P.S. Gurevich

Immunopathology of germinal, embryonic and early fetal periods in human. Allogeneic conflicts.

Immunopathology of germinal, embryonic and early fetal period in human. Allogeneic conflicts. (182 p.)

Author: Gurevich PS - Doctor of Medical Sciences, Professor.

The book is dedicated to the condition of embryos and early fetuses by such diseases as allogeneic conflicts, hemolytic disease of fetus and newborns, sepsis, congenital cytomegalic inclusion disease, and others. The studies are focused on the immune protection of embryos. Particular attention is paid to the state and function of immune factors such as mononuclear phagocytes, extravillous trophoblast and other structures of the placenta (her maternal and fetal parts) and of the embryo, to the functioning of the secretory immune system and the individual immune protection of the vital organs and cells. State of the immune protection of germs is considered at the normal and the pathological conditions.

The author acknowledges:
A. Rosselsa PhD, MD, E. Gurevich, PhD, MD and S. Gershon PhD, MD for their help and support diring the work on the book.

Dear Colleagues,

This book presents the research materials of reproductive pathology and immunology - the formation of immune systems in pre-embryonic, embryonic and in early fetal periods.

It was specified the vigorous activity of mononuclear phagocytes (monocytes and promonocytes) and different types of trophoblast, especially the invasive trophoblast as well as the role of the pregnant in protecting the germ. The secretory immune system (SIS) of the germ begins functioning at very early stages. Then, as we have identified, an individual immune protective system of the vital cells and organs of embryos and fetuses is formed on its basis. It appears along with the formation of organs, accumulating immunoglobulins in the neurons of the brain, in the cells of the endocrine glands, in myocardium, liver and gonads. At the early period of fetal, the immune cells proliferate and form lymphoid organs of the fetus itself and then it comes into effect the overall immune system.

Not yet resolved the problem of repeated early abortions. An important part of them accounts for the early allogeneic conflict between the pregnant woman and her embryo, more or less allogeneic to her. We have investigated the changes of the placental barrier and the embryo in the allogeneic conflicts of early pregnancy, as well as changes in fetuses and newborns with late allogeneic conflicts. We have established the causes of early intrauterine growth retardation (IUGR), which is associated not with antiphospholipid syndrome Hughes, but mostly with a number of other pathogens. We have designed pathomorphology of allogeneic conflicts.

We hope that our studies will contribute to a deeper understanding of the mechanisms pre-embryonic, embryonic and fetal diseases and the creation of methods for their clinical diagnosis and prevention.

Introduction

One of the intriguing problems in modern obstetrics is the ability of the germ to avoid immune rejection (Sargent IL et al., 2006). Indeed, the embryo, which have got half of the genes from its father, for a pregnant woman turns up half-heterogenous (semi-allogeneic) or completely alien (allogeneic) for a surrogate pregnancy. And yet, in most cases the pregnancy is completed successfully for the germ and the pregnant, and in others - there are complications for the mother and the death of the germ. According to some sources, such cases are far not rare: the opinions are expressed that the 20-40 percent of fertilizations are lost in the first few weeks (Kutteh WH, 1999, Кулаков В.И. et al., 2005; Adolfsson A., Larsson PG, 2006). The inadequacy of our knowledge is manifested in the fact that the causes of early spontaneous termination of pregnancy (during the first 8 weeks) in 50% of cases remain unknown (Sargent IL, 1993).

The concept of the immune privilege of the uterus, as well as brain and eyes (Streilein JW, Wegmann TG, 1987; Niederkorn JY, Wang SS, 2005) is based on the assumption that in the uterus there are specific mechanisms that suppress the reaction of rejection. The bases for the opinion on the privileges are some experiments on pregnant animals: when transplanting tissues of the uterus into other tissues of the same animal, then an immune response that rejects the transplanted tissue is developed. Some decrease in the overall immune responsiveness of the pregnant is confirmed by the clinical observations. Some immunosuppressive agents such as progesterone (Piccini MP et al., 1995), prostaglandin E2 (Abe N., et al., 1997), pregnancy early factor (Morton H, 1998), as well as change the balance of Th1 \ Th2 cytokines (Dealtry GB et al., 2000) are identified in pregnant women.

However, there is number of contradictions in the understanding of the immune privilege of the uterus. One of them is the referred experiment on transplantation of the animal germinal tissue into the tissue of its mother. Under such transplantation, not only the genes of germinal envelopes, but the full range of its genes causes the reaction of immune system of the pregnant. An example of such pathology is hemolytic disease of the fetus and newborn (HDFN). While the Rh factor (erythrocytic antigen of the fetus) is isolated from contact with the maternal immune system, the pregnancy is developing normally. But as soon as (usually during birth) erythrocytes get in contact with the mother's immunocompetent cells, an allogeneic conflict is generated.

The question regarding the mechanisms of immune protection of the embryo remains open, and opinion about the "immunoincompetence" of not only the embryo but also the fetus have been still encountered in the recent past (Vetro SW, Bellanti GA, 1989). And what kind of immune defense would seem in the absence of the lymphoid organs and immunocompetent cells at the embryonic period?

In the recent years, there was a significant rise of attention to the field of reproductive immunology - the study of growth and development of the human germs in the earliest stages of its immune self-protection and the ways to fight the pathogens, the preservation of its gene pool.

We are engaged in the study of pathology of the fetal period and the condition of the lymphoid system in a case of such diseases as HDFN, sepsis in fetuses, congenital cytomegaly and others. In recent years we have focused on the study of immune protection of embryos. We are particularly interested in the state and functions of mononuclear phagocytes, extravillous trophoblast and other structures of the placenta (its maternal and fetal parts) and of the embryo itself, the functioning of the secretory immune system (SIS) and the system of individual immune protection of the vital organs and cells. The condition of immune protection of germs has been examined in normal conditions, with fibrin coagulation in the villi, in bacterial infections and allogeneic conflict of the pregnant and her germ.

Current issues in diagnosis, pathology, and thus - the prevention and treatment of pregnancy disorders at its 1st trimester remain largely unclear. This refers to the intrauterine growth restriction (IUGR), to Hughes antiphospholipid syndrome, allogeneic conflict between the pregnant woman and her fetus, to some bacterial infections of the birth canal. Such pathological processes as the emergence of avascular edematous chorionic villi, multiple tissue apoptosis of the placental barrier and the embryo itself, being very far from their physiological nature, remain unclear. We hope to bring some clarity to these issues.

Author wishes to thank Professor M. Moldavsky for the provision of certain fetuses' preparates.

Chapter 1. Organization and functions of immune systems

Immunity is a set of mechanisms to protect the body against pathogens - microorganisms, parasites, foreign antigens and other substances harmful to it. This Chapter provides a summary of the data that will be needed in the future.

1.1. Nonspecific protection

In addition to immune reactions the body possesses different non-specific methods of protection: digestive enzymes and acidity of gastric juice, bile acids and some other factors that create unfavorable conditions for microbial growth. Some importance in the removal of small extraneous substances, as well as microorganisms has the microvilli of the epithelium: their vibrations move them toward the exit, along with mucus, such as happening in respiratory and female genital tracts. Some protein and other biologically active substances, such as a complex of complement, interleukins, interferons and others are involved in the regulation of immune cells. Much attention is paid to the complex substances of apoptosis - the destruction of unnecessary or harmful cells.

Nonspecific cellular defense is realized by cells capable of phagocytosis. Those are granulocytes (mostly neutrophils) and mononuclear phagocytes (macrophages, monocytes, promonocytes, histiocytes, Kupffer cells of liver, osteoblasts, Kashchenko-Hofbauer cells[*] of chorionic villi, microglia of the brain and others). Among the features of nonspecific cellular defense is their action at the early stages of pathological processes, before the development of specific immune responses.

1.2. Specific immune responses

Specific immune response characterized by the fact that their protective actions are directed against a specific pathogen. Cells involved in specific immune response, can distinguish between the normal tissues and the molecular substance of the own body and treat them tolerantly. However, errors may occur, which are expressed in the autoimmune processes.

[*] Kashchenko NF (1855-1935), an embryologist from Kiev, described the placental monocytes (in 1885), as well as other parts of the embryo. Hofbauer J.I.I. (1878-1961), American gynecologist who examined the placenta, described in the stroma of villi cells-phagocytes. These phagocytes belong to the monocytes.

All kinds of specific immune responses are carried out with the participation of various immune cells. They are located in the bone marrow, in lymphoid organs such as thymus, spleen, lymph nodes, tonsils, Peyer's patches of the intestine, as well as throughout the entire body in the form of follicles (a group of different immune cells) or cells traveling alone with the blood, lymph and tissue interstitial fluid (recycling). Thus the commonality of immune responses and the possibility for contact with the pathogen by the antigen-specific cells is realized in any part of the body.

The following types of specific immune systems are specified:

1. overall immune responses - cellular and humoral;
2. secretory immune response - the secretory immune system (SIS) of mucosal and the barrier SIS;
3. personal protection system of the vital cells and organs.

1.2.1. General cellular immune responses

In these reactions a complex of immune cells is involved. Among them the antigen-presenting cells that recognize the presence of enthetic antigens are specified. In the primary contact, those are the mononuclear phagocytes. Their mostly used immunohistochemical marker is CD68. If the same antigen is appeared, T memory cells, which are long-lasting in the body, sometimes during all a carrier-person's life, will represent it. These cells cause the immune response is much faster time course than the mononuclear phagocytes during the initial exposure. Directly involved in the cellular response is a group of T-lymphocytes, whose common marker is CD3. Each type of T-lymphocytes performs its specific functions: helpers regulate the immune response (a marker of them is CD4). The immune response itself is operated by cytotoxic (suppressor) lymphocytes (CD8) and natural killers (NK, the marker is CD56).

Cellular immune reaction occurs in response to foreign tissue containing the major histocompatibility complex (MHC) of class 1 (HLA-A, HLA-B, HLA-C). The MHC molecular complex is unique to each organism and determines its biological identity. Under transplantation of foreign tissue incompatible for MHC class 1, the initial reaction is carried out in two stages. The first stage - sensitization - lasts 7-11 days from the day of initial contact with the antigen. During this time a large number of cytotoxic T lymphocytes and natural killer cells are formed. In the second stage - rejection - these lymphocytes, as well as phagocytes, penetrate into the tissue, caused sensitization, abundantly infiltrate its vessels, it necrotize the tissue which is torn away. An important role in T-cell immune response is played by interleukins (a receptor marker of one of them is IL-2Rα chain CD25). They activate cytotoxic T-lymphocytes and NK, to a lesser extent - B lymphocytes and mononuclear phagocytes.

1.2.2. Humoral overall immune responses

Humoral immune responses occur when foreign antigens of MHC class II (HLA-D, HLA-DP, HLA-DQ, HLA-DR and HLA-G) or other incompatible antigens, such as the Rh antigen, causing hemolytic disease of the fetus and newborn (HDFN) (Gurevich PS et al., 1997) appear.

In the humoral immune responses a group of cells with different functions is involved. These are antigen-presenting mononuclear phagocytes – at the first contact with the pathogen, or memory B-lymphocytes - after repeated contacts. An immune response

is carried out by B lymphocytes (CD20 + and CD79A + markers) and by their descendants - plasma cells (CD20-, CD79A +). The involved in regulating the immune response are T-helper cells and cytokines.

After the initial contact of mononuclear phagocytes with foreign antigen, they convey information about it to B-lymphocytes, which start to multiply (sensitization period). After about 7 days antigen-specific antibodies - immunoglobulins M (IgM) appear in the blood, and two weeks later - a large number of antibodies IgG and IgA.

Activation of B-lymphocytes and their transformation into plasma cells is accompanied by a marker modification: CD20 is lost, but CD79As are saved. Synthesis of antibodies occurs everywhere - in the lymphoid organs: in spleen, lymph nodes and follicles, as well as by B-lymphocytes and plasma cells scattered throughout the body. But the cells themselves are not directly involved in the immune response, as it happens in the case of cellular immune responses. Immunoglobulins are carried by blood, lymph, intercellular fluid where contact with their specific antigens and destroy them, if those are molecule, or cause necrosis or apoptosis in a case of cells.

Repeated contact with antigen develops a much faster immune response - within 3-4 days.

1.2.3. Secretory immune system of mucous membranes

The main function of SIS is the immune protection of organs, getting in contact with the external environment and being contaminated by microorganisms and other pathogens (Brandtzaeg P., 1995; Goldblum RM, et.al. 1996; McGhee JR, Kiyono H., 1999). SIS is located in the epithelium of the mucous membranes and accompanying glands: that is why the name of mucosal immune system is also used. It covers the following structures: the digestive system from the mouth with its salivary glands, esophagus, stomach, intestine (except for goblet cells), tubules and acini of the pancreas (except for its islands), bile ductules of the liver, extrahepatic bile ductules, gallbladder, respiratory system from the nasal cavity to the small bronchi, urinary system from the kidney tubules - collective tubes, pelvis, ureters, bladder, male and female sex organs; lacrimal gland and conjunctiva of the eye, breast, and others.

The significance of the ICU to maintain the organism functionality is extremely large, since the total area of the inner surface of some organisms, and consequently, exposure to pathogens is very high. For example, the surface of the digestive tract mucosa consists of hundreds of square meters. All “cohabitants” as symbiotic microflora and “eating together” commensals have to be constantly restrained. Sterility of some organs, such as alveoli of the lung, the breast, and others, is achieved with several complex mechanisms, among which the SIS plays an important role.

Two stages of SIS are distinguished. The first one is executed by a range of cells which recognize foreign antigens and synthesize immunoglobulins. These are: mononuclear phagocytes, B-lymphocytes and plasma cells, similar to these involved in formation of the general humoral immune system. They are mainly located in the mucosa and submucosa of hollow organs: in the digestive tract - in the Peyer's patches, appendix, in the regional lymph nodes and lymphoid follicles in the pharyngeal tonsils. Close contact of lymphoid organs and pathogens enables the initiation of immunoglobulin

synthesis earlier than it takes place in the overall immune system. This was noted by A. Bezredka (Besredka A., 1919): following an experimental introduction of pathogens with food, antibodies at the intestinal content appear earlier than in the blood.

During the second phase, SIS transports immunoglobulins from intercellular spaces through the epithelium into the lumen of the hollow organ. Three functions are specified in the transport. First one is the capture of immunoglobulins by a receptor on the basal-lateral surface of the epithelium (endocytosis or internalization), the second is the transduction of immunoglobulins through the cytoplasm of the epithelium (transcytosis) and the third one is their excretion at the apical epithelial surface (exocytosis). Here, in the lumen of hollow organ, immunoglobulins neutralize or destroy pathogens (Brandtzaeg P., 1995, 1996; Brandtzaeg P., et al., 1997; Goldblum RM, et al., 1996; McGhee JR, Kiyono H., 1999).

This transport is performed by two receptors: polyimmunoglobulin receptor (pIgR, a part of it is the secretory component - SC) and joining J-chain. In adults mainly IgA and to a lesser extent IgG and IgM are transported (Johansen FE, et al., 2000, 2001).

SC-glycoprotein is found only in vertebrates, especially - in mammals (Peppard JV, Russell MW, 1999). It is believed that it performs all three stages of transport of immunoglobulins through the epithelium (Brandtzaeg P., 1996; Brandtzaeg P., et al., 1997). SC is found at very early stages in human embryo and fetus in the organs of ectodermal, endodermal and in some mesodermal origin organs, namely in the trophoblast, amnion, gallbladder, the epithelium of the skin, in the neuroblasts of the neural tube, the chord, in the epithelium of growing digestive, respiratory and urinary organs, in mesothelioma and in organs formed from it (Gurevich et al., 2001 A, B, 2003 A, B, C). Such early and widespread availability of SC in ontogenesis makes it likely its earlier appearance in phylogenesis.

J-chain is a small (15 kDa) polypeptide that discovered yet in many species of invertebrates, who have neither immunoglobulins nor lymphocytes, and the DNA of J-chain, for example in earthworms and humans reveal a high degree of similarity (Takahashi et al., 1996). In humans, J-chain was detected in T-lymphocytes of bone marrow and thymus, in B-lymphocytes that synthesize IgG, IgD (Brandtzaeg P., 1996), IgA and IgM (Johansen FE et al., 2000). The main function of J-chain seem to be the connection of monomeric immunoglobulin molecules into the polymers by binding of two molecules of IgA or five molecules of IgM via the Fc-fragment.

Mechanisms of transport of immunoglobulins through the epithelium of mucosa and the role in this process of SC and J-chain receptors is not yet fully understood. Some researchers believe that the whole process of transport of immunoglobulins - endocytosis, transcytosis and exocytosis is executed by SC. There are also other opinions: Simada SI. et al., (1999) derived a strain of mice deficient for the gene of polymeric immunoglobulin receptor (pIgR-/ -). Using Western blot analysis and immunohistochemical analysis of intestinal epithelium, the pIgR (SC) was not found, but IgA in that epithelium was present. It was also noted the increased level of IgA in the blood, and its level in bile, intestinal contents and in feces was negligible. Conclusion: the presence of IgA in the intestinal epithelium in the absence of pIgR (SC) shows that endocytosis into the epithelium is performed by some other receptor. Since in pIgR (-/-) mice, in their bile, intestinal contents and fecals the IgA levels is dropped dramatically, while in healthy mice it looked normal, the pIgR (SC) appears to be the leading receptor in exocytosis.

Hendrickson B.A. et al. (1995) studied mice deficient for J-chain (J-chain-/ -). The level of IgA in their serum have been nearly 30 times higher than in wild mice, J-chain + / +, while J-chain content in the bile and feces have been significantly reduced. These conclusions are supported by clinical observations of people living with IgA nephropathy. They have increased levels of IgA in the blood and excretion of them with the urine causes damage to the glomerular mesangium of the kidneys. A detailed examination of the patients showed lack of J-chain content in the intestinal epithelium, an abnormally low content of IgA in it and in the contents of the intestine. Conclusion: J-chain performs endocytosis of immunoglobulins into the intestinal epithelium (Emansipator SN et al., 1999). Ultrastructural study of the epithelium of the small intestine in human revealed the location of J-chain in the basal-lateral compartment of the cells, but not in the apical parts of them where SC is located (Nagura N. et al., 1979). Our investigations presented in Chapters 3 and 5.4 confirm that **in the human embryo and fetus J-chain produces endocytosis of immunoglobulins, while SC executes their exocytosis out of cells.**

The mechanism of exocytosis described is typical for merocrine (eccrine) secretion, which occurs in normal conditions in epithelium of the mucous membranes, salivary and lacrimal glands, and others. Herewith, SC is not separated from the cells with immunoglobulins. Apocrine secretion, during which the apical part of a cell is destroyed, is observed in the mammary gland. Therefore, the SC rejected, together with immunoglobulins, and partly - with J-chain into the colostrum and milk. During holocrine secretion the entire cell is destroyed together with the SC, J-chain and immunoglobulins. This occurs in the sebaceous glands, and - in a case of inflammatory processes - in the bronchial, gastric, intestinal epithelium, the ependyma and epithelium of the cerebral ventricles, and others. But it is unlikely that the contents of these cavities will be positive for immunostaining of SC, J-chain and for immunoglobulins, as they will be destroyed too (Norderhaug IN et al., 1999).

Another function of the SIS have been revealed in the following series of experiments. In the culture of Madin-Darby dog kidney epithelial cells, containing SC, a Sendai or influenza virus and then the appropriate anti-virus IgA were introduced. As a result, virus titers in supernatants were decreased by 1000 times, while in cell lysates there was 10 fold reductions. Mice were injected with mouse hepatitis virus, then with anti-virus IgA, after which the mice remained alive and the virus in their hepatocytes was absent. Authors (Mazanec MB et al., 1992; Nuang DS et al., 1996; North RJ, Convan JW, 1998; Yan H. et al., 2002) emphasize that in SIS, besides the immune effect on pathogens in the lumen of hollow organs, the intracellular action of antibodies is also possible.

The data show that the main function of SIS is to fight pathogens in the organs, related to the environment, by releasing immunoglobulins onto the surface of those organs. Therefore, the SC seems to be the defining feature of the availability of SIS.

1.2.4. Secretory barrier immune system

This version of the SIS, in contrast to the SIS of mucous membranes is not associated with the mucosa, but transports immunoglobulins with the same SC and J-chain through the barriers. They include placental barrier (transport of different substances needed for the embryo and fetus from the pregnant and the reverse transport of waste), the blood-brain barrier (through the ependyma and choroid plexus of the

ventricles of the brain into the cerebral and spinal fluid), preovulatory shell (via ovarian follicular cells to oocytes) serous cavities (peritoneal, pleural, epi- and pericardium) and others. Basically, the function of barrier systems and their mechanisms are identical to the SIS of mucous and can be considered as variants of the SIS. At the same time, there are some differences between them. In the barrier system, not IgA as in the SIS of mucous but IgG and to a lesser extent IgA and IgM are excreted. The barrier system is sometimes great - the area of the placental barrier in the full-term pregnancy is 11,0 ± 1,3 m^2 (Aherne W., Dunnill MS, 1966), and the areas of peritoneum and pleura are also large. But more often it occurs in small structures, such as the follicular cells of ovary, follicles or thyroid gland, and cannot compete to the SIS surface of mucous of gastrointestinal and other tracts. Immunocompetent cells in the barrier system are scarce, and apparently this system uses immunoglobulins of overall immune system - from the blood, lymph or from interstitial fluid.

The barrier SIS should be distinguished from a wide variety of tissue barriers, unrelated to immune responses. These are aerohematic barriers of lungs, filtration barrier of primary urine in the kidneys, perineural barrier, and bicarbonate barriers in the gastric mucosa preventing self-digestion by its own enzymes and acid and others.

1.2.5. The system of individual immune protect the of vital cells and organs

This system is designed to protect parenchymal cells of organs that are of critical strategic importance for the life of the organism and the stem preservation, but not possessing or possessing a small extent of the ability to regenerate. In more details this system will be considered in Chapter 3.

1.3. Protection of the genital tract and gonads of adult

Protection of gametes, the welfare of future generations begins long before fertilization. Besides various non-specific processes it includes all three specific immune systems - general, covering the entire body, secretory that protects the mucous membranes, exocrine glands and part of the barriers, as well as a system of individual immune protection of critical organs and cells. These systems cover all the genital organs of men and women.

1.3.1. Protection of female genital tract and ovaries

Among the nonspecific protective mechanisms in the female genital tract other than those mentioned (Section 1.1) it may be noted the settlement of vagina by Doderlein's bacillus: a commensal that is antagonists of pathogenic bacteria.

Overall immune system in the area of female genitals is represented by the surrounding lymph nodes and various immunocompetent cells, especially T-cells - cytotoxic suppressor and natural killer in the tissues of the fallopian tubes, uterus and vagina. Their number increases considerably during menstrual cycles, menopause, during pregnancy, and infections (Kurman RJ, 1995; White HD, et al., 1997; Corbeil LB et al., 1998).

SIS is presented by SC, J-chain and IgA in the epithelium of the fallopian tubes, uterus, cervix and vagina (Stern JE et al., 1992A; Brandtzaeg P., 1997; MasCasullo V. et al., 2005). Throughout the genital tract there is a moderate amount of B-lymphocytes, mainly containing IgA and to a lesser extent also IgG and IgM (Kutteh WH, 1999).

Our studies have shown that ovarian follicular cells contained SC, J-chain and immunoglobulins, mainly IgA. In the oocytes surrounded by them there found J-chain and immunoglobulins. This indicates that the protection of eggs is realized by the SIS and by the system of self-protection (Fig. 1.1).

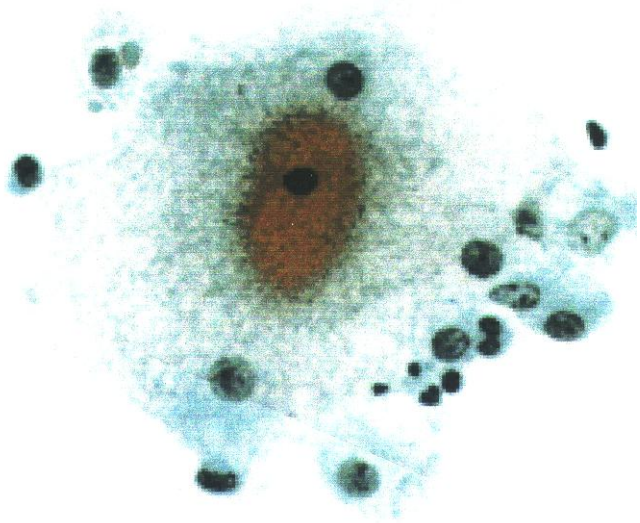


Fig. 1.1. Ovulated egg with nucleus in the center of it. On the periphery of the egg some follicular cells are located, and then the cells of corona radiata. They contain a complex of different enzymes, as well as SC, J-chain and immunoglobulins (see Fig. 3.7.). After fertilization, the dividing oocyte retains the corona radiata cells for one week (see Fig. 2.1. A, B), and then they are replaced by trophoblast. All 5 types of it contain the complex of SIS (see Fig. 4.1) (E. Greenenbaum, 1988).

Ideas about the protection of female genitalia during pregnancy and, in particular, the probability of early allogeneic abortions are not yet clear. It continues to remain an idea of the hypothetical "privileges" in the protection of such diverse organs as the pregnant uterus, the anterior chamber of the eye and the brain. AW Ham and DH Cormack (1983) discuss these privileges for the uterus, which seems to be of particular interest. They have proceeded from the statement that during the allogeneic pregnancy the rejection of the embryo does not occur. By this they decline the possibility of allogeneic conflict in the III trimester - the hemolytic disease of the fetus and newborn. The authors consider a range of theoretical possibilities for immune privilege of the pregnant uterus. The first is the lack in trophoblast cells of any specific antigens of paternal origin, capable to cause an immune response. This possibility is renounced by the authors: the trophoblast contains several variants of HLA-D and HLA-G antigens. The second possibility is that father's antigens are somehow masked and unavailable for recognition; the 0,1 - 2 microns thick fibrinoid layer on the surface of the trophoblast may play a role of masking factor. By this the embryo isolates itself from the hostile environment of maternal cells. But invasive trophoblast is in the direct contact with

decidual cells, while the fibrinoid on its surface is absent. The third group of hypotheses involves the formation by mother of blocking antibodies which isolate antigens on cells of the embryo. As a result, either an inactivation (paralysis) of maternal immune system to the paternal antigens is derived or the maternal immune response is infringed upon hormones. AW Ham and DH Cormack (1983) considered it unlikely that one of these possibilities could explain the fact why the pregnancy is stored without rejection. The concept of immune privilege in the endometrium the authors considered untenable.

Over the next 30 years, questions about the immune privileges of the pregnant uterus, about the possibility of early allogeneic conflict followed by recurrent abortions in trimester I and about the nature of the allogeneic conflict (if it is possible) through cellular or humoral immune response - have not yet been resolved.

1.3.2. Protection of the male genital tract and testicles

Interleukins and other cytokines regulate different stages of cell differentiation, including Leydig and Sertoli cells, which are involved in the development of sperm (Huleihel M., Lunenfeld E., 2004).

Overall immune system in the male genitals all over the course from the network of testicles to the seminal vesicles is represented by T-lymphocytes: by suppressors (CD8) and helpers (CD4), as well as macrophages. They are located in a small amount between the epithelium and basement membrane. B-lymphocytes and plasma cells are rare or absent at certain places. In the prostate and urethra, in which the urinary and genital canals are combined, the number of lymphocytes and macrophages is significantly increased; the cells are arranged in groups. There should also be noted inguinal lymph nodes and those of pelvis, relating to the overall immune system.

SIS covers all areas of sex channel from the network of testicles through the urethra (Mestecky J., Fultz PN, 1999). Plasma cells and B-cells containing IgA, and isolated IgG and IgM are located under the epithelium. SC, J-chain and immunoglobulins are detected in the epithelium throughout the testicular tubules from the network of testicles (Stern JE et al., 1992B; Mestecky J. et al., 2005). A system for the protection of spermatozoa in seminiferous tubules consists of large groups of Leydig cells and Sertoli cells (see 3.6.3).

Chapter 2. The process of fertilization and development pre-embryo. The formation of the secretory immune system of the embryo and fetus

2.1. Fertilization

Unification of a mature oocyte (ovum) and a spermatozoid is a biological event, resulting in the formation of a new organism. Any interference with this process can lead to a breach of the genome, the formation of developmental defects or death of the germ. Therefore, the preservation of the gene pool of future human needs protection from the very beginning of its formation.

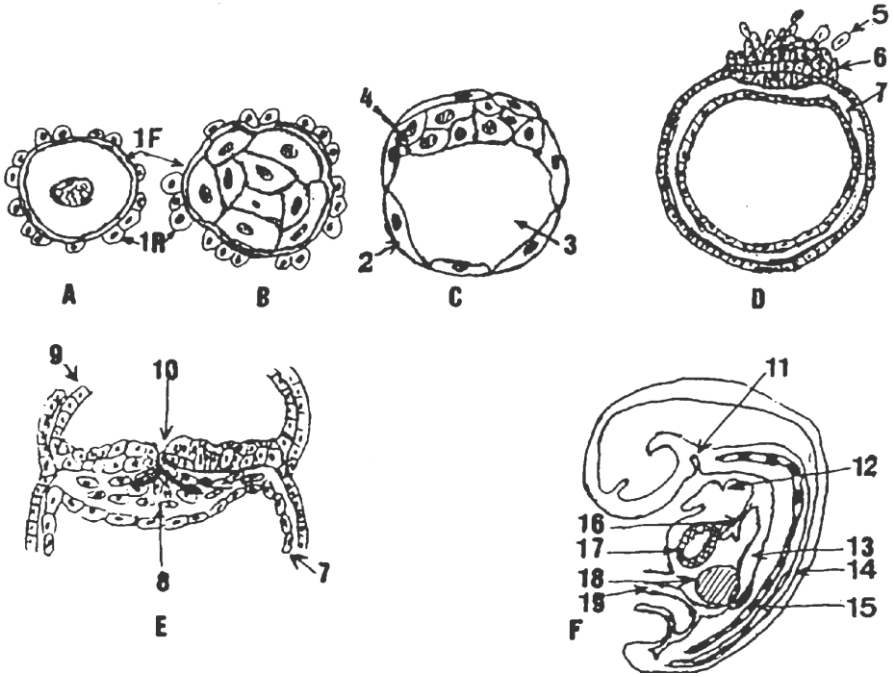
Fertilization - the merging of two sets of DNA - is preceded by a series of processes. Oocyte releases gamones - they enhance the movement of spermatozoid inside it. For its part, the spermatozoid releases its own series of gamones which inhibit the activity of other spermatozoa, and prompt the host to pass through three following barriers. The first barrier is a loose layer of follicular cells, named corona radiata. Then there is a transparent shell (zona pellucida), through which the egg is reached by a large number of protrusions of follicular cells that execute metabolism of the egg. Finally, the egg shell completes the path of sperm. The most significant barrier to sperm is the zona pellucida. Approaching it, the sperm gets weakly attached with its receptors and produces the acrosomal reaction supported by egg cell. At that, from the head membrane of the sperm a set of enzymes is released onto the zona pellucida destroying a small plot of it. Lipids of the sperm membrane fuse with the oocyte membrane lipids and through the formed narrow channel the contents of its head - the nucleus of sperm cell - gets into the egg cytoplasm (Stein KK et al., 2004). Immediately after the penetration of heads of sperm into the egg the cortical reaction occurs. Under the action of enzymes calcium concentration at the liquid portion of the cytoplasm - cytosol increases and as a result of it zona pellucida transforms into fertilization membrane. It is already unable to perform the acrosomal reaction with other spermatozoa which prevents polyspermy.

After the penetration of sperm heads with its nucleus into the egg, both of the nuclei swell, their membranes get lysed, pronuclei are mixed and synkaryon (♀ + ♂) is formed. This process, the syngamy, actually represents the fertilization, resulting in the formation of a new organism, yet one-celled zygote, but with a full set of 46 chromosomes, including the two sex chromosomes.

2.2. Postfertilization processes

During fertilization and immediately after that the ionic composition of the zygote gets changed to a more appropriate with the current situation. The stages of division and movement along the fallopian tube begin then, the zygote becomes morula. Morula, as a zygote, is surrounded by fertilization membrane (fertilization membrane), on the surface of which some follicular cells are preserved, forming the corona radiata. Movement of the morula lasts for three days (see Fig. 2.1, A, B, C, and D).

Fig.2.1. Early stages of human germinal development



A - zygote, the first day after fertilization, at the entrance to the fallopian tube. fertilization membrane is formed **(1F).** Outside of it: follicular cells in the form of corona radiata **(1R)**.

B - morula, 2^{nd} - 3^{d} days, move along the fallopian tube to the entrance to the uterus. Fission occurs with the formation of groups of cells, fertilization membrane (fertilization envelope) and corona are preserved **(1F and 1R).**

C - blastocyst, 4^{th} - 6^{th} days, situated in the uterus, on the 6^{th} day - the beginning of implantation. fertilization membrane and corona radiata get destroyed, a layer of tightly connected cells of the trophoblast is established **(2)**, a cavity - the blastocoel **(3)** and an inner group of cells - germinal disc **(4)** are formed.

D - gastrula, 7^{th} - 13^{th} days, the implantation into the uterine decidual tissue is occurred. The trophoblast intensively proliferates and invades **(5)**, germinal disc **(6)** is divided into several layers of cells hypoblast is formed **(7).** Later it is converted to the yolk sac.

E - continuation of gastrulation, 14^{th} - 21^{st} days, gastrula is set in the decidual tissue, yolk sac **(7)**, mesoderm and endoderm layers **(8),** amnion **(9)** are stood**.** Medullary plate and then neural groove **(10)** are formed. The formation of blood islets, the heart and the liver begin.

F - embryo, 5 weeks. An intensive formation of: the Rathke's pocket **(11),** becoming anterior pituitary in the future; thyroglossal duct **(12),** stomach and intestine **(13),** neurotubule **(14),** chord **(15),** trachea and bronchi rudiments **(16),** heart **(17),** liver **(18),** the yolk stalk **(19)** are formed.

Sources are used (modified): Sadler TW, 1995; Улумбеков Э.Г., Челышев Ю.А. 2007.

In the next stage of development (blastocyst, 4-6 days after fertilization) corona radiata and fertilization membrane - located on the surface structures of maternal origin, which hinder the further development of the embryo, get destroyed. Instead of them, a layer of germinal cells - the trophoblast is formed with organism inherent part of new antigens presented in the main by HLA-G, HLA-D and possibly by their variations. They are intensely divided, penetrate the decidual tissue and form a cavity for implantation of the germ. A group of cells is formed that will form the embryo itself - germinal disc.

At the gastrula stage (7-21 days) implantation of germ into the decidual tissue occurs. Some organs are formed which achieve increasing metabolism - the amnion and yolk sac; cells of germinal disc intensively proliferate, forming the beginnings of tissues of the germ - ectoderm, endoderm and mesoderm (Fig. 2.1D). At the end of gastrula blood islands, blood vessels, heart, liver, and placental villi, as well as the medullary plate, neural groove and then the neurotubule - the future brain begin formation.

In the embryonic period (3-8 weeks of development), the organs of embryo get formed. Fig. 2.1F schematically represents the state of organogenesis at 5^{th} weeks: the Rathke's pocket (future anterior pituitary), the thyroid and thymus formation, stomach and intestine, the neurotubule, chord, trachea, the launching of the bronchi, heart and liver. At this stage also the pancreas, the pronephros, mesonephros and metanephros, adrenal, gonadal ridges and other organs begin their formation.

