 + cells was shown in women with AIT at all stages of the autoimmune process compared to the control, which allows us to speak about the activation of B-cell immune system as a whole. There was noted reliable decrease in the absolute number of cells with the CD25 + marker in patients with AIT at all stages, however the relative value was reduced at the stages of thyrotoxicosis and

euthyroidism. Increased expression of CD95 + receptors indicates an imbalance of activation signals, which leads to the development of immune response along the path of apoptosis. At the same time, in the euthyroidism the increased expression of CD95+ was less pronounced than in thyrotoxicosis and hypothyroidism.

Thus, a comparative analysis of the studied parameters of cellular, humoral and cytokine status in patients with AIT and impaired reproductive function allowed us to establish pronounced changes in the level of indicators. The highest titers of antithyroid antibodies were observed in patients with manifest and subclinical hypothyroidism. Significant deviations of IL-1 $\beta$ , IL-6 and TNF- $\alpha$  were observed in all patients with AIT, the most pronounced activation of proinflammatory cytokines was observed in patients with AIT at the stage of thyrotoxicosis and euthyroidism. Pronounced activation of B-lymphocytes with the CD20 + phenotype was found in women at all stages of the autoimmune process. There was shown decrease in markers of early activation CD25 + in patients with thyrotoxicosis and euthyroidism. The most pronounced expression of CD95 + receptors was registered in patients with thyrotoxicosis and manifest hypothyroidism, less pronounced in euthyroidism. The results obtained allowed us to determine the main criteria for the progression of the autoimmune process and their dynamics depending on the stage of AIT.

Secretion Level  
IL-1 $\beta$  CD95+

Secretion Level  
IL-6 ATPO

Secretion Level  
TNF- $\alpha$  CD20+

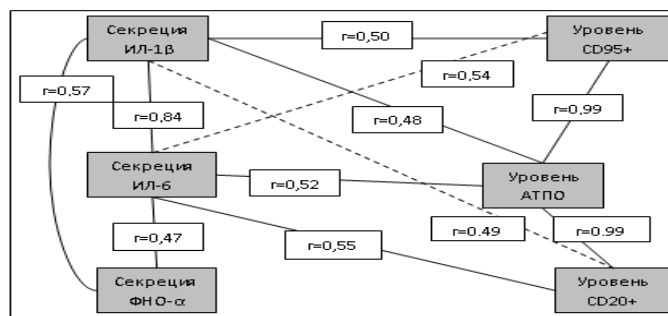


Fig.2. Correlational interrelations of immunologic parameters in patients with AIT at the stage of thyrotoxicosis.

In order to clarify the relationship between the parameters of cellular and humoral immunity and proinflammatory cytokines, we analyzed the correlation of these parameters depending on the stage of AIT.

We have studied the correlation between immune indicators differentially depending on the functional state of the thyroid gland. At the stage of thyrotoxicosis, a direct relationship was found between IL-1 $\beta$  and IL-6 ( $r=0.84$ ,  $p<0.001$ ), IL-1 $\beta$  and TNF- $\alpha$  ( $r=0.57$ ,  $p<0.01$ ), IL-6 and TNF- $\alpha$  ( $r=0.47$ ,  $p<0.05$ ). The study of the correlation between cytokines and markers of lymphocyte activation showed a significant direct relationship between IL-1 $\beta$  and CD20+ ( $r=0.49$ ,  $p<0.05$ ), between IL-1 $\beta$  and ATPO ( $r=0.48$ ,  $p<0.05$ ), characterizing the common mechanisms of inflammatory response and activation of humoral immunity, accompanied by the production of ATPO in patients with AIT at the stage of thyrotoxicosis. The proinflammatory cytokine IL-6 similarly to IL-1 $\beta$  increases B-cell proliferation, as evidenced by significant correlation between IL-6 and CD20+ ( $r=0.55$ ,  $p<0.01$ ), and stimulates the formation of auto-antibodies – IL-6 and ATPO ( $r=0.52$ ,  $p<0.05$ ). The direct correlation between IL-1 $\beta$  and CD95+ ( $r=0.50$ ,  $p<0.05$ ) and IL-6 and CD95+ ( $r=0.54$ ,  $p<0.05$ ) indicates about interrelation between the processes of inflammatory and apoptosis in the patients with AIT at the stage of thyrotoxicosis. The level of ATPO had direct correlation with values CD20+ and CD95+ ( $r=0,99$ ,  $p<0,001$ ) and ( $r=0,99$ ,  $p<001$ ) respectively. The damage of thyrocytes was connected with activation of the process of apoptosis additionally to the effect of complement-fixing cytotoxic autoantibodies.

At the stage of subclinical hypothyroidism there was seen direct correlation between IL-1 $\beta$  and IL-6 ( $r=0,81$ ,  $P<0,001$ ), IL-1 $\beta$  and TNF- $\alpha$  ( $r=0,80$ ,  $p<001$ ) and IL-6 and TNF- $\alpha$  ( $r=0,65$ ,  $p<0,05$ ), showing link between development of inflammatory response and initiation of the process of cytopathic effect on the thyrocytes, as at the stage of thyrotoxicosis. The values of ATPO had a direct connection with the level CD20+ and CD95+ ( $r=0,99$ ,  $p<0,001$ ) and ( $r=0,99$ ,

$p < 0.001$ ), respectively, reflecting activity of the humoral immunity at the stage of subclinical hypothyroidism and cytotoxic orientation of the autoimmune process.

In women with AIT at the stage of manifest hypothyroidism, a similar picture was observed, there was noted direct relationship between IL-1 $\beta$  and IL-6 ( $r = 0.82$ ,  $p < 0.001$ ), IL-1 $\beta$  and TNF- $\alpha$  ( $r = 0.91$ ,  $p < 0.001$ ), between IL-6 and TNF- $\alpha$  ( $r = 0.72$ ,  $p < 0.001$ ), indicating the activity of the autoimmune inflammatory process and at the stage of manifest hypothyroidism. The patients we examined had a direct correlation between IL-6 and CD20+ ( $r = 0.57$ ,  $p < 0.01$ ), indicating an effect on the production of autoantibodies – IL-6 and ATPO ( $r = 0.51$ ,  $p < 0.05$ ), as well as on the induction of apoptosis process – IL-6 and CD95+ ( $r = 0.51$ ,  $p < 0.05$ ). Similarly, as in other stages of the autoimmune process in manifest hypothyroidism, a direct relationship with a high degree of significance was registered between the level of CD20+ and ATPO ( $r = 0.99$ ,  $p < 0.001$ ) and CD95+ and ATPO ( $r = 0.99$ ,  $p < 0.001$ ), characterizing the activation of the synthesis of autoantibodies against their own thyrocytes with subsequent cell destruction and at the stage of manifest hypothyroidism.

The direct relationship at the stage of euthyroidism was noted between IL-1 $\beta$  and IL-6 ( $r = 0.73$ ,  $p < 0.001$ ), IL-1 $\beta$  and TNF- $\alpha$  ( $r = 0.78$ ,  $p < 0.001$ ) and between IL-6 and TNF- $\alpha$  ( $r = 0.54$ ,  $p < 0.05$ ) indicating the activity of the inflammatory process regardless of the stage AIT. The relationship between IL-1 $\beta$  and ATG ( $r = 0.44$ ,  $p < 0.05$ ) was found only in patients with AIT at the stage of euthyroidism. The level of ATPO was directly related to the values of CD20+ ( $r = 0.99$ ,  $p < 0.001$ ) and CD95+ ( $r = 0.91$ ,  $p < 0.001$ ).

Thus, the results of correlation analysis showed a directly proportional relationship between IL-1 $\beta$ , IL-6 and TNF- $\alpha$  in all patients with AIT, which reflects the relationship between the activation of the inflammatory response and subsequent destruction of thyrocytes. However, the analysis revealed a number of features. In patients with AIT at the stage of thyrotoxicosis, there was found a direct significant association of the level of inflammatory response inducers (IL-1 $\beta$  and IL-6) with pronounced stimulation of humoral immunity (CD20+),

accompanied by the production of antithyroid autoantibodies (ATPO) with subsequent activation of the processes of apoptosis of thyroid cells (CD95+). In the stage of manifest hypothyroidism and euthyroidism, direct relationships were observed only between IL-6, ATPO, CD20+ and CD95+. At the stage of euthyroidism, in contrast to other stages of AIT, a correlation between IL-1 $\beta$  and ATG was revealed. In the stage of subclinical hypothyroidism, direct correlations were observed only between CD20+ and ATPO and CD95+ and ATPO.

In the future, we analyzed the comparative effectiveness of various protocols for correcting reproductive disorders in women with autoimmune thyroiditis, where we used the results of study of immunological and hormonal parameters in dynamics of treatment in the patients with AIT, depending on the stage of the disease. In order to correct ovarian hormonal insufficiency the functional state of the thyroid gland was corrected at the first stage. We divided the group of patients with AIT at the stage of thyrotoxicosis into 2 subgroups: subgroup 1 (32 women) was used symptomatic therapy: cardioselective  $\beta$ -blocker metoprolol 25 mg 2 times a day. Subgroup 2 (33 women) - combined therapy was performed: thiamazole 10 mg per day + levothyroxine sodium in a dose of 25-50  $\mu$ g.

The study of the dynamics of immunological parameters in the process of AIT correction was carried out 3 months after in 20 patients: 10 women from subgroup 1 and 10 women from subgroup 2. The study of the thyroid status, levels of gonadotropins and ovarian steroid hormones was carried out after 6 months of treatment.

Analysis of the content of proinflammatory cytokines in patients with autoimmune thyroiditis in the stage of thyrotoxicosis showed a reliable decrease in IL-1 $\beta$ , IL-6 and TNF- $\alpha$  after combined therapy ( $p < 0.001$ ). Thus, the level of IL-1 $\beta$  after correction in subgroup 2 decreased by 8.5 times and averaged  $10.2 \pm 3.6$  pg/ml compared to  $86.5 \pm 44.3$  pg/ml before treatment ( $p < 0.001$ ), the values of IL-6 after correction decreased by 7.1 times and averaged  $14.4 \pm 8.8$  pg/ml compared to  $102.3 \pm 64.5$  pg/ml before correction ( $p < 0.001$ ) and the values of TNF- $\alpha$  decreased by 6.7 times after treatment and averaged  $9.6 \pm 7.4$  pg/ML compared to  $64.2 \pm 40.1$

pg/ml before treatment ( $p < 0.001$ ). Because of symptomatic metoprolol therapy in subgroup 1, in the absence of pathogenic therapy, there was noted a statistically unreliable decrease in the level of IL-1 $\beta$  and IL-6 and an increase in the content of IL-18 and TNF- $\alpha$  relative to the initial values before treatment. IL-18 in subgroup 1 increased to  $193.7 \pm 105.0$  pg / ml, which is probably due to the maintenance of autoimmune inflammatory process activity, confirmed by increased values of IL-1 $\beta$ , IL-6 and TNF- $\alpha$  and after 3 months. In subgroup 2, a different trend was observed in the level of IL-18, which decreased during the correction and amounted to  $147.4 \pm 76.2$  pg / ml ( $p < 0.001$ ), while the content of IL-1 $\beta$ , IL-6 and TNF- $\alpha$  also significantly decreased relatively to the initial values before treatment.

