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METHODOLOGICAL DEVELOPMENT OF TOPIC №3

practical lesson **" Immunology reproduction. Immunodependent infertility of men and women"** (2 hours) in the discipline "Clinical Immunology and Allergology" for 6-th year students of specialty "Medicine"

1. *Theme №3*: Immunology reproduction. Immunodependent infertility of men and women
2. *Relevance of the topic:* At present time it is especially important to understand by the students of the importance of participating immune mechanisms in human reproduction, mechanisms for the functioning of the immune system in reproductive failures, which will determine the choice of modern treatment.
3. *The goals of the class:*
   - educational: students must study basic immunogical factors that determine the correct course of the reproductive process, immunological phenomens in physiological pregnancy, the inpact of immunopathology in their reproductive function in their reproductive functions
   - professionally oriented: students should be based on clinical and laboratory data to diagnose the risk of pregnancy interruption, to establish impressions for immunotropic treatment of miscarrage
   - educational: to form a sense of responsibility for the timeliness and correctness of professional actions.

4. *Equipment* for conducting classes: Presentation for multimedia demonstration, schemes, tables, immunograms, tests, situational tasks, histological and cytological preparations, non-typical situational tasks
5. *Integrative Relations of the theme:*

5.1.Internal Integration: The topic of this practical lesson is associated with the following topics of the cycle "Clinical Immunology and Allergology" classes for students of the 5th year as "Structure and Principles of Functioning of the Immune System" and "Assessment of the Immune System", Fragment "Immunology of infertility" in the topic " Autoimmune diseases: immunopathogenesis, immuniagnostic and treatment ».

5.2. Interdisciplinary integration:

Disciplines	Knowledges	The skills
Histology and embryology	The structure of ovocite and spermatozoon. Meiosis.	Be able to determine the state of maturity and functional activity of gametes.
Therapy	Diagnostic criterias of autoimmune diseases	Differentiation by clinical and laboratory signs organospecific and systemic diseases
Endocrinology	Clinical, laboratory signs of thyroidates, type 1 diabetes, Addison's disease, syndromes of sclerocystosis of the	To diagnose these diseases, to prescribe treatment

	ovaries, premature ovarian failure	
Obstetrics and gynecology	Basic immune-dependent diseases and states in obstetric and gynecological practice	To diagnose these diseases, to prescribe treatment
Pediatrics	Congenital pathology of the genitourinary system in boys (criptorchidism, hypospadia, varicocele)	To appoint clinical and ultrasonographic examination, consultation of a child surgeon, to establish indicators to surgical treatment
Urology	Connecton of chronic inflammatory diseases of the genitourinary system in mens, varicocele, hydrocele with men's infertility	To establish indicators to therapeutic and surgical treatment

6. Contents of the topic of the class:

6.1. Learning questions.
6.1.1. Immunological tolerance and physiological pregnancy. Mechanisms of immunological tolerance to spermatozoons in mens.
6.1.2. Factors that lead to canlerance of tolerance to their own autoantigens, their connection with the development of infertility.
6.1.3. Autoimmune diseases in mens and women, their impact on reproductive function.
6.1.4. Autoimmune diseases of women's reproductive system
6.1.5. Basic principles of laboratory diagnostics of immune-dependent infertility
6.1.6. Classical and modern approaches to the treatment of immune-dependent infertility

Contents of the topic class

Immune-dependent infertility

Over the past decades, several concepts of immune-dependent infertility are formed. The most widespread is the concept of infertility, which is based on autoimmune processes. Such an infertility is basically primary, that is, associated with the impossibility of fertilization and lack of pregnancy in a woman. The causes of immune-dependent primary infertility in women include: antiovarial and antispermunity immunity (conflict of a men-woman), in mens - antisperm immunity.