2.3. Protection of the germ at the postfertilization period

Among the ways of nonspecific germinal protection, an important role is played by anticomplementary proteins. Complement has the ability to destroy cells by immune lysis (classical pathway) or by other enzymatic ways (alternate path). Already the mature sex cells - gametes, and then the germs and their surrounding cells at the preimplantation stage release several proteins that possess anticomplementary activity. One of them is membrane cofactor protein (MCP, a marker antigen CD46) capable of binding complement components C3b and C4b, which makes them accessible to protease cleavage. Another protein - decay acceleration factor (DAF, CD55) accelerates the destruction of complement. Protectin protein (CD59) prevents lysis of cells of the embryo caused by complement. Thus, by MCP, DAF and protectin released by shells, the gamete or the germ rescue themselves (Fenichel P. et al., 1995; Taylor CT, Johnson PM, 1996), and despite the fact that in the uterus, fallopian tubes and in follicular ovarian fluid there are all components of the complementary cascade.

In the preimplantation the germ possesses specific immune responses. As shown by our study, the follicular cells surrounding the egg (and after fertilization also the zygote and morula) contain SC, J-chain and immunoglobulins and, therefore, are related to SIS. Their cytoplasmic processes penetrate the zona pellucida towards the ovum, performing the metabolic reactions, including the commitment of immunoglobulins to it. As part of corona radiata follicular cells are stored in fertilization membrane. This suggests that the metabolism with the germ is continued by them. Inclusively the maternal immunoglobulins arrive for the immune protection of the germ from the microbial and other pathogens.

At the end of the morula stage, after the destruction of corona radiata and fertilization membrane their functions of supply, metabolism and protection of the germ

are transferred to the trophoblast (tropho - nurture, blastos - germ). Stages of development of the own immune protection of germs: pre-embryos, embryos and early fetuses, including the trophoblast, monocytes, and other factors of immune protection are discussed in Chapter 6.

2.4. The formation of the secretory immune system of the embryo and fetus

In the embryonic period, the overall immune system - immunocompetent cells and organs are not yet available. The first preT- and preB-lymphocytes will appear to 4^{th} -5^{th} weeks of development (Labastie MC et al.,. 1998; Melchers F., Rolink A., 1999; Hardy RR, et al., 2000; Khlystova ZS, 2006). However, the trophoblast of chorionic villi contains several types of receptors, providing transport of immunoglobulins of the pregnant to the germ. This poly immunoglobulin receptor (pIgR / SC), J-chain receptor for IgG - Ig-gamma RI (CD64), Ig-gamma RII (CD32), Ig-gamma RIII (CD16), the receptor for IgA - Ig-alpha-R and others (Jauniaux E., et al., 1995; Simister NE, 1998, 2003; Gurevich P., et al., 2003A). There are reports of IgE transport across the placental barrier (Sverremark Extrom E. et al., 2002). This described data and the immunohistochemical detection of IgG, IgA and sometimes also IgM in the tissues of the placental barrier and in the fetus suggest that these immunoglobulins are of maternal origin (Gurevich P., et al., 2003A, 2003B). At the end of the first trimester it begins and then increases the synthesis of immunoglobulins of the fetus, especially IgM, but also IgA, and their overall proportion increases (Ben-Hur H., et al., 2005).

By the beginning of the embryonic period at 3.5 - 4 weeks the components of the SIS: SC, J-chain, IgG, to a lesser extent IgA, and occasionally IgM, become common in all the structures and tissues of ectodermal, endodermal and in some - of mesodermal origin (Fig. 2.2 and several following).

Functional activity of the SIS is already apparent before 3.5 - 4 weeks in a case of infection of the birth canal with its extension to the germinal shell. In a case of chorioamnionitis the infected amniotic fluid is swallowed into the stomach and intestines. This causes the release of immunoglobulins from the epithelium of the stomach and intestines into their clearance, as well as from hepatocytes, and in the 7.4 weeks old embryos - also from the cells of acini and pancreatic ducts, trachea, bronchi and from other organs. The disappearance of immunoglobulins, mainly IgG, less IgA, from the epithelium of the listed organs can be clearly seen with the immunohistochemical staining. For example, the number of epithelial cells of the digestive and respiratory tracts, containing immunoglobulins, decreases in a case of infection to $7.5 \pm 2.3\%$ compared with $77.8 \pm 6.3\%$ in uninfected embryos and fetuses (Gurevich P., et al., 2002).

During organogenesis, composition of the organs containing SC, J-chain and immunoglobulins varies depending on the function of organs and their structure, on the presence of a cavity lined with epithelium and on the capability of immunoglobulins secretion. On 4 - 7 weeks of development the protein components of the SIS

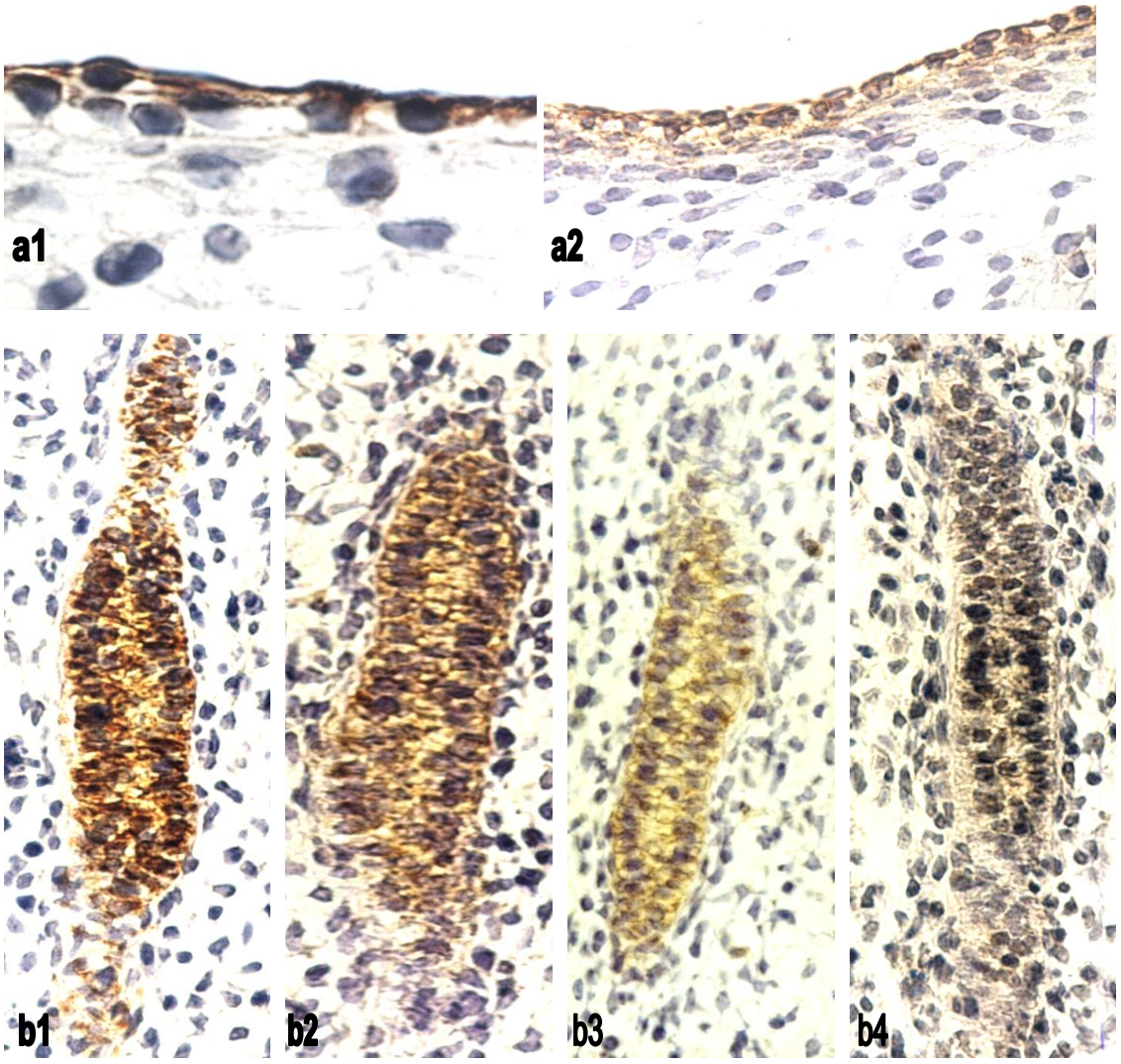


Fig.2.2. All cases of 3.5 - 4 weeks. It draws attention to widespread components of the SIS - SC (+), J-chain (+) and immunoglobulins (+) in the tissues of embryos and even in pre-embryos: for example, in the trophoblast, in the chord, in the cells of the neurotubule, the archenteron and many others. They can stay for life, such as gut and its derivatives, or disappear, in accordance with the functions of the newly formed organ and being converted into other complexes of protection (see Fig. 3.1 - the brain and other organs - Chapter 3). A - skin, **a1** - SC (+) in one row of the epithelium, **a2** - J-chain (±) and immunoglobulins. B - chord, **b1** - SC (+), **b2** - J-chain (+), **b3** - JgG (+), **b4** - IgM (±) - the formation of vertebrae is envisaged. Subsequently skin epithelium retains the SC and J-chain, and the chord with its transformation to the bone, loses the components of SIS. All A x1000, B x400.

are detected in the epithelium of the skin, stomodeum, the initial part of the gut, stomach, intestines, ducts and acini of the pancreas, trachea and bronchi, bile ducts, tubules, in pronephros and mesonephros and their ducts - Muller's paramesonephric and Wolff's mesonephric in the mesothelium of the body cavity, in epicardium, gonadal cylinders, in the epithelium of the Rathke's pocket (the rudiment of the anterior pituitary), ductus thyroglossus (rudiment of the thyroid) and in pockets of the pharynx (the beginnings of

the parathyroid glands and thymus) as well as in the epithelium of extracorporeal tissues, including trophoblast of villi, the trophoblast lining the lacunae of the placenta, in the invasive trophoblast of decidual tissue, in the epithelium of the yolk sac. The high reactivity of SC, J-chain and immunoglobulins in the chord, in neuroblasts of superficial and deep layers is noted (Fig. 2.2).

At 8-9 weeks of development in connection with the ongoing organogenesis and specialization of cells, some changes in the system of SIS of individual organs are observed. The complex of protein components of the SIS (SC, J-chain, immunoglobulins) is preserved in the epithelium of the skin, the epithelium of the gastrointestinal tract, salivary glands, ducts and acini of the pancreas, bile ducts in the liver and outside of it, in the gall bladder, the trachea and bronchi and in developing tubules of metanephros (kidney), in the pelvis, ureters and bladder, in the mesothelium in the oral cavity, of epicardium and pericardium, in the pleura in the spleen capsule, developing gonadal ridges, the epithelium of thyroid follicles. Complex of SIS proteins is preserved in the extracorporeal membranes - in the epithelium of the amnion, of yolk sac, of chorion, and in villous trophoblast and extravillous trophoblast as well as in the ependyma and choroid plexus of the brain ventricles. In the cells of certain organs in the restructuring process all the components of the SIS are gradually disappearing. These are vessel walls of some organs and disintegrating organs of embryos, such as pronephros and others. In the newly forming organs the SIS has occurred in a body. It happens, for example, in the epithelium of metanephros, urinary and genital (male and female, except the gonads) organs. But in some organs SC could disappear, while J-chain and immunoglobulins remained. It is noted in the neuroblasts of the brain; parenchymal cells of the anterior pituitary gland, parathyroid glands, and the islets of the pancreas (see Chapter 3). In the liver, SC remains in the peripheral hepatocytes of the lobules but disappear in the cells of their center.

In the subsequent second and third trimesters the cell structure of SIS is gradually formed. It consists of B-cells that synthesize the own fetal immunoglobulins. Antigen-recognizing cells - mononuclear phagocytes (monocytes and pre monocytes) begin operating already before 3.5 - 4 weeks. Pre-B-cells begin to function in the II trimester: IgM appears not only in fetal blood and cells, which is noted during the transport of maternal IgM in embryos, but also in the cytoplasm of B-lymphocytes in the liver at the beginning and then in clusters of different locations (Asma GEM et al., 1984).

2.4.1. Barrier version of secretory immune system

Embryos and fetuses develop their barrier SIS in parallel with the formation of shells and organs. This includes different types of trophoblast of the placental barrier (cytotrophoblast, syncytiotrophoblast, proliferating villous trophoblast and extravillous types: invasive trophoblast in the decidual tissue and trophoblast lining the blood lacunae of the pregnant), epithelium of amnion and of the yolk sac, mesothelium of serous membranes and its derivatives (epicardium, pericardium, pleura, spleen shells, and gonadal ridges), ventricular ependyma, as well as other blood-tissue and tissue-tissue SIS (ovarian follicular cells, neurons of the brain and others). Their main function is to

transport immunoglobulins, mainly IgG between the embryo - and the pregnant, and in addition it at the II trimester the immunoglobulins of fetal origin are synthesized.

Cell structure of SIS is developed in parallel with the formation of common cellular and humoral immune systems in the II trimester.

2.5. Conclusion

Protein components - receptors of the secretory immune system - SC and J-chain - are widespread in the ectoderm, endoderm and partly in mesoderm of the forming germ and its shells already before 3.5 - 4 weeks of development. The simultaneous presence of antibodies indicates the functionality of the SIS. Such a wide and early dissemination suggests that the SC and J-chain appear much earlier - perhaps during the blastocyst, when follicular cells, corona radiata as well as fertilization membrane, serving to protect the zygote and morula, are destroyed. The probability of such an early development is further confirmed by the phylogenesis: J-chain exists already in invertebrates. Apparently, also the SC appears much earlier than with vertebrates, and all the more not only in mammals.

Chapter 3. Formation of individual immune protection of vital cells and organs in human embryos and fetuses

3.1. The development of the secretory immune system and its transformations

The previous Chapter presented the data on the formation of the SIS: its transport elements: polyimmunoglobulin receptor - the secretory component (SC) and J-chain in pre-embryonic and embryonic periods. These receptors transport immunoglobulins of the pregnant through the barriers of the trophoblast, and then by other means of transportation, they are spread throughout the germ and widely trapped into the structure of the ectodermal, endodermic and mesodermal origin. In that way, upon the beginning of the embryonic period, SIS functions: a system that provides immune protection of the pre-embryo, the embryo and its membranes.

In the process of organogenesis, some newly-formed structures are losing their previous structure and relationship with the superficial epithelium and organ cavities, and therefore losing the possibility to excrete immunoglobulins. As a result, such organs lose SC, but in some of the newly formed organs preserve J-chain and immunoglobulins. The composition of these organs is typical: the neurons of the brain and spinal ganglia, parenchymal cells of the endocrine system and of the liver, cells of the gonads. In the myocardium and adrenal SC has been apparently absent from the beginning of their development, but the J-chain and immunoglobulins still appear during the formation of these bodies.

3.2. Protection of the developing brain

3.2.1. Nonimmune mechanisms of brain protection

Protective barrier mechanisms of the developing brain are largely different from the blood-brain barrier of the adult brain. This corresponds to the initial stage of brain development. One of such non-immune defense mechanisms is an increase in the capillary network. Angiogenesis occurs through the introduction of the endothelium in early neuroectoderm, which is then transformed into the capillaries and the larger vessels. Vascular endothelial growth factor (VEGF) controls the growth of blood vessels. Massive angiogenesis in the embryo is accompanied with a large amount of VEGF. In adults, when angiogenesis is negligible or absent, VEGF correlates with vascular permeability factor (VPF), which is responsible for the permeability of capillary blood-brain barrier

(BBB). Thus, the ratio of VEGF / VPF is a key factor regulating the growth of capillaries and their permeability, important for the protection of the brain from harmful substances (Risau W., 1994).

3.2.2. Secretory and other immune systems of the brain of embryos and fetuses

Numbers of examined embryos and fetuses are listed in Table 3.1. Applied methods are presented in Chapter 4.2. The first immune defense mechanism in the forming tubus neuralis is the SIS. Embryos at 3.5 - 4 weeks of development reveal a positive immunochemical staining of SC and J-chain throughout the neural tube. The staining for IgG is more significant, for IgA - is somewhat weaker, while absent for IgM. The SC staining is more intense for superficial neuroblast and for basal neuroepithelial cells (Fig. 3.1).

At 5 - 6 weeks SC staining in neuroblast significantly weakens, but remains at the narrowings of the neural tube. Staining of J-chain and immunoglobulins remain more or less evenly distributed in all areas. By the end of the first and early second trimester, SC disappears from significantly large areas. Some poor staining is only available at the narrowings of the tube and in the cells related to the forming eye, but J-chain and immunoglobulins are still preserved. Intensive immunostaining of SC, J-chain and immunoglobulins is observed in ventricular ependyma and in epithelium of the vascular plexus.

In the second and third trimesters in the forming medulla oblongata, cerebellar cortex and in cerebral hemispheres several groups of neurons appear. They contain no SC, but in the neuronal bodies and their dendrites, as well as in the neurons of spinal ganglia J-chain, IgA (a more intense staining), IgG, sometimes IgM are revealed. This is the beginning of formation of another immune reaction type - the intracellular accumulation of immunoglobulins with J-chain outside the SIS in the vital cells. In the epithelium of the vascular plexus and the ependyma of ventricles a complete set of proteins of the SIS, including the SC is preserved. Neuroglia cells do not contain components of the SIS.

Table 3.1. Number of embryos and fetuses examined

	Embryos			Fetuses				Total
Term of development	**3.5-4 weeks**	**5-8 weeks**	**All**	**9-12 weeks**	**Second trimester**	**Third trimester**	**All**	
Uninfected	3	15	18	6	12	5	23	41
Infected	3	12	15	4	8	10	22	37
Total	6	27	33	10	20	15	45	78

An important role in maintaining the normal state of brain, including its immune protection, is played by the cerebral ventricles and by their choroid plexus. The ventricles are formed in the following order: the fourth, the two laterals and the median (Dziegielewska KM, et al., 2001). The functions of ependyma, lining the ventricular

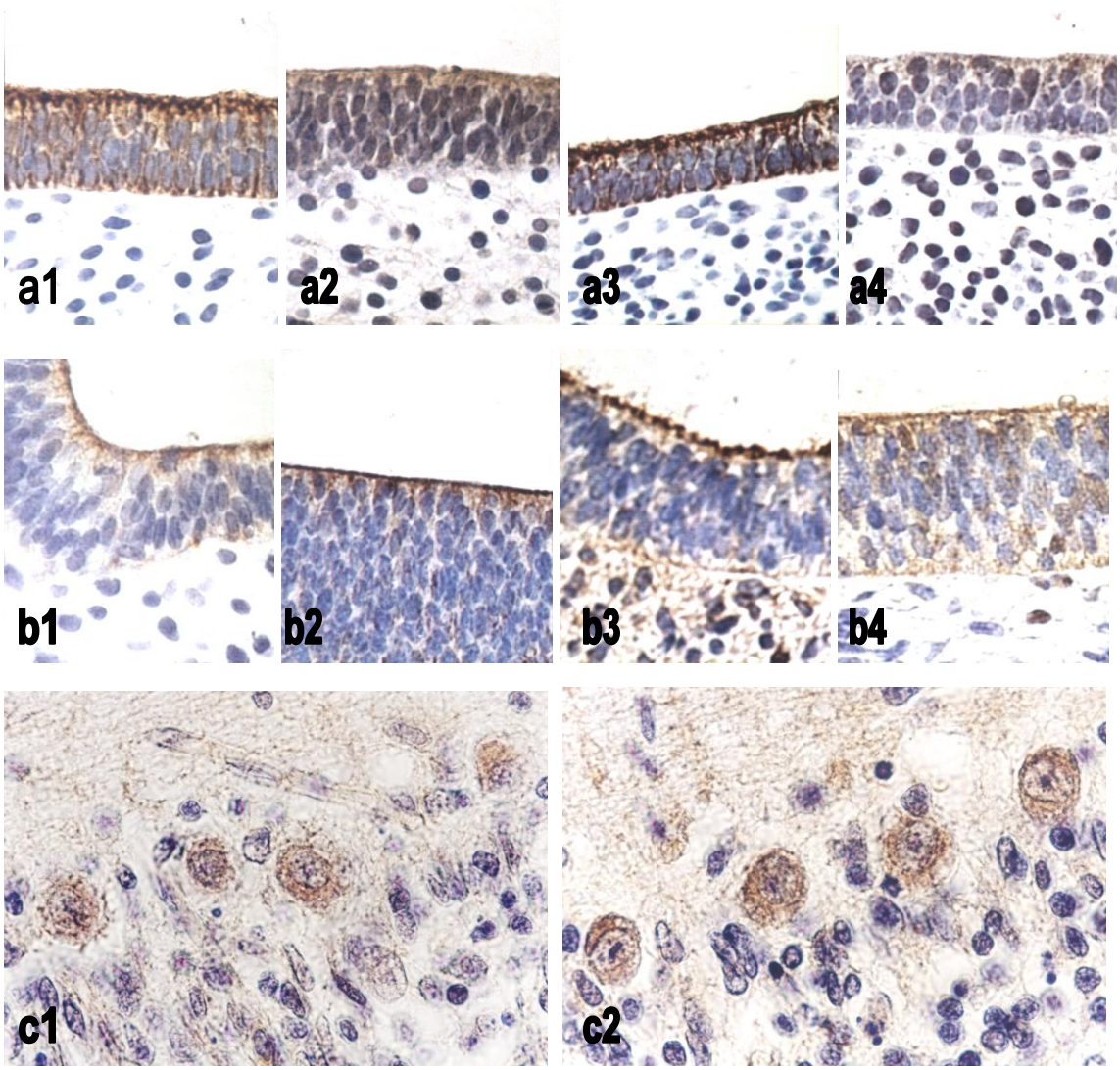


Fig. 3.1. Development of SIS of the neural structures. In all cases there is no infection. **A** - embryos at 3.5 - 4 weeks. Neural tube. The whole complex of neuroblasts: **a1** - SC (+), **a2** - J-chain, **a3** - JgG(+), **a4** - IgA (±). **B** - brain structures. 9 weeks of development. **b1** - the superficial layer of neuroblasts still exists: SC (+), **b2** - J-chain (+), **b3** - JgG (+), **b4** - IgA (±), while in the other layers - SC, J-chain and immunoglobulins (-) or (±). All the cases **a** and **b** are x400. **C** - the fetus at 22 weeks of development. Cerebellum: **c1**-J-chain (+) in Purkinje cells and their protrusions, **c2** - IgA(+). Cells of granular layer and glia cells: J-chain and IgA (-), as well as in all structures SC, J-chain and immunoglobulins (-). In all cases, **c** is x1000. The comparison of the state of nerve tissues at the later terms indicates the disappearance of SC form forming neurons and from some other types of brain cells.

walls and covering the choroid plexus, account for the transport of immunoglobulins and water to the cerebrospinal fluid. Maintenance of the proper water conditions is especially important to preserve the brain tissue. This is not only the physical preservation of delicate brain tissue in its bone case by a hydraulic cushioning. Cerebrospinal fluid contains complex of salts and very little of proteins, while at the second and third

trimesters the immunocompetent cells, including various types of lymphocytes, the elements of the overall immune system are also present. In the ventricular ependyma and in ependyma of vascular plexus a complete set of SIS receptors - SC, J-chain and immunoglobulins is preserved (Fig. 3.2).

In addition, the neurons themselves, forming the nuclei of the medulla oblongata, cerebellum and spinal ganglia and at later stages the neurons of other brain areas contain J-chain and immunoglobulins without SC. By that a different system of immune protection of parenchymal cells in the vital organs is formed.

Inflammatory processes at the embryonic period differ from the morphological development of inflammation in the fetus. This is due to the absence of immune cells (only some mononuclear phagocytes and single lymphocytes in the liver are observed). Overall immune system in fetuses begins its formation at the late first - early second trimester.

Inflammatory processes in fetuses of the second and third trimesters, which are more often caused by the ascending infection from the birth canal with a complication of chorioamnionitis (see Chapter 4.5, group 3A), are usually not complicated by inflammation of the brain and do not cause significant changes in the state of SC, J-chain and immunoglobulins in the ependyma of vascular plexus, in the walls of the ventricles and in the neurons of different ganglia. When ascending infection of the birth canal with hematogenous spread and development of meningoventriculitis occur (see Chapter 4.6, group 3B) a significant number of neutrophils, monocytes, and various types of lymphocytes of fetal origin appear in the cerebrospinal fluid, which is an indication of increased activity of the overall immune system of the fetus. In the ventricular ependyma and that of vascular plexus the immunochemical staining of SC and J-chain becomes weak, staining for IgG, IgA, IgM sometimes may be missing. Ependyma cells may be rejected. These changes are related to the SIS activity. In the ganglion neurons of the medulla oblongata and the neurons of cerebellum the J-chain staining is preserved. The staining of IgG, IgA and IgM is also maintained or somewhat weakened while the structure of neurons is not altered.

Neurons of the brain relate to the static population of cells: shortly after the birth the formation of new neurons is terminated, and regardless of the conditions in vivo they are not subject to recovery, proliferation or replacement (Ham AW, Cormark DH, 1983). An impression appears that the additional intracellular accumulation of immunoglobulins, except for the general and the secretory immune systems, is aimed at individual protecting of each of these so important cells.

3.3. Protection of the endocrine glands

Functions of the endocrine glands are extremely important during the prenatal period for normal development and survival of the fetus (Filiushkin IV et al., 1998; Harrell G., Murray P., 1998). Cytokines and, in particular interleukins (IL1 and IL4) have been found in the front and intermediate pituitary (Spangelo BL, Gorospe WG, 1995). These intracellular mediators are aimed to realize the relationship between

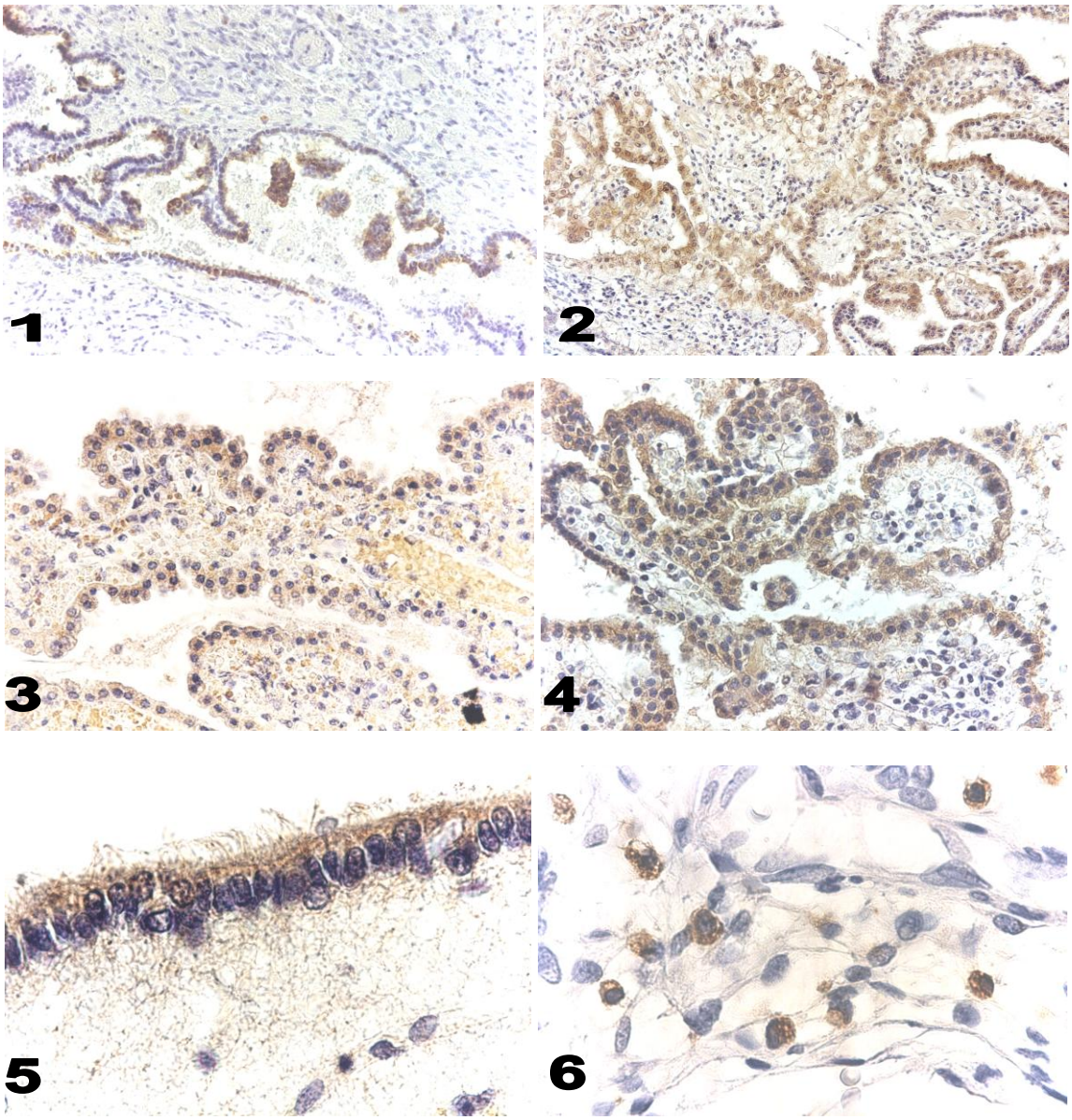


Fig.3.2. The vascular tissues of the ventricles of the brain retain the SIS with its complete structures - SC, J-chain and immunoglobulins. This is due to metabolism through the brain ventricles and their liquid. Fetuses 16 - 19 - 22 weeks old at their vascular plexuses in the ependyma contain: **1** - SC (+), **2** - J-chain, **3** - IgG (+), **4** - IgA (+), **5** - IgM (+ in some cases), **6** - in cases without inflammation, and allogeneic conflicts in the stroma there are monocytes. Magnification at 1, 2, 3, and 4: x400; at 5 and 6: x1000.

cells involved in protective responses, including immune and inflammatory ones. Gurevich P. et al. (2001a) have described the manifestations of immune protection in the parenchymal cells of human embryos and fetuses from 3.5 to 38 weeks of development in the pituitary gland, thyroid and parathyroid glands, in pancreatic islets and their predecessors.

3.3.1. Pituitary

In the epithelium of stomodeum, pharynx and gut tube of embryos, from which it most of the major endocrine glands are formed, SC, J-chain and IgG antibodies are found in 92-98% of all cells. Then throughout the second and third trimesters in the listed organs, preserving their structure and functions, the components of SIS are kept, even the content of IgA and IgM in them is inconsiderable, which manifests itself in a weaker immune staining.

All the mentioned protein components of the SIS are also contained in the Rathke's pocket and in the epithelium of pharynx, from which it has grown. When the upper part of the Rathke's pocket reaches sella turcica and begins to relocate itself to the front pituitary, its clearance disappears by forming clusters of parenchymal cells, separated by stromal cells and capillaries. Gradually in the emerging pituitary gland, the number of cells containing SC decreases: at 8-9 weeks of development SC is detected in 60.5 ± 7.2% of parenchymal cells; in the second trimester - in 19.0 ± 2.9%, while in the third trimester - only in 5.5 ± 0.8% (in all three groups the differences are highly significant: $p < 0.001$) (Fig. 3.3). Here at, the intensity of immunochemical staining of J-chain of IgG, IgA, and after 10 weeks and in the second and third trimesters - also IgM, in not just persists, but increases in the parenchymal (non-stromal) cells. In the cells of neurohypophysis SC is not revealed, but the J-chain and immunoglobulins show weak immunostaining. Intermediate pituitary contains several small glands lined by cubical epithelium, in which SC, J-chain and immunoglobulins are identified. This segment of pituitary is regarded as a rudiment without any endocrine activity, but it contains the interleukins IL1 and IL6, and possibly plays a role of intercellular mediators' producer.

3.3.2. Thyroid gland

The thyroid gland is laid at the end of 4^{th} week of development using the epithelium of ductus thyroglossus in the form of two protrusions. By 7^{th} weeks they shape two acini connected by an isthmus. At 10 - 11 weeks they are composed of trabecular and alveolar epithelium groups, 85-97% of which contains SC, J-chain and immunoglobulins. After the 11^{th} week follicles are formed thick with colloid and lined with a single layer of thyreocytes. Thyreocytes for the rest of the fetal period will contain SC, J-chain and immunoglobulins (Fig. 3.4). Thus, the thyroid is an exception among the major endocrine glands: in its cells, as in the cells of the intermediate pituitary glands, SC does not disappear. This is due to some specialties of thyroglobulin transportation: thyrocytes secrete it into the colloid, where it matures and then is reabsorbed into thyrocytes and is excreted from their basal side to the capillaries (Kirsten D., 2000). In colloid SC and J-chain are absent, and the staining of immunoglobulins in it is more intense than in the thyrocytes.

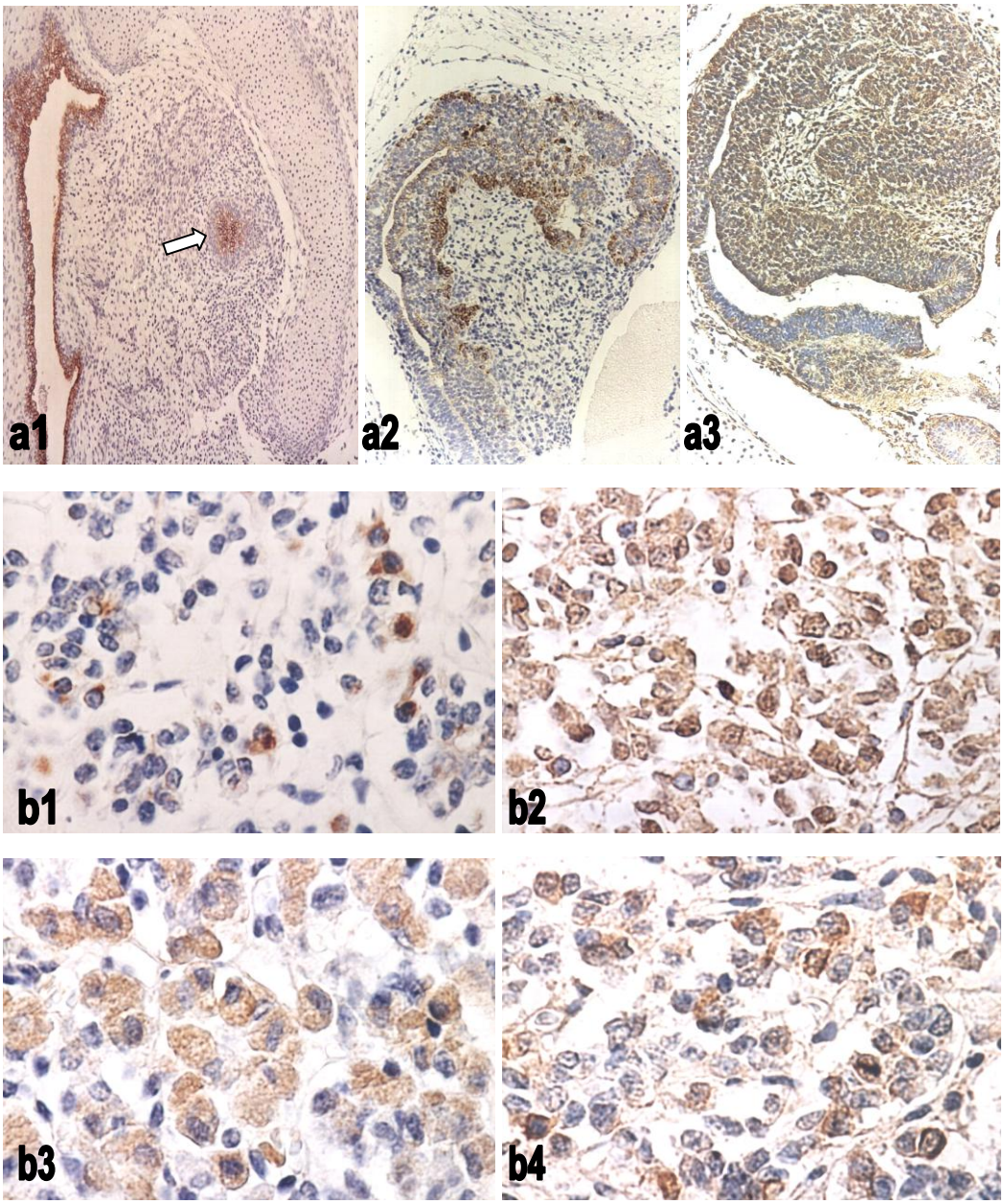


Fig.3.3. The formation of the adenohypophysis. A - the second half of the first trimester (7 weeks): **a1** - in the sella turcica of the skull the pharyngeal Rathke's pocket, the front part of the adenohypophysis (arrow) is formed, almost all of its cells are SC (+), **a2** - 10 weeks, the number of SC (+) cells is significantly decreased, **a3** - J-chain is already contained in almost all parenchymal cells. B - by the end of the second period: **b1** - there are only a few SC (+) cells, **b2, b3, b4** - parenchymal cells containing J-chain, IgG and IgA. Magnification of a1 x100, a2 and a3 - x200, b1, b2, b3, b4 - x1000.