The study of the relative and absolute number of markers of lymphocyte activation in women with AIT at the stage of thyrotoxicosis showed that the level of CD20+lymphocytes did not reliably change in subgroup 1 of patients who received monotherapy. However, because of combined therapy in subgroup 2 there was a decrease to  $20.5 \pm 3.4\%$  compared to  $24.1 \pm 3.0\%$  before treatment ( $p < 0.001$ ). The content of CD25+lymphocytes significantly increased only in subgroup 2 and averaged  $20.6 \pm 2.3\%$  compared to  $19.1 \pm 2.0\%$  before treatment ( $p < 0.05$ ). The number of lymphocytes expressing Fas-antigen (CD95+) in the blood of patients after treatment in subgroup 2 significantly decreased and amounted to  $24.2 \pm 5.2\%$  compared to  $36,0 \pm 6.2\%$  before treatment ( $p < 0.001$ ). The results of studying the level of antithyroid antibodies showed that in subgroup 1 there was an increase in the concentration of ATG and ATPO, while in subgroup 2 there was noted a significant decrease in ATG by 3.5 times, and ATPO by 2.6 times.

The results of study on the level of gonadotropins in women with AIT at the stage of thyrotoxicosis after 6 months of correction showed a reliable increase in the average value of FSH in patients in subgroup 2 – from  $5.6 \pm 3.2$  IU / l to  $7.4 \pm 1.5$  IU / l ( $p < 0.001$ ), in subgroup 1 this parameter tended to decrease. Against the background of AIT correction, there was a tendency to decrease the level of LH in both subgroups. The content of prolactin decreased reliably in subgroup 2 and averaged  $9.4 \pm 3.7$  pg / ml compared to  $14.6 \pm 7.3$  pg / ml before treatment ( $p < 0.001$ ),



in subgroup 1 the level of prolactin did not significantly change. Against the background of treatment, there was a positive dynamics in the level of progesterone in patients who received combined AIT therapy ( $9.6 \pm 4.3$  pg / ml compared to  $5.4 \pm 4.0$  pg / ml before treatment,  $p < 0.001$ ).

The results of the evaluation of the ovarian functional state showed that in patients of subgroup 1 the anovulatory cycle was preserved in 13 (40.6%) patients, while SLNF was observed in 3 women, ovulation occurred in 19 (59.4%) women, of which NLF was observed in 12 patients, and pregnancy occurred in one woman. In the 2nd subgroup of women anovulation was preserved in 8 (25.8%), ovulation occurred in 23 (74.2%) women, while NLF was observed in 9, pregnancy occurred in 5 women.

The next stage of the reproductive function restoration was the correction of the ovarian functional state. All patients (42) of the examined subgroups with preserved anovulation and NLF were prescribed a drug containing ethinylestradiol 30 µg/drospirenone 3mg from the 5th day of the menstrual cycle, 1 tablet for 21 days, for a period of 3 cycles, followed by an assessment of the functional state of the ovaries. Because of the use of a combined oral contraceptive (COC), pregnancy occurred in 3 patients. In patients with preserved anovulation (22), ovulation was induced with clomiphene citrate 50 mg from 5 to 9 days of the menstrual cycle. Because of induction in the 1st cycle, pregnancy occurred in 3 women, in the 2nd cycle-in 1 woman and in the 3rd cycle-in 3 women.

The study of immunological and hormonal parameters in the dynamics of treatment in patients with AIT at the stage of subclinical hypothyroidism showed the following. At the first stage of reproductive function restoration we performed a correction of the thyroid status in women with AIT at the stage of subclinical hypothyroidism. Subgroup 1 consisted of 50 people who were prescribed levothyroxine in a dose of 50-75 µg. Subgroup 2 consisted of 56 people who received thiamazole in a dose of 10 mg per day and levothyroxine-50-100 µg. Immunological studies in dynamics after 3 months of treatment were performed in 20 patients, selected by 10 women from each subgroup. Evaluation of the thyroid

status and levels of gonadotropins and ovarian steroid hormones was performed after 6 months of AIT treatment.

The dynamics of pro-inflammatory cytokines content depending on the type of correction showed a reliable decrease in IL-1 $\beta$  in both subgroups, from  $75.8 \pm 49, 6$  pg/ml to  $32.7 \pm 36, 3$  pg/ml ( $p < 0.01$ ) after monotherapy and from  $74.1 \pm 56, 5$  pg/ml to  $24.7 \pm 43, 4$  pg/ml ( $p < 0.05$ ) after combination therapy. The IL-6 values decreased in both groups after treatment, from  $123.1 \pm 67.6$  pg/ml to  $57.2 \pm 43.5$  pg/ml ( $p < 0.01$ ) after monotherapy and from  $144.7 \pm 66.3$  pg/ml to  $25.2 \pm 59.2$  pg/ml ( $p < 0.001$ ) after combination therapy. Mean IL-18 values in treatment dynamics increased and TNF- $\alpha$  levels decreased, although differences in outcomes were significant. Because of AIT correction in the stage of subclinical hypothyroidism, significant changes in the level of CD20 + -lymphocytes were obtained after combined therapy from  $24.8 \pm 2.9\%$  to  $21.0 \pm 2.7\%$ , while the absolute number of cells decreased in both subgroups. The content of CD25 + -lymphocytes, early activation markers increased significantly after treatment in the 1st subgroup and amounted to  $21.9 \pm 1.7\%$  relatively to  $20.4 \pm 1.3\%$  before treatment ( $p < 0.05$ ), while the absolute number increased significantly in both subgroups. The level of CD95 + -lymphocytes, markers of apoptosis processes activation, significantly decreased in both subgroups after treatment and amounted in the 1st subgroup to  $26.4 \pm 6.7\%$  relatively to  $33.7 \pm 6.3\%$  before treatment ( $p < 0.05$ ) and  $26.2 \pm 5.6\%$  relatively to  $33.9 \pm 6.4\%$  before treatment in 2nd subgroup ( $p < 0.01$ ). The ATH level after 6-month correction in the 1st subgroup decreased by 1.1 times ( $p < 0.05$ ), in the 2nd subgroup decreased by 3.7 times ( $p < 0.001$ ). The level of ATPO because of treatment in the 1<sup>st</sup> subgroup decreased by 1.2 times ( $p < 0.01$ ), in the 2nd subgroup decreased by 3 times ( $p < 0.001$ ).

During 6-month AIT correction at the stage of subclinical hypothyroidism, positive dynamics in the level of gonadotropins was noted. The FSH level in 1 subgroup increased from  $4.0 \pm 2.5$  ME/l to  $4.4 \pm 2,0$  ME/l ( $p < 0,001$ ), in 2 subgroup increased more significantly from  $4.3 \pm 2.5$  ME/l to  $6.8 \pm 2.3$  ME/l ( $p < 0,001$ ). In the LG level, against the background of the AIT correction, a downward

trend was noted, significant in the 2 subgroup - from  $6.5 \pm 4.6$  ME/l to  $5.3 \pm 0,9$  ME/l ( $p < 0.05$ ). The AIT correction, resulted in the prolactin level decrease from  $16.0 \pm 7.6$  pg/ml to  $14.6 \pm 6.8$  pg/ml in the 1 subgroup ( $p < 0.01$ ) and from  $15.7 \pm 7.9$  pg/ml to  $10.2 \pm 5.2$  pg/ml in the 2 subgroup ( $p < 0,001$ ). Estradiol and progesterone values were significantly higher in the 2 subgroup. The level of estradiol increased from  $48.0 \pm 29,5$  pg/ml to  $54.6 \pm 24,7$  pg/ml in 1 subgroup ( $p < 0.01$ ) and from  $48.8 \pm 28.5$  pg/ml to  $75.5 \pm 26.6$  pg/ml in 2 subgroup ( $p < 0,001$ ). The level of progesterone increased from  $4.3 \pm 2.5$  pg/ml to  $5.1 \pm 1.7$  pg/ml in the 1 subgroup ( $p < 0.01$ ) and from  $4.6 \pm 2.3$  pg/ml to  $9.2 \pm 3.9$  pg/ml in the 2 subgroup ( $p < 0,001$ ).

Assessment of the ovarian functional state with use of the functional diagnostic test showed that in 1 subgroup anovulation was preserved in 17 (35.4%), of which SLNF was observed in 7, ovulation occurred in 31 (64.6%) women, of which NLF was noted in 9, pregnancy occurred in 5 patients. In the 2nd subgroup anovulation was preserved in 15 (27.8%) patients, of which SLNF was noted in 4, ovulation occurred in 39 (72.2%) women, of which NLF was observed in 8, pregnancy occurred in 6 patients.

The following stage of the reproductive function restoration was correction of the ovarian function state. All the patients of the studied subgroups with preserved anovulation and NLF (49) were prescribed a drug containing ethinylestradiol 30µg/drospirenone 3mg receiving from the 5th day of the menstrual cycle, in dose 1 tablet per day for 21 days, for a period of 3 cycles, followed by an assessment of the functional state of the ovaries. While taking a combined oral contraceptive in intermittent mode, ovulation occurred in 64.8%, pregnancy occurred in 4 patients. In patients with preserved anovulation (25), ovulation was induced with clomiphene citrate 50 mg from 5 to 9 days of the menstrual cycle. Because of the induction in the 1st cycle pregnancy occurred in 2 patients, in the 2nd cycle-there were no cases of pregnancy, in the 3rd cycle – in 6 patients.

The study of immunological and hormonal parameters in the dynamics of treatment in patients with AIT at the stage of manifest hypothyroidism was performed in 2 groups formed according to the Protocol of treatment. Subgroup 1 (n=38) was given levothyroxine in a dose of 75-150 µg, Subgroup 2 (n=42) received thiamazole in a dose of 10 mg per day and levothyroxine – 100-200 µg. Immunological parameters in dynamics after 3 months of treatment were studied in 20 patients with AIT at the stage of manifest hypothyroidism, 10 women were selected from each subgroup. The assessment of the thyroid status and the level of gonadotropins and steroid hormones of the ovaries was carried out after 6 months of treatment.