Antiovarial immunity develops when implementing of immune response in woman who is directed against the autoantigens of her reproductive system. *The*

antigens of the brilliant membane (Zona Pellucida) are particularly interesting because Zona Pellucida is one of the most important structures that provides a normal course of fertilization and early embryonic development processes. The zona Pellucida antigen mosaic is a complex, comprising cross-reactive and probably species-specific antigens. Antibodies against Zona Pellucida (antizonal antibodies) are one of the etiological infertillity factors of immune genesis in women. Investigations are proved that in women with immune-dependent infirtility there is no antibodies against Zona Pellucida and antispermal antibodies. The determination of antibodies to the transparent zone of the ovocite is complicated by ethical considerations due to the impossibility of using human ovocytes as a diagnostic material. In 2.5 - 5,6% of infertious women are antibodies against the transparent zone antigens. The presence of these antibodies in fertilization can inhibit the connection of sperms and ovocytes. The combination of sperm with an ovocite is an example of the most important biological contact. Initiates fertilization of sperm contact and transparent zone of ovocite - Zona Pellucida (ZP). ZP consists a small number of glycoproteins that mediate the interconnection with sperm, prevent polyspermia and promote the development of the embryon after implantation. There are three glycoproteins: ZP1, ZP2 and ZP3, or ZPB, ZPA and ZPC. Investigations have found an increase of more than 2 times variations of ZP1 and ZP3 sequences of genes in women with infertility.

The cytotoxic effect against functional ovocite bubbles is carried out by proinflammatory T-lymphocytes that infiltrate the tissue. The ovary can be a target of an autoimmune attack in various pathological conditions, including organospecific and systemic autoimmune diseases. Clinically, this is most often manifested by **premature ovarian failure – POF**), but as a result of an antiovarial autoimmune response can develop such diseases as **syndrome of polycystic ovaries and endometriosis.** The first message about the syndrome of POF showed its association with autoimmune illnesses of the adrenal glands and concerned the detection of antibodies in such patients to various types of steroid-producing cells of the adrenal, testicles, placenta and ovary tissues (they received a general name antibodies to SCA steroid cells). With a POF syndrome, autoantibodies are fixed in various histological compartments of the ovocite and directed against various tissue elements and steroidogenic enzymes, for example, against 21-hydroxylase and 3β-hydroxyteroid dehydrogenase (associated with HLA-DQB1). In autoimmune diseases of ovacytes, complex defects of regulation of the immune system are revealed. Thus, in women with a POF syndrome was detected increasing of the levels of CD4 + lymphocytes, the ratio of CD4 + / CD8 +, CD19 + / CD5 + lymphocytes (B2 lymphocytes responsible for autoimmunity), in 35-50% of women T-lymphocytes were activated and expressed a large number of MHC class II molecules, especially HLA-DR.

The **premature ovarian failure** is the primary manifestation of insufficiency of ovaries, which manifests itself in women, younger 40 years. This syndrome is irreversible, although there were cases of spontaneous remission and pregnancy in such patients. On the development of the syndrome of POF, genetic, enzymatic, infectious, yatrogenic factors (e.g., chemo- radiotherapy) are influenced. Syndrome often coexists with such autoimmune diseases such as autoimmune thyroiditis, Grave's disease, insulin-dependent diabetes, multiple sclerosis, rheumatoid arthritis. In patients with the

syndrome of POF autoantibodies against antigens of endocrine glands (thyroid glands, adrenal glands, pancreas), antibodies that are cross-reacted with the receptor for gonadotropins, and most importantly - antibodies against tissue elements of the ovaries.

P**olycystic ovarian syndrome – PCOS** clinically manifests itself by the termination of menstruation, the disappearance of ovulation and hirsutism. In the examination of 50% of these women, the existence of antibodies directed against grainy cells and / or other elements of the ovary and infiltration of the tissue of the ovary lymphocytes are found. It is found connection between the level of these antibodies and the appearance of cysts in ovaries. Antiovarial antibodies (AOA) do not have a great value in the pathogenesis of syndrome of polycystic ovarian, but confirmed the presence of organonespecific heterogeneous antibodies. Currently this syndrome is considered genetically determinated (CYP 17A gene encoding a P450C 17α-enzyme, and a CYP 11A gene encoding the metabolism of the P450 enzyme, the insulin gene and VNTR sequences variants). In addition, this syndrome is considered to be metabolic aberration; its development is associated with an increase in the level of immunological factors that are characteristic of chronic inflammatory process (inhibitor of plasminogen PAI-1, C-reactive protein, endothelin-1, molecule of cell adhesion sICAM-1, sVCAM-1, sE-SELECTIN).