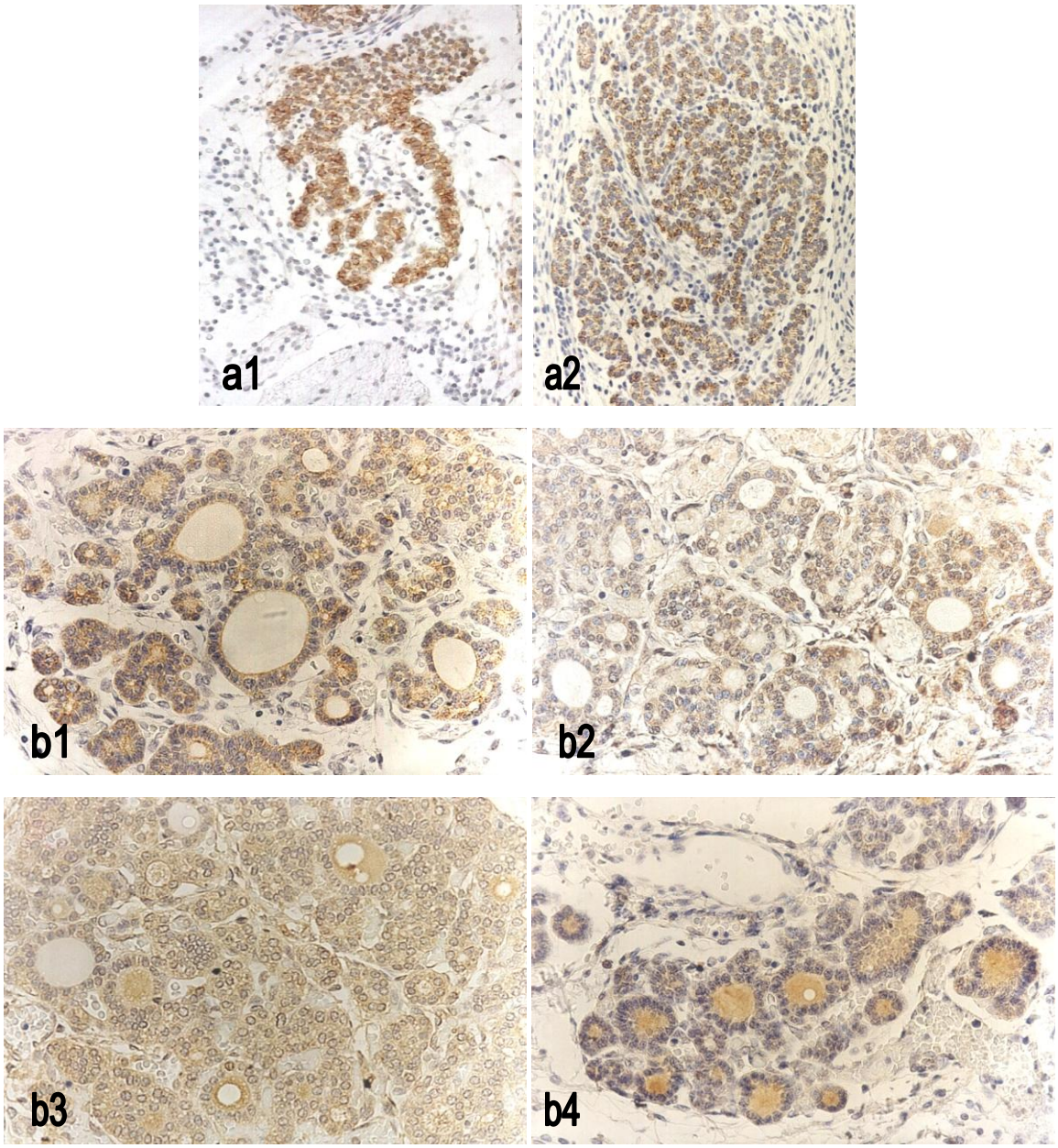


Fig..3.4. A - thyroid gland in its development, **a1** - one of the two lobules, 5 weeks of development. Most of cells are SC (+), **a2** - same at 10 weeks. All parenchymal cells are SC (+). Magnification is x200. B - thyroid at 15 weeks, **b1** - SC (±) glandular epithelium. Colloid of small glands is SC (±), and of the big ones - SC (-), **b2** - J-chain (+) glandular epithelium, most of the colloid is (-), **b3** - IgG (+) epithelium of glands, and (+) most of the colloid, **b4** - IgM (+) colloid, (±) epithelium. Magnification is x400. The change in the content of glands in the colloid and less in the epithelium is a result of its secretion.

3.3.3. Parathyroid glands

These small glands we have seen as the strands of epithelium at the fifth week of development. Their epithelium contains SC, J-chain, IgG and IgA in all cells. In this

epithelium of fetuses at the middle of the second trimester the J-chain and immunoglobulins is identified. The weak immunostaining of SC is noted in 39.9% of the cases in main brighter cells.

3.3.4. Pancreatic islets

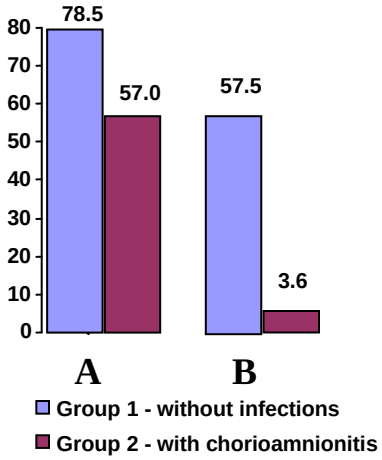
Pancreas, its exocrine part, by the 4 weeks of development represents by itself a group of lobules. Their epithelium at the early embryonic period and then in the second and third trimesters contains SC, J-chain, IgG, IgA, and less, IgM. The endocrine part of the gland consists of Langerhans islets, which are formed from cells of the exocrine part. At the beginning of the second trimester they contain J-chain and immunoglobulins, and their immunostaining is more intense than in the adjacent epithelium of the exocrine acini and ducts. SC in the islets is detected in single cells, and as early as at 18th week

Table 3.2. The content of immunoglobulins in the cells of pancreatic acini and islets of Langerhans with and without infection in fetuses from the end of II to III trimester (% of total cells)

Immunoglobulins	Cases with infection (in the islets and acini are the same), %	Cases with infection	
		% of islet cells	% of acini and duct cells
IgG	83.9 ± 7.3	72.7 ± 6.9	5.2 ± 1.7 [a b]
IgA	54.3 ± 5.1	42.9 ± 3.9	1.3 ± 0.6 [a b]
IgM	37.5 ± 3.4	22.8 ± 2.8 [a]	2.4 ± 1.1 [a b]

[a, b] - significant difference (p <0.01 - 0.001) in number (%) of cells containing immunoglobulins

Diagram 3.1. The number of cells containing immunoglobulins in the structures of pancreas: hormonal cells of Langerhans islets (A) and the epithelium of acini and ducts (B) in normal conditions and during infections in fetuses (%)



of development it disappears from these cells. J-chain and immunoglobulins of the islets are preserved even in a case of infections when they almost completely disappear from the epithelium of acini and tubules (Table 3.2, Diagram 3.1, and Fig. 3.5).

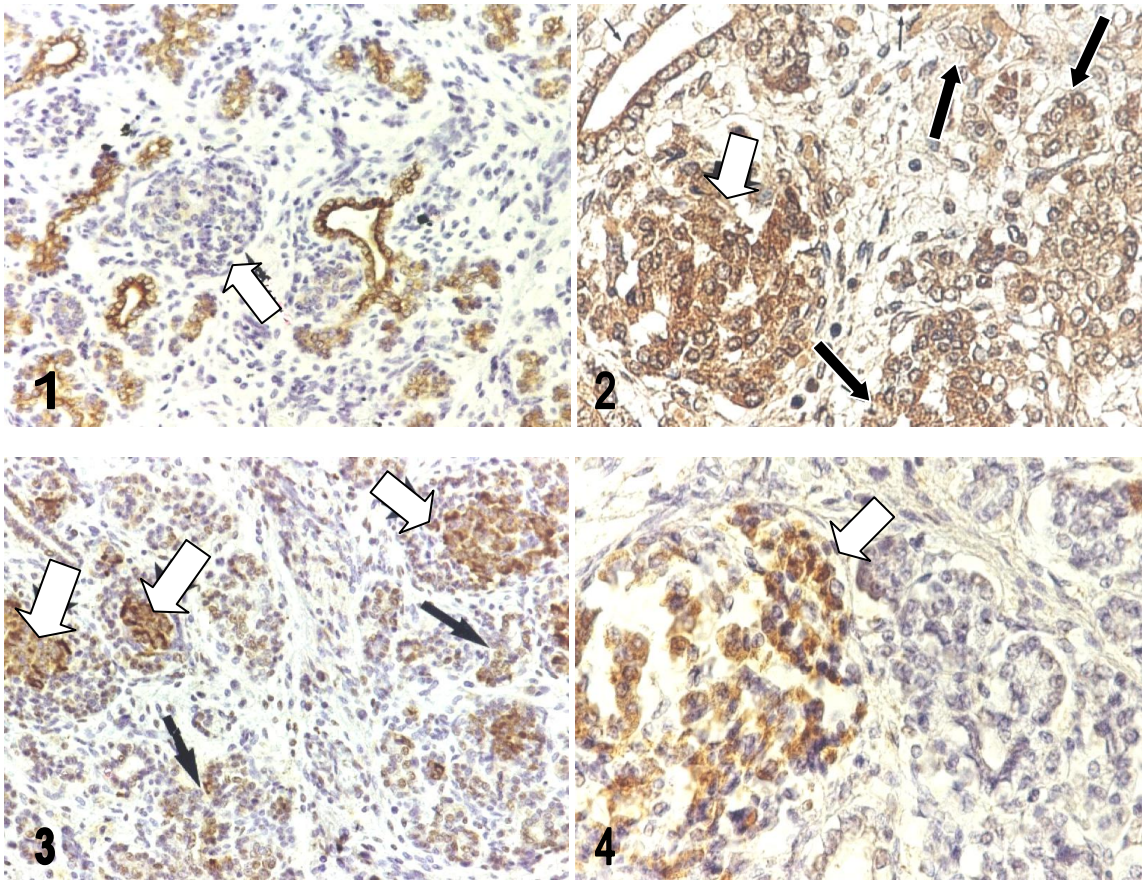


Fig.3.5. The pancreas at 18 - 23 weeks. With lighter arrows islets of Langerhans are marked, with darker arrows - epithelium of acini and ducts. **1** - normal state: SC (-) islets of Langerhans, SC (+) acini and ducts. **2** - the same case: J-chain, IgA, IgG and IgM (+) cells in the islets, acini and ducts. **3** - moderate inflammation (Group 3A): J-chain, IgG, IgA and IgM (+) remain in the islets, but they are few in the epithelium of acini and ducts. **4** - severe inflammation (Group 3B): the cell of islets, J-chain and immunoglobulins (+), in the acini and ducts these components are found only in single cells. Magnification: at 1 and 3 - x200, at 2 and 4 - x400.

3.3.5. Adrenals

They are formed at 4-5 weeks of development as clusters of large cells related to coelomic epithelium of mesodermal origin. The coelomic epithelium itself, the gonadal ridges derived from it and the future adrenal cells located next to it contain SC, J-chain and immunoglobulins. Cells proliferate rapidly and form the two zones of the adrenal cortex: the definitive zone (under the capsule) and the embryonic one, later becoming fetal (located deeper). Already in the embryonic period SC is not found, while only J-chain, IgG and IgA (see Fig. 5.3.1). At the end of the second and at third trimesters the cells of fetal cortex reveal weak positive immunochemical reactions for J-chain, IgG, IgA and sometimes IgM. SC is absent.

Table 3.3. The average number of B-lymphocytes containing immunoglobulins in the stroma of the endocrine glands (per 50000 μm^2 of the sample area area)

Organs	IgG	IgA	IgM	IgG	IgA	IgM
	Group - without pathogenic effects					
	I trimester			II-III trimesters		
Pituitary	-	-	-	0.3±0.2	0.2±0.2	0.6±0.2 [a]
Thyroid	-	-	-	0.3±0.2	0.1±0.1	0.5±0.2 [a]
Pancreas	-	-	-	0.8±0.4	0.5±0.2 [a]	1.5±0.5 [a]
Adrenal glands	-	-	-	0.2±0.2	0.1±0.1	0.3±0.2
	Group with infections or allogeneic conflict					
Pituitary	sporadic	-	sporadic	0.1±0.1	-	0.1±0.1 [b]
Thyroid	sporadic	-	sporadic	0.2±0.1	0.1±0.1	0.2±0.1
Pancreas	sporadic	0.2±0.2	0.8±0.2 [b]	0.7±0.3	0.4±0.2	0.7±0.3
Adrenal glands	sporadic	-	0.3±0.2	-	-	-

[a] - significant differences of the same group in the first trimester, p <0.05-0.001
[b] - significant differences of group 1 in the same trimester, p <0.05-0.001
Explanations are given in the Table 3.4.

3.3.6. Three immune systems in the endocrine glands

In the immune defense of the endocrine glands during the prenatal human development all three types of immune system are involved. First enters the SIS: it begins functioning already before the 4th week in the precursors of the endocrine glands. The sign if SIS functioning is a significant reduction in amount of immunoglobulins or their complete release in cases such as inflammation, to neutralize extracellular pathogens. That is noted in the pituitary gland in meningoencephalitis, but to a lesser extent. In other endocrine glands during inflammatory processes around their area the disappearance of immunoglobulins in the parenchymal cells is insignificant or absent. The reason for this is that in the process of organogenesis at 5-8 weeks of development another defense system is formed: the individual defense system of parenchymal cells, particularly of endocrine glands in the given case. Mainly this system is formed by the restructuring of the SIS. The meaning of restructuring is in elimination of SC and thereby in the cessation of exocytosis of immunoglobulins and in their accumulation in parenchymal cells that carried out by keeping J-chain, fulfilling the function of endocytosis of immunoglobulins. This is particularly evident in the pancreas, where under infections in the gastrointestinal tract the immunoglobulins disappear from the cells of acini and ductal epithelium (the secretory system zone),

while remain at the same or slightly decreased amount in the Langerhans islets (zone of the individual protection system) (see Fig. 3.1, Table 3.2).

Formation of the overall immune system begins from 8-10 weeks of development, when the first lymphocytes appear, including B-lymphocytes (CD20 +, CD79A +). We could not detect the transformation of B-cells into plasma cells (CD20-, CD79A +) during intrauterine fetal life, even under infection. In endocrine glands, the presence of overall immune system is revealed by presence of moderate number of T-lymphocytes (CD3), T-suppressors (CD8) and B-lymphocytes referred above (see Table 3.4). Much earlier, with the beginning of the function of yolk sac monocytes appear, while T-helper cells (CD4) appear later and show weaker response to pathogenic influences than the other types of lymphocytes. The highest activity of component of the overall immune system is observed in the pancreas, which is situated near the intestine: the areas of high probability pathogenic influences. In contrast, the smallest reaction occurs in both two groups in the adrenal glands.

3.4. Immune protection of the myocardium of embryos and fetuses

The heart is laid from the mesenchyme at the third week of development. At the beginning of the fourth week in a two-chamber heart three layers of its wall are separated: epicardium, myocardium and endocardium. The mesothelium of the coelom at this time contains transport components of SIS - SC, J-chain, IgG and IgA, which are also identified in the epicardium. Myocardium and endocardium do not contain SC, but there is J-chain as well as immunoglobulin inside them (Fig. 3.6). With the formation of the endocardium, J-chain and immunoglobulins also appear in it. These states are saved in the first, second and third trimesters, although somewhat weaker immunostaining of immunoglobulins is seen, and sometimes the presence of IgM is noted. Even in cases of significant inflammation no major changes in immunostaining of J-chain and immunoglobulins in the myocardium and endocardium is observed. After 9-10 weeks of development some isolated lymphocytes (CD3 (+) and CD20 (+)) and monocytes (CD68 (+)) at 1.02 ± 0.02 per 50000 μm2 of the section area of the myocardium appear.

The above data indicate the presence of a small number of elements of the overall immune system as lymphocytes and monocytes in the tissues of the heart. Presence in the epicardium and pericardium of SC, J-chain and immunoglobulins can refer them to the barrier type of SIS. In the myocardium and endocardium, the presence of J-chain and immunoglobulins demonstrate a complete system of the self-immune protection.

Table 3.4. The mean number of immunocompetent cells in the stroma of the endocrine glands (per 50000 μm^2 of the sample area)

Organs	CD68	CD3	CD4	CD8	CD20	CD68	CD3	CD4	CD8	CD20
	Group without pathogenic effects									
	I trimester					II-III trimesters				
Pituitary gland	1.3±0.4	sporadic	-	-	-	3.4±0.8 [a]	1.5±0.4 [a]	sporadic	1.6±0.5 [a]	0.5±02[a]
Thyroid	1.5±0.4	sporadic	-	-	sporadic	3.1±0.9 [a]	2.1±0.8 [a]	sporadic	1.9±0.6 [a]	0.9±0.3 [a]
Pancreas	3.8±0.7	sporadic	-	sporadic	sporadic	6.8±1.1[a]	3.3±1.6 [a]	0.2±0.1	3.8±1.3 [a]	0.7±0.3 [a]
Adrenals	1.9±0.6	sporadic	-	sporadic	sporadic	2.1±0.6	0.8±0.3 [a]	sporadic	0.7±0.2 [a]	0.1±0.1
	Group with infection or allogeneic conflict									
Pituitary gland	3.2±0.7[b]	0.8±0.3[b]	sporadic	0.9±0.5	0.3±0.2	5.4±1.2	1.1±0.4	0.1±0.1	0.8±0.3	0.1±0.1
Thyroid	2.2±0.6	0.9±0.4[b]	sporadic	0.7±0.3[b]	0.3±0.2	9.2±1.6 [a,b]	1.6±0.6	0.3±0.2	1.2±0.4	0.4±0.2
Pancreas	4.9±1.1	2.1±0.8[b]	sporadic	1.8±0.8[b]	0.8±0.4	12.9±2.2[a,b]	2.8±1.1	1.0±0.4 [a]	2.1±1.2	2.2±0.9
Adrenals	1.1±0.4	0.7±0.3[b]	sporadic	0.7±0.2[b]	0.3±0.2	3.4±1.0[a]	0.2±0.2	0.1±0.1	0.1±0.1	0.1±0.1

[a] - significance of difference from the same group at the first trimester: p <0.05-0.001
[b] - significance of differences in the group 2 at the same trimester as compared with group 1: p <0.05-0.001

Lymphocytes of the Group without infection at I trimester are in initial development. Pathogenic influences in the I trimester cause their accelerated development. Lymphocytes of II-III trimesters are moderately developed (except for T-helper cells, CD4). But in the pathogenic group, the reaction of lymphocytes tends to decrease which is a sign of failure.

Monocytes have been developed earlier and more extensively, they are not signs of failure in pathogenic Group of I and II-III trimesters.

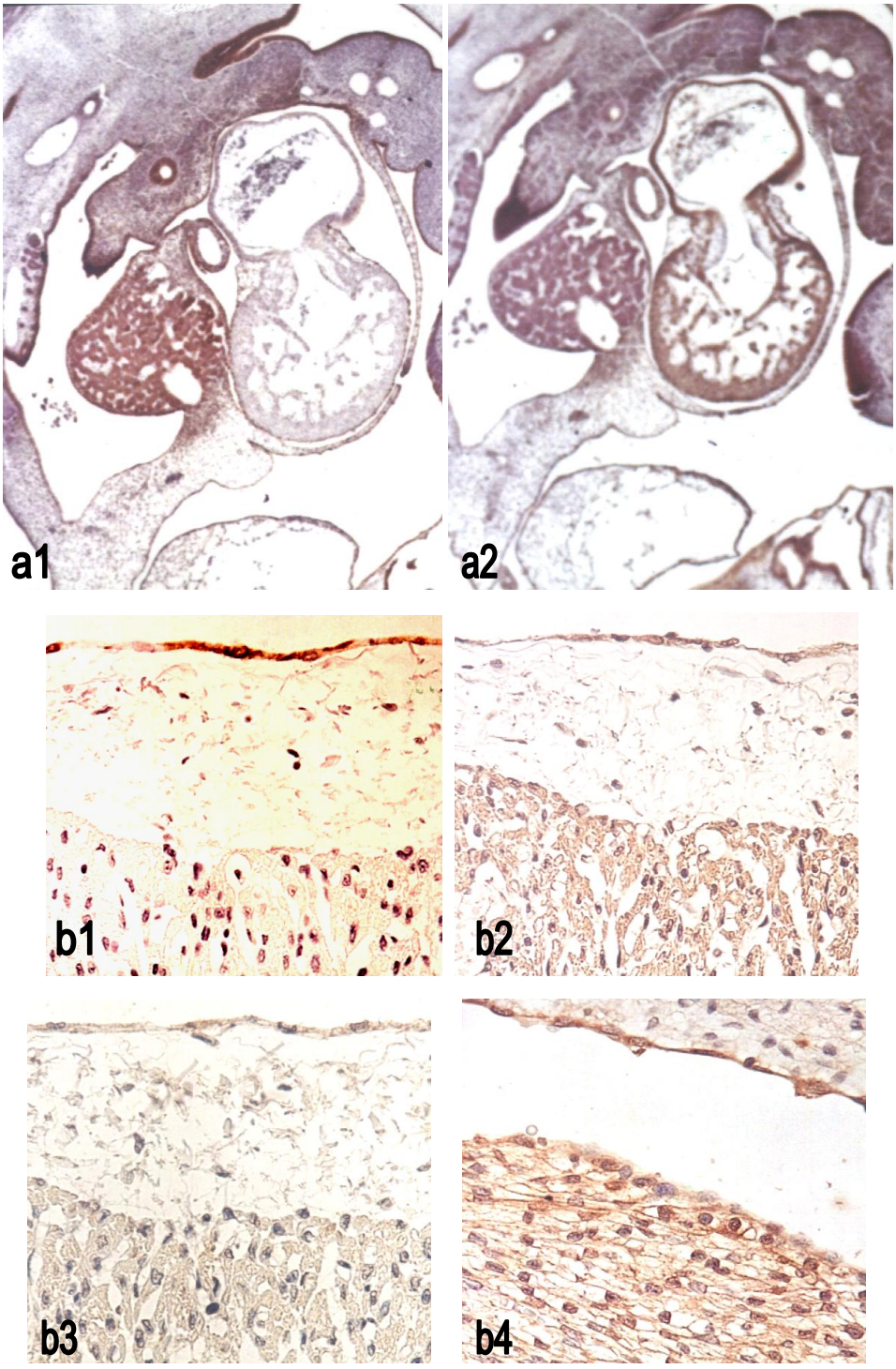


Fig. 3.6. Organs of the embryo. 4 weeks of development. Widespread SC. **a1** - SC (+) epicardium and pericardium, bronchial and gut epithelium, gonadal cushion, hepatocytes, tubules of mesonephros, mesothelium, SC (-) myocardium; **a2** - the same embryo: J-chain (+) for all of mentioned organs and formations, but J-chain (+) myocardium and endocardium; x40. **b1** - SC (+) epicardium, SC (-) myocardium, **b2** - J-chain (+) epicardium and myocardium, **b3** - IgA (+) epicardium and myocardium, **b4** - IgG (+) myocardium and endothelium of the valve; x400.

3.5. Immune protection of the bile ducts and the hepatocytes

The liver is laid in the middle of the third week in the form of outgrowth of intestine epithelium. Cells of the upper part of the outgrowth, especially at 4-6 weeks, of proliferate intensively turning into hepatocytes and form the liver, while from the bottom of the outgrowth the bile ducts and the bladder are formed. Like all derivatives of the epithelium, initially they include the intensely colored components of the SIS - SC, J-chain and immunoglobulins. But by the middle of the second trimester the intense staining of SC remains only in the epithelium of the bile duct, bladder, and in hepatocytes at the periphery of lobules. To the center of lobules SC in hepatocytes is almost completely lost, but the J-chain and immunoglobulins are preserved. From 5-8 weeks in the liver lobules some immunocompetent cells appear, including pro-CD3(+) T-lymphocytes and pro-CD20 (+) B-lymphocytes, as well as intensive synthesis of erythroblasts and mononuclear phagocytes is deployed . Thus in the liver all three systems of immune defense are getting in contact.

3.6. Protection of the developing reproductive tracts, gonads and gametes

Various non-immune and immune systems are involved in the protection of gametes during their development. Cytokines regulate both the development and the regression of the ovaries (Bukovsky A., 2006). They are related to the development of oocytes and the formation of follicles in the ovaries of the human fetus ant to the functions of the corpus luteum. In the ovaries some lymphocytes are found (Suzuki T., et al., 1998). Macrophages are involved in immune reactions in the gonads. (Cooper T.G., 1999).

3.6.1. Formation of immune systems of genitals

Precursors of germinal cells are formed in the yolk sac from the extragerminal endoderm. At 5-6 weeks of development, they migrate to the gonadal ridges, which are located in the body cavity of the embryo to medial site of mesonephros. Next to each gonadal ridge the two pairs of channels are situated - to the left and right. Two external channels extending from the pronephros are the paramesonephric ducts of Muller; subsequently they are transformed into the female extragonadal genital organs - two fallopian tubes, which are merged in the caudal part, forming the uterus and upper vagina. Two internal channels - mesonephric ducts of Wolff, extend from the mesonephros. Later on they serve the basis of male epididymis and vas deferens. With the development of male sexual organs, the Muller ducts atrophy, while with the formation of female organs - the ducts of Wolff atrophy. Ductal Muller and Wolff epithelium already before the transformation into a reproductive tract contain components of the SIS - SC, J-chain and immunoglobulins. In addition to the SIS, at later stages in the retroperitoneal tissue a congestion of lymphocytes is formed, being transformed then into the lymph nodes, the plasma vessels, which are the components of the main immune system. In that way in the genital area different immune systems are formed.

3.6.2. Formation of immune protection of female gametes

Although the sex of an embryo genetically determined already during fertilization, the transformation of the gonads into ovaries or testes is happening only at 8th week of development. Prior to that, the surface mesothelium of gonadal ridges already contains SC, J-chain and immunoglobulins. Mesothelium cells penetrate into the future ovary and proliferate intensely. By the approach of primordial oocyte, they are transformed into follicular cells. This process happens at the end of the I trimester. Follicular cells contain SC, J-chain and IgG and to a lesser extent IgA and IgM. Oocyte are surrounded by the follicular cells contain J-chain, IgG, and less - IgA and IgM; SC is missing. It that way the primordial follicles are formed. At 19th weeks the egg cells divide, they have no SC, but contain a J-chain and immunoglobulins (Fig. 3.7.)

By the seventh month of fetal development about 10 million primordial follicles are already formed, each containing one egg cell. Reproduction of oocytes at this time is terminated. Much of them are dying, so that to the birth of a girl their number is about 2 millions, to puberty - about 400,000; the stage of primary and secondary follicles is reached by only two thousands, and the stage of the Graafian follicles (mature) and ovulation - only about 400 for all sexually life of a women (Ham AW, Corm DM, 1983). What causes such wastefulness of nature is unclear. Although the consumptions of sperm cells are much higher: 1 ml of semen contains more than 100 million of them.

During the formation of female genital tract (fallopian tubes, uterus and upper vagina) from Muller ducts, and the organization of the lower part of vagina and external genitalia from the epithelium of the skin, the protein components of the SIS - SC, J-chain, IgG, IgA and to the lesser extent, IgM are kept.

The stroma of the gonads of the embryos contains monocytes (3.9 ± 0.6 per 50000 μm^2 of a slice), a small number of lymphocytes CD3, CD4, CD8, CD20. Pelvic lymph nodes related to the main immune system, are formed in the second trimester of development.

3.6.3. Formation of immune protection of male gametes

Formed from Wolff mesonephric duct, male genital tract, beginning from the rete testis and further - to the vas deferens, in its epithelium inherits the transport complex of SIS - SC, J-chain, and immunoglobulins. In the testes the immune protection is organized differently form the ovaries. SC, except the rete testis at the inlet, is absent from all other cells of the testes. In the epithelium of the straight and convoluted tubules all components of the SIS - SC, J-chain and immunoglobulins are completely absent. Protection of each gamete through the spermatogenic series is impossible: they are too numerous and small in size. The main line of defense is the Leydig cells (Fig. 3.8). By a wide zone they surround the seminiferous tubules; their cytoplasm contains a large amount of J-chain, IgG, slightly smaller amount of IgA and IgM. Sertoli cells in tubules get a weak staining, and the spermatogenic cells - very weak. Thus, the cells of Leydig, and Sertoli, by a broad layer protect the whole mass of spermatogenic series of cells, which are filling the straight and convoluted seminiferous tubules. This is not the SIS, but another system of immune defense. Its function - using J-chain and mass of Leydig and Sertoli cells create a shaft of different immunoglobulins around the mass of sperm cells and their precursors. In the embryonic period these are the immunoglobulins of a pregnant. Stroma of the testes of embryos contains 3.6 ± 0.5 monocytes per 50000 μm^2 of section; after 9 weeks of development some isolated lymphocytes appear.

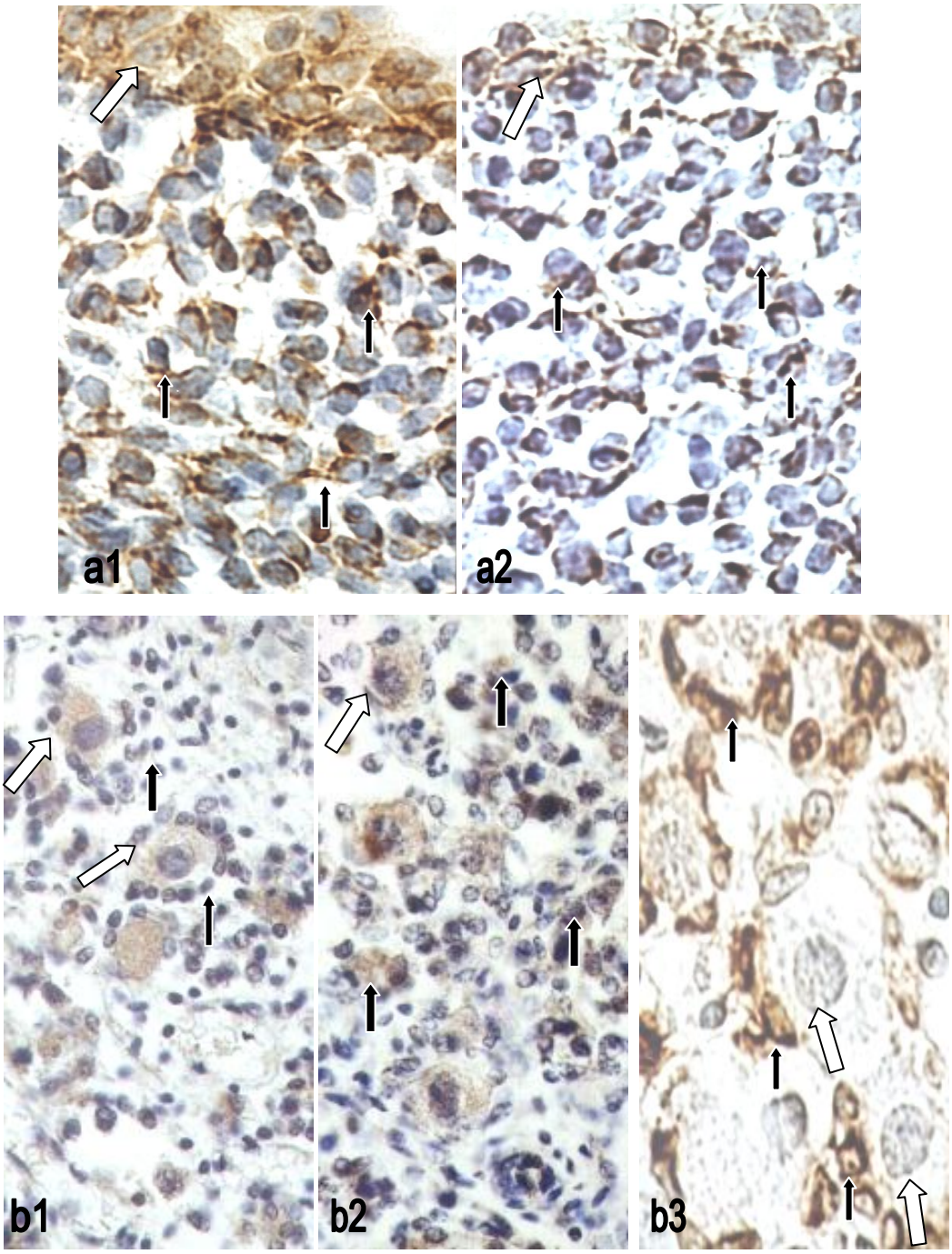


Fig. 3.7. A - gonadal ridge, 5 - 6 weeks of development: **a1** - mesothelium (white arrows) and stromal cells (black arrows) SC (+), **a2** - the same, J-chain (+), immunoglobulins (±). Magnification is x1000. B - ovary: **b1**-19th week. Oocytes (open arrows) are small in number, they contain J-chain (+). Follicular cells (black arrow) J-chain (±), **b2** - the same case, in eggs IgG (+) (light arrow), in some follicular cells the IgG (+) (black arrows). Magnification is x400; **b3** - ovary, 23 weeks. Number of eggs increases, they are SC (-), follicular cells are SC (+) (black arrows). Magnification is x1000

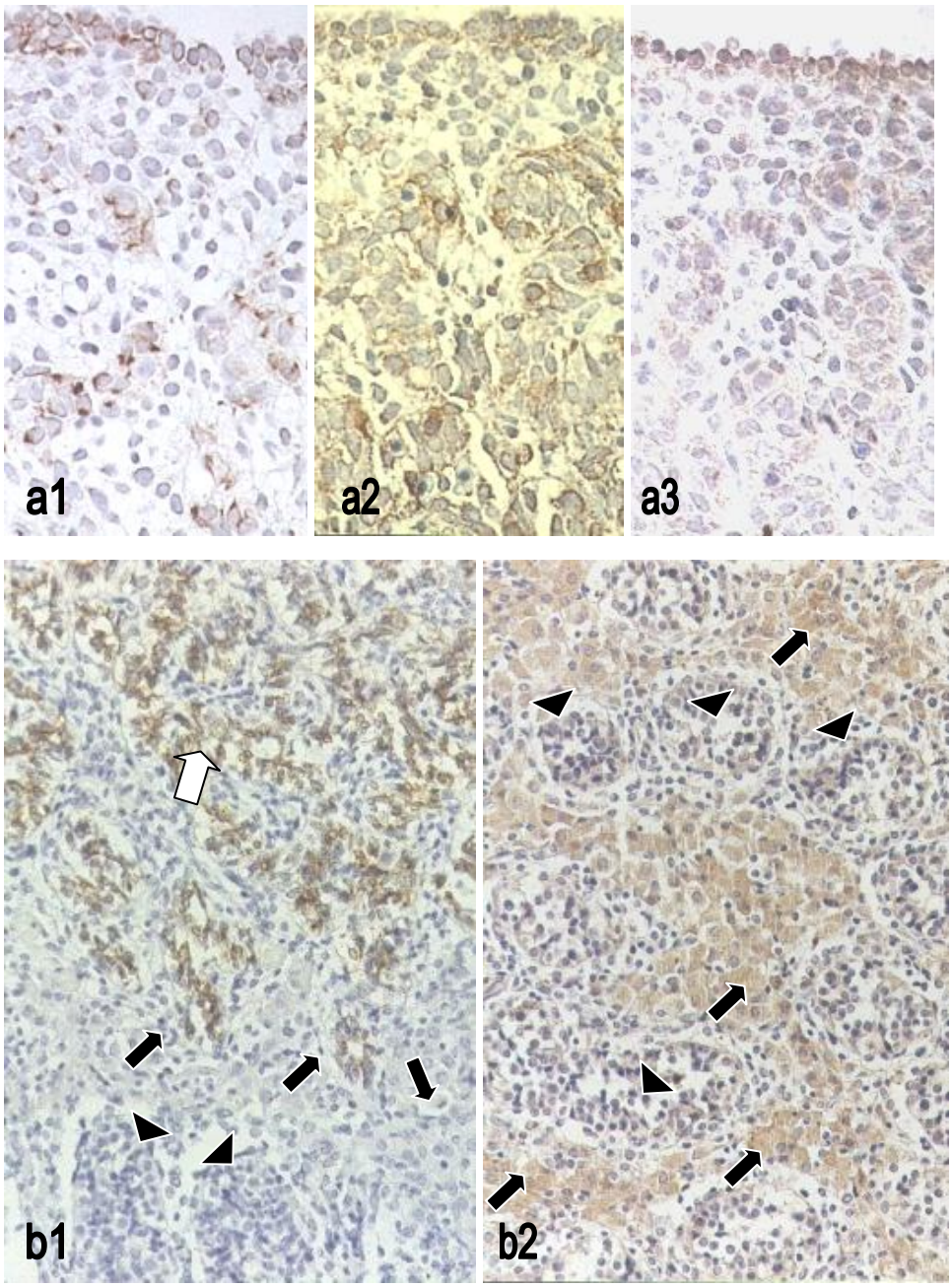


Fig. 3.8. Development of immune systems of male genital organs. A - 8th weeks of development: **a1** - in gonadal bands seminiferous tubules are formed, in which diminishes and then disappears SC, but remain J-chain **(a2)** and immunoglobulins **(a3)**, particularly IgA (+). Images in A are x400. B - 16th - 19th weeks of development: **b1** - the network of testicle - rete testis is developed (light arrow). This is a large number of small ducts, which bind to the efferent tubules and on, with the vas deferens. This entire system, including the network of testicle contains SC, J-chain and immunoglobulins, and, therefore, is considered as SIS. **b2** - in the ovary there are large clumps of Leydig cells (black arrows). They surround the seminiferous tubules (▲), and under their capsules of them the Sertoli cells are found. Both types of these cells do not contain SC, but have a set of J-chain and immunoglobulins in large amount. Images in B are x200.