Against the background of correction of AIT at the stage of manifest hypothyroidism, a significant reliable decrease in the level of IL-1β was achieved as a result of combined therapy – from 69.4±48.7 pg/ml to 7.0±2.6 pg / ml after treatment (p<0.01). Similarly, there was a significant decrease in IL-6 levels as a result of complex therapy – from 111.3±73.4 pg / ml to 16.8±10.9 pg / ml (p<0.01). As for TNF-α, its content significantly decreased due to result of combined therapy and amounted to 4.9±1.9 pg / ml compared to 66.9±64.7 pg / ml before treatment (p<0.01). The content of IL-18 in both groups before and after treatment did not significantly change in both subgroups and remained within the standard parameters. Against the background of monotherapy with levothyroxine, there was noted tendency to increase the level of IL-1β in subgroup 1 of patients with AIT, though the difference in the mean values before and after treatment was not reliable. The average values of IL-6, IL-18 and TNF-α decreased slightly, while the obtained values were many times higher than the standard values indicated in the test kits, probably indicating that there was no significant effect of levothyroxine on the concentration of proinflammatory cytokines in women with AIT at the stage of manifest hypothyroidism. The level of CD20+lymphocytes decreased significantly in subgroup 2 and averaged 24.2±3.0% compared to 25.8±2.2% before treatment (p<0.01). The content of CD25 + lymphocytes in the blood increased reliably in subgroup 2 and averaged 25.7±2.3% compared to

22.3±1.7% before treatment ( $p<0.001$ ). The significant decrease in CD95+lymphocyte levels was observed only in subgroup 2 and averaged 27.3±6.6% compared to 36.3±7.0% before treatment ( $p<0.01$ ). In the level of antithyroid antibodies the significant level was not achieved in the result of treatment in the subgroup 1. The reliable reduction of ATG was registered in subgroup 2 from 384±356.1 IU / ml to 129.9±197.8 IU / ml ( $p<0.001$ ) and ATPO, which decreased from 756.9±797.1 IU/ml to 202.9±411.6 IU/ml ( $p<0.001$ ).

Against the background of 6-month correction of the thyroid status in patients with manifest hypothyroidism, a positive dynamics in the level of gonadotropins was revealed. The level of FSH in subgroup 1 increased and averaged 4.0±1.3 IU/l relative to 2.6±0.7 IU / l before correction ( $p<0.001$ ), in subgroup 2 it increased from 2.7±0.9 IU/l to 5.3±1.2 IU/l ( $p<0.001$ ). There was a decrease in the level of LH in both subgroups relative to the values obtained before the correction, but there were not found reliable differences in the results of both subgroups. The content of prolactin decreased in both subgroups, more significant decrease was observed in patients who received combined therapy in subgroup 2. Thus, the level of prolactin in subgroup 1 decreased from 23.5±13.8 pg/ml to 19.8±8.8 pg/ml ( $p<0.01$ ), in subgroup 2 – from 21.0±13.6 pg/ml to 15.0±8.0 pg/ml ( $p<0.001$ ). Estradiol levels increased significantly in both subgroups, from 36.3±18.9 pg / ml to 49.7±18.9 pg/ml ( $p<0.001$ ) in subgroup 1, and from 37.9±15.5 pg/ml to 63.0±18.3 pg / ml ( $p<0.001$ ) in subgroup 2. A similar trend was observed in the level of progesterone, which in subgroup 1 increased from 3.5±2.0 pg / ml to 5.2±2.4 pg / ml after treatment ( $p<0.001$ ), in subgroup 2 there was shown more significant increase from 3.7±1.8 pg/ml to 7.2±3.1 pg/ml after 6-month treatment ( $p<0.001$ ).

An assessment of the ovarian function state in patients with manifest hypothyroidism showed that in subgroup 1 anovulation was preserved in 18 (47.4%) patients, of which SLNF was observed in 6, ovulation occurred in 20 (52.6%), while NLF was noted in 9, pregnancy occurred in 2 women. In subgroup 2 anovulation was preserved in 14 (33.3%), SLNF was observed in 4, ovulation

occurred in 28 (66.7%), while NLF was observed in 5, pregnancy occurred in 3 women.

In order to restore reproductive function and to induce ovulation, all patients with preserved anovulation and NLF (46) were prescribed a drug containing ethinylestradiol 30 µg/drospirenone 3mg from the 5th day of the menstrual cycle for 21 days, during 3 cycles. Because of a result of the use of COC in intermittent mode, the recovery of the two-phase ovulatory cycle was observed in 25 (45.4%), pregnancy occurred in 2 patients. Patients with preserved anovulation (30) were prescribed clomiphene citrate 50 mg from 5 to 9 days of the cycle. Because of ovulation induction in the 1st cycle, pregnancy occurred in 2 patients, in the 2nd cycle-in 2 and in the 3rd cycle-in 2 patients.

In the future, we studied immunological and hormonal parameters in dynamics of treatment in patients with AIT at the stage of euthyroidism. We formed 2 subgroups of patients with euthyroidism, depending on the Treatment Protocol: subgroup 1 (n=35) received levothyroxine in a dose of 25 µg, subgroup 2 (n=39) used thiamazole in a dose of 5 mg per day and levothyroxine – 25-50 µg. The study of immunological parameters in dynamics of AIT correction was performed in 20 patients with euthyroidism, selected from each subgroup of 10 women.

The results of the study of cytokine status in patients with AIT at the stage of euthyroidism showed a significant decrease in the level of IL-1 $\beta$ , IL-6, IL-18 and TNF- $\alpha$  in patients receiving combined therapy. The concentration of IL-1 $\beta$  because of the correction in subgroup 2 decreased by 14.9 times and averaged 5.4 $\pm$ 1.9 pg / ml compared to 80.7 $\pm$ 50.3 pg / ml before treatment (p<0.001). The level of IL-6 decreased 4.4 times and averaged 20.2 $\pm$ 27.0 pg / ml compared to 89.8 $\pm$ 66.5 pg / ml before treatment (p<0.01). The IL-18 content as a result of treatment decreased by 1.4 times and averaged 103.2 $\pm$ 24.9 pg / ml compared to 142.4 $\pm$ 52.8 pg/ml before treatment (p<0.01). The level of TNF- $\alpha$  decreased by 12.2 times and averaged 7.7 $\pm$ 6.7 pg / ml compared to 94.0 $\pm$ 64.9 pg / ml before treatment (p<0.01). Against the background of monotherapy, a reliable decrease

was observed only in the level of IL-1 $\beta$ , IL-6 and TNF- $\alpha$ . The level of IL-1 $\beta$  decreased by 2.5 times and averaged 32.8 $\pm$ 46.0 pg / ml compared to 81.4 $\pm$ 51.5 pg / ml before treatment ( $p < 0.05$ ). The level of IL-6 decreased by 2.2 times and averaged 59.7 $\pm$ 80.6 pg / ml compared to 132.0 $\pm$ 78.4 pg/ml before treatment ( $p < 0.05$ ). The level of TNF- $\alpha$  decreased by 3,8 times and averaged 17.5 $\pm$ 28.5 pg / ml compared to 65.8 $\pm$ 58.7pg / ml before treatment ( $p < 0.05$ ). The average value of IL-18 did not significantly change because of treatment in subgroup 1.

Against the background of AIT correction, a significant decrease in the relative number of CD20+lymphocytes was observed in subgroup 2, while the absolute number of cells reliably decreased in both subgroups. The content of CD25 + lymphocytes in the blood increased because of treatment in both subgroups, significantly after combined therapy and averaged 22.6 $\pm$ 1.1% compared to 20.7 $\pm$ 0.9% before treatment ( $p < 0.001$ ). A significant decrease in the relative and absolute number of CD95 + lymphocytes in the blood of patients was observed because of result of AIT correction in subgroup 2. The level of ATG reliably decreased after combined therapy and amounted to 62.6 $\pm$ 135.4 IU / ml compared to 198.9 $\pm$ 201.7 IU/ml before correction ( $p < 0.001$ ). The level of ATPO significantly decreased in both subgroups, in subgroup 1 from 476.2 $\pm$ 630.1 IU / ml to 386.8 $\pm$ 464.7 IU / ml ( $p < 0.01$ ) and in subgroup 2 from 476.3 $\pm$ 504.3 IU/ml to 175.9 $\pm$ 284.1 IU/ml ( $p < 0.001$ ).

AIT correction resulted in the significant increase of the level of FSH in the subgroup 2 and averaged 6.4  $\pm$  2.0 ME/L relatively to 5.0  $\pm$  2.7 ME/L before treatment ( $p < 0,001$ ). The level of prolactin reliably reduced in the both subgroups, in subgroup 1 from 11.4  $\pm$  6.2 pg/ml to 8.8  $\pm$  4, 9 pg/ml ( $p < 0,001$ ), in subgroup 2 from 12.6  $\pm$  6.7 pg/ml to 7.7  $\pm$  4.1 pg/ml ( $p < 0,001$ ). Estradiol and progesterone levels increased in the both subgroups. So, the level of estradiol in the 1<sup>st</sup> subgroup after correction of hypothyroidism averaged from 62.5  $\pm$  34.0 pg/ml compared to 55.1  $\pm$  36.4 pg/ml before treatment ( $p < 0,001$ ), the level of progesterone increased and averaged 7.0  $\pm$  4.1 pg/ml relatively to 4.9  $\pm$  4.5 pg/ml before treatment ( $p < 0,001$ ). In the 2nd subgroup the estradiol level reached higher

values and averaged  $74.7 \pm 27.5$  pg/ml relative to  $64.7 \pm 42.5$  pg/ml before correction ( $p < 0.05$ ), and the progesterone level averaged from  $9.2 \pm 5.0$  pg/ml in relation to  $5.7 \pm 5.6$  pg/ml before treatment ( $p < 0,001$ ).

Assessment of ovarian functional state with use of functional diagnosis tests showed that in subgroup 1 of patients anovulation was preserved in 9 (28.1%) women, SLNF was observed in 3, ovulation occurred in 23 (71.9%), NLF was noted in 4, pregnancy occurred in 4 women. In subgroup 2 anovulation was preserved in 9 (25.7%) women, SLNF was observed in 2 women, ovulation occurred in 26 (74.3%), while NLF was noted in 4, pregnancy occurred in 5 women.

In order to restore reproductive function, all patients with preserved anovulation and NLF (26) were prescribed a preparation containing ethinyl estradiol 30 µg/drospirenone 3 mg from 5 to 21 days of menstrual cycle for 3 menstrual cycles. Assessment of the ovarian functional state showed recovery of the two-phase cycle in 14 (53.8%) patients, pregnancy occurred in 6 women. In the patients with preserved anovulation (12), clomiphene citrate 50 mg using from 5 to 9 days of the cycle was prescribed to induce ovulation. The stimulation of ovulation resulted in pregnancy occurred the 1st cycle in 2 patients, in the 2nd cycle there were no cases of pregnancy, in the 3rd cycle - in 2 patients. Thus, because of AIT correction alone and recovery of thyroid imbalance in 325 women with impaired reproductive function and AIT pregnancy occurred in 44 women. The ovarian stimulation promoted the pregnancy occurrence in 40 women.