In patients with **endometriosis,** cellular immune factors along with cytokines also affect fertility and can lead to sterility. In such patients the level of IL-6 and CD 8+ and CD11b + in peritoneal fluid is substantially elevated, indicating an important role of a specific immune response with this disease.

Antispermal immunity in women. There are risk factors that cause an immune response to sperm in a women's reproductive system. These factors include: 1) local infections of the woman's reproductive system; 2) diseases of the cervix (erosion, novoval) or chronic crack.

Immune-dependent reasons for secondary infertility.

Autoimmune reactions are one of the main causes of infertility of the immune genesis. These reactions include the presence of anti-phospholipid antibodies, anti-tureoid antibodies, antinuclear antibodies, antispermal and antiovarial ABs.

Changing functions and phenotypes of natural killer cells (NK). In a healthy pregnant woman in peripheral blood, NK cells (CD56 dim CD16 +) should be (CD56 DIM CD16 +), and in the uterine circulation - uNK (Uterine) cells (CD 56 bright CD16).

1. Development of cytokine imbalance, which arises as a result of activation of congenital immunity in maternal and fruit circulation. In connection with this distinguish two main groups of cytokines with the opposite influence on the course of gestation: unfavorable (TNF, IFN-γ, IL-2 - may cause maternity activities, inflammatory reactions that can cause spontaneous miscarriage or premature births depending on Stages of gestation) and favorable (TGF-β, Lif, CSF-1, GM-CSF, IL-1, IL-3, IL-4, IL-6, IL-10, IL-13, IFN-κ).
2. Defects of vascularization of placenta, developing due to dysfunction of factors of innate immunity, which manifests itself in the insufficient synthesis of growth factors - granulocyte-macrophage colony stimulating factor GM-CSF and macrophage colony stimulating factor CSF-1.

3. Defective metabolism of indolaminooxygenase enzyme (IDO)
4. Urogenital infections.
5. Bacterial vaginosis.

6. Rhesus (Rh) D-Immunization

Hypothetical models of immunopathogenesis of habitual miscarriages (recurrent miscarriage - RM)

	Model A	**Model B**	**Model C**
For the theory	**Miscarriage caused by graft rejection-like alloimmunity**	**Miscarriage caused by the innate immune system** **Abundant presence of $CD56^+$ cells in the decidua** ***In-vitro* evidence that lymphokine-stimulated $CD56^+$ cells can kill trophoblast** **Increased NK cell number and activity in RM patients indicate a poor prognosis**	**Miscarriage caused by organ-specific autoimmunity** **Increased prevalence oa autoantibodies in RM patients Predominant T_h1-type immunological response to trophoblast in women with RM** **Association between RM and particular maternal class II HLA alleles**
Against the theory	**The HLA antigens responsible for strong graft-rejections are not expressed on trophoblast** **The degree of HLA antigen sharing between spouses has no impact on the probability of fetal survival** **No correlation between in-vitro reactions of maternal T cells or antibodies against paternal HLA and pregnancy outcome**		
HLA = human leukocyte antigen; NK = natural killer; T_h = T helper			

The miscarriage of pregnancy is caused by: 1) autoimmune pathology in the mother (the presence of an organospecific or systemic autoimmune disease with high titres of antifospholipid, antinuclear, antineutrophilic acid, anti-tireoid and antispermal antibodies); 2) a change in the phenotype of cytotoxic lymphocytes (a decrease in the number of so-called uNK (uterine NK), or uterine natural killers CD16- / 56 +, which lose a cytotoxic function, but have an increased ability to produce cytokines of a regulatory nature); 3) disregulatory changes in the immune system of the pregnant women (increased number of T-lymphocytes-cytotoxic, predominance of T-lymphocytes-helper-layers of the 1st order over T-lymphocytes-heelper 2-th order, overproduction of proinflammatory cytokines); 4) immune-dependent mechanisms for the development of preeclampsia.

In this case, treatment often carries an immunoregulating character. *To improve implantation,* prescribe: 1) low doses of gonadotropins, anticoagulants, corticosteroids, progesterone; 2) stimulation of the endometrial cytokines (in particular, G-CSF drugs); 3) the intrauterine feed of PBMC (autologous cells of the peripheral blood of the woman). If a woman was extracorporeal fertilization, then they are used: 1) acupuncture on the day of fence of oocytes; 2) additional hatching (laying a laser of peculiar "rivots" on the embrion). *For patients with late miscarriage of immune genesis recomend*:1) monoclonal antibodies - TNF-α blockers; 2) intravenous immunoglobulins and monoclonal antibodies - TNF-α blockers (separately or together). Aspirin and heparin do not always give effect, even if women have positive ANA (antinuclear) and APA (antiphosphate) antibodies.