3.6.4. Immune response to infection of the gonads

Development of inflammatory processes in fetuses of the second trimester, often with chorioamnionitis, extending to the gastro-intestinal tract, differently affects the gonads. Even with the development of the generalized process the staining intensity of SC and J-chain does not change, for IgG and IgA, the staining decreases slightly if at all, IgM usually shows a moderate stain. Do not become different the immune staining of J-chain and immunoglobulins in the cells of Leydig. In the ovaries the immune staining of SC, J-chain and immunoglobulins in the follicular cells and J-chain and immunoglobulins in the egg cells are unaffected. Thus, the individual immune protection of male and female gametes can be considered sufficient.

Number of monocytes in the gonads increases with a large spread: from 6.7 to 35.2 per 50000 μm^2 of the area of the specimen, which is 20.9 ± 7.6 per 50000 μm^2 of sections, in average (compared to the fruits without infection, $p < 0.001$). The number of lymphocytes changes only slightly.

3.7. Self-protective immune system of the vital cells and organs

Among the states of the immune protection of human embryos and fetuses, discussed in this Chapter and along with the SIS and the main immune system there are changes that are not included in their scope. SIS appears first in ontogeny and is already functioning prior to the embryonic period. It protects the organ systems: digestive, respiratory, urinary, and individual organs: eyes and mammary gland which are associated with the external environment and therefore will be subject to intense attacks of microorganisms, foreign antigens and other pathogens. For SIS the transport receptors are typical: polyimmunoglobulin receptor, also known as secretory component (SC) and connecting (joining) - J-chain. They provide the transport of immunoglobulin antibodies on the mucosal surfaces through the epithelium, so the SIS is sometimes called a system of mucous membranes (mucosal immune system).

Barrier immune system is a version of the SIS. Its function is in the transport of immunoglobulins through the interstitial structures, such as the placenta barrier, choroid plexus of the ventricles of the brain, serous membranes and other tissue-tissue communications. Receptors of the barrier system, transporting immunoglobulins - SC and J-chain are the same as in the mucosal immune system. They begin to function along with the formation of a certain organ.

Main immune system protects the entire body. Its range of immune cells and organs is highly complex and largely distributed, forming from the beginning of the fetal period.

Our studies of the state of SIS during organogenesis revealed various changes in it. In the organs, which keep their mucous membranes, the excretory gland or new barriers of communication have been formed; SC, J-chain and immunoglobulins are found. Another option: neuroblasts of the neural tube contain SC, J-chain and immunoglobulins. Neuroblasts serve an origin for glial cells and neurons, but in the glial cells the whole complex of SIS completely disappears, while in neurons only SC is gone

but J-chain and immunoglobulins are preserved. Similar changes occur during the formation of the anterior pituitary, in the parathyroid glands, in the islets of the pancreas and adrenal gland - in their predecessors SC, J-chain and immunoglobulins have been held, and in the newly formed organs SC is absent, but the J-chain and immunoglobulins are preserved. The third option: the myocardium, formed from splanchnotom, apparently has not contained the SC from the beginning as at 3.5 weeks of development any signs of SC are not found there while J-chain and immunoglobulins are available. Intricate constructions are found in the gonads. Moved from the yolk sac, female gametes meet follicular cells containing SC, J-chain and immunoglobulins. They surround each gamete with a dense ring. In the future eggs themselves there are no SC while J-chain and immunoglobulins are available. The male gametes future billions of sperm cells are protected even in more complicated way. Cells which containing SC, J-chain and immunoglobulins and, therefore, related to the SIS, are located in the rete testis, closing the entrance to the testicles. Further, around the straight and convoluted tubules the large clumps of Leydig cells are formed, whose cytoplasm is intensely stained with J-chain and immunoglobulins-sensitive reagents. This powerful barrier of antibodies protects fully each single tubule, filled with lots of small precursors of sperm cells. Sertoli cells, located inside the tubules and weaker stained under the processing with reagents sensitive to the J-chain and immunoglobulins, provide some extra protection.

Organs, whose parenchymatous cells contain J-chain and immunoglobulins, but not the SC, are rare. These are neurons of the brain and spinal ganglions, myocardium, gametes, cells of the main endocrine glands. There are two organs which should be referred to ones dealt with, although they contain the SC together with J-chain and immunoglobulins. These are the thyroid gland and the liver. Thyrocytes of thyroid contain this complex, but this is due to the peculiarities of its physiology - the need to withdraw the thyroglobulin in the colloid of follicles, enter it again, and repeatedly remove from thyrocytes to the adjacent capillaries. Hepatocytes of embryos contain SC, J-chain and immunoglobulins. But gradually the SC disappears in hepatocytes located in the central parts of lobules, and persists in the peripheral ones. This reflects the joint activity of the SIS and the individual immune system.

All of these organs have two features. They all are vital organs, their safety and proper functioning is necessary to preserve human life. Herewith they have no or very little ability to proliferate (by cell divisions) for their parenchymal cells, to repair or replacement of the victims. The regions of dead neurons in the brain, the areas of myocardial infarction and damaged places of the majority of endocrine glands are replaced only by connective tissue - by scar. Removal or destruction of the gonads does not seem to threaten human life, but the gonads provide a continuation of the family, the entire set of people. This allows us to legitimately relate the gonads to the strategically vital organs. Again and again one can marvel at the foresight of nature that has created the local immune defense mechanisms of the gene pool already at the embryonic period - from the earliest stages of formation of the gonads.

The question arises - where should be place the observed system of immune protection of individual cells? The main body's immune system is excluded, as it will emerge later while in the embryonic period it does not exist yet, its organs and the ways of functioning are different. Loss of the SC or the initial lack of it (in the gonads, perhaps in the myocardium) excludes the primary function of the SIS - the protection of major systems and organs from the constant attacks of a large number of different pathogens. It excludes immune protection of individual cells from the SIS.

The investigated here protection of each individual, but very important for the whole organism, cells suggests us to call it **the immune system of an individual cell protection in the vital organs of embryos and fetuses.** It is characterized by the presence of J-chains which is receptor for immunoglobulin influx into a cell. Immunoglobulins at the embryonic period are received from the pregnant mother body through the placenta and later on the main fetal immune system is activated through the blood, lymph or through the extracellular fluid. In all organs of the individual immune system: in the endocrine glands (Tables 3.2; 3.3), in the heart, liver and gonads, along with J-chain and immunoglobulins, from the beginning of the fetal period, it appears a limited number of different immune cells, including B-lymphocytes, which are able to synthesize immunoglobulins. In a case of inflammation the number of these cells is significantly increased. Perhaps these cells actually represent the cellular part of the individual immune system.

In the organs of individual immune system: in the zones of the ventricles of the brain, in pancreas, liver and, most clearly in the ovaries and testes there are manifestations of all three immune systems, operating together in a case of inflammatory processes (Tables 3.2; 3.3; Diagram 3.1). Around the ovary central immune system is presented by pelvic lymph nodes with a set of immunocompetent cells, including B-lymphocytes. Then the SIS in a form of SC, J-chain and immunoglobulins is found in follicular cells surrounding each egg cell. And in the oocyte itself, the individual immune system of vital cells and organs is presented by J-chain and immunoglobulins.

Chapter 4. Pathology of the placental barrier in early abortion

4.1. Placental barrier

Development, growth and the existence of the embryo depends on the surrounding it another organism - a pregnant woman. In order to carry out metabolism - to get what it needs and get rid of the waste - special structures are formed in the embryo - chorionic villi. Chorion is the first structure that comes into contact with the environment - with decidual tissue, blood of pregnant women in the lacunae, with various immunocompetent cells in these structures and with a variety of hormonal and other biologically active substances. Chorion and its derivatives - various types of trophoblast, are the first to come in defense of the germ and, unfortunately, the first to suffer defeat. Pathological processes at first develop in the chorion and the trophoblast, and in the embryo itself more often the dysfunction of the placental barrier are manifested.

Often the concept of "placental barrier" is rather simplified so that it refers only to tissue located between the blood of pregnant women in the gaps and fetal blood in the capillaries of the villi. These tissues of the barrier are - syncytiotrophoblast, cytotrophoblast, and their basal membrane, stroma of villi, the basal membrane and endothelium of the capillary. No mention is made of monocytes (Kashchenko-Hofbauer cells), which are often located between the trophoblast and villous capillaries, i.e., within the placental barrier. Little attention is paid to types of trophoblast - except syncytiotrophoblast and cytotrophoblast, to the groups of proliferating cells at the ends of the villi, to invasive trophoblast in the decidual tissue and to trophoblast lining the lacunae. All of mentioned are to some extent differ from each other on the functions and properties. Proliferating trophoblast is actively propagated from cytotrophoblast on villi, and then moves to the decidual tissue, which turns into invasive trophoblast. These cells, except for forming a blastocyst base, are involved in the restructuring of blood vessels, forming a cavity for the blood of pregnant women - the lacunae (Evain-Biron D., 2001), and perform the role of the endothelium of these vessels. They are capable of phagocytosis (Red-Horse K. et al., 2004). Continuations of the placental barrier are the chorion and amnion. Through them, for example, the immunoglobulins are transposed to the fetus (Cleveland MG et al., 1991), and urine with the waste is passed from the fetus to pregnant woman, for example, indirect bilirubin in hemolytic disease of the fetus (Gurevich P. et al., 1995).

On the other hand the body of a pregnant woman reacts to the appearance of antigens of the germ. Already during the implantation the macrophages are concentrated in the basal part of the placenta - a place of the contact with the germ, and in particular with its invasive trophoblast. Macrophages of a pregnant woman actively phagocytize (Bulmer JN, Johnson PM, 1984) and secrete tumor-necrotizing factor alpha - TNFα, also known as FasLigand (Chen HL et al., 1991; Todt JC et al., 1996), which can cause apoptosis in the trophoblast (Yui I. et al., 1996). It has been suggested that macrophages of decidual tissue phagocytize alloantigens of germ in a case of incompatibility (Abrahams VM et al., 2004).

During early pregnancy (week 5) and subsequently, throughout the decidual tissue there is a huge number of natural killer (NK, CD56 +), and T-lymphocytes (CD3 +) form groups. Other types of lymphocytes are scattered as single cells (Slukvin II et al., 2004). The reasons for the accumulation of so many NK and CD3 lymphocytes are unclear. The assumption that by this a cooperation of NK mother with invasive trophoblast in the process of restructuring the blood supply during the introduction of the germ into decidual tissue is manifested (Parham P., 2004) is not sufficiently substantiated.

Along with morphological changes, the implantation of the germ is accompanied by significant biochemical changes. Trophoblast releases membrane cofactor protein (MCP, CD45 +), decay acceleration factor (DAF, CD55 +) and protectin (CD59 +), which affect the components of complement (Weetman AP, 1999). Decidual tissue itself releases a wide variety of cytokines, also involved in the regulation of immune response (Saito S., 2000). These reactions, aimed primarily at reducing the potential for conflict of pregnant - fetus, actually reduce the overall immunoreactivity of the pregnant woman, making it more accessible to viral or bacterial infections.

In recent years, much attention is paid to preeclampsia. This is a heavy gestosis, which usually occurs in primigravidas - teenagers or in women over 35 years. Due to spasm of blood vessels the stroke, hypertension or proteinuria appear at once. In the pathology of preeclampsia much is unclear, but some disruptions caused by the invasive trophoblast are suggested (Genbacev O., et al., 1999; Reister F. et al., 1999, 2001; Burk MR et al., 2001; Gross JG, 2003; Cetin J., et al., 2004; Fisher SJ, 2004; Austgulen R., 2004).

Thrombosis of intervillous spaces with intrauterine growth retardation (IUGR) is a frequent cause of death of embryos and fetuses; 27-42% of early abortion is associated with this disorder (Beer AE, Kwak J., 2000; Сидельникова В.М., 2002; Viero S., et al., 2004). It is found in a variety of diseases of the mother: during connective tissue disease (Ackerman J., et al., 1999), in malaria (Crocker JP, et al., 2004), diabetes (Mayhew TM, Sampson C., 2003B), in smoking women (Mayhew TM, et al., 2003A; Vogt Isaksen C., 2004), women living in mountainous areas (Mayhew TM, et al., 2002) and for many other reasons (Cetin J., et al., 2004). Intervillous thrombosis frequently complicates the artificial donor insemination (Perni SC, et al., 2005). IUGR is associated with antiphospholipid Hughes syndrome - the thrombotic vasculopathy of different localization in adults, as well as IUGR at early (before 9 weeks) abortions, repeated in subsequent pregnancies. Such diversity of causes of IUGR indicates that it is not actually a defined disease but just a symptom of various diseases.

With IUGR, besides the thrombosis of intervillous spaces, there is considerable damage to the syncytio- and cytotrophoblast in the large areas or in the form of small foci (Милованов А.П and others, 2005). They are associated with the isolation of trophoblast villi with fibrin coming from the blood of pregnant women and with the termination of metabolism (Svensson AM, et al., 2004). It is also suggested that the primary stage is the damaging of the villi trophoblast and then at the damaged sites the clotting of blood fibrin appears (Mayhew TM, Barker BL, 2001).

Apoptosis of the villi, mainly the syncytiotrophoblast, can be observed in the normal development of the embryo, but it abruptly becomes more frequent in cases of spontaneous abortions (Kokawa K., et al., 1998). Jerzak M. and Bischof P. (2002) particularly analyze the apoptosis in the first trimester of pregnancy in terms of

maintaining privilege in the opposition between the pregnant and the embryo as a normal physiological process during pregnancy.

Currently, early abortion followed with thrombosis of intervillous spaces remains an unsolved problem. The roots of its causes, pathology of injuries (Chaddha V., et al., 2004) and repetition of such abortions are not jet defined. As a result the diagnosis, prevention and treatment of this disease are not jet developed (Sun CC, et al., 2002).

Equally important and unresolved issue is the early allogeneic conflict of pregnant - germ. The possibility of its occurrence is not in doubt. Apparently, it is mainly developed at the interface of two organisms - in the area of the placental barrier. However, everything remains unknown: the genetic factor, which causes an incompatibility followed by allogeneic reaction of pregnant; manifestations of this conflict: clinical, immunological and pathological cause the death of the germ. That makes impossible to develop diagnosis, prevention and treatment of this not a rare disease.

All mentioned above shows that the processes that cause early abortions occur not only in the placental barrier as it is now understood - from the blood of the pregnant to the blood of the germ. Apparently, its boundaries should be expanded to structures involved in the protection of two organisms - of the germ and the pregnant woman.

4.2. Materials and methods

We conducted a comprehensive study of tissue obtained at an early abortion, including the placental barrier - its embryonic part (chorionic villi), provisory bodies (chorion, amnion, yolk sac), self-tissues of the embryo and parts of the pregnant (decidua tissue and intervillous lacuna with blood of pregnant women). Surveyed is full incoming material of early abortion (3.5-8 weeks of development) coming from four clinics in Israel. Along the way, we examined the second-trimester abortions. All the cases received were taken into account except for a small number of congenital malformations and chromosomal abnormalities. A total of 129 cases including 82 in the embryonic period (Diagram 4.1) and 47 in the fetal period were studied.

4.2.1. Materials

Embryos were divided into 5 groups based on clinical, pathologic, and other detailed data (Table 4.1). In group 1 (control - 18.3%) we included cases of ectopic pregnancy, bicornuate uterine horn rupture, induced abortions on medical or social reasons, and several cases of clinically unclear reasons, in which detailed examination revealed no inflammatory or pathologic immune processes. In group 2 we included cases where there was developmental delay (IUGR) due to significant thrombotic events around the chorionic villi. Initially, this group included 23 (28%) fetal cases, but after careful examination, they were moved in groups 3B and 4 (see Chapter 4.4.), while in group 2, only 2 (2.4%) embryonic cases and 3 fetal ones, 5 in total (3.9%) (see Tab. 4.1). In group 3A (15.9%) we included cases of ascending infection of the birth canal with deciduitis and its extension to the shell - chorioamnionitis with infection of amniotic fluid, bypassing the placental barrier. In cases of group 3B (11.0%), infection of decidual tissue is distributed in the blood of intervillous spaces and farther to chorionic villi and

anchoring villi directly, connected to the decidual tissue. In these cases, hematogenous spread is accompanied by a lesion of the capillaries of terminal villi, stem villi vessels and vessels of the embryo with the possible meningoencephalitis and inflammation of the brain ventricles - ventriculitis. Group 4 (52.4%) was formed by cases with recurrent miscarriages and widespread lesions, distinguished by significant, sometimes multiple apoptosis of trophoblast and other tissues of the embryo. There were no fetuses in group 4. Such changes are linked by some researchers with allogeneic conflict of the pregnant woman and the germ (Jerzak M., Bischof P., 2002; Bulla R., et al, 2003).

Table 4.1. Number of studied abortions at embryonic and early fetal periods in different groups

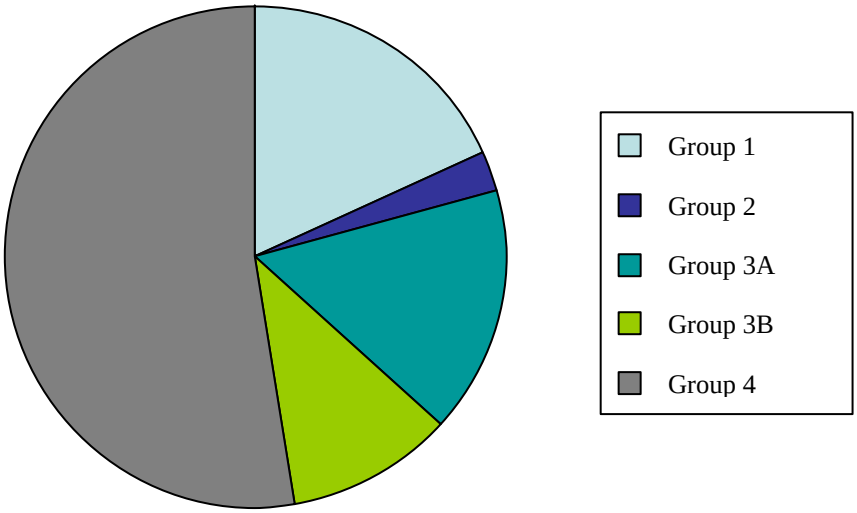
Periods		groups					Total
		1	2	3A	3B	4	
Embryonic 3.5-8 weeks	number	15	2	13	9	43	82
	%	18.3	2.4	15.9	11.0	52.4	100
Fetal 9-22 weeks	number	18	3	22	4	0	47
	%	38.3	6.4	46.8	8.5	0	100
Total	number	33	5	35	13	43	129
	%	25.6	3.9	27.1	10.1	33.3	100

Explanations are given in the text

4.2.2. Methodology

Functional state of organs, tissues and whole body is connected to and is manifested in pathological, immunohistochemical and morphometric changes. We have applied the appropriate complex of research methods. The following was used: general hematoxylin-eosin staining, techniques of van Gieson (detection of fibrosis), Gram (identification of microorganisms), and Perls (compounds of iron, hemosiderin). Used immunohistochemical methods (including Dobbs D., 2006) were: the avidin-biotin complex and peroxidase method with commercial markers (antibodies) to detect SC, J-chain, IgG, IgA and IgM, monocytes (CD68), promonocytes (CD14), T-lymphocytes (CD3), T-helper lymphocytes (CD4), cytotoxic T lymphocytes (CD8), natural killers - NK (CD56 and partly CD3), B lymphocytes (CD20 and CD79A), a common leukocyte antigen (lymphocytes, monocytes, etc. - CD45LCA), a common antigen to detect endothelium, macrophages, NK et al (CD31), receptor IgG-Fc-gamma RIII (CD16) and Fc gamma RII (CD32), interleukin IL2Rα (CD25), myelomonocytic and lymphoid stem cells and capillary endothelium (CD34), cells activated and preparing for division (Ki67), involved in apoptosis, Fas, FasLigand (FasL), bcl-2, p53.

Diagram 4.1 The distribution of cases studied of the embryonic period of pregnancy (3.5-8 weeks)



Group 1 - control, no cases of infection and pathological immune processes (18.3%)
Group 2 - multiple perivillous thrombosis without infection (2.4%)
Group 3A - acute ascending infection of the birth canal, complicated by deciduitis, chorioamnionitis and by deglutition of infected amniotic fluid (15.9%)
Group 3B - the same infection that spreads through the blood of lacunae in the villi and fetal circulatory system (11.0%)
Group 4 - early allogeneic conflict of the pregnant and the embryo (52.4%)

Apoptosis was determined by AopTag-peroxidase reaction of TUNEL. Smooth muscle cells of villous stroma were detected by desmin; the trophoblast (including invasive one) is very clearly revealed by the reagent for SC, as well as by reagent for cytokeratin 8/18. Quality control of immunostaining of the embryo cells and its membranes served staining of the same cells of the pregnant decidual tissue and blood of intervillous spaces on the same slide. Some difficulties existed in the differentiation between monocytes and promonocytes. They are similar in size, in the presence of phagocytic particles and other structures. Clusters of CD14, CD33, CD111, and others, are positive for a monocyte and a promonocyte; CD68 is positive for monocytes, but negative for promonocyte. Comparison of two slides of CD68 and CD14 in the same preparation helps distinguishing between them. All the cells of embryos, including mononuclear phagocytes, are negative in the processing of CD45 LCA. This allows us to distinguish them from the leukocyte cells and from lacunae blood of the pregnant woman, which are CD45LCA (+) and thus predict the probability of the cellular immune response of the pregnant against her embryo.

4.2.3. Morphometric methods

The number of mononuclear phagocytes (macrophages of pregnant, monocytes and promonocytes of embryo) and the number of different lymphocytes, as well as the

number of cells containing different immunoglobulins, some receptors and biologically active components were calculated on the area of 50,000 μm^2 of a section using an ocular grid (Olimpus) under magnification of x400 on 20-50 fields. The number of positively stained monocytes and promonocytes were converted to % of the total number of mononuclear phagocytes. Average number of phagolysosomes in slices of mononuclear phagocytes and invasive trophoblast determined for 100-150 cells in immersion magnification microscope Olimpus BX60, coupled with a digital color video system Nakafugi increases h1500, and counted the average number of them to cut. Number of those or other changes CHORIONIC SAMPLING villi was determined with increasing x200 or X400% of the total number of villi at 20-30 fields of view of the drug. The average number of capillaries and villi was determined by changes in the 100-120 villi stained with CD31 and CD34 increases x200 or X400. All calculations performed by the author.

4.3. Group 1, control. Placental barrier without bacterial and allogeneic damage

This group addresses the state of chorionic villi in early abortion, as well as the state of decidual tissue and intervillous lacunae of pregnant - 15 cases of embryonic period (3.5-8 weeks), and 18 fetuses (9-22 weeks).

4.3.1. Chorionic villi

Shown in Table 4.2 morphometric data demonstrate the normal state of chorionic villi in a period of 3.5-8 weeks of development: a large number of capillaries filled with erythroblasts (76.4%), the presence of a small (4.8%) number of avascular villi and the average number of vessels per one villus in the slice (5.3). There are no signs of pathological processes: thrombosis of capillaries, necrosis, calcification, fibrosis of the stroma. Apoptosis is very rare: no more than 1.8% of the villi in single cells; TUNEL positive reaction is seen for single cells. The data in Table 4.3 confirm the normal physiologic state of the villi vessels: along with germinal development increases the average number of capillaries per villus, decreases the number of swollen and avascular villi, villous vascularization is nearing completion at 10-11 weeks.

Trophoblast at the placental barrier is represented by five kinds of cells. On chorionic villi there are 3 types: syncytiotrophoblast, cytotrophoblast and groups of the proliferating trophoblast. Extravillous trophoblast has two types: invasive trophoblast in the decidual tissue and trophoblast that covers the surface of lacunae filled with the blood of pregnant women (Fig. 4.1). In the embryonic period syncytiotrophoblast forms a layer of core cells. Underneath is a layer of cubic cells - cytotrophoblast. Proliferating trophoblast forms groups of cells at the ends of some of the villi. In a section of each group, many cells are found. Cytotrophoblast and especially proliferating trophoblast actively divide, i.e. proliferate. This reaction is detected with Ki67. Positive reaction determines that a cell is prepared for division, or already divides. At 3.5-6 weeks this proliferation is more active than at 7-8 weeks of development (see Table 4.4). Sometimes on the preparates it is seen how the groups of cells of proliferating trophoblast get in touch with the maternal part of placenta and distribute in the decidual tissue, becoming

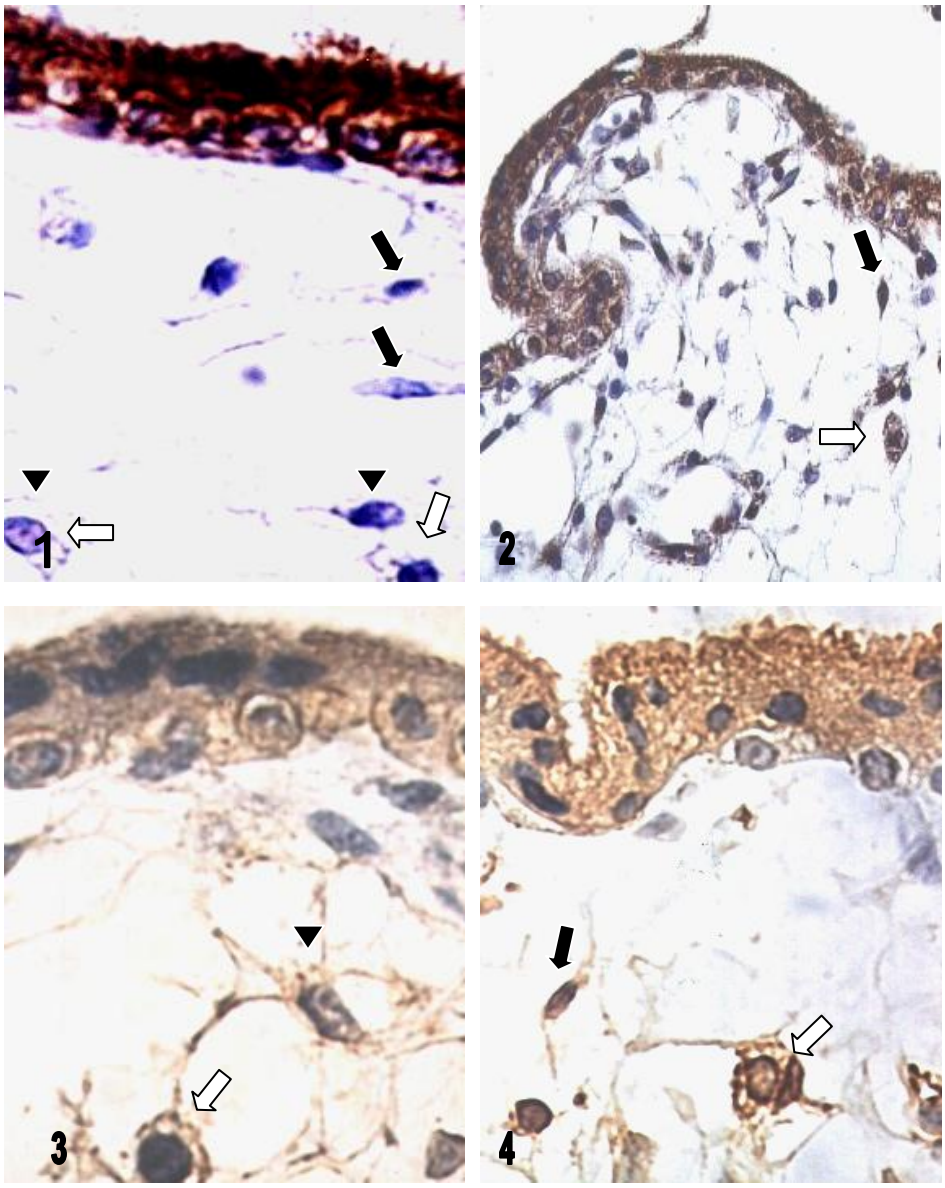


Fig. 4.1. Embryos of 3.5-5 weeks of development without infections or other disorders. On the surface of the villi normally two layers are found: the syncytiotrophoblast with microvilli and cytotrophoblast under it. Their main function is the transport to and from the embryo. **1** - SC (+), 2 - J-chain (+) involved in this function, **3** - IgA are transported, **4** - IgG are transported. The pertain stroma is somewhat swollen. Inside of it, away from the trophoblast, monocytes are seen (open arrows) with a small number of phagosomes and phagolysosomes, capillaries, few fibroblasts (▲) and single myocytes (black arrows) For Figs 1, 3, 4 magnification is x1000, for Fig. 2 - x400.