### **Conclusions**

1.The results of a comprehensive clinical, immunological and hormonal examination of 465 women with reproductive failure indicate a high incidence (69.9%) and a significant role for autoimmune thyroiditis in the formation of reproductive disorders.

2.Patients with all stages of autoimmune thyroiditis were found to have immune status disorders expressed by increased values of CD20+ and CD95+-lymphocytes, ATA level, proinflammatory cytokines, as well as a decrease in the



level of IL-18 and CD25<sup>+</sup>-lymphocytes, indicating activation of the autoimmune process in all autoimmune thyroiditis stages.

3.The results of the correlation analysis indicated a directly proportional relationship between the proinflammatory cytokines IL-1 $\beta$ , IL-6 and TNF- $\alpha$ , CD20<sup>+</sup> and CD95<sup>+</sup>-lymphocytes, and the level of ATA in all autoimmune thyroiditis stages.

4.The study of the features of the functional state of the hypothalamic-pituitary-ovarian system in women with autoimmune thyroiditis approved a decrease in the level of gonadotropins and steroid hormones of the ovaries, in the stage of thyrotoxicosis luteal phase insufficiency was more often noted (46.2%), subclinical and manifest hypothyroidism anovulation was predominant (56.6% and 73.7%, respectively) and luteinization syndrome of the unovulated follicle (48.6%) was more frequently observed in the euthyroid stage.

5.Correction of autoimmune thyroiditis with thiamazole in combination with levothyroxine compared with monotherapy with levothyroxine led to a leveling of thyroid and immune imbalances, a significant decrease in the level of ATA, a significant increase in the level of gonadotropins and steroid hormones of the ovaries and a 1.5-fold recovery of reproductive function.

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## IMMUNO-ENDOCRINE MECHANISMS OF REPRODUCTIVE FUNCTION DISORDERS IN WOMEN WITH AUTOIMMUNE THYROIDITIS AND APPROACHES TO THE THERAPY

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**IMMUNO-ENDOCRINE MECHANISMS OF REPRODUCTIVE  
FUNCTION DISORDERS IN WOMEN WITH AUTOIMMUNE  
THYROIDITIS AND APPROACHES TO THE THERAPY**

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**ABSTRACT**

**Objective:** to study the features of the immune and neuroendocrine links of the reproductive function disorder pathogenesis in women with autoimmune thyroiditis and on this basis to substantiate fertility reproduction.

**Material and Methods:** The research presents the analysis of the dynamic monitoring results and the evidences of clinical, immunologic and hormonal examinations of 325 women with reproductive disorders and autoimmune thyroiditis. The control group included 30 healthy women of maternal age.

**Results and conclusions:** clarified and expanded conceptions on the immunological and neuroendocrine links of the reproductive disorders formation pathogenesis in women with AIT; presented data of immunoregulatory and effector role cytokines in the progression of immune system dysregulation in various clinical events of AIT in women with reproductive failure; the relationship between immune and endocrine changes was investigated for the first time and progression of reproductive disorders was shown in association with a number of unidirectional changes in the immune system: imbalance of cytokines, a decrease in the number of activation markers of early activation lymphocytes, increase of late activation lymphocytes, increase in the number of the B-lymphocytes quantity, antibodies to thyroperoxidase and antibodies to thyroglobulin; it is established that

the pathogenesis of menstrual cycle and reproductive disorders in women with AIT is caused by a combination of endocrine changes and immunological abnormalities, the nature and severity of immunological and endocrine disorders depends on the stage of AIT progress; the features of the formation of ovarian insufficiency syndrome in patients with AIT were studied taking into account the stage of development of the autoimmune process.

**Key words:** autoimmune thyroiditis, antithyroid antibodies, immune system, cytokines, hypothalamic-pituitary-ovarian system, reproductive disorders.

### **Introduction**

Diseases of the thyroid gland occupy the first place among the causes of reproductive disorders. The prevalence of thyroid pathology in the world is 10-15%, of which hypothyroidism accounts for 7 to 14%. The most common cause of hypothyroidism is autoimmune thyroiditis (AIT). According to the WHO AIT occurs in 5-26% of women of reproductive age and in 13-20% of pregnant women.

In women with autoimmune thyroiditis, reproductive disorders are manifested by early termination of pregnancy and the development of infertility, which are a significant medical and social problem. Various exogenous and endogenous factors influence on the course of AIT, including hereditary factors, endocrine diseases, adverse environmental and other factors. The relevance of AIT is due to the lack of study of etiological factors, the lack of clear understanding pathogenic mechanisms of disease development. The priority directions of modern scientific researches are the study of cellular and humoral immunity, changes in cytokine status, early detection of the disease, the use of modern methods of treatment and improvement of preventive measures.

Currently, there is an accumulation of sometimes quite contradictory clinical and experimental data on the participation of various cytokines in the pathogenesis of AIT. The authors provide contradictory data on the participation of cellular and humoral factors of the immune system in the occurrence and progressing of the autoimmune process, which may be the result of insufficient consideration of the stage of the disease (Gataulina R. G., 2001; Andreeva A.V. et al., 2011). This



makes it necessary to study the features of humoral and cellular immunity depending on the stage of the disease (Diez J. J. et al., 2003; Yunusov A. A., 2014). The study of the dynamics of cytokine production, immunocompetent blood cells and their relationship will complement the current information on the pathogenesis of AIT, and will contribute to the development of treatment protocols for reproductive disorders.

The pathogenic mechanisms of the relationship between AIT and reproductive disorders have not been fully studied. Analysis of the results of clinical studies shows that underestimation of the mechanisms of development of menstrual cycle disorders, ovulation in patients with AIT, the lack of clear protocols for restoring fertility, leads to an increase in the frequency of miscarriage and infertility (Jones R. L. et al., 2004; Fang Y, 2008; Yunusov A. A., 2014).

Currently, there is no single point of view on the role of antithyroid antibodies to the thyroid gland in the pathogenesis of infertility and miscarriage. Many authors agree that the risk of spontaneous termination of pregnancy at an early stage in women with an increased titer of antithyroid antibodies exceeds that of women without them by 2-4 times, so carriers of antibodies to thyroperoxidase (ATP) they are a group of risk for early reproductive loss, which requires special monitoring of this category of women by obstetricians and gynecologists at the stage of pregnancy planning (Yukhnovets A. A., 2004; Abalovich M. et al., 2007). According to other researchers it makes no sense to determine the level of thyroid antibodies in order to assess the dynamics of treatment and predict AIT (Fadeev V. V., 2010).

Available data in the literature suggest that changes in the hormone-producing function of the thyroid gland lead to a pronounced violation of the production of gonadotropins, which may result in various disorders in the functioning of the female reproductive system (Tatarchuk T. F. et al., 2003; Potin V. V., 2008; Petunina N. A., 2011). Literature data on the dynamics of gonadotropins depending on the stage of AIT are contradictory. However, knowledge of the content of gonadotropins, hormones of the peripheral glands,

could shed light on the pathological mechanisms of anovulation and lutein phase insufficiency in AIT. This problem is particularly relevant in women with AIT and reproductive dysfunction, when the doctor faces the issue of restoring fertility and he limits to time parameters.

### **Purpose of the study**

To study the features of the immune and neuroendocrine components of the pathogenesis of reproductive functional disorders in women with autoimmune thyroiditis and on this basis to justify the principles of the fertility restoration.

### **Material and methods**

Modern methods of instrumental diagnosis, hormonal and immunological research methods were used. The analysis of the structure of causes of reproductive disorders in 465 patients with infertility and miscarriage was carried out. Because of the screening study, 325 women with AIT were identified and groups were formed depending on the functional state of the thyroid gland, the control group consisted of 30 healthy women of fertile age. The diagnosis of AIT was formed on the basis of characteristic complaints, data from Doppler studies of the thyroid gland, and the detection of elevated ATPO and ATG titers in the blood serum. All patients were divided into groups depending on TG activity and phase of AIT at the time of the examination: 65 patients with AIT in a stage of hyperthyroidism (group 1), 106 patients with AIT in a stage of subclinical hypothyroidism (group 2), 80 women with AIT in a stage of manifest hypothyroidism (group 3) and 74 women with AIT in a stage of euthyroidism (group 4).

To assess the state of the immune system there was used determination of the level of antithyroid antibodies of class G: ATPO and ATG were identified using standard sets of the company "Insep" (Russia). The study of cytokine levels: IL-1 $\beta$ , IL-6, IL-18 and TNF- $\alpha$  was performed on a StatFax-2100 device using standard kits from firm Vector-best (Russia). The relative and absolute number of B-lymphocytes (CD20+), markers of lymphocyte activation (CD25+, CD95+) were determined using monoclonal antibodies produced by Sorbent LLC (Russia) with method of indirect rosette formation using stabilized erythrocytes.

Hormonal studies included determining the level of gonadotropins (FSH, LH), prolactin, TSH, ovarian steroid hormones (progesterone, estradiol, testosterone), thyroid hormones (T3C, T4C) in peripheral blood serum was performed on the 3rd-5th days of the menstrual cycle, and progesterone levels were evaluated on the 20th-22nd days of the cycle. We used standard kits for enzyme immunoassay of hormones of the German company "Human".

Ultrasound examination showed the position of the uterus, evaluated the nature of its contours and internal structure. The median uterine echo (m-echo), correspondence to the day and phase of the menstrual cycle, and cyclic changes were studied. Ultrasound echometry of the uterus and ovaries was performed on the device "MindrayDC-3". Mode of operation: image in module B, ultrasound head 5.0 MHz, electronic convex sensor, 384 elements, radius 40 mm, secretory angle 60°. Ultrasound examination of the thyroid gland was performed using a 7.5 MHz multi-frequency linear sensor using various scanning modes. The examination of patients included gray-scale echography (the thyroid gland linear sizes determination, the tissue volume calculation, the echostructure and echogeneity evaluation). Such criteria as diffuse heterogeneity of the echostructure and reduced echogeneity of the parenchyma were used as the main ultrasound signs allowing establishment of the autoimmune nature of the thyroid gland lesion in gray-scale echography.