Basic immunotherapeutic strategies in the treatment of miscarriage

Active immunotherapy	Production of anti-paternal antibodies
▶ (lymphocyte transfusions)	Dampening of NK cell activity
▶	Modification of cytokine production
▶	Establishment of microchimerism
Passive immunotherapy	Suppression of autoantibodies
▶ (intravenous immunoglobulin)	Neutralization of autoantibodies
▶	Dampening of NK cell activity
▶	Modification of cytokine production
	Inhibition of complement binding and activation
▶	Fc receptor modulation and blockade
▶	Inhibition of superantigens
	Modulation of adhesion molecules on T lymphocytes
	Induction of apoptosis of activated cytotoxic lymphocytes

Antispermal immunity in men's.

The synthesis of antisperal antibodies (ASA) may be the cause of the development of an autoimmune reaction, which leads to an immune-dependent infertility. This condition is formed as a result of a violation of passive and active immunological tolerance to sperm, which in physiological conditions maintains and protects spermatozoids on the reactivity of the immune system.

ASA in male people are most often detected: 1) on the surface of spermatozoids (linked antibodies); 2) in a seminal fluid and serum of peripheral blood (free antibodies).

Control questions.

6.2.1. Antigameting immunity as a reason for the primary infertility of men's and women.

6.2.2. Differences in immunological mechanisms of early and late miscarriage in women

6.2.3. Diagnosis and treatment of early and late gestosis of immune genesis.

6.2.4. The main reasons for immune-dependent male infertility

6.2.5. Therapeutic strategies for the treatment of immune-dependent female and male infertility.

6.3. Practical skills and skills.

6.3.1. Overcome knowledge about the participation of the immune system factors in physiological reproductive processes

6.3.2. To know methods of clinical and laboratory diagnostics of autoimmune diseases as leading risk factors of immune-dependent infertility of men's and women

6.3.3. Be able to work with immunological observation cards and other medical documentation under patients with reproductive losses

6.3.4. To know the main groups of immunotropic and hormonal drugs for the curation patients with immunodependent infertility, be able to determine the correct treatment

6.2. 6.4. *Plan and organizational structure of class (2 academic hours or 90 min.).*

Main stages of classes, their functions and content	Levels of knowledge	Methods of control and training	Methods of methodical supporting	Time in min
1. The first stage Organization of classes Educational goals Control of the primaryt level of knowledge and skills: -regulation of the immune response	I	Frontal poll Express-poll Test control	Tests Schemes	10

- the causes of violations of immune response regulation				
2. Basic stage Formation of professional knowledge and skills: - To describe the types of tumor antigens; - to determine the main mechanisms of antitumor immune protection; - to identify the main factors of immuno-resistance of tumors; - to form the main directions of immunotherapy of tumors	II II III, IV	Individual control tests Professional training in typical tasks ("step-2")	Tables Schemes Immunological observation cards Typical situational tasks Histological and cytological preparations, immunograms	70
Final stage -Control and correction of professional knowledge, skills	III IV	Testing (Output Level) Individual survey Solving of non-typical situational tasks	Schemes Tests Non-typical situational tasks	10
Conducting a summary of classes. Homework for the next topic				

6.5. Conclusions:

6.5.1.Formed knowledge of the main causes of primary infertility of men's and women, immune-dependent infertility

6.5.2. Modern information on the reasons for early and late miscarriage

6.5.3. Formed the main approaches to clinical and laboratory diagnostics

6.5.4.Systematized knowledge of modern methods of immunotropic treatment of immune-dependent infertility of men's and women.

7. *Materials for control*

8. *Tasks for individual work on this topic*

6.7.1. Make a table of auto-antibodies that are most often found in systemic and organospecific autoimmune diseases of women and men with reproductive problems
6.7.2. Make up an algorithm of laboratory and instrumental examination of patients with suspicion of immune-dependent infertility.