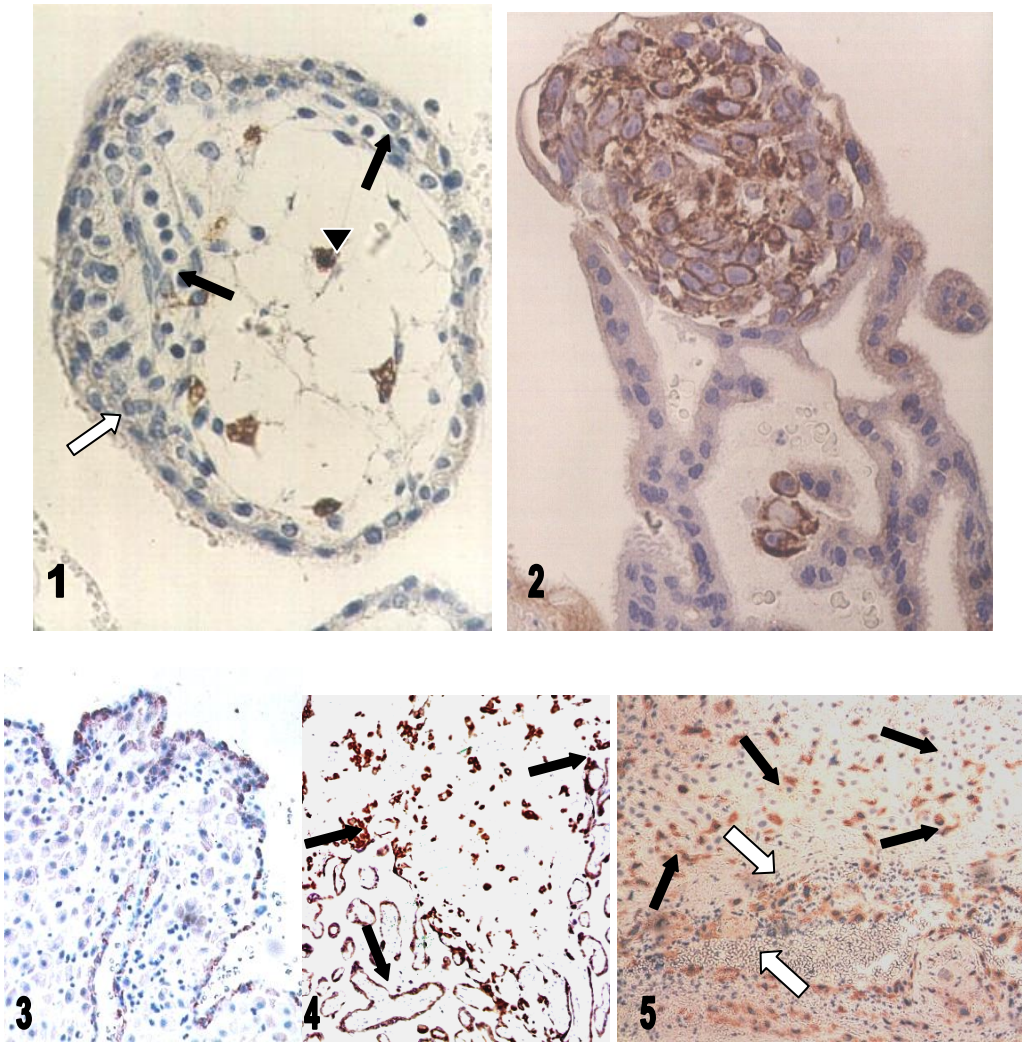


Fig.4.2. Proliferating trophoblast. Group 1, 3.5 - 5 weeks of development, normal villi. **1** - a villus: in the cytoplasm some CD68 (+) monocytes and CD69 (±) promonocytes (▲) are observed, the capillaries (black arrows), syncytio- and cytotrophoblast are normal, at certain place trophoblast forms a few layers (light arrow). **2** - a knot of SC (+) proliferating trophoblast is formed on a villus. **3** - decidual tissue of the pregnant: its lacunae are covered with a layer of trophoblast, the reaction IL2Rα-CD25 (+). **4** - small nodes of the proliferating trophoblast leave the villi for the decidual tissue (black arrows) in which their cells become the invasive trophoblast. They are clearly identified by SC (+). **5** - two types of extravillous trophoblast: one covering the lacunae (white arrows) and the invasive, spreading inside of the decidual tissue (black arrows). Reactions with keratin AE1, AE2. 1& 2 are magnified x400, 3&5 - x200, 4 - x100.

an invasive trophoblast (Fig.4.2.). Along with it the germinal maturation, the need in invasive trophoblast decreases and so the activity of proliferative trophoblast falls. Proliferative abilities of these cell types are different. Syncytiotrophoblast does not

proliferate (Mazur MT, Kurman RJ, 1965): the reaction of Ki67 in all the groups studied is negative. This suggests that their multi-core property is the result of a merger of mononuclear cytotrophoblast cells, rather than division of their nuclei. Cytotrophoblast proliferates moderately, and the proliferative trophoblast when getting in contact with the decidual tissue ("nuclear villi") spreads in and turns into invasive trophoblast.

Table 4.2. The state of chorionic villi of embryos in various pathological processes

Condition of villi	Groups				
	1	2	3A	3B	4
Normal capillaries (%)	76.37±2.72	22.13±4.86 [a]	60.01±4.1 [a b]	18.5 ± 6.81 [a c]	28.53 ± 2.97 [a c]
Obliteration of the capillaries (%)	18.83 ± 1.63	56.7 ± 5.0 [a]	33.25 ± 2.6 [a b]	62.6 ± 7.76 [a c]	56.73 ± 3.33 [a c]
Avascular villi (%)	4.82 ± 0.28	21.17 ± 1.41 [a]	6.71 ± 0.83 [a b]	18.9 ±1.28 [a b c]	14.74±1.31 [a b c]
Thrombosis of the capillaries (%)	0	0	0	10.3 ± 2.87 [a bc]	12.53±1.31 [a b c]
Average number of vessels per villus	5.29 ±0.83	2.19 ± 0.31 [a]	3.92 ±0.34 [b]	2.12 ± 0.11 [a c]	2.07 ± 0.68 [a c]
Edematous villi (%)	26.91 ±3.01	30.49 ± 8.07	39.97 ±5.12	37.48 ± 3.11	47.12 ± 3.35 [a d]
Hemorrhage in the villi (%)	0.77 ±0.51	2.77 ± 1.38	2.63 ±0.43 [a]	1.21 ± 0.53 [c]	1.22 ± 0.21 [c]
Necrosis of the villi (% of cases)	0	15.64 ± 4.56 [a]	0 [b]	33.33±1.32 [a b c]	0 [b d]
Petrifaction of the villi (% of cases)	0	2.43 ± 1.81 [a]	0	22.22±0.3 [a b c]	0 [d]
Villous fibrosis (% of cases)	0	10.54 ± 1.99 [a]	0 [b]	22.22 ± 0.8 [a b c]	0 [b d]
Apoptosis of capillary (% of villi)	1.52 ± 0.04	sporadic	2.03 ± 0.51	9.26 ± 2.36 [a b c]	18.53±4.01 [a b c]

a, b, a, d - significant differences ($p < 0.05 - 0.001$) follow-up groups to groups 1, 2, 3A and 3B.
Explanations are given in the text.

Immunochemical reactions are not always similar in different types of trophoblast. In the process of transport of immunoglobulins SC is more often positive in the syncytiotrophoblast or villous cytotrophoblast, and sometimes - in both, while occasionally only in the apical microvilli of syncytiotrophoblast. With reaction to SC the cells in clusters of proliferating trophoblast get stained (Fig.4.2.). Very intensely get the SC staining: the invasive and the lacunae lining types of trophoblast in the decidual tissue. J-chain is more prevalent in all types of trophoblast. The location of IgG and IgA may change due to their migration from one cell layer to another. IgM in group 1 are found only in isolated cases. Receptors of IgG-Fc gamma RIIIA (CD16) and Fc gamma RII (CD32) are positive in the trophoblast villi. Interleukin-2 receptor (CD25) is positive

in all types of trophoblast. The regulators of apoptosis reaction: Fas and bcl-2 are occasionally found in the apical syncytiotrophoblast microvilli. FasL and p53 are observed rarely, as small loci or missing. Apoptosis or necrosis of small plots of trophoblast occurs in 1.2 ± 0.7 per 50000 μm^2.

Table 4.3. State of normal vessels (group 1) of chorionic villi in the embryonic and early fetal periods

	The embryonic period		The fetal period
	3.5 – 5 weeks	**6 – 8 weeks**	**9 – 22 weeks**
Normal capillaries (%)	72.64 ± 3.75	78.61 ± 5.71	79.19 ± 7.92
Vasospasm (%)	21.81 ± 3.04	18.31 ± 3.26	19.44 ± 2.85
Avascular villi (%)	5.54 ± 0.75	3.08 ± 0.82 [a]	1.37 ± 0.51 [a]
Edematous villi (%)	35.03 ± 3.78	21.48 ± 2.42 [a]	15.44 ± 1.48 [a b]
The average number of capillaries in the villi	4.83 ± 0.86	5.82 ± 0.56	6.58 ± 0.92

[a, b] - significant ($p < 0.05 - 0.001$) differences compared to the previous columns

Stromal cells of chorionic villi. In the stroma of the villi there is a bit of monocytes and a very small amount of promonocytes (Table 4.5). At the embryonic period in the chorionic villi all types of lymphocytes NK, granulocytes, all CD45LCA-positive cells, including all the cells of the pregnant are absent, Monocytes of embryos, in contrast to macrophages from the pregnant decidual tissue are CD45LCA-negative and somewhat weakly stained by CD68. Promonocytes are CD68 negative and CD14 positive. In mononuclear phagocytes SC is missing; there are receptors for IgG - Fc gamma RIII (CD16) and Fc gamma RII (CD32), J-chain, IgG, IgA, and rarely IgM (Fig. 4.1) With the appropriate immunostaining immunoglobulins are located in the cell capsule as some droplets of staining in the amount of IgG - 15.92 ± 1.52, IgA - 10.89 ± 1.18 and in some cases IgM - 1.93 ± 0.99 on a section of one cell (see Fig.6). These are immune complexes in which receptors are bound to immunoglobulins with their Fc-fragments, or Fab-fragments stand out and are able to get in contact with the specific antigen (Simister NE, 1998, 2003). In this state, monocyte passes into the capillary and carries immunoglobulins over the body of the embryo. In addition to immunoglobulins on the surface of phagocytes capsules, they are found in phagolysosomes of the cytoplasm. Their average number in the section of monocytes is given in Table 4.9 and in Diagram 4.2. In mononuclear phagocytes and in endothelium of capillary IL2Rα (CD25 +) receptor is revealed. Phagocytes moderately proliferate (Ki67 + cells: $13.2 \pm 1.7\%$, see Table 4.5). Involved in apoptosis, Fas, FasLigand, bcl 2, p53 are not detected, apoptosis of monocytes consists 1.52 ± 0.04 per 50000 μm^2.

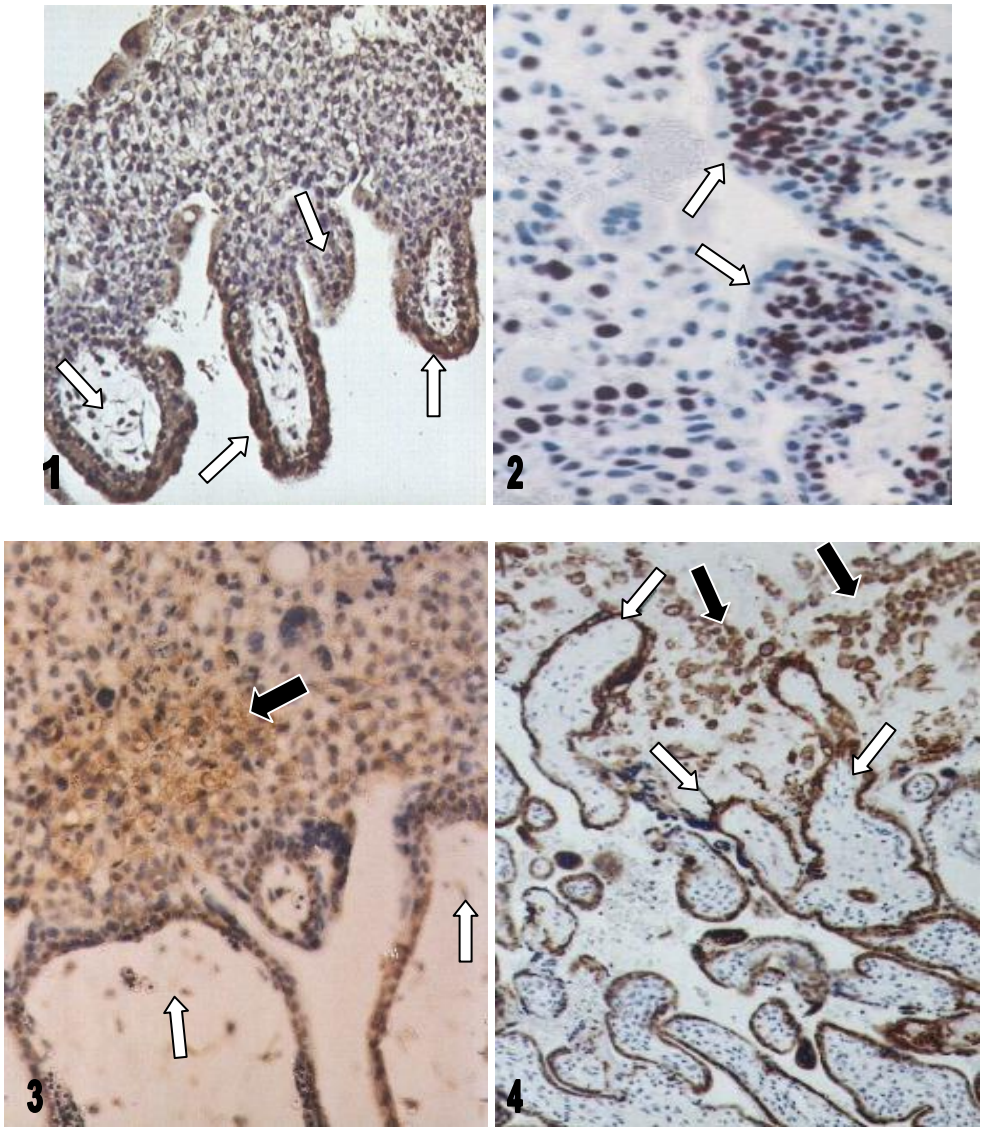


Fig.4.3. Proliferative trophoblast. **1** - in 4 neighboring villi (white arrows), there was an intense proliferation of the cytotrophoblast, **2** - proliferative trophoblast (white arrow) rapidly proliferates: Ki67 (+) cells consist 70-80%; left: Ki67 (+) monocytes or villous cytotrophoblast. **3** - Several neighboring villi with proliferative areas (white arrows) came into contact with the decidual tissue ("anchoring villi) and proliferates move on it (CD25 - IL2Rα staining); (dark arrow) - a group of red cells. **4** - Villi (white arrows) with the trophoblast in the decidual tissue - it is already an invasive trophoblast: it is an intense phagocyte containing SC (+) (black arrows). 1, 2, 3 are magnified x200 and 4 - x100.

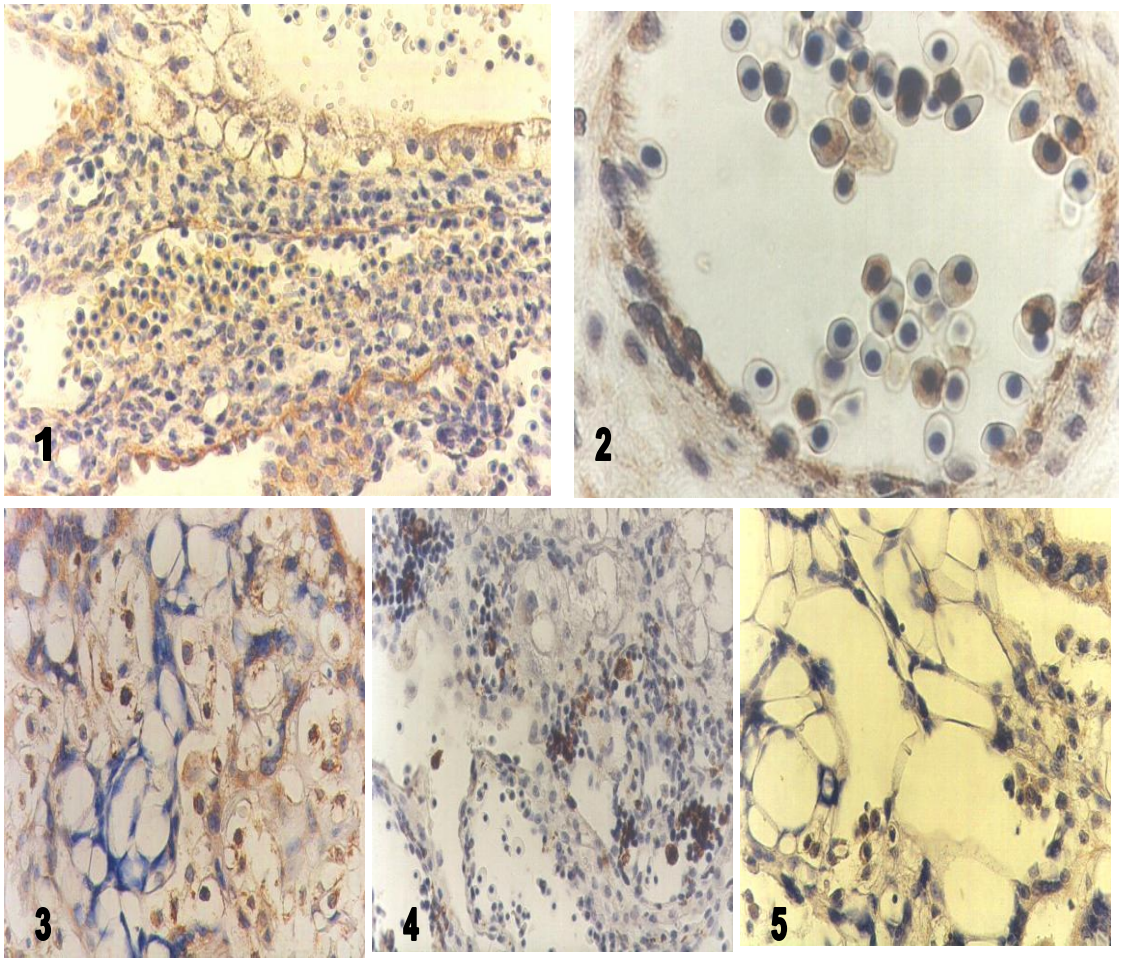


Fig.4.4. Yolk sac. Group 1 is at 3.5 -5 weeks of development. **1** - abundant accumulation of erythroblasts, some of them with IgA (+), **2** - more clearly erythroblasts with IgA (+) are visible at x1000. These erythroblasts are not formed in the yolk sac, and arrive in a small amount from the main circulating system of the embryo (Chapter 5.3, table 5.1). **3** - in the vessels of the yolk sac there are monocytes CD68 (+) and promonocytes CD14 (+) which also contain IgA (+). **4** - in the yolk sac at 4th week of development there is a large amount of CD3 (+) pro-T lymphocytes. **5** - there are also a few CD20 (+) cells from a group of B-pro-lymphocytes. Preparations 1, 3, 4, 5 are magnified x400, 2 - x1000.

Table 4.4. The intensity of proliferation of different types of trophoblast and villous capillary endothelium during the embryonic period by groups

Number of (Ki67+) proliferating cells (%)	Term of develop-ment (weeks)	Group 1	Group 3A	Group 3B	Group 4
in syncytial trophoblast	**3.5 - 8**	0	0	0	0
in cytotrophoblast	**3.5 - 6**	69.19±8.34	31.9±3.36 a	6.84±0.89 a b	11.12±1.63 a b c
	7 - 8	30.6±2.29	24.81±1.98	14.16±2.91 a b	16.78±1.4 a b
the number of groups of proliferating trophoblast (% of villi)	**3.5 - 6**	29.39±3.41	9.56±1.12 a	3.63±0.24 a b	8.62±0.84 a c
	7 - 8	32.41±3.17	8.23±0.72 a	8.53±1.48 a	6.65±0.32 a b
in proliferating trophoblast	**3.5 - 6**	76.1±6.53	85.94±6.39	81.02±5.85	56.59±4.62 a b c
	7 - 8	34.54±3.94	20.55±2.31 a	56.3±5.79 a b	49.31±5.12 a b
in invasive trophoblast	**3.5 - 8**	76.34±1.3	13.23±2.44 a	11.94±2.32 a	12.1±2.13 a
endothelium of the capillaries of villi	**3.5 - 8**	26.5±2.4	13.67±1.42 a	6.71±1.82 a b	1.96±0.84 a b c

[a, b, c] - significant differences ($p < 0.05$-0.001) the next group versus groups 1, 3A and 3B. Under normal conditions (group 1), villous cytotrophoblast cell proliferation in the first half of the embryonic period (3.5 - 6 weeks) is twice higher than in the second half (7 - 8 weeks). This reflects the continuous maturation of the villi. The same number of groups of proliferating trophoblast in both parts of the embryonic period shows the continuing growth of the invasive trophoblast. State of proliferation of trophoblast in groups 3A, 3B and 4 are considered in the text. Group 2 is not included because of the small number of cases.

The capillary endothelium of chorionic villi contains J-chain, and sometimes a weak immunostaining of SC (in 20-40% of cases) is observed. The number of proliferating (Ki67 +) endothelial cells is 26.5 ± 3.9%. Part of erythroblasts in the capillaries of the villi contains J-chain and immunoglobulins. This means that the erythroblasts, together with monocytes, transport immunoglobulins (see Tables 5.1, 5.2 and 5.3; Fig.4.4.2).

In the stroma of the villi a small amount of fibroblasts and some isolated smooth muscle cells are observed, when stained with desmin. In the intermediate part of the villi they consist of 5-10, while in the stem considerable bundles are seen

Yolk sac: (see Fig.4.4).

4.3.2. Placenta of the pregnant

In the I-st and the beginning of the II trimester of pregnancy the placenta is composed of decidual tissue and intervillous spaces - lacunae. Along with them there are tissues of the embryonic origin - invasive trophoblast and trophoblast that covers the lacunae.

Decidual tissue is formed with large light decidual cells rich in glycogen and lipids. They do not contain SC, J-chain and immunoglobulins and do not get stained by CD68, but they and the glandular epithelium of decidual tissue are positive for CD25

(IL2Rα). In the decidual tissue there are different types of immune cells (Table 4.6). Most of them are in group 1 consist just a small amount: 2 - 4 cells in the area of 50,000 μm^2 of

Table 4.5. Mononuclear phagocytes in chorionic villi of embryos in various pathological processes

The number		Group 1	Group 2	Group 3A	Group 3B	Group 4
Mononuclear phagocytes (per 50000 μm^2)		7.85 ± 0.73	6.67 ± 0.65	15.78±1.86 a b	7.2±2.36 c	6.48 ± 0.57 c
out of them, promonocytes (per 50000 μm^2)		0.16 ± 0.01	0.13 ± 0.01	2.07 ± 0.17 a b	2.15 ± 0.09 a b	3.51 ± 0.42 a b c d
promonocytes (% of phagocytes number)		2.04 ± 0.34	2.01 ± 0.41	18.47± 3.85 a b	25.61± 3.42 a b	54.17± 3.61 a b c d
Proliferating (Ki67+) phagocytes (%)		13.16± 1.67	7.92 ± 0.85 a	9.92 ± 1.06	6.13 ± 0.73 a c	6.45 ± 0.98 a c
Interleukin IL2Rα (CD25+) (% of phagocytes)		57.0 ± 1.32	-	13.13± 4.13 a	13.68± 2.91 a	10.86± 1.48 a
Phagocytes containing receptors: (% of phagocytes)	**J-chain**	73.86± 2.77	72.57± 3.11	79.45 ± 4.0	77.54± 2.71	67.84± 3.35 c d
	Fc gamma RII(CD32)	82.29± 2.01	10.21± 1.19 a	24.47± 1.65 a b	25.44± 1.47 a b	10.58± 2.32 a c d
	Fc gamma RIII(CD16)	80.16± 3.75	25.0 ± 3.12 a	18.4 ± 2.01 a	27.51± 1.55 a c	12.74± 1.57 a b c d

a, b, c, d - significant differences (p <0.05-0.001) the following groups versus groups 1, 2, 3A, 3B. Explanations are given in the text. Decrease of IL2A (CD25), Fc gamma RII (CD32) and Fc gamma RI (CD16) is associated with an increased number of promonocytes that do not contain these receptors.

the preparate, only CD3-positive lymphocytes and natural killers (NK) are found in large numbers - a few dozen cells per the noted area of the preparation. But the distribution of various types of cells in the decidual tissue is not the same. T-lymphocytes (CD3, CD4, and CD8), NK and macrophages are more or less uniformly detected in both zones of decidual tissue - in the compact, where they have direct contact with invasive trophoblast of the embryo, and in the spongy zone, somewhat distant from them. These cells are involved in general cellular immune response; they realize an immune response and are located close to the subject of reaction. B lymphocytes and plasma cells are involved in humoral immune response, their task is to obtain the information about foreign antigens from macrophages to develop immunoglobulins and send them into a free flight; in the contact with the object these cells are not involved. Therefore, in a compact area of CD20 and CD79A only single cells are found per dozens fields of view (x400), while in the spongy area there is lot of them along with follicles - clusters of several dozen cells. It should be emphasized that the plasma cells in the decidual tissue are the cells of the pregnant woman. In macrophages CD16, CD32, CD45LCA are positive. IL2Rα (CD25) are detected in the part of lymphocytes, as well as in the epithelium of the glands. In some cases, in decidual tissue several small fresh hemorrhages are found. Spiral arteries stay without changes. Microflora is not detected.

Invasive trophoblast in the decidual tissue is composed of cubic or elongated cells with a dark nucleus; multinuclear cells of syncytiotrophoblast type are rare. Trophoblast, while getting contact with the decidual tissue, extends deep into it by strands or by single cells. Its cells contain SC, J-chain and immunoglobulins in the form phagolysosomes in small quantities (Table 4.9 and Fig.4.2). SC gets stained very intensely, making it easy to distinguish between invasive trophoblast from decidual cells and macrophages. The intensity of the proliferation of invasive trophoblast (Ki67 +) is very high (Table 4.4.), and apoptosis (TUNEL and hematoxylin-eosin) occurs in 0.7-1.9 cells per 50,000 μm^2, which roughly corresponds to the data of Chan CC, et al. (1998). Invasive trophoblast phagocytosis by macrophages is equally rare.

Intervillous spaces in the histological preparations are free or contain a little of blood, while clots of fibrin are lacking. The blood contains a bit of immunocompetent cells (10-20 per 50,000 μm^2, see Table 4.7.). Trophoblast cells that cover the lacunae are cubic ones and contain SC, J-chain and immunoglobulins.

Table 4.6. Immunocompetent cells in decidual tissue of the pregnant in various pathological processes at 3-8 weeks. (Number of cells per 50,000 μm^2 at x400)

		Group 1	Group 2	Group 3A	Group 3B	Group 4
T-lymphocytes (CD3)		28.86± 2.24	31.83± 6.88	21.15± 2.38	30.25±3.43	25.42± 4.5
T-helpers (CD4)		2.23± 0.7	2.08± 0.47	1.84± 0.23	1.15± 0.32	1.38± 0.45
T- cytotoxic (CD8)		3.29± 0.51.	2.48± 0.29	4.33± 0.86	4.23± 1.3	2.63± 0.5
Natural killers (CD56)		48.55±7.04	68.67± 7.09	56.39± 7.34	47.8± 6.71	48.22±7.42
B-lymphocytes (CD20)	**spongy zone**	8.2± 1.81	0.39± 0.18 a	1.96± 0.42 a	0.52± 0.12 a	4.01± 0.81 a b c d
	compact zone	0.15±0.01	-	0.07± 0.01 a	0.17± 0.05	0.32± 0.04 a c d
Plasma cells (CD79A+, CD20-)	**spongy zone**	5.05±1.33	-	3.39± 0.61	10.64±1.39 a c	6.04± 1.36 d
	compact zone	0.24± 0.02	-	0.09± 0.02 a	2.07± 0.39 a,c	0.43± 0.04 a,c,d
Macrophages (CD68)		2.3±0.46	2.5± 0.41	5.62± 1.24 a	8.19± 1.72 a,b	4.2± 0.71 a,b,d
Leukocytes (CD45LCA)		48.85±3.25	10.86±2.43 a	17.92± 1.86 a,b	16.78±3.54 a	20.45± 2.97 a,b

a, b, c, d - significant differences ($p < 0.05$-0.001) the following groups versus groups 1, 2, 3A and 3B. Different immunocompetent cells of decidual tissues react to the pathological effects not in the same way. T-lymphocytes (CD3, CD4, CD8, and NK), normally involved in cellular immune reactions, during bacterial infections (groups 3A and 3B) and allogeneic conflict (group 4) are not changed when there is a humoral immune response. Cells of humoral responses CD20 are significantly reduced in number in the groups 3A and 3B, and less in the group 4, due to their transformation into plasma cells. Their number increases dramatically in group 3B, stays the same in group 3A and shows a small increase in group 4. Number of macrophages is increased in groups 3A, 3B and 4, but becomes twice greater in group 3B. This shows that the antigenic effect on the pregnant women in a case of a bacterial infection is much stronger than in a cases of early allogeneic conflict.

4.3.3. Conclusion for group 1

In the cases included in the group 1, the signs of inflammation, necrosis, fibrosis and calcification of villi are absent. Apoptosis has been observed in single cells of no more than in 1.8% of the villi. In the course of normal development in II compared to the I trimester, the number of villi with edematous stroma and avascular villi is significantly reduced and a tendency to increase the average number of capillaries per villus is observed. Monocytes, cells of cytotrophoblast and especially of proliferating and invasive trophoblast have a pronounced tendency to proliferate. Through the villous trophoblast the transport of immunoglobulins is realized, namely: IgG, IgA (weaker) and in some cases (weakly) IgM.

Table 4.7. Immunocompetent cells of pregnant women in the intervillous blood in various pathological processes at 3-8 weeks after fertilization (Number of cells per 50,000 μm^2)

	Group 1	Group 2	Group 3A	Group 3B	Group 4
T-lymphocytes (CD3)	4.48 ± 0.3	3.87 ± 0.62	3.8 ± 0.29	3.42 ±0.27 a	4.41 ± 0.93
T-helpers (CD4)	2.46 ± 0.41	2.07 ± 0.94	1.55 ± 0.26	0.57 ± 0.2 a c	0.59 ± 0.17 a c
T-cytotoxic (CD8)	2.1 ± 0.27	2.48 ± 0.28	2.25 ± 0.79	3.41 ± 0.81	1.71 ± 0.41
Natural killers (CD56)	0.85 ± 0.22	2.17± 0.21 a	1.69 ± 0.35	1.48 ± 0.22 b	0.85 ± 0.08 b d
B-lymphocytes (CD20+, CD79+)	1.68 ± 0.27	0.56 ± 0.21 a	1.03 ± 0.2	1.25 ± 0.11 b	1.43 ± 0.35 b
Plasma cells (CD20-, CD79+)	1.86 ± 0.2	-	1.21 ± 0.29	2.22 ± 0.2 c	2.81 ± 0.31 a c
Macrophages (CD68)	1.51 ± 0.4	0.92 ± 0.31	5.84 ± 1.31 a b	4.84 ± 0.84 a b	0.99 ± 0.39 c d
Leukocytes (CD45LCA)	5.6 ± 1.01	4.07 ± 0.91	12.0 ± 1.87 a b	9.23 ± 1.28 a b	2.49 ± 0.25 a c d

a, b, c, d - significant differences ($p < 0.05$-0.001) the following groups versus groups 1, 2, 3A and 3B. Changes in maternal immune cells in decidual tissue and in blood of lacunae are substantially similar, although the number of cells in the decidual tissue is usually much greater. Also the number of T-lymphocytes in all groups is not changed, but the number of helpers (CD4) in groups 3B and 4 decreases. The number of B-lymphocytes and plasma cells stays constant. The number of macrophages increases in groups of bacterial infections (3A and 3B).

In villi, along with monocytes, there is about 2% of promonocytes. Monocytes and promonocytes, as well as invasive trophoblast are capable of phagocytosis. About half of the monocytes, as well as all types of trophoblast and villous capillary endothelium contain receptors IL2α. Given the absence of lymphocytes in the tissues of the embryo's villi which synthesize IL2Rα, we can assume that it is transported from the pregnant woman. It also confirms that only a fraction of monocytes of embryo contains IL2Rα. Monocytes of embryo are CD45LCA negative, while the same type of macrophages of the pregnant women are positive. This is due to the fact that monocytes are somewhat less matured than macrophages.

In decidual tissue (the part of the placental barrier of the pregnant), there are all kinds of T-and B-lymphocytes, NK, plasma cells, macrophages. In this case, CD4 (+) helper cells, CD8 (+) cytotoxic T lymphocytes and macrophages are represented a very small amount - 0.6-3 cells to 50,000 μm^2 area of the preparation. In contrast, CD3 (+) T cells, CD56 (+) NK and the total staining of lymphocytes CD45LCA (+) are detected as 30-50 cells per specified area of the slide. CD20 B lymphocytes and CD79A plasma cells occupy an intermediate position: in the compact zone of the decidual tissue they are rare, but the spongy zone contains 8-10 cells per 50,000 μm2 of the preparation area, often forming groups - follicles of 20-50 or more cells. Listed types of leukocytes in the blood of intervillous lacunae of the pregnant in the cases of group 1 are contained in a small amount (0.85-5.6 to 50,000 μm^2).

Conducted morphological, immunohistochemical and morphometric study of group 1 shows that the state of the placental barrier: its pregnant and germinal sides, corresponds to the normal development at the embryonic period. These changes (increased number of capillaries in the villi, reduced number of the swollen and avascular villi, and intensive proliferation of certain types of cells) are quite physiological. Signs of pathological processes are absent.

4.4. Group 2. Disruption of the placental barrier, due to deposition of fibrin on the villi of the embryo

This group consisted of intrauterine growth restriction (IUGR) and antiphospholipid Hughes syndrome. The disease is expressed in the deposition of fibrin in the chorionic villi and in the spiral arteries of decidual tissue. These changes lead to dysfunction of the placental barrier and to repeated spontaneous abortions. However, the causes of IUGR and repeat abortions remain unclear. The narrowness of knowledge about the disease was noted repeatedly (Cetin J. et al., 2004; Chaddha V., et al., 2004).

In our material, these cases are initially has accounted for 23 embryos (28%), which corresponds to the literature (Kutteh WH, 1999; Сидельникова В.М., 2002; Adolfsson A., Larrson PG, 2006). Preliminary morphological examination showed deposition of fibrin around groups of villi, or of small fibrin clots on some specific villi. There have been damages of syncytiotrophoblast and to a lesser extent - cytotrophoblast. With some major damages there were CD45LCA-positive small clusters of a variety of leukocytes on the surface of fibrin, which were not in contact with the trophoblast. Spiral arteries in some cases had no changes. Tissues of embryos in all cases were absent; fetal tissues were preserved only in two cases.

Subsequent detailed studies have revealed the true causes of diseases which caused early abortions. In five cases there was marked inflammation of the decidual tissue along with necrosis, leukocyte surrounding and with the presence of microorganisms (in four cases - cocci, and in one - coli). Germs spread on some villi, forming areas of necrosis (and not apoptosis) of cells and stroma on them and then spreading through the capillaries of the villi to the umbilical vein towards the embryo. The noted changes have suggested moving these 5 cases into group 3B - infection with hematogenous spread in the area of the placental barrier (see Chapter4.6.).

In 16 other cases, signs of infection were absent. There were apoptotic lesions of some sections of syncytio- and cytotrophoblast, of the endothelium and erythroblasts of capillaries and of villous monocytes. In the surviving monocytes, in replacing them promonocytes and in invasive trophoblast of the decidual tissue there was a very large number of phagolysosomes containing IgG, IgA and IgM. These and other changes allowed us to move these 16 cases into group 4 - a possible allogeneic conflict of pregnant woman and the embryo (for details see Chapter 4.7.).

It remained 2 cases of embryonic (6 and 8 weeks of development) and 3 - of fetal (12, 14 and 21 weeks) periods (Table 4.1., Diagram 4.1).

4.4.1. Chorionic villi

Number of villi is dramatically reduced: in all preparations it ranges from 6 to 18. Only in 22.13% of villi there were functioning capillaries with erythroblasts, the remaining villi did not contain capillaries, they were constricted or obliterated (Table 4.2). The average number of capillaries per a villus was 2.19 ± 0.31 (less than in group 1 $p < 0.01$). 30.49% of villi were edematous, 15.64% - necrotic, 12.97% were fibrous and limy.

Trophoblast. Syncytiotrophoblast, in the areas covered by fibrin, is absent or revealed as a structureless mass in which sometimes p53 and FasLigand are identified, while the microflora is absent. Elsewhere in the trophoblast Fas and bcl2 are positive, more intensively in the apical parts. In cytotrophoblast the reaction Ki67 (+) 14.65 ± 1.94% (reduced by almost 2-fold) in invasive trophoblast 30.88 ± 4.22% compared with group 1 also reduced by half. SC in the syncytium and cytotrophoblast is weakly positive, J-chain is positive, CD25 (receptor IL2Rα) is weakly positive in the syncytiotrophoblast and is positive in the cytotrophoblast. Receptors CD16 and CD32 are positive.