At the first stage of reproductive function restoration we performed correction of AIT by two known methods: levothyroxine monotherapy and combined therapy of levothyroxine with thiamazole. Against the background of AIT correction there was carried out the comparative assessment of thyroid status, levels of gonadotropins, prolactin and ovarian steroid hormones as well as the functional state of the ovaries in 6 months after treatment. All women who, in the process of correcting AIT alone (6 months) did not have a pregnancy, in order to restore reproductive function and induce ovulation, a preparation was prescribed, containing ethinylestradiol to 30 µg/drospirenone 3 mg from day 5 of the

menstrual cycle 21 days for 3 months. Patients with preserved ovulation received clomiphene citrate 50 mg from 5 to 9 days of the cycle, maximum 3 courses.

Statistical processing of the obtained results carried out on a personal computer using standard packages of applied statistical analysis programs (Statistical Package for Social Science-22, Microsoft Excel). Correlation analysis was performed by Pearson.

### **Results and discussion**

The menstrual disorders and infertility were monitored in 465 women suffering from menstrual cycle disorders and infertility. After a primary screening examination of the total number of patients 103 (22.2%) were diagnosed with early pregnancy failure (PF), 362 (77.8%) had infertility. Of the women with infertility 261 (72.1%) had primary and 101 (27.9%) had secondary infertility. Of 103 patients with PF up to 12 weeks, 30 (29.1%) had one spontaneous miscarriage, 44 (42.7%) had two miscarriages, 29 (28.2%) had three or more. Of 103 patients with PF, spontaneous interruption at 4-6 weeks was noted in 17 (16.5%), at 6-8 weeks in 45 (43.7%), 8-10 weeks in 28 (27.2%) and at 10-12 weeks in 13 (12.6%).

In order to study the functional state of the hypothalamic-pituitary-ovarian system and clarify the pathogenic mechanisms of formation of menstrual cycle disorders and ovulation processes we studied the concentration of gonadotropins, ovarian steroid hormones and thyroid status in 465 patients with impaired reproductive function. The disturbance of reproductive function in the examined patients in 62.2% of cases was associated with a hypogonadotropic state, caused in 25.2% by hyperprolactinemia, in 54.0% by thyroid dysfunction, in 29.0% by hyperandrogenia and in 4.7% by hypergonadotropic ovarian insufficiency. Elevated ATPO and ATG titers were found in 67.5% and 55.5% of women with impaired reproductive function, respectively. A comparative study of the results of clinical examination, the nature of menstrual cycle disorders and hormone levels led to the conclusion that disturbances of the gonadotropic function of the pituitary gland can be caused by insufficient function of the pituitary gonadotrophs as well as by hyperprolactinemia, hyperandrogenism and thyroid gland dysfunction.

Comparison the results of studying the thyroid status allowed us to form 4 groups of patients with AIT depending on the functional state of the thyroid gland:

Group 1 - 65 patients with complaints of palpitations, lack of air, sweating, sleep disorders, irritability, tremor, puffiness of the face and hands. In this group of patients, the presence of an increased serum titer of ATPO (93.8%), ATG (73.8%), and characteristic changes in the hormonal status in the form of low TSH values in combination with an increased or normal content of the free thyroxine fraction (T4C) was noted. Based on the data obtained, the diagnosis of AIT at the stage of thyrotoxicosis was made.

The criteria for inclusion of patients in group 2 (106 patients) were complaints of dry skin, hair loss, drowsiness, fatigue, irritability, periodic heartbeat, a feeling of "a lump in the throat". Changes in the hormonal status manifested in isolated increase in the level of TSH, with normal parameters of T4C. ATG values were increased in 76 (71.7%) and ATPO in 99 (93.4%) patients. These parameters corresponded to AIT at the stage of subclinical hypothyroidism.

Group 3 included 80 patients with complaints of dry skin, weight gain, headaches, irritability, drowsiness, rapid fatigue, lack of air, constipation. Along with an increase in the level of TSH, a decrease in the value of T4C observed. APO values increased in 100% (80) of women, and APG-in 88.8% (71). This group included patients with AIT at the stage of manifested hypothyroidism.

Among patients with impaired reproductive function and normal values of TSH and thyroid hormones, 74 (34.6%) showed an increase in the level of ATPO and 62 (29.0%) an increase in the titer of ATG. We included these patients in group 4 (n=74). These parameters corresponded to AIT at the stage of euthyroidism.

It seemed appropriate to perform clinical and laboratory comparisons depending on the phase of development of the disease and the nature of reproductive dysfunction in women with AIT. In patients with AIT at the stage of thyrotoxicosis, the level of FSH was 1.2 times lower, and the level of LH was increased 1.3 times, the level of estradiol was 1.1 times higher, and the level of

progesterone was 2.9 times lower compared to similar parameters obtained in the control group and in these patients NLF 30 (46.2%) was more often observed, anovulation was noted in 26 (40.0%), SLNF in 9 (13.8%).

In patients with AIT at the stage of subclinical hypothyroidism, the level of FSH was 1.5 times lower, estradiol was 1.5 times lower, progesterone was 3.6 times lower, and the level of LH was 1.2 times higher relatively to similar parameters obtained in the control group and anovulation was observed with a higher frequency (56.6%), less often NLF – 25 (23.6%) and SLNF – 21 (19.8%).

In the group of patients with AIT at the stage of manifest hypothyroidism, the level of FSH was lower by 2.4 times, estradiol was reduced by 2.1 times and progesterone was reduced by 4.5 times compared to similar values obtained in the control group and this group of patients was mainly characterized by anovulation - 59 (73.7%), NLF was noted in 12 (15.0%) and SLNF in 9 (11.3%) ones.

In patients with AIT at the stage of euthyroidism, the level of FSH was 1.3 times lower, estradiol was reduced by 1.2 times and progesterone was reduced by 2.9 times compared to the similar values obtained in the control group, with a higher frequency there was found SLNF 36 (48.6%), anovulation was noted in 23 (31.1%), NLF in 15 (20.3%) patients..

At the next stage of research, we studied the features of cellular and humoral immunity in women with impaired reproductive function and AIT, depending on the stage of the disease.

It was necessary to determine the features of cytokine-mediated mechanisms of dysregulation in the immune system and to characterize changes in the subpopulation composition of lymphocytes in patients with AIT and impaired reproductive function.

To achieve this purpose, 80 women were selected among all patients with impaired reproductive function and AIT (465), whose levels of ATPO and ATG were more than 5 times higher than the standard parameters specified in the test set: 20 patients with AIT at the stage of thyrotoxicosis, 20 patients with AIT at the

stage of subclinical hypothyroidism, 20 patients with AIT at the stage of manifested hypothyroidism and 20 patients with AIT at the stage of euthyroidism.

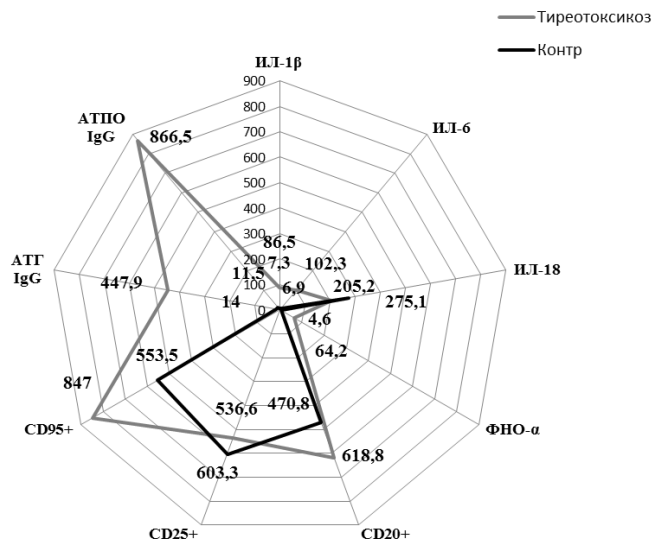


Fig.1. Peculiarities of the cellular and humoral immunity in the patients with AIT at the stage of thyrotoxicosis.

The control group consisted of 10 women without thyroid pathology.

The highest values of IL-1β were found in patients with thyrotoxicosis ( $83.2 \pm 44.2$  pg / ml) and euthyroidism ( $81.1 \pm 49.6$  pg/ml). A less pronounced reaction was found in manifest hypothyroidism and the concentration of IL-1β was  $63.1 \pm 47.7$  pg / ml. A different picture was observed in the IL-6 level. The highest values were found in subclinical hypothyroidism ( $133.9 \pm 66.1$  pg/ml), and relatively low values were found in thyrotoxicosis ( $88.9 \pm 62.5$  pg/ml). All patients with AIT had significantly high levels of TNF-α, the highest values were obtained in the group of women with euthyroidism ( $79.9 \pm 62.0$  pg / ml) and thyrotoxicosis ( $61.4 \pm 39.6$  pg/ml). IL-18 values remained within the standard parameters in all patients with AIT, but were significantly low relative to control.

When analyzing the content of lymphocytes in the blood of patients expressing activation antigens, a significant increase in the relative and absolute number of CD20 + cells was shown in women with AIT at all stages of the autoimmune process compared to the control, which allows us to speak about the activation of B-cell immune system as a whole. There was noted reliable decrease in the absolute number of cells with the CD25 + marker in patients with AIT at all stages, however the relative value was reduced at the stages of thyrotoxicosis and

euthyroidism. Increased expression of CD95 + receptors indicates an imbalance of activation signals, which leads to the development of immune response along the path of apoptosis. At the same time, in the euthyroidism the increased expression of CD95+ was less pronounced than in thyrotoxicosis and hypothyroidism.

Thus, a comparative analysis of the studied parameters of cellular, humoral and cytokine status in patients with AIT and impaired reproductive function allowed us to establish pronounced changes in the level of indicators. The highest titers of antithyroid antibodies were observed in patients with manifest and subclinical hypothyroidism. Significant deviations of IL-1 $\beta$ , IL-6 and TNF- $\alpha$  were observed in all patients with AIT, the most pronounced activation of proinflammatory cytokines was observed in patients with AIT at the stage of thyrotoxicosis and euthyroidism. Pronounced activation of B-lymphocytes with the CD20 + phenotype was found in women at all stages of the autoimmune process. There was shown decrease in markers of early activation CD25 + in patients with thyrotoxicosis and euthyroidism. The most pronounced expression of CD95 + receptors was registered in patients with thyrotoxicosis and manifest hypothyroidism, less pronounced in euthyroidism. The results obtained allowed us to determine the main criteria for the progression of the autoimmune process and their dynamics depending on the stage of AIT.