Villous stromal cells. Number of mononuclear phagocytes is somewhat reduced in comparison with group 1 ($p > 0.1$), the number of promonocytes per area unit of measurements and their percentage regarding phagocytes does not differ from group 1. Phagocytes (Ki67 +) proliferate to less extend than in group 1 ($p < 0.05$). Monocytes and promonocytes contain phagolysosomes with IgG, IgA and IgM in small amounts (Table 4.9, Diagram 4.2). CD16 (FcRIIIα) and CD32 (FcRIIα) - IgG receptors in monocytes are much less found ($p < 0.001$) than in group 1 (Tabl.4.5.).

4.4.2. Placenta of the pregnant

In decidual tissue in the case of abortion on day 6 pathological changes are not recorded, in second trimester abortion (12-21 weeks) in two cases there were small areas of necrosis with slight infiltration of neutrophils, the microflora is absent. In all cases, many spiral arteries are obliterated by endothelial proliferation.

The complex of immunocompetent cells in the decidual tissue is shown in Table 4.6., significant deviations from group 1 were not observed, but B-lymphocytes (CD20) and complex reaction to leukocytes (CD45LCA) were decreased significantly compared with groups 1, 3A, 3B and 4.

In the fibrin covering the villi, in all cases there are white blood cells (4.3 ± 0.3 per 50000 μm^2 of slice surface, in group 1 their content is 4.48 ($p > 0.1$).

Blood of intervillous spaces contains different types of immune cells that are different from group 1 only by a large number of NK ($p < 0.001$) and by lower number of

CD20 B-lymphocytes (p <0.001) (see Table 4.7.). Fibrin in the intervillous spaces is available in all cases.

4.4.3. Conclusion on the Group 2

Thrombosis of the intervillous spaces of the placental barrier is a common pathology in the embryonic period (32.9% of cases observed by us, see Table 4.8.). It is not a specific disease - IUGR, antiphospholipid syndrome, or any other. Often, this thrombosis is a complication of early allogeneic conflict, when the action of antibodies of pregnant destroys the trophoblast and other structures of the placental barrier. As a result, some plaques are formed on the surface of the villi. Another cause of blood clots in the villi is the local inflammatory processes. We will return to the consideration of these processes in groups 3B and 4.

Table 4.8. Blood clots in the intervillous spaces in various pathological processes at the 3.5 - 8 weeks of development

	Group 1	Group 2	Group 3A	Group 3B	Group 4	Total number
Total cases	15	2	13	9	43	82
The cases of thrombosis	0	2	4	5	16	27
%	0	100	30.77	55.55	37.21	32.93

Explanations are given in section 4.4.3.

These five cases which remain in group 2, at the time of abortion have no signs of allogeneic conflict or serious inflammation. Changes in all these 5 cases - in embryos and fetuses at II trimester are of the same type. Disruption of the placental barrier could be due to obliteration of a large number of spiral arteries of decidual tissues. But the obliteration of the spiral arteries itself may be due to inflammation with subsequent necrosis of the villi, fibrosis, calcification and a decrease in the number of villi. The average number of capillaries per one of the remaining villi decreased to 2.19 ± 0.31 in group 2 with an average number of capillaries in group 1 - 5.29 ± 0.43 ($p < 0.001$). Naturally, such a strong decrease in the placental barrier volume results in the death of germs. Also proliferative activity of cyto- and invasive trophoblast and the number of monocytes containing receptor CD16 (Fc gamma RIII) and CD32 (Fc gamma RII) were decreased. But at the same time, compared with group 1, the number of monocytes in villi and of immunocompetent cells in decidual tissue and intervillous blood in group 2 did not change significantly. This, as well as the absence of microflora and other characteristics excludes the possibility of acute and other pathological immune processes at the time of abortion, but it is quite possible that in the recent past the inflammation was there. Cases of obliteration of a single or small number of spiral arteries in group 1 were not observed, but in all other groups they were found.

It is doubtful that 5 cases (3.88%) observed by us can be attributed to antiphospholipid Hughes syndrome. More likely sounds an assumption that changes in the villi - necrosis with subsequent fibrosis and petrification, reduction in the number of B-lymphocytes in decidual tissue and in blood of lacunae and an increase of NK there, obliteration of the spiral arteries may be a residual manifestation of the previously existing bacterial inflammatory process presented in Chapters 4.6 and 5.7 (Group 3B).

This is also indicated by a significant similarity of almost all indicators of the chorionic villi in groups 2 and 3B, listed in Table 4.2.

Problems of diagnosis and treatment of antiphospholipid syndrome, IUGR, PR and other similar situations remain obscure (Sun CC et al., 2002; Cetin I., et al., 2004). Fully justified seems the decision of the seminar «Fetal & Neonatal Medicine», that "the use of heparin for the treatment of IUGR in the future remains debatable» (Chaddha V. et al., 2004), (see Section 4.1).

4.5. Group 3A. Ascending infection of the birth canal, extending through the chorion and amnion, bypassing the placental barrier

Stages of development of ascending infection in group 3A (Fig.4.5 and 4.6) are reflected in morphological changes. Bacterial infection, penetrating into the uterus during pregnancy, causes inflammation of the decidual tissue (deciduitis), and then spreads to the chorion, which is closely in contact with the amnion (chorioamnionitis) and in amniotic fluid. Embryo ingests the infected water in the digestive tube, and with the development of respiratory organs aspirates also into them.

In group 3A included 13 cases of early abortions (5-8 weeks) and 22 – of second trimester (Table 4.1). Tissues of embryos were preserved in 8 cases, and the fetuses - in 12 cases.

4.5.1. Chorionic villi

Morphometric changes in the villi (Table 4.2.) are moderate compared with group 1: somewhat reduced is the number of normal villi with functioning capillaries, increased is spasm of the capillaries and the number of avascular villi. But the major defeat of the villi: necrosis, fibrosis, calcification, and massive apoptosis are absent.

Villous trophoblast in many places is devoid of syncytiotrophoblast; sometimes areas of caseous necrosis are visible. Cases of shallow cytotrophoblast necrosis, proliferating trophoblast villi and invasive trophoblast in the decidual tissue are frequent, as well as the number of groups of proliferative trophoblast and their sizes are strictly reduced (Table 4.4., Fig.4.5). State of SC, J-chain, IgG and IgA in trophoblast is not distinguished from their content in group 1, but there is transport of IgM: it is detected in trophoblast, in monocytes and stroma of the villi (Table 4.9.). Phagolysosomal activity of invasive trophoblast is increased (Fig.4.8.3).

In chorion and amnion there are areas of necrosis, sometimes on slides it is visible their contact with the inflamed decidua tissue (Fig.4.7). At the areas of necrosis microflora, mainly cocci, is observed. Apoptosis is rare: individual or in small groups.

Stromal cells of chorionic villi. Number of mononuclear phagocytes is doubled (Table 4.5.) in account of monocytes and promonocytes; number of the letter increases by more than 10 times. The number of proliferating (Ki67 +) monocytes is reduced slightly, but the receptors Fc gamma RII (CD32), Fc gamma RIII (CD16), as well as the receptors

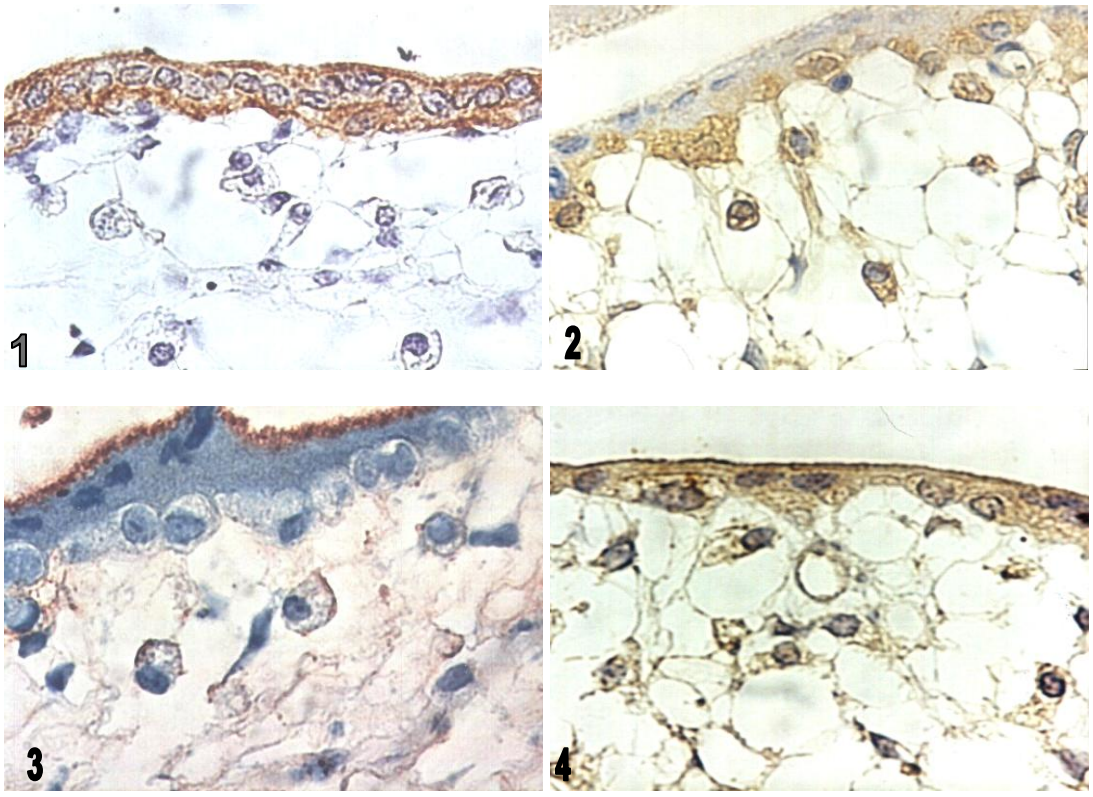
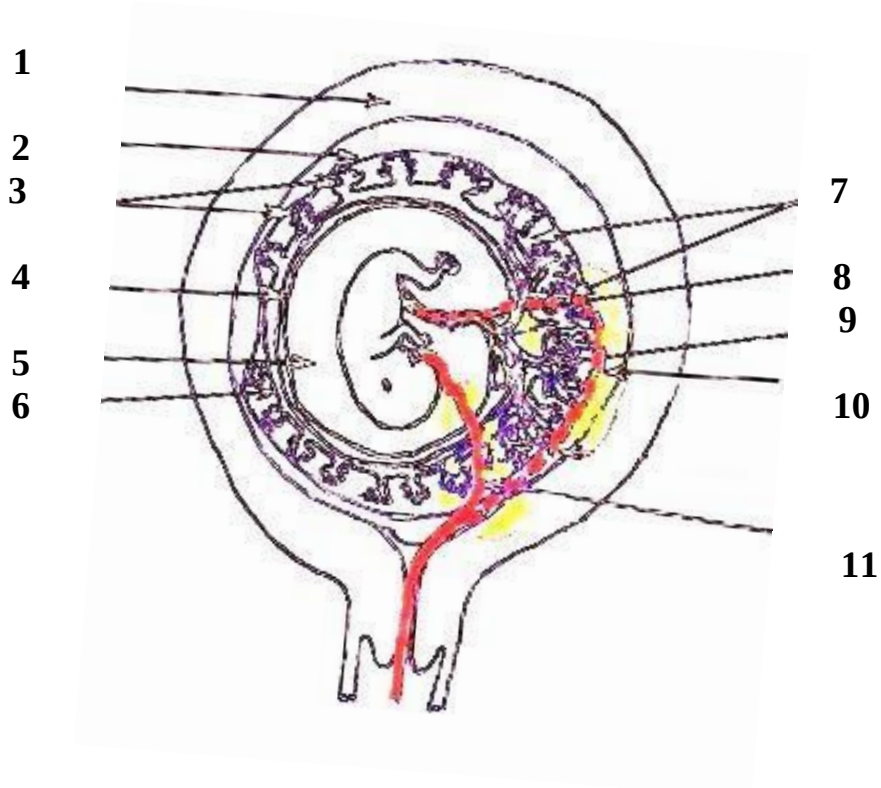


Fig.4.5. State of the villi in group 3A at 5 - 8 weeks of development. Syncytiotrophoblast is absent in 1, 2, 3, 4. In 3 there is a section of caseous necrosis. At 2, 3, 4 the cytotrophoblast cells are necrotic. **1** - SC (+), **2** - J-chain (+), **3** - IgG (+) only microvilli of trophoblast, **4** - IgA (±). Mononuclear phagocytes are closer to the trophoblast; the number of phagolysosomes is increased. Stroma is somewhat swollen. All is taken at x1000.

of interleukin-2α (CD25) dramatically decreased (p <0.001). Phagocytic activity of mononuclear phagocytes against IgG and IgA did not change (Table 4.9.), while for IgM increased (p <0.01) due to activated IgM transport through the placental barrier. SC in phagocytes is not detected, J-chain is seen, Fas, FasLigand, bcl2 and p53 are absent. Number of fibroblasts - 2.03 ± 0.48/50000 μm^2, smooth muscle cells and fibers of the villi stroma are not changed. All types of immune cells of pregnant CD3-, CD4-, and CD8 are positive. T cells, CD56 (+) NK cells, CD20 (+), CD79A (+) B-lymphocytes and plasma cells, CD45LCA (+) leukocytes, including macrophages in the stroma of the villi are not detected. Apoptosis of stromal cells is not increased, the number of capillaries differs from group 1 moderately (Table 4.2).

Fig. 4.6. Ways of acute bacterial infection in the pregnant uterus



1 - myometrium, **2** - decidual part, **3** - chorionic villi, **4** - amnion, **5** - amniotic water, **6** - chorion, **7** - lacunae with the blood of pregnant, **8** - umbilical cord, **9** - yolk sac, **10** - hematogenous spread of infection (group 3B): endometritis - deciduitis - to the blood of lacunae - villi - umbilical cord - the blood of the embryo, **11** - another way of infection (group 3A): endometritis - deciduitis - chorioamnionitis - amniotic water - ingestion of infected water.
Yellow highlighted the areas inflammation, red - the two ways of infection.

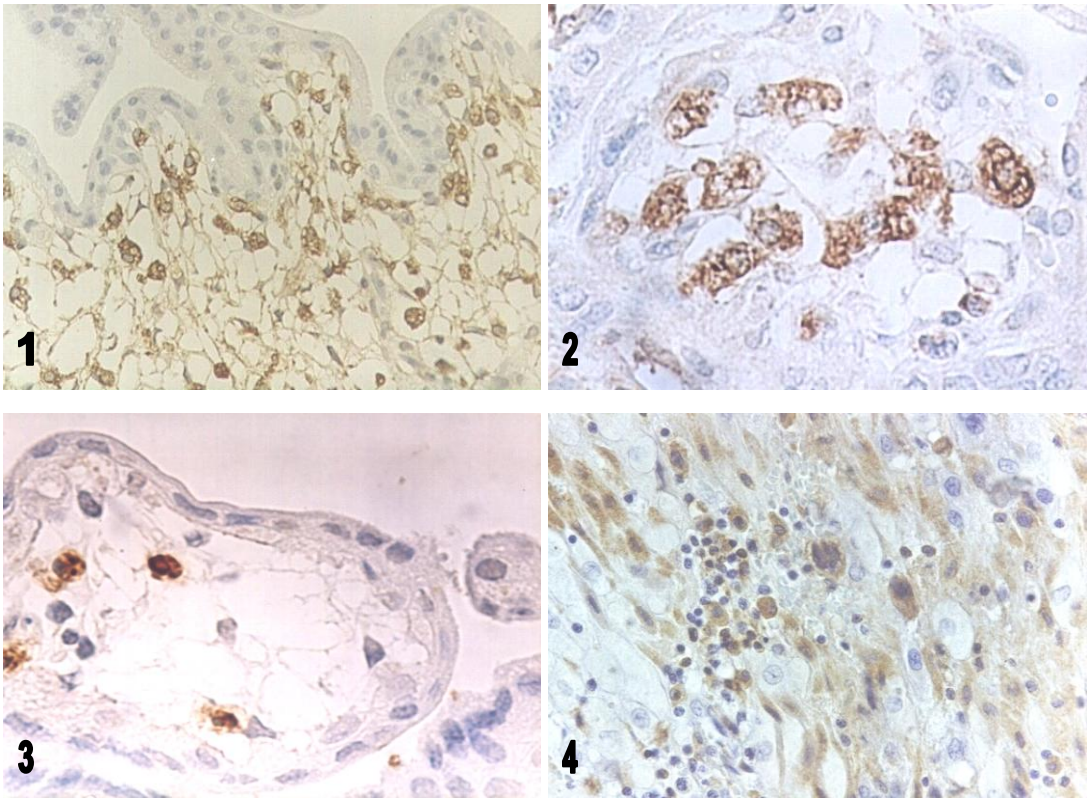
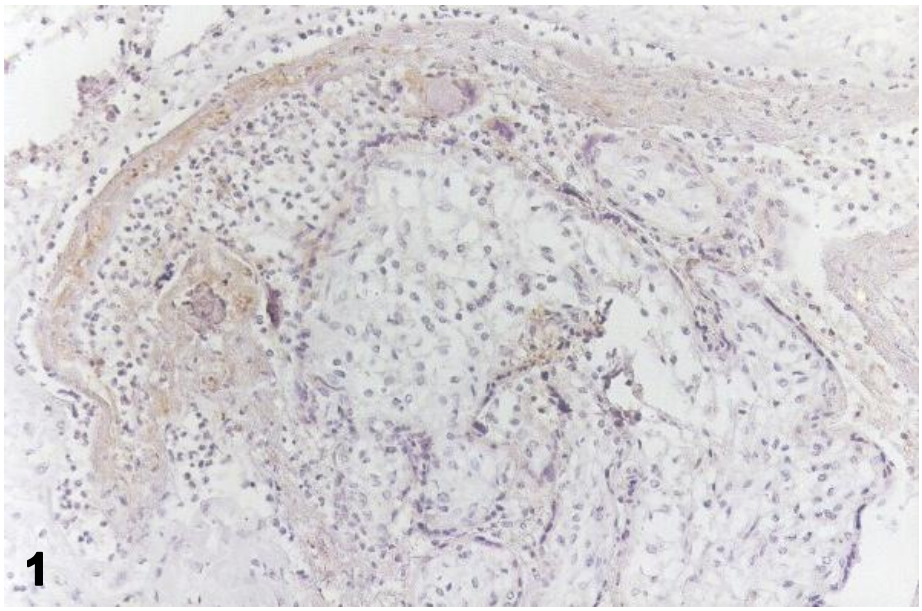


Fig. 4.7. **1**, **2** - in the villi of group 3A at 13 - 20 weeks, the number of phagocytes and the phagocytic activity significantly increases (compare with Fig. 4.1., Table 4.9.). **3** -CD3 (+) T-pro-lymphocytes appear in villi. **4** - increased movement of groups of proliferative trophoblast in the decidual tissue and, as a result, increased the amount of invasive trophoblast, which contains a lot of IgM. There are IgM (+) B cells. Magnification in 1, 4 are x400, 2, and 3 - x1000.



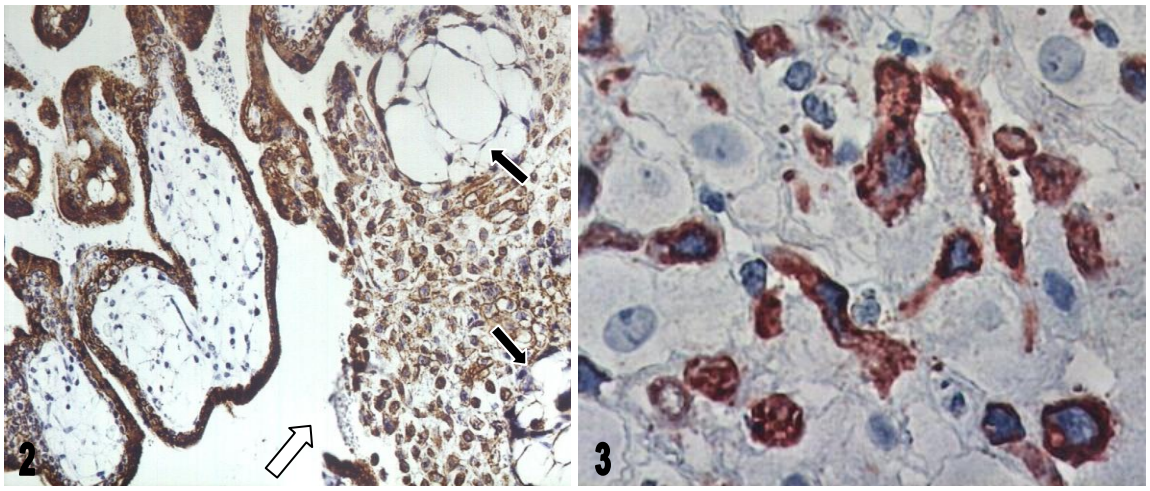


Fig. 4.8. Group 3A, 17 - 20 weeks of development. **1** - villi: syncytiotrophoblast and at some places cytotrophoblast are destroyed. Some vessels are thrombosed, while others are free and do not contain red blood cells. Many phagocytes are seen. On the surface of the villi there is a large number of white blood cells and fibrin. In the surrounding shell white blood cells are seen, x200. **2** – in the villi there is a lot of proliferating trophoblast, there are regions in contact with the decidual tissue. Trophoblast of lacunae in some places is destroyed (light arrow). In decidual tissue there are sections of colliquative necrosis (black arrows). All types of trophoblast, including lacunae located and invasive are SC (+). Invasive trophoblast is multiple; it does not contain too many phagocytized particles, x200. **3** - among the decidual cells, in large SC (+) invasive trophoblast there is a little number of phagolysosomes. x1000.

Table 4.9. Averaged number of phagolysosomes contained in mononuclear phagocytes of villi, in invasive trophoblast and macrophages of the decidual tissue

The number of phagolysosomes in the cell section		Group 1	Group 2	Group 3A	Group 3B	Group 4
Mononuclear phagocytes	**IgG**	10.2 ± 0.67	8.75 ± 0.88	9.47 ± 0.37	14.05 ± 1.69 b c	47.58±2.66 a,b,c,d
	IgA	8.84 ± 0.72	6.07 ± 0.57 a	8.95 ± 0.23 b	13.26 ± 0.81 a b c	34.21 ± 2.71 a,b,c,d
	IgM	0.96 ± 0.25	3.1 ± 0.25 a	4.54 ± 1.14 a	7.41 ± 1.13 a b	15.37 ± 1.34 a,b,c,d
Invasive trophoblast	**IgG**	12.69±0.69	12 22±0.84	8.58 ± 0.47 a b	13.31 ± 1.52 c	51.73 ± 2.98 a,b,c,d
	IgA	12.76±1.15	12.02±1.13	7.59 ± 1.17 a b	12.53 ± 0.79 c	47.11 ± 3.38 a,b,c,d
	IgM	10.14±0.99	9.67±0.88	9.33 ± 0.59	10.48 ± 1.27	42.45 ± 3.16 a,b,c,d
Decidual tissue macrophages	**IgG**	5.73±0.41	4.84±0.33	12.7±1.44 a b	14.0±1.97 a b	18.09±2.03 a b
	IgA	4.35 ± 1.12	3.11 ± 0.72	10.15 ± 1.32 a b	13.19 ± 1.21 a b	18.59 ± 2.95 a,b,c,
	IgM	3.67 ± 1.04	2.23 ± 0.46	5.41 ± 0.83 b	11.04 ± 1.42 a,b,c,	17.39 ± 2.28 a,b,c,d

$^{a, b, a, d}$ - significant differences ($p < 0.05$-0.001) of the next group to the groups 1, 2, 3A and 3B.

Table 4.9 is illustrated by Diagram 4.2. Immunoglobulins in groups 1, 2, 3A and 3B of mononuclear phagocytes in the villi in the invasive trophoblast and macrophages in the decidual tissue show a small amount of alloantibodies. In group 4 phagocytosis increases dramatically (for all immunoglobulins $p < 0.001$), which is a sign of the large number of allogeneic immunoglobulins. Lower rates of phagocytosis by phagocytes of the villi in comparison with invasive trophoblast are associated with no lower activity of phagocytes. They phagocytize the matter and as much of it as passes through the placental barrier. For example, IgM, which in group 1 is only occasionally and in small amount passes through the barrier, is close to zero. While the invasive trophoblast is in the decidual tissue of pregnant where there are a lot of immunoglobulins from a large number of B-lymphocytes situated there. A large number of IgM, phagocytized in group 4 by invasive trophoblast in the decidual tissue does not differ significantly ($p > 0.05$) from the number of phagocytized IgG and IgA. This shows that in group 4 the new, just newly synthesized antibodies are phagocytized. Number of phagolysosomes in macrophages of decidual tissue during the embryonic period in the first and second groups, where inflammatory processes are not occurring, is small. In groups 3A, 3B and 4 the inflammatory and immune processes are happening, and the number of phagolysosomes in them is significantly higher than in groups 1 and 2 ($p < 0.02$-0.001).

4.5.2. Placenta of the pregnant

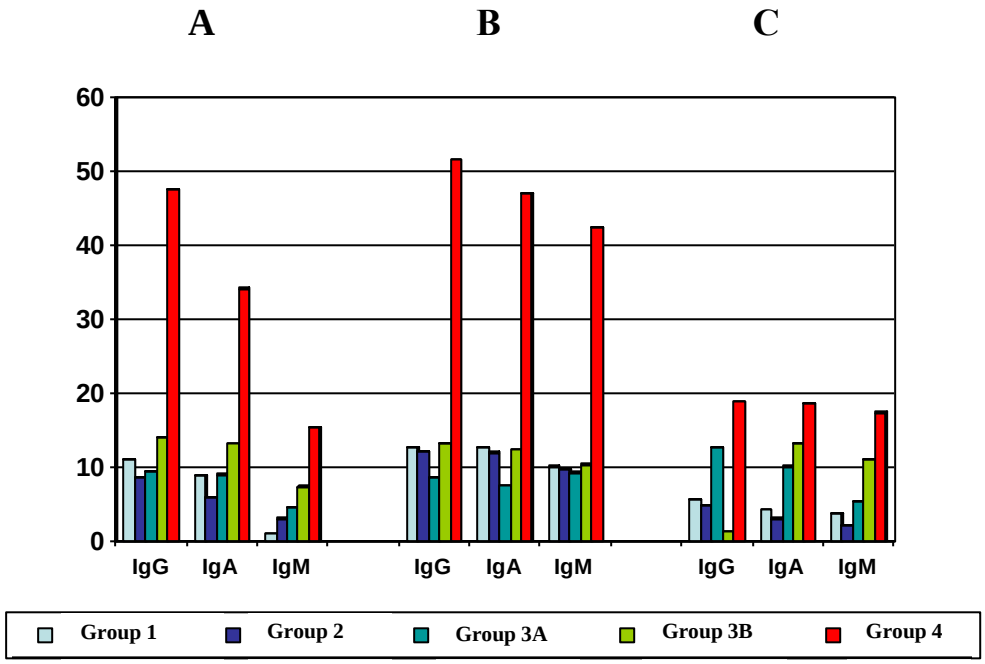
Placenta in cases of group 3A is undergoing significant changes due to acute bacterial action.

In the decidual tissue there are significant areas of necrosis with abundant infiltration by leukocytes, mainly neutrophils, as well as by a small number of CD68 (+), CD16 (+) and CD32 macrophages. Sometimes it can be seen the spread of necrosis in the chorion and amnion. Along the edge of the necrosis at the side of decidual tissue there is a small number of neutrophils. In necrotic tissue the microflora is found, often - cocci, sometimes rods. In some cases, in areas of inflammation marked thrombosis of spiral

vessels is observed: together with obliteration of individual spiral arteries - in one case. Number of leukocytes outside areas of necrosis in the decidual tissue is changed differently. Number of T-lymphocytes (CD3, CD4, CD8, and CD56 NK) has not changed in comparison with group 1 (Table 4.6.). The quantity of CD20 (+) B-lymphocytes is decreased, while increased the number of plasma cells CD79A (+), in which B lymphocytes have been transformed. It significantly increases the number of CD68 (+) macrophages and the number of phagolysosomes in them (but not containing immunoglobulins see Table 4.9.). Also it increased the number of CD45LCA (+) leukocytes (Table 4.7).

Invasive trophoblast, along with a drastic drop in proliferative activity (Table 4.4.) also slightly reduces the phagocytosis of immunoglobulins (Table 4.9., Diagram 4.2). But apparently, this is not a sign of reduction of their phagocytic function, it is more likely a consequence of increased formation of antimicrobial antibodies, which are not harmful to the embryo and are not subject to destruction. Apoptosis of the invasive trophoblast and phagocytosis of it are minor.

Diagram 4.2. Average number of phagolysosomes with immunoglobulins in mononuclear phagocytes of villi (A), invasive trophoblast in the decidual tissue (B) and of macrophages of pregnant (C) (see Table 4.9)



Intervillous space does not undergo significant changes. They contain a little blood and fibrin on the villi (Table 4.8.). Small fibrin clots were available in six cases. Bacterial flora is absent. Number of neutrophils in the blood in 75.5% of 10-20 cells per 50,000 μm^2 of slice area and in 24.5% of cases the number rises to 30-60 per 50000 μm^2. Composition of T-lymphocytes is not significantly altered (Table4.7): it increases slightly the number of NK ($p < 0.05$), macrophages and CD45LCA (in both cases $p < 0.01$).

4.5.3. Conclusion for the group 3A

Noted changes in placental barrier of pregnant and, in particular, the state of immune cells indicate an acute inflammatory process that has recently begun initiated, and having a compensatory character. In the embryonic part of the placental barrier, this version of a bacterial infection also occurs with compensatory responses. This is due to the fact that in group 3A acute bacterial infection of the birth canal passes the placental barrier and is distributed in the embryo through the chorion and amnion (Fig. 4.6). Through these membranes the immunoglobulins of the pregnant enter into the amniotic fluid. They are ingested by the embryo, together with bacteria into the stomach and intestines. So in their epithelium and in the adjacent pancreas and liver by the early embryonic period are already found the operating elements of the ICU: SC, J-chain and immunoglobulins (Israel EI, et al., 1993; Gurevich P., et al., 2003a; Ben -Hur H., et al., 2004). Therefore, this variant of a bacterial infection of the birth canal progresses softer than the other versions of the same disease which extends through the placental barrier and beyond, through the hematogenous ways (Group 3B). And yet, this process is difficult to call easy because of reduced cell proliferation of cytotrophoblast, the number of Ki67 + cells in groups of proliferating trophoblast, in invasive trophoblast and capillary endothelium villi (Table 4.4.), IL2Rα, receptors of trophoblast and monocyte Fc gamma RII and RIII (Table 4.5) and others. At the same time, there are processes of a positive nature: in proliferating trophoblast the number of Ki67 + does not decrease. In the yolk sac, separated from the placental barrier, it increases the proliferation of monocytes and promonocytes, so that their total number is doubled and for promonocytes - even by13 times (Table 4.5, $p < 0.001$). Significant changes occur in the embryo or fetus (see 5.6.1.). Cause of their death is in heavy pathogenic effects resulting from bacterial infection and metabolic disorders due to inflammation of the placenta of the pregnant.

4.6. Group 3B. Ascending infection of the birth canal with hematogenous spread

It should be noted at once that in group 3B it is not spoken on a hematogenous infection of pregnant - it is hematogenous spread to the embryo or fetus. In the initial stages in the cases of acute 3B ascending bacterial infection of genital tract of pregnant progresses in the same way as in group 3A: there is an inflammation of the decidual tissue (deciduitis). But the spread of inflammation may change - transforming not to the trophoblast and amnion, but the walls of the lacunae and on to villi – the regular or anchor ones contacting with the decidual tissue (Fig. 4.6). Thus the placental barrier is affected and the bacterial infection spreads in hematogenous way to the embryo or fetus. Nine cases of embryonic period and 4 – of fetal are investigated (Table 4.1).

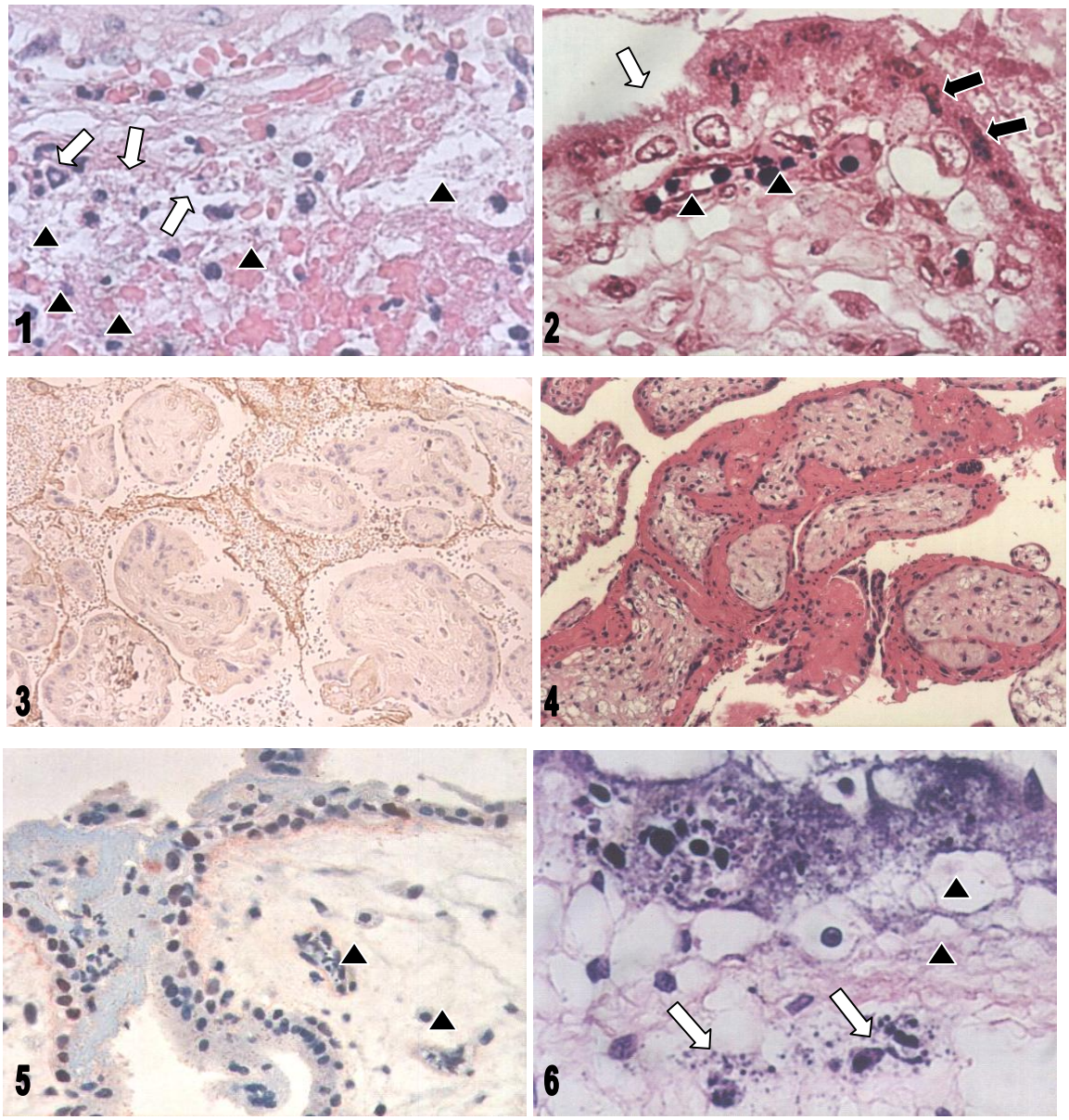


Fig. 4.9. Group 3B. Cases of 6 - 10 weeks. **1** - hemorrhage and necrosis in the stroma of villi. Most of the nuclei are in pyknosis, some in apoptosis (white arrows). Everywhere micrococci (▲) are seen. **2** - villus. It is destroyed the part of the syncytiotrophoblast (light arrow), on the other side - apoptosis (black arrows). In the cytotrophoblast part of the cells is destroyed. There are cocci. In capillary the apoptosis of erythroblasts (▲) is seen. **3** - between the villi threads of fibrin with blood clot. Trophoblast is quite destroyed. **4** – a group of villi with destroyed trophoblast is surrounded by fibrin with leukocytes. Capillaries are rare. **5** - the destruction of syncytiotrophoblast, the formation of fibrin with neutrophils. Damage of the two capillaries (▲) is shown. **6** - 16 weeks of development. Villus with large colonies of cocci (Gram +) at the colliquative necrosis of the stroma (▲). Outside of necrosis - the vessels in a state of apoptosis (white arrows). The magnification in 1, 2 x1000, in 3, 4 x200, in 5, X400, in 6 x1200.

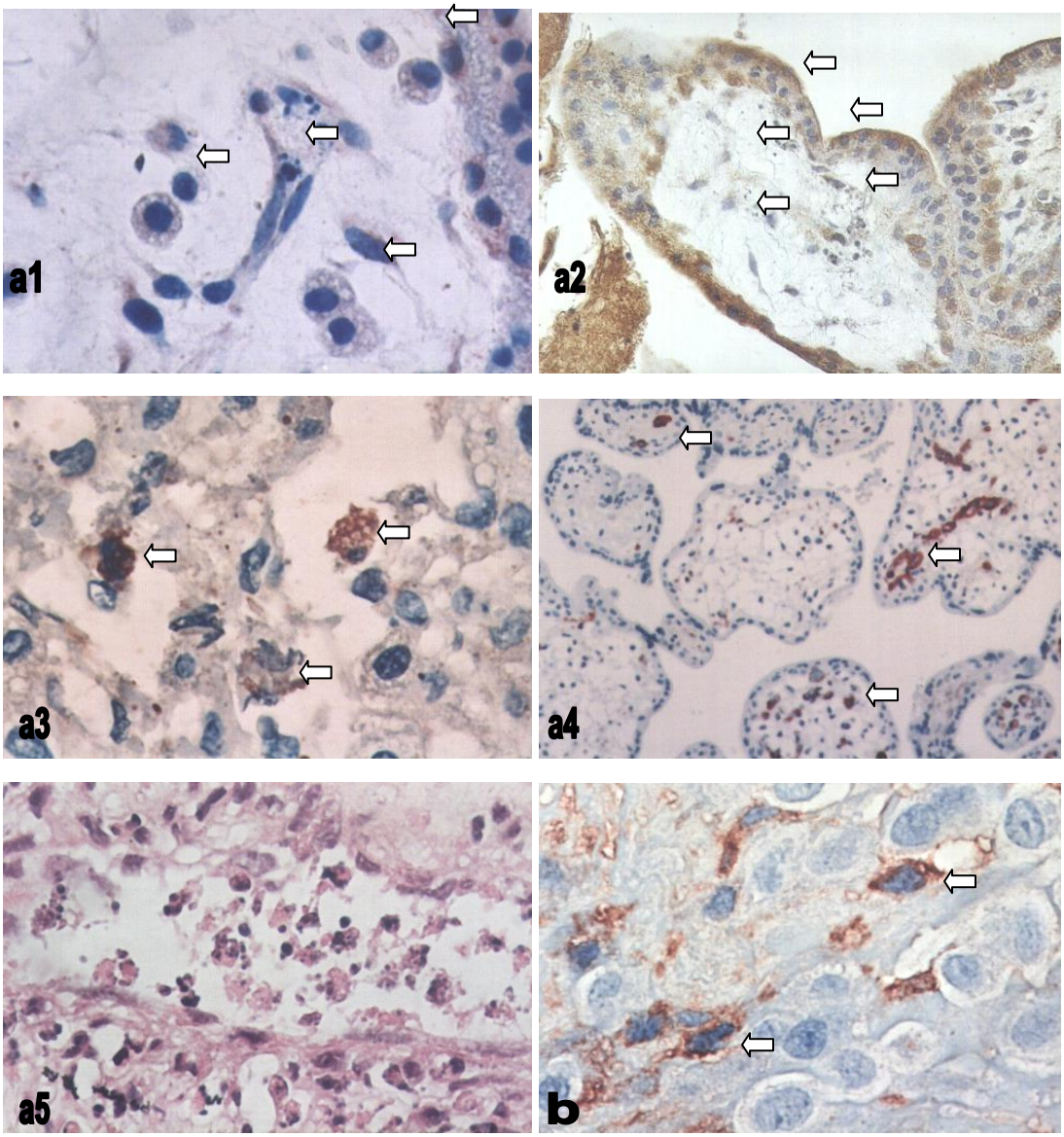


Fig.4.10. Group 3B. In the vessels of the villi the vessels the apoptosis of capillaries is often found, and sometimes the same of monocytes in the stroma. **a1** - apoptosis has recently developed: erythroblasts are destroyed, fibrin clots are in the lumen of the capillary and the capillary while endothelium and monocytes in the stroma of villi are still intact (white arrows), **a2** - apoptosis has affected all capillaries of villus entirely, leaving only small lumps (white arrows) in trophoblast; J -chain (+); **a3** - monocyte apoptosis occurs with the participation FasLigand (+) (light arrows), along with the degradation of cells, **a4** - in many villi the capillaries are completely destroyed by apoptosis, CD34 (+) reveals just a preserved part (only in three villi - light arrows) and the others are without capillaries; **a5** - in collecting vessel, in which blood flows away from a few villi, sometimes one can see multiple apoptosis of erythroblasts and monocytes; **b** - invasive trophoblast in the decidual tissue of a pregnant contains IgG (+) and a small amount of phago-endosomes (open arrows). a1, a3, a5 and b are x1000, a2 is x400, a4 is x200.

4.6.1. Chorionic villi

Small groups of villi or portions of some villi are covered with fibrin in the destruction of places of the syncytium and, by less - cytotrophoblast. Sometimes the necrosis extends to the stroma of the villi (Fig.4.9). In the fibrin at the surface and in areas of necrosis of the stroma there are colonies of cocci and rarely - bacilli, and in some cases - both. Destructions of the villi of 3B group causes changes far greater than of not only the control group 1, but also of group 3A (see Table 4.2. and Fig.4.7). Less than 20% of the villi operate, in the others the capillaries are at a state of spasm, obliteration or thrombosis. The average number of capillaries among the villi of group 3B is lower than in groups 1 and 3A ($p < 0.001$). In 33% of cases the necrosis of the stroma is observed, being sometimes converted to fibrosis, calcification of the placenta, or to hemosiderosis in the fields of ex-hemorrhage.

Trophoblast. In the syncytiotrophoblast and cytotrophoblast outside the fracture zone there are SC; J-chain, IgG, IgA, weak staining of IgM. TUNEL, Fas, FasLigand and p53 are positive in the areas of destruction of the trophoblast; bcl2 weakly stains the apical villi of the syncytiotrophoblast in the intact areas. Suddenly, by 4 - 10 times, cell proliferation is reduced (indicator Ki67+) in all types of trophoblast (syncytiotrophoblast, as always, is Ki67 negative)) (see Table 4.4.).

Cells and stroma of chorionic villi. Necrosis of the stromal areas of villi and its consequences - fibrosis, calcification or hemosiderin deposition is observed in 55.5% of cases. In the fetuses at II trimester of this group the necrotic villi and their consequences were not found. Colonies of bacteria were found in 84.6% of cases. Along with necrosis of the stroma, of numerous fibroblasts and of smooth muscle cells there is apoptosis of mononuclear phagocytes p53 positive ($9.26 \pm 2.36\%$ of total) and villous capillary endothelium, and sometimes also of the veins of stem villi (Fig.4.9, 4.10). Number of mononuclear phagocytes compared with control group 1 is not changed, but dramatically increased the number of promonocytes (up to 25.6%), while reduced the number of proliferating Ki67 (+) of phagocytes, as well as phagocytes containing different receptors (Table 4.5). In the blood single myelomonocytic stem cells appear. The complex of these changes is a sign of failure of mononuclear phagocytes. The content of IgG and IgA in the phagolysosomes is not that large and it is not significantly different from groups 1, 2 and 3A, only IgM is increased slightly (Diagram 4.2, Table 4.9, Fig.4.10), which transportation through the placental barrier is enhanced.

4.6.2. Placenta of the pregnant

State of the placenta in the pregnant is basically similar to those observed in group 3A, since both groups have similar pathogens - bacteria. However, there are some differences.

Decidual tissue. Acute deciduitis is represented by the large areas of necrosis, surrounded by a moderate infiltration of leukocytes (Fig.4.9), mainly neutrophils. There are also invasive trophoblasts, some natural killer cells and macrophages – their significant concentration. There is microflora, mainly cocci. Sometimes the necrosis reaches the surface of blood lacunae, trophoblast lacunae covering the in these sites is destroyed. Sometimes the contact areas of necrosis with anchor villi is seen, with no passage of leukocytes from the maternal decidual tissue necrosis to the villi, but the

invasive trophoblast actively protrudes not only into the decidual tissue, but also in leukocyte shaft and into necrosis, where white blood cells do not penetrate to (Fig.6.2). In the 7.7% endothelial proliferation in some spiral arteries is mentioned. Decidual cells do not contain SC, J-chain and immunoglobulins; it clearly distinguishes them from the invasive trophoblast. In the invasive trophoblast it is significantly reduced the number of proliferating Ki67 (+) cells (Table 4.4). Invasive trophoblast apoptosis is at 1.12 ± 0.08 per 50000 μm^2. Number of phagolysosomes with immunoglobulins did not differ from group 1 (Table 4.9; Fig. 4.2). Number of T-lymphocytes (CD3, CD4, CD8, NK-CD56) in group 3B differs slightly from that of other study groups (Table 4.6). The strong decline in the number of B-lymphocytes and plasma cells increase reflects the transformation of the first into the plasma cells, which is a manifestation of the humoral immune response. It is significantly increased compared with all groups the number of CD68 macrophages, indicating the highly pathogenic effects (Table 4.6).

Intervillous spaces. Clots around the small groups of villi of embryos and fetuses are noted in 33.06% of the cases and in addition, in 15.4% of cases there were small fibrin clots. In embryos, the frequency of thrombosis was noted in 55.55% of cases (Table 4.8). In blood and fibrin in all cases there are microflora - cocci, in one case - cocci and bacilli, colonies of microflora are detected in the blood lacunae. The number of neutrophils in the blood is increased: from 30 to 50 cells per 50,000 square μm^2 of the slice in half the cases; in the second half - from 50 to 100 and even 300 cells, on average, 78.9 ± 12.3 per 50000 μm^2 of the section area. In the blood of intervillous spaces and in fibrin there are all kinds of immune cells (Table 4.7) In addition, T-lymphocytes (CD3, CD4, and CD8), B-lymphocytes and plasma cells do not deviate significantly from the number of these cells in all experimental groups. The content of NK (CD56) is increased in comparison with group 1 ($p < 0.001$). The number of macrophages (CD68) is increased.

4.6.3. Conclusion for the group 3B

Separation of acute bacterial infection of the genital tract during early pregnancy into two ways is based on their different distribution, complications and severity. This is because the group 3B in the hematogenous tract in embryos there are not pregnant immunoglobulins, which are found on the path at the same infection in the group 3A in the amniotic fluid and the intestinal tube. Moreover, hematogenous spread of infection in group 3B passes through the placental barrier, where the capillaries of villi and the villi themselves are severely damaged. The destruction of the villous mononuclear phagocytes has a distinct character of apoptosis with presence of p53. In the areas of destruction of the syncytiotrophoblast and partly cytotrophoblast, there are positive reactions for TUNEL, Fas and FasLigand, which indicate apoptosis. But some partly changed areas of trophoblast and villous stroma areas should be attributed to necrosis, as referred there TUNEL, Fas, FasLigand and p53 are negative. In addition, in necrosis of the stroma and in the stroma itself, except for mononuclear phagocytes, cells (fibroblasts and myocytes) are few. Less than a fifth of the capillaries remain functional, more than half of the villi (apoptosis, necrosis, fibrosis, and petrification) is destroyed. Further, spread of infectious pathogens (microbes themselves, their toxins) with the blood gives them entry permission into the vital organs of the embryos - the brain, endocrine organs, heart, and others. Heavy damage to tissues and organs is manifested in a critical decrease in their proliferative (regenerative) capacity (trophoblast, mononuclear phagocytes). Biologically

active substances (interleukin IL2Rα, Fc gamma RIII and RII) in phagocytes are reduced (Table 4.5). All this leads to the death and destruction of the germ: thus in group 3A tissues of embryos have been preserved until the abortion in 61.54%, and for fetus - in 90.91% of cases (in total 80.0%), whereas in group 3B only 44.44% of embryos has been preserved and 25%, of fetuses which is 38.46% in average (Table 5.1).

States of maternal part of placenta in groups 3A and 3B for the majority of immune cells are similar. But in group 3B there is a significant increase in plasma cells compared with the group 3A in decidual tissue (Table 4.6) and blood lacunae (Table 4.7), ($p < 0.01 - 0.001$). This indicates a more intense effect of pathogens on the organism of pregnant women in group 3B: the passage of blood lacunae containing microflora to the bloodstream of the pregnant may cause in her body some pathological processes during the first 4 months of pregnancy.

The apoptosis progressed considerably, affecting tissue of $9.26 \pm 2.36\%$ of villi, mainly for mononuclear phagocytes, capillaries and syncytiotrophoblast, to less extend. This caused a significant increase in the number of avascular villi and a decrease in the average number of capillaries in them, although a significant increase in villous edema wasn't noted. The number of functioning capillaries was highly decreased (Table 4.2).

4.7. Group 4. Early allogeneic conflict of the pregnant and the embryo

The group includes 43 cases (52.4%) of embryos at 3.5-8 weeks, later stages haven't been found. Tissues of embryos have been found in 15 cases (Table 4.10).

In the area of the placental barrier in cases of group 4, there are typical changes different in many respects from the normal condition (Group 1) and from the other pathological (including inflammatory) processes (Groups 2, 3A and 3B) although there are some similar changes. Chapter 4 will address the changes in placental barrier.

4.7.1. Chorionic villi

The main process that causes in the group 4 heavy damages is a multiple apoptosis of all types of trophoblast, mononuclear phagocytes, capillary villi and erythroblasts. As a result of apoptosis of capillaries in group 4 the number of villi with functioning capillaries decreased by 2.5 times, the number of avascular villi increased by 3 times, the average number of capillaries per a villus decreased by 2.5 times (all $p < 0.001$). There was also a spasm, obliteration, thrombosis and complete destruction of the capillaries (Table 4.2). The number of edematous villi is dramatically increased. Necrosis, fibrosis and petrification of the villi are absent.

Apoptosis simultaneously affects a small number of villi: in 83.7% of villi only 3-18% are damaged. More significant number - 20-33% is noted only in 16.3% of cases. In average, the number of villi, containing TUNEL-positive syncytiotrophoblast (of an entire villus or just parts of it) as well as in the capillaries and monocytes of those and other villi, was 26.39 ± 4.75. The new villi are gradually involved. For each of the weeks: 3.5-4, 5 and 6, the number of villi with apoptosis was the same (Table 4.10), the number of villi with normal capillaries and avascular villi also similar during the mentioned weeks. And then at the 7th and 8th final weeks the number of villi with normal capillaries is reduced while the number of avascular villi increases. And only the number of villi

with apoptosis of blood vessels and of the swollen ones remains the same. This distribution of apoptosis and its consequences indicates the progress of the pathogenesis gradually, in small doses. In the past two weeks the minimum possible number of still-functioning villi comes to the end.

Fibrin clots in the nap are calling some attention. At 3.5th-4th week their formation is small, but every 5-8 weeks, their number is increasing by 3.3 - 4.1 times (Fig. 4.10, Table 4.10). This shows that the beginning of a pathological process in these cases is not in the clotting of blood proteins, but in the destruction of the trophoblast villi by alloantibodies. This creates the conditions for subsequent coagulation: coagulation of proteins is a complication of the process that causes damage to the trophoblast.

Table 4.10. Terms of abortion and the changes of chorionic villi in allogeneic conflict (Group 4)

	Weeks				
	3.5-4	**5**	**6**	**7**	**8**
Number of abortions (43 in total)	11	11	10	8	3
Out of them - embryonic tissue (15 in total)	8	2	2	2	1
Remain for the next week	32	21	11	3	0
Villi with normal capillaries (%)	38.45±3.29	36.07±3.52	32.0±3.76	16.44±4.57	19.68±5.63
Villi with apoptosis (%)	11.04±2.78	10.36±2.1	13.63±2.67	13.85±2.12	12.82±3.07
Avascular villi (%)	13.64±1.36	13.99±1.78	13.85±2.01	22.94±3.33	7.27± 0.99
The number of cases with fibrin clots (%)	9.1	36.4	30.0	37.5	33.0
Edematous villi (%)	45.58±5.87	57.64±4.38	41.35±5.76	48.88±5.85	38.81±2.86

Trophoblast. In the syncytiotrophoblast, and less in cytotrophoblast of some villi there is destruction into small particles in small or larger areas. Positive reaction of TUNEL, the presence of FasLigand and p53 indicate the ongoing apoptosis; although the disintegration of cells in pathological apoptosis is rapidly completed by necrosis (see Chapter 5.2). In syncytiotrophoblast outside the areas of apoptosis some proteins involved in apoptosis as a receptor - Fas, and antagonist - bcl2 are identified. The intensity of cytotrophoblast proliferation, proliferating trophoblast and the number of its groups are dramatically reduced (Table 4.4).

Immunoglobulin receptor - SC in different villi changes its position according to the stage of transport of immunoglobulins. J-chain, IgG and IgA are detected in the syncytium and cytotrophoblast, IgM is stained weakly. Some groups of cells of the proliferating trophoblast do not contain J-chain and immunoglobulins. Receptors Fc gamma RIIIα (CD16) and Fc gamma RII (CD32) here and there show a weak positivity in the syncytiotrophoblast microvilli.

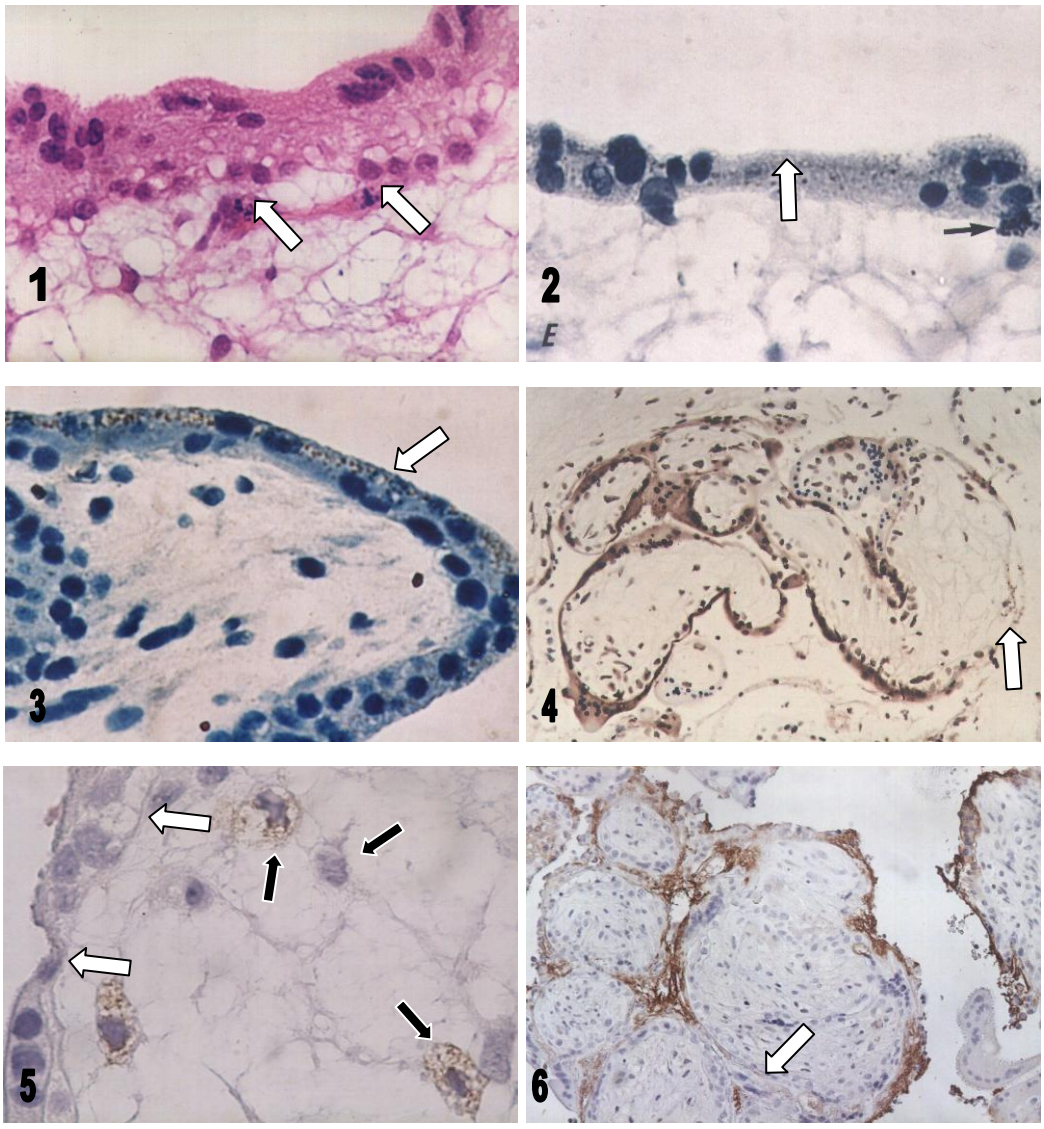


Fig.4.11. Group 4. **1** - one of the first significant damage is in syncytiotrophoblast. Destruction occurs by apoptosis: at first nuclei become homogeneous, and then break into small grains. Cytotrophoblast under it has not yet changed, but the capillary under the cytotrophoblast are also destroyed (open arrows). Stroma looks edematous. **2** - syncytiotrophoblast has already been destroyed and disintegrated cytotrophoblast is being disintegrated (light arrow). Many nuclei of it become homogeneous, dense, and then break up into small grains (dark arrow). **3** – syncytio- and cytotrophoblast get destroyed, dark brown granules in them are FasLigand (+), reactions induced apoptosis is marked with light arrow. **4** - TUNEL reaction shows the apoptosis beginning and occurring in trophoblast, monocytes, and capillaries of the villi (brown). Ruined trophoblast loses its brown color (light arrow). **5** - syncytiotrophoblast is completely destroyed, cytotrophoblast – at some places (open arrows); and some promonocytes (CD68 ±) (black arrows) get also destroyed. **6** - the remnants of the group of villi, connected with fibrin. Trophoblasts and the phagocytes are absent. Single traces of capillaries are found in only one villus (light arrow). In the stroma of the villi fibroblasts and rare myocytes are retained. Cases 1 – 5: damage at 3.5 - 4 weeks, the case of 6: 5-6 weeks. 1, 2, 3, 5 is x1000, 4 - x100 and 6 - x200.

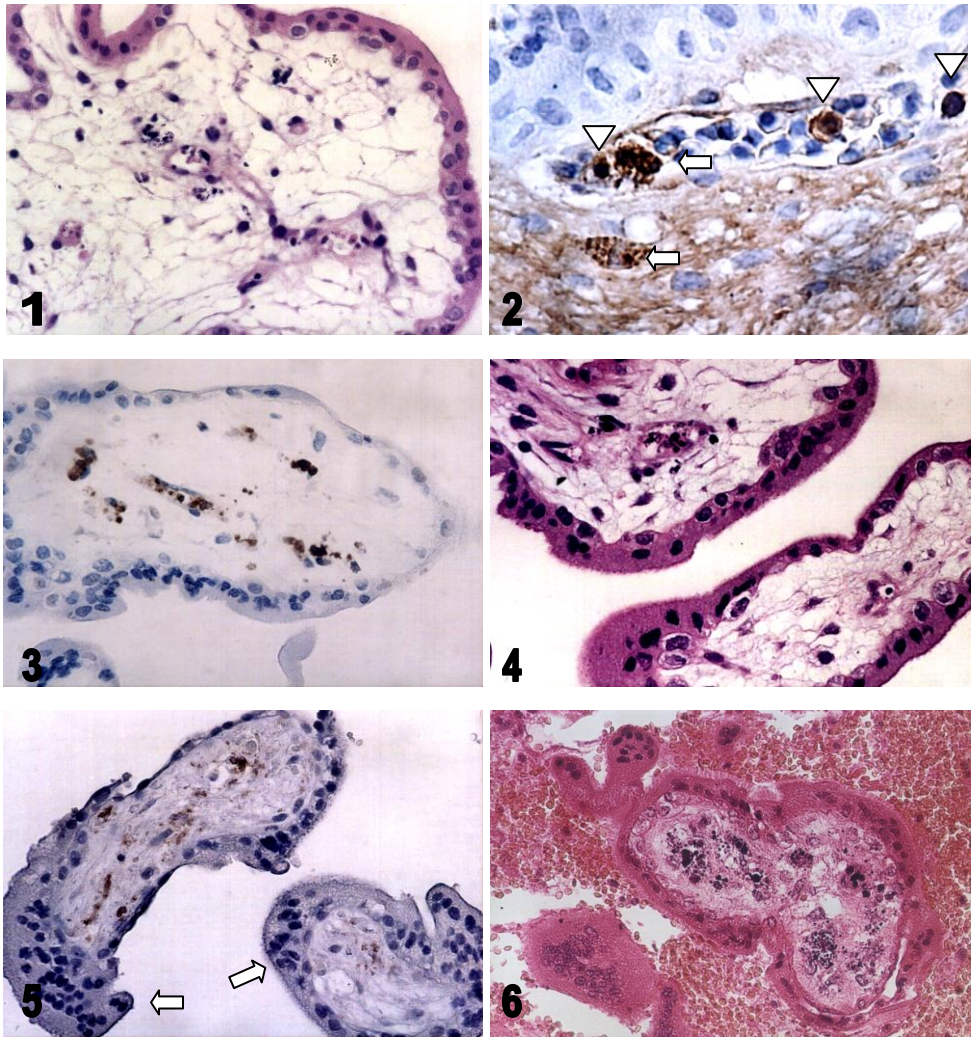


Fig.4.12. Group 4. Cases 1-4: 3.5 weeks of development. **1** - syncytiotrophoblast, endothelium of capillary are almost completely destroyed;, apoptosis of erythroblasts, monocytes in capillaries and in the stroma. A small swelling is seen. **2** - IgA is found in the capillary endothelium, but in a little amount, only in three erythroblasts (Δ) and in two monocytes (white arrows) located in the capillary and the stroma of villi in apoptosis. **3** - the destruction of the syncytiotrophoblast and cytotrophoblast. Erythroblasts and monocytes are in apoptosis, TUNEL (+). **4** - the same picture: the destruction of syncytiotrophoblast, pyknosis of the nuclei of cytotrophoblast, the disintegration of capillary endothelium, apoptosis of erythroblasts. **5** - 5 week development: the decayed part of the syncytium and cytotrophoblast. There are areas of proliferation (white arrows). Apoptosis of capillaries and their cells is seen. **6** - the seventh week. Complications are developed: thickening and clotting of blood around the villi, intense apoptosis in almost all tissues. 1, 3, 4, 5 and 6 are x400; 2 is x1000.

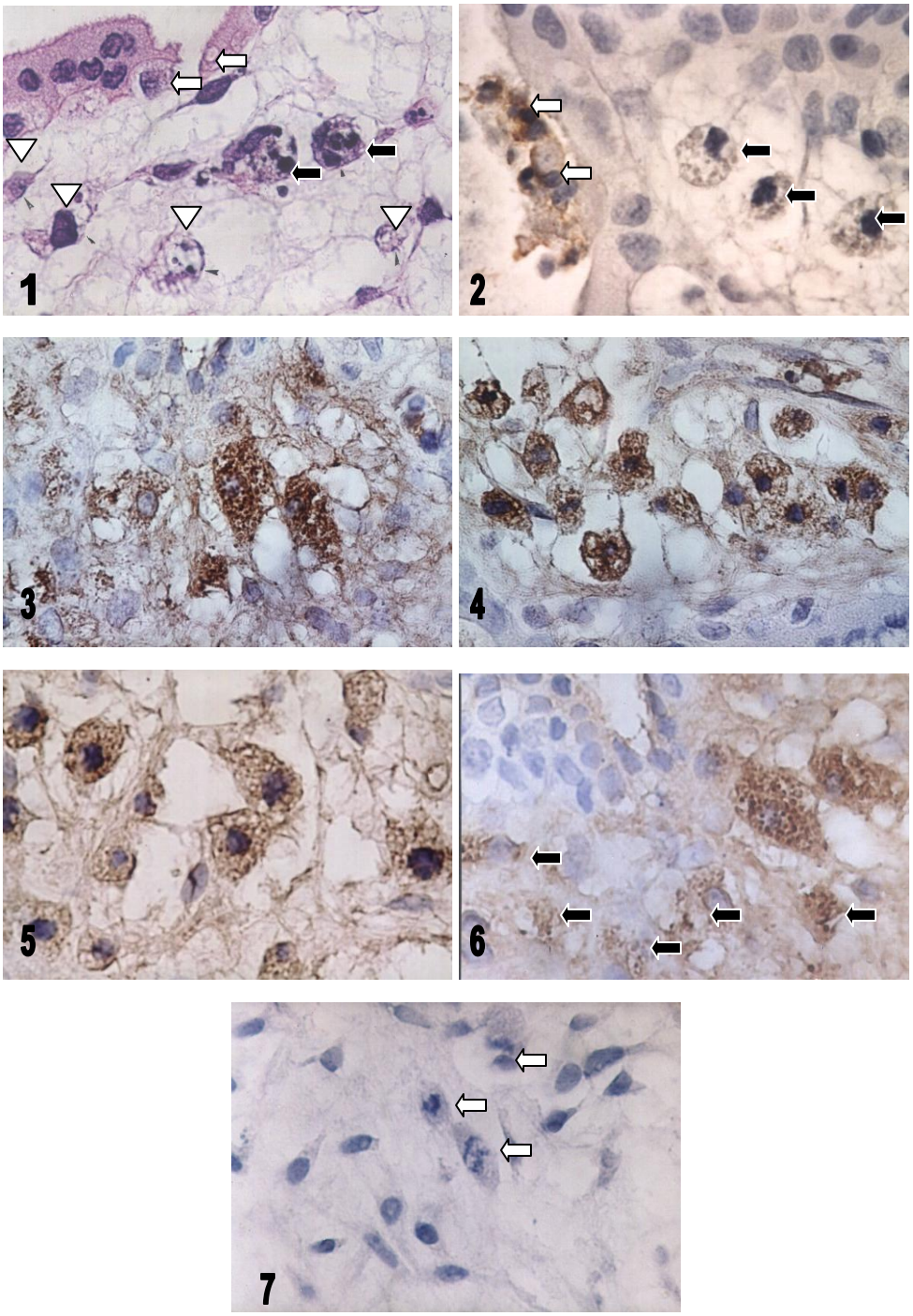


Fig. 4.13. Group 4. All cases of 3.5 - 5 weeks. **1** - syncytiotrophoblast is destroyed, cytotrophoblast decays at several places (white arrows). In the capillaries apoptosis is seen (black arrows); in stroma, apoptosis of monocytes is observed (Δ). **2** - promonocytes Fc-gamma RIII (CD16 ± or CD16-) (black arrows) compared with macrophages of the pregnant differ by CD16 (+) (light arrows). **3, 4, 5**-promonocytes of embryos function as active phagocytes, the most intense are IgA, IgM, and significantly weaker - IgG, which is associated with the pathogenicity of antibodies and their quantity. **6** – seven out of five phagocytes are in apoptosis (black arrows). **7** - villous fibroblasts do not undergo apoptosis and sometimes even proliferate (white arrows). All panels are x1000.

Stromal cells of chorionic villi. Mononuclear phagocytes, although being represented almost at the same amount as in control (group 1, Table 4.5), but reveal composition which is changed dramatically. Number of monocytes decreased, while the bulk of these cells (54.17 ± 3.61%) are promonocytes. This is the result of the death of a significant part of the monocytes. In some cases, 90-100% of monocytes are destroyed, they are replaced by promonocytes. In the blood capillaries of the villi in the blood vessels and heart cavities of embryos sometimes single CD34+ stem cells of proerythroblastic or myelomonocytic cells are seen as a consequence of their proliferation enhancement. Monocytes were positive with CD68, but their color is less intense than the staining of CD68 + macrophages in the decidual tissue of the pregnant on the same slide. Promonocytes are not stained with CD68, but are positive with CD14. In addition, promonocytes of group 4 contain a very large number of phagolysosomes with immunoglobulins, which are clearly distinguishable when stained with IgG, IgA and IgM. A very large number of phagolysosomes with immunoglobulins is also available in monocytes. In some sections of phagocytes it reaches 100-150. In mononuclear phagocytes in group 4 the ratio of immunoglobulin-containing immune complexes (in the membranes of cells) and of phagolysosomes (in cytoplasm) was 21.52: 78.48% (in group 1 it constituted 57.77: 46.23%). All this means a dramatic increase in the number of pathogenic immunoglobulins in cases of group 4. Average number of phagolysosomes in the slice of monocytes is 49.58 ± 2.86, their number in the slice of promonocytes is 37.24 ± 2.68 ($p < 0.01$). This means that the phagocytic ability of promonocytes is only slightly lower than that of monocytes. Average number of IgG, IgA and IgM in mononuclear phagocytes of group 4 dramatically exceed it in all investigated groups 1, 2, 3A and 3B (Table 4.9, Diagram 4.2). In the cytoplasm of phagocytes there is a J-chain in 67.84 ± 3.35% cells (Table 4.5), which is somewhat less than in groups 3A and 3B ($p < 0.05$). The number of proliferating Ki67 (+) mononuclear phagocytes in the villi is reduced compared with groups 1 and 3A. It also decreases the number of phagocytes containing IL2Rα (21.32 ± 1.24%), receptors Fc gamma RIIIα (CD16) and Fc gamma RII (CD32). This is a result of increased number of promonocytes that do not contain these receptors.

In some villi a significant part of monocytes and promonocytes (up to 35.7 ± 6.42%) contain p53, Fas and sometimes FasLigand, TUNEL positive, the number of decaying phagocytes is lower. Part of erythroblasts in the capillaries contains IgG, IgA, and less IgM. These erythroblasts are at different stages of apoptosis. At the end of apoptosis the cells of the capillaries break down into smaller particles, gradually disappearing without phagocytosis. Proliferating Ki67 (+) endothelial cells of capillaries are observed only in 15.38% of the cases, their mean number is 1.96 ± 0.84% (Table 4.4). The consequence of this is the regeneration of capillaries and an increase in the number of swollen and large avascular villi (Table 4.2, 4.3). Fibroblasts and isolated monocytes in the stroma of villi do not undergo apoptosis, the collagen fibers of the stroma stay without any changes. In avascular and swollen (in which blood vessels are not functioning), villi the apoptosis is absent (Fig. 4.15).