Secretion Level  
IL-1 $\beta$  CD95+

Secretion Level  
IL-6 ATPO

Secretion Level  
TNF- $\alpha$  CD20+

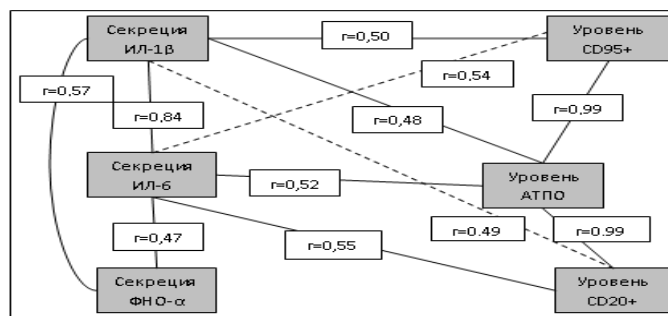


Fig.2. Correlational interrelations of immunologic parameters in patients with AIT at the stage of thyrotoxicosis.



In order to clarify the relationship between the parameters of cellular and humoral immunity and proinflammatory cytokines, we analyzed the correlation of these parameters depending on the stage of AIT.

We have studied the correlation between immune indicators differentially depending on the functional state of the thyroid gland. At the stage of thyrotoxicosis, a direct relationship was found between IL-1 $\beta$  and IL-6 ( $r=0.84$ ,  $p<0.001$ ), IL-1 $\beta$  and TNF- $\alpha$  ( $r=0.57$ ,  $p<0.01$ ), IL-6 and TNF- $\alpha$  ( $r=0.47$ ,  $p<0.05$ ). The study of the correlation between cytokines and markers of lymphocyte activation showed a significant direct relationship between IL-1 $\beta$  and CD20+ ( $r=0.49$ ,  $p<0.05$ ), between IL-1 $\beta$  and ATPO ( $r=0.48$ ,  $p<0.05$ ), characterizing the common mechanisms of inflammatory response and activation of humoral immunity, accompanied by the production of ATPO in patients with AIT at the stage of thyrotoxicosis. The proinflammatory cytokine IL-6 similarly to IL-1 $\beta$  increases B-cell proliferation, as evidenced by significant correlation between IL-6 and CD20+ ( $r=0.55$ ,  $p<0.01$ ), and stimulates the formation of auto-antibodies – IL-6 and ATPO ( $r=0.52$ ,  $p<0.05$ ). The direct correlation between IL-1 $\beta$  and CD95+ ( $r=0.50$ ,  $p<0.05$ ) and IL-6 and CD95+ ( $r=0.54$ ,  $p<0.05$ ) indicates about interrelation between the processes of inflammatory and apoptosis in the patients with AIT at the stage of thyrotoxicosis. The level of ATPO had direct correlation with values CD20+ and CD95+ ( $r=0,99$ ,  $p<0,001$ ) and ( $r=0,99$ ,  $p<001$ ) respectively. The damage of thyrocytes was connected with activation of the process of apoptosis additionally to the effect of complement-fixing cytotoxic autoantibodies.

At the stage of subclinical hypothyroidism there was seen direct correlation between IL-1 $\beta$  and IL-6 ( $r=0,81$ ,  $P<0,001$ ), IL-1 $\beta$  and TNF- $\alpha$  ( $r=0,80$ ,  $p<001$ ) and IL-6 and TNF- $\alpha$  ( $r=0,65$ ,  $p<0,05$ ), showing link between development of inflammatory response and initiation of the process of cytopathic effect on the thyrocytes, as at the stage of thyrotoxicosis. The values of ATPO had a direct connection with the level CD20+ and CD95+ ( $r=0,99$ ,  $p<0,001$ ) and ( $r=0,99$ ,

$p < 0.001$ ), respectively, reflecting activity of the humoral immunity at the stage of subclinical hypothyroidism and cytotoxic orientation of the autoimmune process.

In women with AIT at the stage of manifest hypothyroidism, a similar picture was observed, there was noted direct relationship between IL-1 $\beta$  and IL-6 ( $r = 0.82$ ,  $p < 0.001$ ), IL-1 $\beta$  and TNF- $\alpha$  ( $r = 0.91$ ,  $p < 0.001$ ), between IL-6 and TNF- $\alpha$  ( $r = 0.72$ ,  $p < 0.001$ ), indicating the activity of the autoimmune inflammatory process and at the stage of manifest hypothyroidism. The patients we examined had a direct correlation between IL-6 and CD20+ ( $r = 0.57$ ,  $p < 0.01$ ), indicating an effect on the production of autoantibodies – IL-6 and ATPO ( $r = 0.51$ ,  $p < 0.05$ ), as well as on the induction of apoptosis process – IL-6 and CD95+ ( $r = 0.51$ ,  $p < 0.05$ ). Similarly, as in other stages of the autoimmune process in manifest hypothyroidism, a direct relationship with a high degree of significance was registered between the level of CD20+ and ATPO ( $r = 0.99$ ,  $p < 0.001$ ) and CD95+ and ATPO ( $r = 0.99$ ,  $p < 0.001$ ), characterizing the activation of the synthesis of autoantibodies against their own thyrocytes with subsequent cell destruction and at the stage of manifest hypothyroidism.

The direct relationship at the stage of euthyroidism was noted between IL-1 $\beta$  and IL-6 ( $r = 0.73$ ,  $p < 0.001$ ), IL-1 $\beta$  and TNF- $\alpha$  ( $r = 0.78$ ,  $p < 0.001$ ) and between IL-6 and TNF- $\alpha$  ( $r = 0.54$ ,  $p < 0.05$ ) indicating the activity of the inflammatory process regardless of the stage AIT. The relationship between IL-1 $\beta$  and ATG ( $r = 0.44$ ,  $p < 0.05$ ) was found only in patients with AIT at the stage of euthyroidism. The level of ATPO was directly related to the values of CD20+ ( $r = 0.99$ ,  $p < 0.001$ ) and CD95+ ( $r = 0.91$ ,  $p < 0.001$ ).

Thus, the results of correlation analysis showed a directly proportional relationship between IL-1 $\beta$ , IL-6 and TNF- $\alpha$  in all patients with AIT, which reflects the relationship between the activation of the inflammatory response and subsequent destruction of thyrocytes. However, the analysis revealed a number of features. In patients with AIT at the stage of thyrotoxicosis, there was found a direct significant association of the level of inflammatory response inducers (IL-1 $\beta$  and IL-6) with pronounced stimulation of humoral immunity (CD20+),

accompanied by the production of antithyroid autoantibodies (ATPO) with subsequent activation of the processes of apoptosis of thyroid cells (CD95+). In the stage of manifest hypothyroidism and euthyroidism, direct relationships were observed only between IL-6, ATPO, CD20+ and CD95+. At the stage of euthyroidism, in contrast to other stages of AIT, a correlation between IL-1 $\beta$  and ATG was revealed. In the stage of subclinical hypothyroidism, direct correlations were observed only between CD20+ and ATPO and CD95+ and ATPO.

In the future, we analyzed the comparative effectiveness of various protocols for correcting reproductive disorders in women with autoimmune thyroiditis, where we used the results of study of immunological and hormonal parameters in dynamics of treatment in the patients with AIT, depending on the stage of the disease. In order to correct ovarian hormonal insufficiency the functional state of the thyroid gland was corrected at the first stage. We divided the group of patients with AIT at the stage of thyrotoxicosis into 2 subgroups: subgroup 1 (32 women) was used symptomatic therapy: cardioselective  $\beta$ -blocker metoprolol 25 mg 2 times a day. Subgroup 2 (33 women) - combined therapy was performed: thiamazole 10 mg per day + levothyroxine sodium in a dose of 25-50  $\mu$ g.

The study of the dynamics of immunological parameters in the process of AIT correction was carried out 3 months after in 20 patients: 10 women from subgroup 1 and 10 women from subgroup 2. The study of the thyroid status, levels of gonadotropins and ovarian steroid hormones was carried out after 6 months of treatment.

Analysis of the content of proinflammatory cytokines in patients with autoimmune thyroiditis in the stage of thyrotoxicosis showed a reliable decrease in IL-1 $\beta$ , IL-6 and TNF- $\alpha$  after combined therapy ( $p < 0.001$ ). Thus, the level of IL-1 $\beta$  after correction in subgroup 2 decreased by 8.5 times and averaged  $10.2 \pm 3.6$  pg/ml compared to  $86.5 \pm 44.3$  pg/ml before treatment ( $p < 0.001$ ), the values of IL-6 after correction decreased by 7.1 times and averaged  $14.4 \pm 8.8$  pg/ml compared to  $102.3 \pm 64.5$  pg/ml before correction ( $p < 0.001$ ) and the values of TNF- $\alpha$  decreased by 6.7 times after treatment and averaged  $9.6 \pm 7.4$  pg/ML compared to  $64.2 \pm 40.1$

pg/ml before treatment ( $p < 0.001$ ). Because of symptomatic metoprolol therapy in subgroup 1, in the absence of pathogenic therapy, there was noted a statistically unreliable decrease in the level of IL-1 $\beta$  and IL-6 and an increase in the content of IL-18 and TNF- $\alpha$  relative to the initial values before treatment. IL-18 in subgroup 1 increased to  $193.7 \pm 105.0$  pg / ml, which is probably due to the maintenance of autoimmune inflammatory process activity, confirmed by increased values of IL-1 $\beta$ , IL-6 and TNF- $\alpha$  and after 3 months. In subgroup 2, a different trend was observed in the level of IL-18, which decreased during the correction and amounted to  $147.4 \pm 76.2$  pg / ml ( $p < 0.001$ ), while the content of IL-1 $\beta$ , IL-6 and TNF- $\alpha$  also significantly decreased relatively to the initial values before treatment.

The study of the relative and absolute number of markers of lymphocyte activation in women with AIT at the stage of thyrotoxicosis showed that the level of CD20+lymphocytes did not reliably change in subgroup 1 of patients who received monotherapy. However, because of combined therapy in subgroup 2 there was a decrease to  $20.5 \pm 3.4\%$  compared to  $24.1 \pm 3.0\%$  before treatment ( $p < 0.001$ ). The content of CD25+lymphocytes significantly increased only in subgroup 2 and averaged  $20.6 \pm 2.3\%$  compared to  $19.1 \pm 2.0\%$  before treatment ( $p < 0.05$ ). The number of lymphocytes expressing Fas-antigen (CD95+) in the blood of patients after treatment in subgroup 2 significantly decreased and amounted to  $24.2 \pm 5.2\%$  compared to  $36,0 \pm 6.2\%$  before treatment ( $p < 0.001$ ). The results of studying the level of antithyroid antibodies showed that in subgroup 1 there was an increase in the concentration of ATG and ATPO, while in subgroup 2 there was noted a significant decrease in ATG by 3.5 times, and ATPO by 2.6 times.

The results of study on the level of gonadotropins in women with AIT at the stage of thyrotoxicosis after 6 months of correction showed a reliable increase in the average value of FSH in patients in subgroup 2 – from  $5.6 \pm 3.2$  IU / l to  $7.4 \pm 1.5$  IU / l ( $p < 0.001$ ), in subgroup 1 this parameter tended to decrease. Against the background of AIT correction, there was a tendency to decrease the level of LH in both subgroups. The content of prolactin decreased reliably in subgroup 2 and averaged  $9.4 \pm 3.7$  pg / ml compared to  $14.6 \pm 7.3$  pg / ml before treatment ( $p < 0.001$ ),

in subgroup 1 the level of prolactin did not significantly change. Against the background of treatment, there was a positive dynamics in the level of progesterone in patients who received combined AIT therapy ( $9.6 \pm 4.3$  pg / ml compared to  $5.4 \pm 4.0$  pg / ml before treatment,  $p < 0.001$ ).

The results of the evaluation of the ovarian functional state showed that in patients of subgroup 1 the anovulatory cycle was preserved in 13 (40.6%) patients, while SLNF was observed in 3 women, ovulation occurred in 19 (59.4%) women, of which NLF was observed in 12 patients, and pregnancy occurred in one woman. In the 2nd subgroup of women anovulation was preserved in 8 (25.8%), ovulation occurred in 23 (74.2%) women, while NLF was observed in 9, pregnancy occurred in 5 women.

The next stage of the reproductive function restoration was the correction of the ovarian functional state. All patients (42) of the examined subgroups with preserved anovulation and NLF were prescribed a drug containing ethinylestradiol 30 µg/drospirenone 3mg from the 5th day of the menstrual cycle, 1 tablet for 21 days, for a period of 3 cycles, followed by an assessment of the functional state of the ovaries. Because of the use of a combined oral contraceptive (COC), pregnancy occurred in 3 patients. In patients with preserved anovulation (22), ovulation was induced with clomiphene citrate 50 mg from 5 to 9 days of the menstrual cycle. Because of induction in the 1st cycle, pregnancy occurred in 3 women, in the 2nd cycle-in 1 woman and in the 3rd cycle-in 3 women.

The study of immunological and hormonal parameters in the dynamics of treatment in patients with AIT at the stage of subclinical hypothyroidism showed the following. At the first stage of reproductive function restoration we performed a correction of the thyroid status in women with AIT at the stage of subclinical hypothyroidism. Subgroup 1 consisted of 50 people who were prescribed levothyroxine in a dose of 50-75 µg. Subgroup 2 consisted of 56 people who received thiamazole in a dose of 10 mg per day and levothyroxine-50-100 µg. Immunological studies in dynamics after 3 months of treatment were performed in 20 patients, selected by 10 women from each subgroup. Evaluation of the thyroid

status and levels of gonadotropins and ovarian steroid hormones was performed after 6 months of AIT treatment.

The dynamics of pro-inflammatory cytokines content depending on the type of correction showed a reliable decrease in IL-1 $\beta$  in both subgroups, from  $75.8 \pm 49, 6$  pg/ml to  $32.7 \pm 36, 3$  pg/ml ( $p < 0.01$ ) after monotherapy and from  $74.1 \pm 56, 5$  pg/ml to  $24.7 \pm 43, 4$  pg/ml ( $p < 0.05$ ) after combination therapy. The IL-6 values decreased in both groups after treatment, from  $123.1 \pm 67.6$  pg/ml to  $57.2 \pm 43.5$  pg/ml ( $p < 0.01$ ) after monotherapy and from  $144.7 \pm 66.3$  pg/ml to  $25.2 \pm 59.2$  pg/ml ( $p < 0.001$ ) after combination therapy. Mean IL-18 values in treatment dynamics increased and TNF- $\alpha$  levels decreased, although differences in outcomes were significant. Because of AIT correction in the stage of subclinical hypothyroidism, significant changes in the level of CD20 + -lymphocytes were obtained after combined therapy from  $24.8 \pm 2.9\%$  to  $21.0 \pm 2.7\%$ , while the absolute number of cells decreased in both subgroups. The content of CD25 + -lymphocytes, early activation markers increased significantly after treatment in the 1st subgroup and amounted to  $21.9 \pm 1.7\%$  relatively to  $20.4 \pm 1.3\%$  before treatment ( $p < 0.05$ ), while the absolute number increased significantly in both subgroups. The level of CD95 + -lymphocytes, markers of apoptosis processes activation, significantly decreased in both subgroups after treatment and amounted in the 1st subgroup to  $26.4 \pm 6.7\%$  relatively to  $33.7 \pm 6.3\%$  before treatment ( $p < 0.05$ ) and  $26.2 \pm 5.6\%$  relatively to  $33.9 \pm 6.4\%$  before treatment in 2nd subgroup ( $p < 0.01$ ). The ATH level after 6-month correction in the 1st subgroup decreased by 1.1 times ( $p < 0.05$ ), in the 2nd subgroup decreased by 3.7 times ( $p < 0.001$ ). The level of ATPO because of treatment in the 1<sup>st</sup> subgroup decreased by 1.2 times ( $p < 0.01$ ), in the 2nd subgroup decreased by 3 times ( $p < 0.001$ ).

During 6-month AIT correction at the stage of subclinical hypothyroidism, positive dynamics in the level of gonadotropins was noted. The FSH level in 1 subgroup increased from  $4.0 \pm 2.5$  ME/l to  $4.4 \pm 2,0$  ME/l ( $p < 0,001$ ), in 2 subgroup increased more significantly from  $4.3 \pm 2.5$  ME/l to  $6.8 \pm 2.3$  ME/l ( $p < 0,001$ ). In the LG level, against the background of the AIT correction, a downward

trend was noted, significant in the 2 subgroup - from  $6.5 \pm 4.6$  ME/l to  $5.3 \pm 0,9$  ME/l ( $p < 0.05$ ). The AIT correction, resulted in the prolactin level decrease from  $16.0 \pm 7.6$  pg/ml to  $14.6 \pm 6.8$  pg/ml in the 1 subgroup ( $p < 0.01$ ) and from  $15.7 \pm 7.9$  pg/ml to  $10.2 \pm 5.2$  pg/ml in the 2 subgroup ( $p < 0,001$ ). Estradiol and progesterone values were significantly higher in the 2 subgroup. The level of estradiol increased from  $48.0 \pm 29,5$  pg/ml to  $54.6 \pm 24,7$  pg/ml in 1 subgroup ( $p < 0.01$ ) and from  $48.8 \pm 28.5$  pg/ml to  $75.5 \pm 26.6$  pg/ml in 2 subgroup ( $p < 0,001$ ). The level of progesterone increased from  $4.3 \pm 2.5$  pg/ml to  $5.1 \pm 1.7$  pg/ml in the 1 subgroup ( $p < 0.01$ ) and from  $4.6 \pm 2.3$  pg/ml to  $9.2 \pm 3.9$  pg/ml in the 2 subgroup ( $p < 0,001$ ).

Assessment of the ovarian functional state with use of the functional diagnostic test showed that in 1 subgroup anovulation was preserved in 17 (35.4%), of which SLNF was observed in 7, ovulation occurred in 31 (64.6%) women, of which NLF was noted in 9, pregnancy occurred in 5 patients. In the 2nd subgroup anovulation was preserved in 15 (27.8%) patients, of which SLNF was noted in 4, ovulation occurred in 39 (72.2%) women, of which NLF was observed in 8, pregnancy occurred in 6 patients.

The following stage of the reproductive function restoration was correction of the ovarian function state. All the patients of the studied subgroups with preserved anovulation and NLF (49) were prescribed a drug containing ethinylestradiol 30µg/drospirenone 3mg receiving from the 5th day of the menstrual cycle, in dose 1 tablet per day for 21 days, for a period of 3 cycles, followed by an assessment of the functional state of the ovaries. While taking a combined oral contraceptive in intermittent mode, ovulation occurred in 64.8%, pregnancy occurred in 4 patients. In patients with preserved anovulation (25), ovulation was induced with clomiphene citrate 50 mg from 5 to 9 days of the menstrual cycle. Because of the induction in the 1st cycle pregnancy occurred in 2 patients, in the 2nd cycle-there were no cases of pregnancy, in the 3rd cycle – in 6 patients.

The study of immunological and hormonal parameters in the dynamics of treatment in patients with AIT at the stage of manifest hypothyroidism was performed in 2 groups formed according to the Protocol of treatment. Subgroup 1 (n=38) was given levothyroxine in a dose of 75-150 µg, Subgroup 2 (n=42) received thiamazole in a dose of 10 mg per day and levothyroxine – 100-200 µg. Immunological parameters in dynamics after 3 months of treatment were studied in 20 patients with AIT at the stage of manifest hypothyroidism, 10 women were selected from each subgroup. The assessment of the thyroid status and the level of gonadotropins and steroid hormones of the ovaries was carried out after 6 months of treatment.

Against the background of correction of AIT at the stage of manifest hypothyroidism, a significant reliable decrease in the level of IL-1β was achieved as a result of combined therapy – from 69.4±48.7 pg/ml to 7.0±2.6 pg / ml after treatment (p<0.01). Similarly, there was a significant decrease in IL-6 levels as a result of complex therapy – from 111.3±73.4 pg / ml to 16.8±10.9 pg / ml (p<0.01). As for TNF-α, its content significantly decreased due to result of combined therapy and amounted to 4.9±1.9 pg / ml compared to 66.9±64.7 pg / ml before treatment (p<0.01). The content of IL-18 in both groups before and after treatment did not significantly change in both subgroups and remained within the standard parameters. Against the background of monotherapy with levothyroxine, there was noted tendency to increase the level of IL-1β in subgroup 1 of patients with AIT, though the difference in the mean values before and after treatment was not reliable. The average values of IL-6, IL-18 and TNF-α decreased slightly, while the obtained values were many times higher than the standard values indicated in the test kits, probably indicating that there was no significant effect of levothyroxine on the concentration of proinflammatory cytokines in women with AIT at the stage of manifest hypothyroidism. The level of CD20+lymphocytes decreased significantly in subgroup 2 and averaged 24.2±3.0% compared to 25.8±2.2% before treatment (p<0.01). The content of CD25 + lymphocytes in the blood increased reliably in subgroup 2 and averaged 25.7±2.3% compared to



22.3±1.7% before treatment ( $p<0.001$ ). The significant decrease in CD95+lymphocyte levels was observed only in subgroup 2 and averaged 27.3±6.6% compared to 36.3±7.0% before treatment ( $p<0.01$ ). In the level of antithyroid antibodies the significant level was not achieved in the result of treatment in the subgroup 1. The reliable reduction of ATG was registered in subgroup 2 from 384±356.1 IU / ml to 129.9±197.8 IU / ml ( $p<0.001$ ) and ATPO, which decreased from 756.9±797.1 IU/ml to 202.9±411.6 IU/ml ( $p<0.001$ ).

Against the background of 6-month correction of the thyroid status in patients with manifest hypothyroidism, a positive dynamics in the level of gonadotropins was revealed. The level of FSH in subgroup 1 increased and averaged 4.0±1.3 IU/l relative to 2.6±0.7 IU / l before correction ( $p<0.001$ ), in subgroup 2 it increased from 2.7±0.9 IU/l to 5.3±1.2 IU/l ( $p<0.001$ ). There was a decrease in the level of LH in both subgroups relative to the values obtained before the correction, but there were not found reliable differences in the results of both subgroups. The content of prolactin decreased in both subgroups, more significant decrease was observed in patients who received combined therapy in subgroup 2. Thus, the level of prolactin in subgroup 1 decreased from 23.5±13.8 pg/ml to 19.8±8.8 pg/ml ( $p<0.01$ ), in subgroup 2 – from 21.0±13.6 pg/ml to 15.0±8.0 pg/ml ( $p<0.001$ ). Estradiol levels increased significantly in both subgroups, from 36.3±18.9 pg / ml to 49.7±18.9 pg/ml ( $p<0.001$ ) in subgroup 1, and from 37.9±15.5 pg/ml to 63.0±18.3 pg / ml ( $p<0.001$ ) in subgroup 2. A similar trend was observed in the level of progesterone, which in subgroup 1 increased from 3.5±2.0 pg / ml to 5.2±2.4 pg / ml after treatment ( $p<0.001$ ), in subgroup 2 there was shown more significant increase from 3.7±1.8 pg/ml to 7.2±3.1 pg/ml after 6-month treatment ( $p<0.001$ ).

An assessment of the ovarian function state in patients with manifest hypothyroidism showed that in subgroup 1 anovulation was preserved in 18 (47.4%) patients, of which SLNF was observed in 6, ovulation occurred in 20 (52.6%), while NLF was noted in 9, pregnancy occurred in 2 women. In subgroup 2 anovulation was preserved in 14 (33.3%), SLNF was observed in 4, ovulation

occurred in 28 (66.7%), while NLF was observed in 5, pregnancy occurred in 3 women.

In order to restore reproductive function and to induce ovulation, all patients with preserved anovulation and NLF (46) were prescribed a drug containing ethinylestradiol 30 µg/drospirenone 3mg from the 5th day of the menstrual cycle for 21 days, during 3 cycles. Because of a result of the use of COC in intermittent mode, the recovery of the two-phase ovulatory cycle was observed in 25 (45.4%), pregnancy occurred in 2 patients. Patients with preserved anovulation (30) were prescribed clomiphene citrate 50 mg from 5 to 9 days of the cycle. Because of ovulation induction in the 1st cycle, pregnancy occurred in 2 patients, in the 2nd cycle-in 2 and in the 3rd cycle-in 2 patients.

In the future, we studied immunological and hormonal parameters in dynamics of treatment in patients with AIT at the stage of euthyroidism. We formed 2 subgroups of patients with euthyroidism, depending on the Treatment Protocol: subgroup 1 (n=35) received levothyroxine in a dose of 25 µg, subgroup 2 (n=39) used thiamazole in a dose of 5 mg per day and levothyroxine – 25-50 µg. The study of immunological parameters in dynamics of AIT correction was performed in 20 patients with euthyroidism, selected from each subgroup of 10 women.

The results of the study of cytokine status in patients with AIT at the stage of euthyroidism showed a significant decrease in the level of IL-1 $\beta$ , IL-6, IL-18 and TNF- $\alpha$  in patients receiving combined therapy. The concentration of IL-1 $\beta$  because of the correction in subgroup 2 decreased by 14.9 times and averaged 5.4 $\pm$ 1.9 pg / ml compared to 80.7 $\pm$ 50.3 pg / ml before treatment (p<0.001). The level of IL-6 decreased 4.4 times and averaged 20.2 $\pm$ 27.0 pg / ml compared to 89.8 $\pm$ 66.5 pg / ml before treatment (p<0.01). The IL-18 content as a result of treatment decreased by 1.4 times and averaged 103.2 $\pm$ 24.9 pg / ml compared to 142.4 $\pm$ 52.8 pg/ml before treatment (p<0.01). The level of TNF- $\alpha$  decreased by 12.2 times and averaged 7.7 $\pm$ 6.7 pg / ml compared to 94.0 $\pm$ 64.9 pg / ml before treatment (p<0.01). Against the background of monotherapy, a reliable decrease

was observed only in the level of IL-1 $\beta$ , IL-6 and TNF- $\alpha$ . The level of IL-1 $\beta$  decreased by 2.5 times and averaged 32.8 $\pm$ 46.0 pg / ml compared to 81.4 $\pm$ 51.5 pg / ml before treatment ( $p < 0.05$ ). The level of IL-6 decreased by 2.2 times and averaged 59.7 $\pm$ 80.6 pg / ml compared to 132.0 $\pm$ 78.4 pg/ml before treatment ( $p < 0.05$ ). The level of TNF- $\alpha$  decreased by 3,8 times and averaged 17.5 $\pm$ 28.5 pg / ml compared to 65.8 $\pm$ 58.7pg / ml before treatment ( $p < 0.05$ ). The average value of IL-18 did not significantly change because of treatment in subgroup 1.

Against the background of AIT correction, a significant decrease in the relative number of CD20+lymphocytes was observed in subgroup 2, while the absolute number of cells reliably decreased in both subgroups. The content of CD25 + lymphocytes in the blood increased because of treatment in both subgroups, significantly after combined therapy and averaged 22.6 $\pm$ 1.1% compared to 20.7 $\pm$ 0.9% before treatment ( $p < 0.001$ ). A significant decrease in the relative and absolute number of CD95 + lymphocytes in the blood of patients was observed because of result of AIT correction in subgroup 2. The level of ATG reliably decreased after combined therapy and amounted to 62.6 $\pm$ 135.4 IU / ml compared to 198.9 $\pm$ 201.7 IU/ml before correction ( $p < 0.001$ ). The level of ATPO significantly decreased in both subgroups, in subgroup 1 from 476.2 $\pm$ 630.1 IU / ml to 386.8 $\pm$ 464.7 IU / ml ( $p < 0.01$ ) and in subgroup 2 from 476.3 $\pm$ 504.3 IU/ml to 175.9 $\pm$ 284.1 IU/ml ( $p < 0.001$ ).

AIT correction resulted in the significant increase of the level of FSH in the subgroup 2 and averaged 6.4  $\pm$  2.0 ME/L relatively to 5.0  $\pm$  2.7 ME/L before treatment ( $p < 0,001$ ). The level of prolactin reliably reduced in the both subgroups, in subgroup 1 from 11.4  $\pm$  6.2 pg/ml to 8.8  $\pm$  4, 9 pg/ml ( $p < 0,001$ ), in subgroup 2 from 12.6  $\pm$  6.7 pg/ml to 7.7  $\pm$  4.1 pg/ml ( $p < 0,001$ ). Estradiol and progesterone levels increased in the both subgroups. So, the level of estradiol in the 1<sup>st</sup> subgroup after correction of hypothyroidism averaged from 62.5  $\pm$  34.0 pg/ml compared to 55.1  $\pm$  36.4 pg/ml before treatment ( $p < 0,001$ ), the level of progesterone increased and averaged 7.0  $\pm$  4.1 pg/ml relatively to 4.9  $\pm$  4.5 pg/ml before treatment ( $p < 0,001$ ). In the 2nd subgroup the estradiol level reached higher

values and averaged  $74.7 \pm 27.5$  pg/ml relative to  $64.7 \pm 42.5$  pg/ml before correction ( $p < 0.05$ ), and the progesterone level averaged from  $9.2 \pm 5.0$  pg/ml in relation to  $5.7 \pm 5.6$  pg/ml before treatment ( $p < 0,001$ ).

Assessment of ovarian functional state with use of functional diagnosis tests showed that in subgroup 1 of patients anovulation was preserved in 9 (28.1%) women, SLNF was observed in 3, ovulation occurred in 23 (71.9%), NLF was noted in 4, pregnancy occurred in 4 women. In subgroup 2 anovulation was preserved in 9 (25.7%) women, SLNF was observed in 2 women, ovulation occurred in 26 (74.3%), while NLF was noted in 4, pregnancy occurred in 5 women.

In order to restore reproductive function, all patients with preserved anovulation and NLF (26) were prescribed a preparation containing ethinyl estradiol 30 µg/drospirenone 3 mg from 5 to 21 days of menstrual cycle for 3 menstrual cycles. Assessment of the ovarian functional state showed recovery of the two-phase cycle in 14 (53.8%) patients, pregnancy occurred in 6 women. In the patients with preserved anovulation (12), clomiphene citrate 50 mg using from 5 to 9 days of the cycle was prescribed to induce ovulation. The stimulation of ovulation resulted in pregnancy occurred the 1st cycle in 2 patients, in the 2nd cycle there were no cases of pregnancy, in the 3rd cycle - in 2 patients. Thus, because of AIT correction alone and recovery of thyroid imbalance in 325 women with impaired reproductive function and AIT pregnancy occurred in 44 women. The ovarian stimulation promoted the pregnancy occurrence in 40 women.

### **Conclusions**

1.The results of a comprehensive clinical, immunological and hormonal examination of 465 women with reproductive failure indicate a high incidence (69.9%) and a significant role for autoimmune thyroiditis in the formation of reproductive disorders.

2.Patients with all stages of autoimmune thyroiditis were found to have immune status disorders expressed by increased values of CD20+ and CD95+-lymphocytes, ATA level, proinflammatory cytokines, as well as a decrease in the

level of IL-18 and CD25+-lymphocytes, indicating activation of the autoimmune process in all autoimmune thyroiditis stages.

3.The results of the correlation analysis indicated a directly proportional relationship between the proinflammatory cytokines IL-1 $\beta$ , IL-6 and TNF- $\alpha$ , CD20+ and CD95+-lymphocytes, and the level of ATA in all autoimmune thyroiditis stages.

4.The study of the features of the functional state of the hypothalamic-pituitary-ovarian system in women with autoimmune thyroiditis approved a decrease in the level of gonadotropins and steroid hormones of the ovaries, in the stage of thyrotoxicosis luteal phase insufficiency was more often noted (46.2%), subclinical and manifest hypothyroidism anovulation was predominant (56.6% and 73.7%, respectively) and luteinization syndrome of the unovulated follicle (48.6%) was more frequently observed in the euthyroid stage.

5.Correction of autoimmune thyroiditis with thiamazole in combination with levothyroxine compared with monotherapy with levothyroxine led to a leveling of thyroid and immune imbalances, a significant decrease in the level of ATA, a significant increase in the level of gonadotropins and steroid hormones of the ovaries and a 1.5-fold recovery of reproductive function.

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