4.7.2. Placenta of the pregnant

In decidual tissue in 67.4% of cases there are small necrotic areas with moderate leukocyte infiltration around. Microflora was found in two cases: in one there was a small group of nodules of actinomyces and in the other – some cocci. Small new hemorrhages,

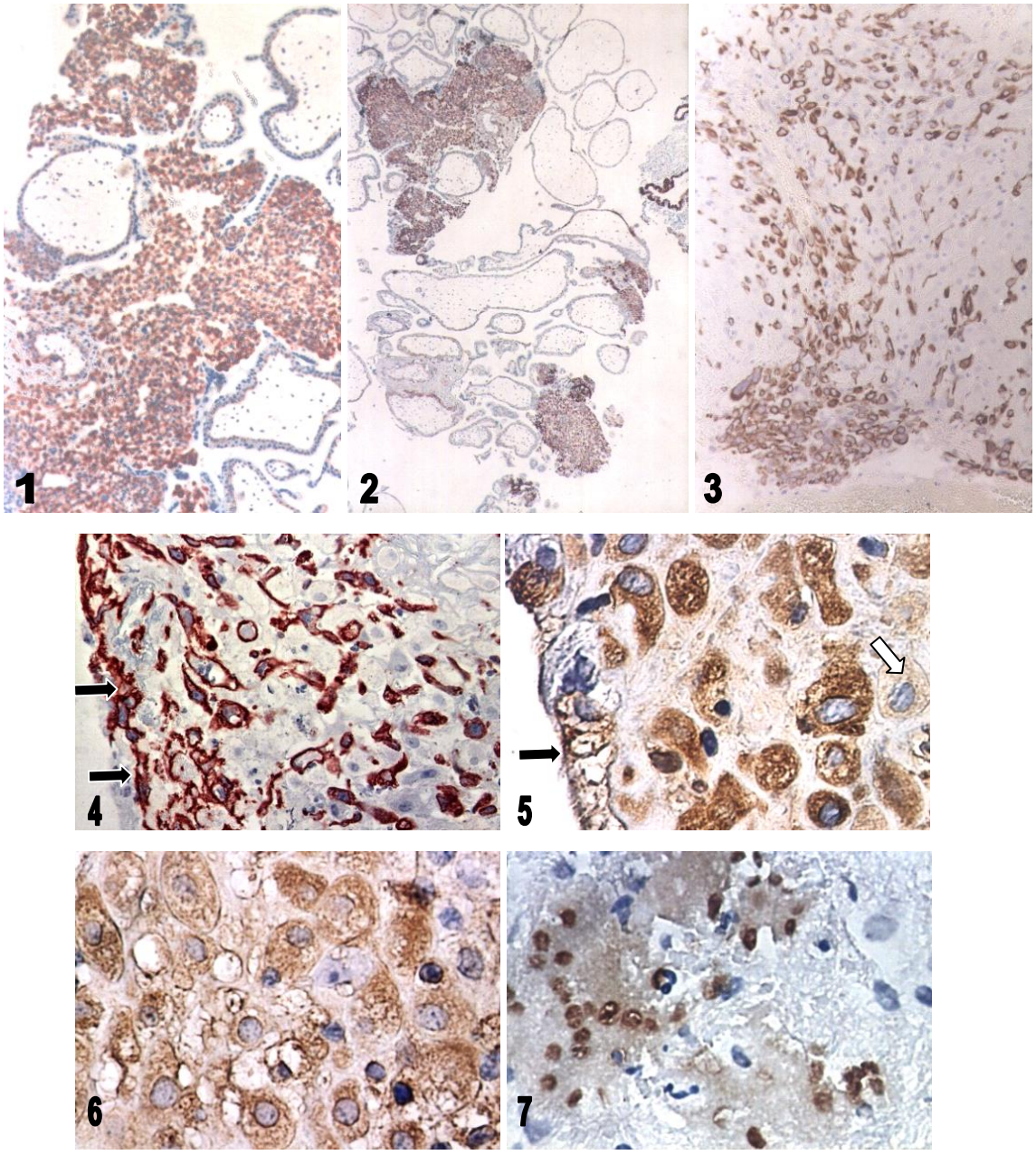


Fig.4.14. Group 4. **1, 2** - a significant strengthening of proliferating trophoblast. It is formed from cytotrophoblast (see Fig. 4.2.1, 4.2.2, Fig. 4.3.1-4, Fig. 4.12.5). The number of proliferating cells increases: Ki67 (+) to more than 80%. **3** - Villi with groups of proliferating cells attached to the decidual tissue ("anchoring villi"). SC (+) proliferative cells are introduced into the tissue of the pregnant woman and become invasive trophoblast. **4** – trophoblast of lacunae is also formed of proliferative trophoblast (dark arrow). **5** - trophoblast of lacunae is destroyed, almost entirely (dark arrow), invasive trophoblast contains SC and J-chain, it uptakes a lot of immune globulins. Decidual cells are replaced (light arrow). **6** - many of the invasive cells are destroyed by apoptosis. **7** - TUNEL reaction shows the apoptosis of many cells, but the decidual cells of pregnant. Magnification for 1 is x100, for 2 - x40, for 3 - x200, for 4-7 - x1000.

apparently formed during the abortion, were found in 20.9% of cases. In the spiral arteries in 11.7% some single cases of marked endothelial proliferation were observed.

Cellular compositions of T-lymphocytes and NK were not significantly different from the other groups (Table 4.6). Number of macrophages is somewhat higher than in groups 1 and 2 ($p < 0.05$), does not differ from group 3A, but less than half of the group 3B. Phagolysosomes in macrophages are few. B lymphocytes and plasma cells have undergone significant changes. Number of B-lymphocytes decreased in spongy zone of decidual tissue by transforming into plasma cells and moving into a compact area. Part of the plasma cells is also moving into a compact area. A small number of B-lymphocytes and plasma cells suggest a moderate humoral immune response in the decidual tissue.

Invasive trophoblast actively phagocytize IgG, IgA and IgM, the number of phagolysosomes in the cytoplasm of cellular sections reaches 100-150, being in average 51.73 ± 2.98 IgG, 47.11 ± 3.38 IgA and 42.45 ± 3.16 IgM (Table 4.9) - 3-5 times more than is contained in sections of invasive trophoblast cells in 1, 2, 3A and 3B groups. More significantly than in phagocytes villi, invasive trophoblast contains IgA and IgM. More frequent invasive trophoblast cell apoptosis and phagocytosis of its multinucleated cells are noted. For this reason it decreases in amount (Table 4.4). And all that, despite the significant movement of proliferating trophoblast into the decidual tissue, where it turns into invasive trophoblast

Intervillous spaces are free or contain a little blood. There are small fibrin clots in 12 cases; multiple perivillous fibrin is seen in one case. At 3.5-4 weeks of development fibrin clots are found only in 9.1% of cases, but at 5, 6, 7, 8 weeks, the number of cases with small clots of fibrin occurs in 30.0-37.5% (Table 4.10). This shows that the coagulation of fibrin in these cases of group 4 is a complication, as a consequence of the destruction of syncytiotrophoblast and to a lesser extent the cytotrophoblast by apoptosis.

Number of neutrophils in the blood of intervillous spaces ranges from a few to 30 per 50,000 square μm^2 of slide in 83.7% of cases: from 40 to 200 neutrophils in 16.3%, while the flora is not found. The composition of immune cells has changed little (Table 4.7). One can only note the decrease in the number of T-helpers (CD4) and no change in NK (CD56). Macrophage numbers (CD68 +) compared with group 1 are somewhat fewer ($p > 0.1$), but dramatically lower than in groups 3A and 3B ($p < 0.001$). This demonstrates the weakness of the antigenic stimulus in group 4.

4.7.3. Conclusion for the group 4

In the study of cases of group 4 two main problems arise in front of us there: first is to identify the common pathologic, immunologic and morphometric changes of placental barrier, its complications and comorbidities, and the second is an attempt to identify the pathogenic factors causing these changes (see Chapter 5), taking into account the changes in the embryos themselves.

Studies have shown that leading change is the multiple apoptosis. This is not the destruction of single cells, as happens in physiological apoptosis. In group 4 apoptosis covers various sites in the placental barrier, in contact with the tissues of pregnant woman

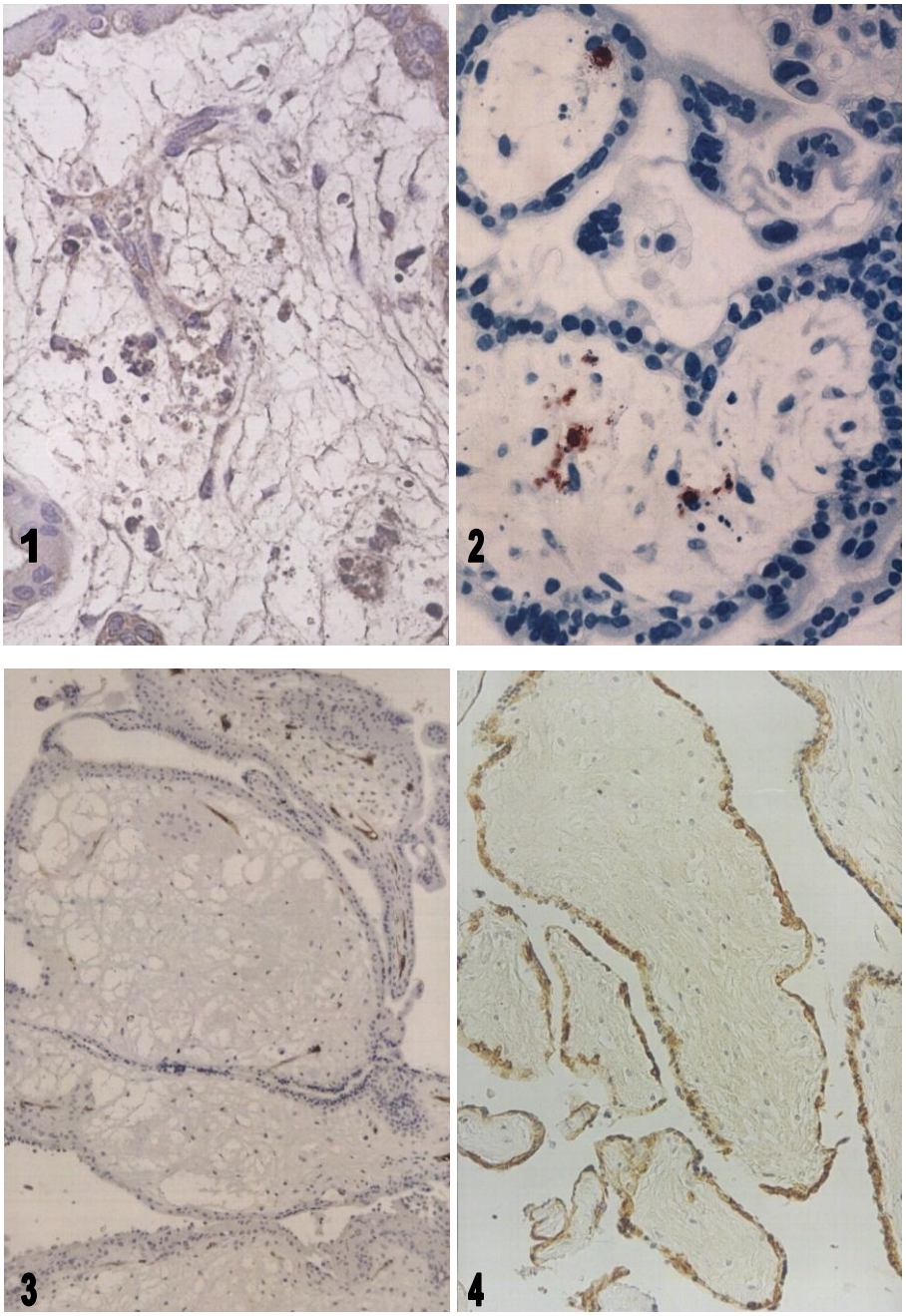


Fig. 4.15. Group 4. 3.5 - 7 weeks. End of avascular villi functioning. **1** - apoptosis, and further destruction of the trophoblast, capillaries and phagocytes. **2** - CD34 (+) reveals the remnants of red capillary endothelium. **3** - in the upper villus the capillaries are mainly preserved (CD34 (+)) as well as the other structures. Three other villi are enlarged, swollen, and sometimes CD34 (+) residues of capillaries collapsed without erythroblasts can be seen. Phagocytes are not available, while fibroblasts are preserved. **4** – villi are reduced, covered by SC (+) trophoblast. The stroma is fibrous, not edematous, containing some fibroblasts only, while the other cells and traces of the capillaries are absent. 1, 2, are x400, 3, 4, are x100.

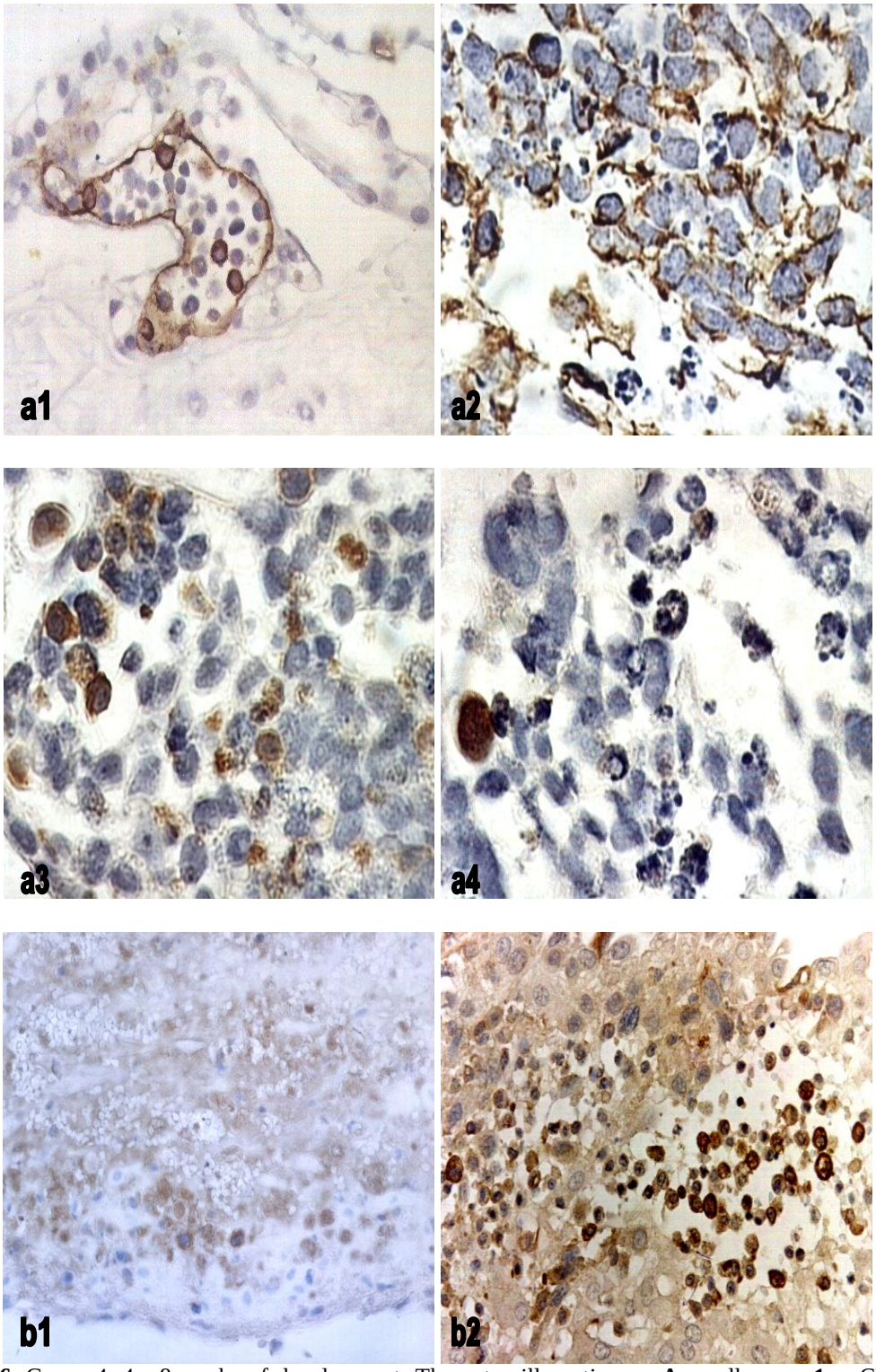


Fig. 4.16. Group 4. 4 - 8 weeks of development. The extravillous tissues. **A** - yolk sac: **a1** - CD34 (+) BFU-E - precursors of erythroblasts have appeared. **a2, a3, a4** - contain hematopoietic cells and other primary cells of monocyte group, 37 - 45% of them are at the state of apoptosis. Many of them contain IgG (+), significantly less - IgA (+). **B** - decidual tissue: **b1** - many of trophoblasts are at the stage of apoptosis and compressed decidual cells die: the large areas of destroyed cells of both species are seen, **b2** - congestion of the B-lymphocyte IgA (+) and other cells in the area of inflammation. a1 is x400, a2-4 are x1000, b1 is x200, b2 is x400.

i.e. with her decidual tissue and blood. They are the five types of trophoblast: 1) - invasive, 2) - covering the trophoblast lacunae with blood of pregnant women, 3) - syncytiotrophoblast, 4) - proliferating trophoblast villi and weaker - 5) - cytotrophoblast, covered by syncytiotrophoblast. The cells in the chorionic villi participating in the transport of immunoglobulins, together with other substances are also affected. This is the endothelium and erythroblasts of the capillaries, and mononuclear phagocytes, which are located in the stroma of the villi. In all these structures and in areas of apoptosis, Fas, FasLigand and p53 as well as positive reaction to TUNEL are identified (Fig.4.11 - 4.14).

At the same time only part of the villi is affected, in average, 18.53 ± 4.01% of the total. In the villi usually only a portion of the trophoblast, segments of some capillaries along with erythroblasts inside of them and some mononuclear phagocytes are destroyed. Stroma of the villi: fibroblasts, myocytes and collagen fibers do not change (Fig. 4.13, 4.15).

According to the literature and our data (Cotran RS et al., 1999; Mor G. et al., 2003; Abrahams VM, 2004; Halperin R., et al., 2008; see also Chapter 5.2) the duration of the complete destruction of every cell at the apoptosis continues for 2 - 18 hours. Apoptosis of villous tissue begins at 3.5-4 weeks of development and continues to 8 weeks. As shown by morphometric estimates (Table 4.10) the number of villi injured by apoptosis in all these 5 weeks does not change significantly: from 10.36 ± 2.1 to 13.85 ± 2.12 ($p > 0.1$). This means that the action of the pathogen that causes apoptosis continues without interruption until abortion with almost the same low intensity, covering new and emerging areas of villi as well as other villi.

Apoptosis is a destructive process, it leads to several complications. Destruction of sites of syncytiotrophoblast and less cytotrophoblast, as well as the trophoblast, which covers the lacunae, create defects, ulceration, which can partly be covered fibrin clots (see Fig. 4.12). For 3.5^{th} -4^{th} week, at the beginning of the process, fibrin clots are in the 9.1% of the total number of cases, additionally in 2.33% small groups of villi with trophoblast destroyed and covered with fibrin have been seen. In all subsequent weeks (5, 6, 7, 8) clotted fibrin was observed in 30-37.5% of cases (Table 4.10). This means that apoptosis is preceded by the formation of fibrin: fibrin covers the defects in trophoblast villi remaining after apoptosis. We can conclude that early abortions have nothing to do with Hughes antiphospholipid syndrome.

Apoptosis also destroys some parts of villous capillaries, sometimes several loops of capillaries in a villus at the same time, leading to their extinction, along with erythroblasts. Under normal circumstances, villous capillaries at the end of I and at II trimesters continue to proliferate, but the capillaries destroyed by apoptosis cannot be restored (Fig. 4.12, 4.15). This is associated with profound suppression of endothelial cell proliferation; single Ki67-positive cells were found only in 15.4% of cases. As a result, the number of avascular villi increases by 3 times and the average number of capillaries in the villi is reduced by 2.6 times. Number of villi with preserved capillaries at 3.5-6 weeks gradually decreases from 38.4% to 32% ($p > 0.1$), at the 7th and 8th weeks it dramatically falls to 16.44% and 19.68%, respectively (see Table 4.10).

In this laid yet another complication of apoptosis in the villi: increase of the number of large avascular and edematous villi. The destruction of the entire trophoblast on the villus is rare, so that cytotrophoblast under syncytiotrophoblast damaged areas can be preserved. Through these sites of cytotrophoblast the villus receives water and other

components of the metabolism. But the outflow of water from avascular villi is discontinued and from ones with the partially preserved capillaries it is highly reduced. As a result, the stroma of these villi is greatly swollen, the dimensions are increased significantly (Fig. 4.15). Their number increases at the beginning and at 6th-8th weeks gets somewhat reduced. This decrease is due to the rejection by abortion of significantly affected placentas and to the persistence of the less affected ones. A large number of dormant villi: avascular, swollen ones, with a reduced number of capillaries, where a combination of them reaches 70% and sometimes even 100%, leads to the cessation of function of the placenta and to its removal.

Thus, apoptosis is followed by a series of complications. This includes formation of clot and fibrin films on the ruined sections of syncytia and, at least, cytotrophoblast, which simulates the antiphospholipid syndrome. Destruction of villus capillaries without restoring of them leads to the formation of large swollen or non-vascular or rare-vascular villi. Add to this the destruction of erythroblasts, the death of monocytes with the lack of their replacement with promonocytes and the mass mortality of invasive trophoblast: all this is a consequence of apoptosis. The result is a severe disruption of the placental barrier, not only in the metabolism through it, but also in immune defense, which is carried by mononuclear phagocytes and by invasive trophoblast (see Chapter 5.2) (Fig. 4.11, 4.12, 4.15).

In addition to complications through apoptosis in the developing disease sometimes (67.4% of cases in group 4) the inflammatory processes in the form of small areas of necrosis of decidual tissue with insignificant leukocyte surrounding have been found (see Fig. 4.16.b1). In one of them there were a few nodules of actinomyces. In the lacunae in 16.3% of cases there was an elevated white blood cell number (70 to 50000 μm^2 per slice area). Microflora in all these cases was not found. Inflammatory and necrotic processes in the villi were also absent. These changes were significantly different from those observed in the groups 3A and 3B and did not cause the massive apoptosis in group 4. Significant effect of these moderate processes on the state of disease in group 4 was not observed and therefore could be referred to the concomitant disease conditions.

* * *

In the processes occurring in the cases of group 4, immune mechanisms actively participate both in the embryonic part of the placental barrier and in the part of the pregnant. Feature of group 4 is a very big phagocytosis of immunoglobulins by monocytes, promonocytes and by extravillous trophoblast (Diagram 4.2; Table. 4.9 and 5.3, Fig. 4.13, 4.14, 4.16). In groups 1, 2, 3A, 3B amount of phagolysosomes per section of a phagocyte in its cytoplasm (excluding the transport immune complexes in the capsules: Simister NE, 1998, 2003) constitutes for each: IgG, IgA and IgM from 0.96 ± 0.25 to 14.5 ± 1.69. In group 4 under the same conditions in phagocytes can be found from 15.37 ± 1.34 to 47.58 ± 2.66 phagolysosomes. In the preparates of some phagocytes of group 4 can often be seen 100-150 phagolysosomes with each of: IgG, IgA, and somewhat less IgM. In terms of area of all sections of 3 microns thick the number of phagolysosomes containing IgG, IgA and IgM in a single cell can reach 1200-2200.

Immune responses of the embryo are manifested in a series of processes. In the yolk sac and in the stroma of villi, a dramatic increase in the number of promonocytes compared with monocytes (Table 4.5, Fig. 4.13). Significantly increases the phagocytic activity of both types of mononuclear cells (Fig. 4.13 and 4.14). Also significantly

increases the proliferative abilities of trophoblast on the villi: the number and size of proliferative nodes, linking them with the decidual tissue of the pregnant ("anchoring villi") (Table 4.9, Diagram 4.2, Fig. 4.14), and then move of the trophoblast cells on lacunae and inside the decidual tissue. Here they are actively phagocytizing immunoglobulin and then get destroyed themselves by massive apoptosis (Fig. 4.6).

Phagocytosis of IgG by these kinds of trophoblast only slightly more intensive than phagocytosis of monocytes and promonocytes, but IgA and especially IgM are phagocytized much more (Fig. 4.13). This is due to the fact that the immune conflict significantly increases the production of IgM antibodies by B-lymphocytes (Table 4.6., 4.7). It is also important that the phagocytes of villi get as much immunoglobulins as being passed through the placenta barrier, so the trophoblast contacting with blood and with decidual tissue of the pregnant, has more possibilities in this regard.

In decidual tissue of immunocompetent cells of the pregnant T-lymphocytes (CD3, CD4 helper cells, CD8 cytotoxic, and CD56 NK), involved in the cellular immune response, do not change significantly their number in all the groups - from 1 control to group 4 (Table 4.6). Reaction of cytotoxic cells and NK occur in contact with the cells which are subject to elimination. But in the tissues of the embryo in the chorionic villi and even at the damaged surface of the villi, these cells are absent while the absence of contacts of cytotoxic T cells and NK cells with ones prepared for destruction and elimination, let us deny the existence of the cellular immune response.

Changing number of immune cells of the pregnant involved in humoral immune responses: B-lymphocytes, plasma cells and macrophages, in conjunction with the subsequent pathological processes and the development of complications let us determine the intensity of ongoing immune reactions. For example, a large number of cells in the cases referred to the group 3B, hematogenous complications, necrosis, massive apoptosis, give grounds to consider these cases of the group 3B the most severe of the listed above. Less intense accumulation of the same immune cells of the group 3A and fewer complications indicate less severe course of the bacterial infection.

In group 4 changes of CD20, CD68 and CD79A are less significant than in group 3B but more intense than in group 3A. It shows a moderate synthesis of immunoglobulins in group 4. This may be due to a small antigenic excitation from pre-embryos and embryos that have at this period the size of some fractions of millimeter or a single millimeter. Small amount of CD20 in the spongy and compact regions of decidual tissue and especially in group 3B is associated with its transformation into plasma cells. In group 4 these transformations are moderate, which also confirms the small antigenic stimulation.

IgM is produced first out of other immunoglobulins in the humoral immune responses, but then its synthesis decreases while the formation of IgG and IgA goes up. Significant amount of IgM compared with IgG and IgA, which are trapped by invasive trophoblast in the decidual tissue (Table 4.9) suggests the recent launch of an immune response.

State of the immunocompetent cells of group 4 shows that in decidual tissue of the pregnant a humoral immune response is going on. Its result is the formation of IgG, IgA and IgM in almost the equal amounts. This lets us to suggest that the synthesis of these immunoglobulins has been launched recently. They are actively phagocytized by invasive trophoblast and mononuclear phagocytes in a much larger amount (4 times more) than in groups 1, 2, 3A and 3B (Table 4.9). And all this despite the fact that in group 3B during the bacterial infection, the number of plasma cells is about twice of the

group 4. All the above suggests that immunoglobulins phagocytized in group 4, are perceived by the phagocytizing cells as immunologically incompatible. Immunoglobulins of the pregnant in the first instance hit all kinds of trophoblast. Somewhat weaker it happens in cytotrophoblast, but it is partly covered by syncytiotrophoblast. This shows that one of the antigens of the trophoblast serves the cause of the early allogeneic conflict.

4.8. Conclusion for Chapter 4

1. Placental barrier includes not only "from the blood of the pregnant to the blood of the embryo" and is not limited to the exchange from the pregnant women to the germ and from the germ to a pregnant woman. The elements of prime importance are acting there working on the selection and destruction of pathogenic agents for the germ. This is the invasive trophoblast, situated in the decidual tissue of the pregnant and the trophoblast covering the lacunae with the blood of pregnant women. They are at the front line of defense of the placental barrier of the embryo. Immunocompetent cells in decidual tissue of the pregnant are also involved in the protection of placental barrier from the site of pregnant woman, but only within certain limits. Mononuclear phagocytes of the villi also intensively protect the border barrier, but this time from the fetal side.
2. Clotting of fibrin on the surface of chorionic villi and in clusters in the cavity of the lacunae is mostly represents the damage of the superficial villous tissue from various bacterial infections and pathogenic immune processes. Number of cases of Hughes antiphospholipid syndrome in obstetric practice has been greatly exaggerated.
3. In the cases studied, bacterial infection and allogeneic conflicts occur with the participation of the humoral immune response and immunoglobulins. Signs of cellular immune responses were not recorded.
4. Out of all the investigated cases, the acute bacterial infection as a primary or concomitant illness was available in the embryonic period in 63.4% of cases and in early fetal period – in 55.3%. Such a large number of acute infections suggest that in I and II trimesters of pregnancy it is a consequence of reduced immune status of the uterus and whole organism of the pregnant.
5. IgG, IgA, IgM in cases of group 4 were synthesized by B-lymphocytes and plasmocytes of the pregnant, and then rapidly phagocytized and destroyed by invasive trophoblast, trophoblast on the surface of the lacunae and by mononuclear phagocytes 3-5 times more rapidly than in a case of bacterial infections. After this, phagocytes themselves and other cells that come into contact with immunoglobulins (erythroblasts and capillary endothelium) are subject to multiple apoptosis. The whole complex of these reactions gives reason to believe that these immunoglobulins of the pregnant are pathological for the phagocytic cells and for many other types of cells of pro-embryos, causing an early allogeneic conflict.

Chapter 5. State of embryos and fetuses at an early abortion. Allogeneic conflict

5.1. Early spontaneous abortion

Currently, early spontaneous abortions constitute a significant problem not only for obstetrics and not only in medicine. This is a great social problem of loss of future generations. There are data on abortion before the implantation, during and after it (Clark DA, 2003) with a frequency of 15-31% (Wilcox A., et al., 1988; Kutteh WH, 1999), and 40% (Кулаков В.И. et al, 2005), 50% (Волощук И.Н., 2002) and even 70% (Girardi G., Salmon JB, 2003) of the total number of fertilizations. Many uncertainties exist in the occurrence of early abortion in the embryonic period. In the United States 1-3% of women suffer from repeated miscarriages (Girardi G., Salmon JB, 2003). Lack of knowledge in this area is shown by a fact that the cause of abortion in 50% of cases remains unknown (Sargent IL, 1993). In the 17 years since then, no significant changes have occurred. Such a common diagnosis as intrauterine growth restriction (IUGR), early loss of pregnancy (missed abortion, pregnancy rests and blight), and others are still "in vogue". They do not determine the cause of abortion, its prevention and treatment. Some researchers link them with allogeneic conflicts (Jerzan M., Bischof P., 2002; Bulla R. et al., 2003).

The etiology of early spontaneous abortions is omnigenous. We only list the less frequent ones. They may be a consequence of congenital uterine anomalies due to the Muller duct anomalies (Dendrinos S. et al., 2005), pathology of the placenta (Burton GJ, Jauniaux E., 2004), genetic disorders arising from abnormalities of mitosis or meiosis of sperm, eggs or pre-embryos. The inheritance of genetic defects of a parent is possible. The dramatic increase in the incidence of Down's syndrome is associated with increasing age of the mother or rather, the age of her eggs, following the too long wait for the turn. Possible cause of early abortion may apparently become a chronic disease of the pregnant (lupus erythematosis, heart and kidney disease, diabetes, HIV) (Brocklehurst P., French P., 1998; Wenstrom KD, et al., 1998). The early recurrent abortions of unknown pathogenesis may constitute the complications of endocrine diseases (Li TS, et al., 2000; Lazarus JH, 2005) or biochemical disturbances and disorders (George L., et al., 2002).

One of these diseases is the Hughes antiphospholipid syndrome, the main manifestation of which is the appearance of antibodies as IgG, IgA, IgM to a phospholipid - cardiolipin (von Landenberg P. et al., 2003). Their appearance causes a dramatic increase in blood clotting in the vessels of various organs, including the placental vessels, which is compounded by repeated (3 or more frequent) ultra-early and early abortion or fetal death before 34 weeks in the absence of morphological, hormonal or chromosomal abnormalities in the mother (Cowchook S., 1998; Milovanov AP et al, 2005; Shoenfeld Y., et al., 2006;). Nevertheless, there are some doubts. Perivilleous thrombosis has been observed in women smokers (Mayhew TM., Et al., 2003A; Vogt Isaksen C., 2004), in diabetic pregnants (Mayhew TM, Sampson C., 2003B), in women living at high altitude (Mayhew TM, Bowles C., Yucel F., 2002). Out H.J., et al. (1991) has found that for the diagnosis of antiphospholipid syndrome, except perivillous

thrombosis during pregnancy, the presence of lupus and phospholipid (cardiolipin) antibodies are required. However, out of 102 examined patients positive results were observed in only 21. The other conflicting reasons for the outcome of pregnancy remained unknown. Seminar on "Medicine of fetuses and newborns" in its conclusion has noted that the use of anticoagulant - heparin is considered to be controversial in such cases, (Chaddha V., et al., 2004). Similar conclusions have been made by several authors. They cite concerns about the dubious links of antiphospholipid syndrome with all cases of recurrent early abortions, and inappropriate use of any anticoagulant serum, heparin and aspirin (Coulam CB, et al., 1995; Daya S., et al., 1998; Sher G., et al., 1998; Branch DW, et al., 2000; Carp HJA, et al., 2001; Vaquero E., et al., 2001; Clark DA, et al., 2006; Carp HJA, 2007). Carp H.J.A. (2007) believes the main cause of recurrent early pregnancy losses is in chromosomal aberrations. Yet supporters of aspirin and heparin still exist: "This therapy may be useful for cases of unexplained recurrent abortions» (Levi AJ, 2008).

The above information from the literature shows that the formation of fibrin in the chorionic villi can occur for various reasons. According to our data (Chapter 4) in control cases (group 1), fibrin formation in villi is not observed. In group 2 perivillous fibrin is found in all five cases. In the groups of inflammation (3A and 3B) fibrin clots existed respectively, in 46.1% and 22.2%, and fibrin in groups of villi has been found in 30.8% and 33.3%. With allogeneic conflict (group 4), fibrin clots, mainly in the apoptotic syncytiotrophoblast are seen in 27.9% of cases and clusters around groups of villi - in 13.9%. Thus, the formation of fibrin in a form of restricted or large regions around the groups of villi is noted in group 2 in 100% of the cases in groups 3A and 3B - in 76.9% and 55.5%, respectively, in group 4 - in 41.9%. These changes are the complications of major diseases – the bacterial inflammations in groups 3A and 3B or the allogeneic conflict in group 4. Syncytiotrophoblast along that is damaged within small areas or groups of villi. Increased IgG, IgA and IgM (von Landenberg P., et al., 2003) may be due to Hughes syndrome, but also a consequence of inflammatory or immunological processes following allogeneic conflict. Concluding, the connection of early spontaneous abortions with Hughes antiphospholipid syndrome, if exists at all, is a rare case.

5.2. Physiological and pathological apoptosis in early abortions

Apoptosis is a unique form of cell destruction. It is characterized by the programmed destruction and by the possible formation of new cells in a place of destroyed ones. Apoptosis has been examined and separated from necrosis by Kerr JE, et al. in 1972. Destruction occurs according to a specific plan and is regulated by some groups of proteins. One of them is Fas and FasLigand, and the other - p53, a complex of bcl-2 prevents the progress of apoptosis, particularly in embryos of humans

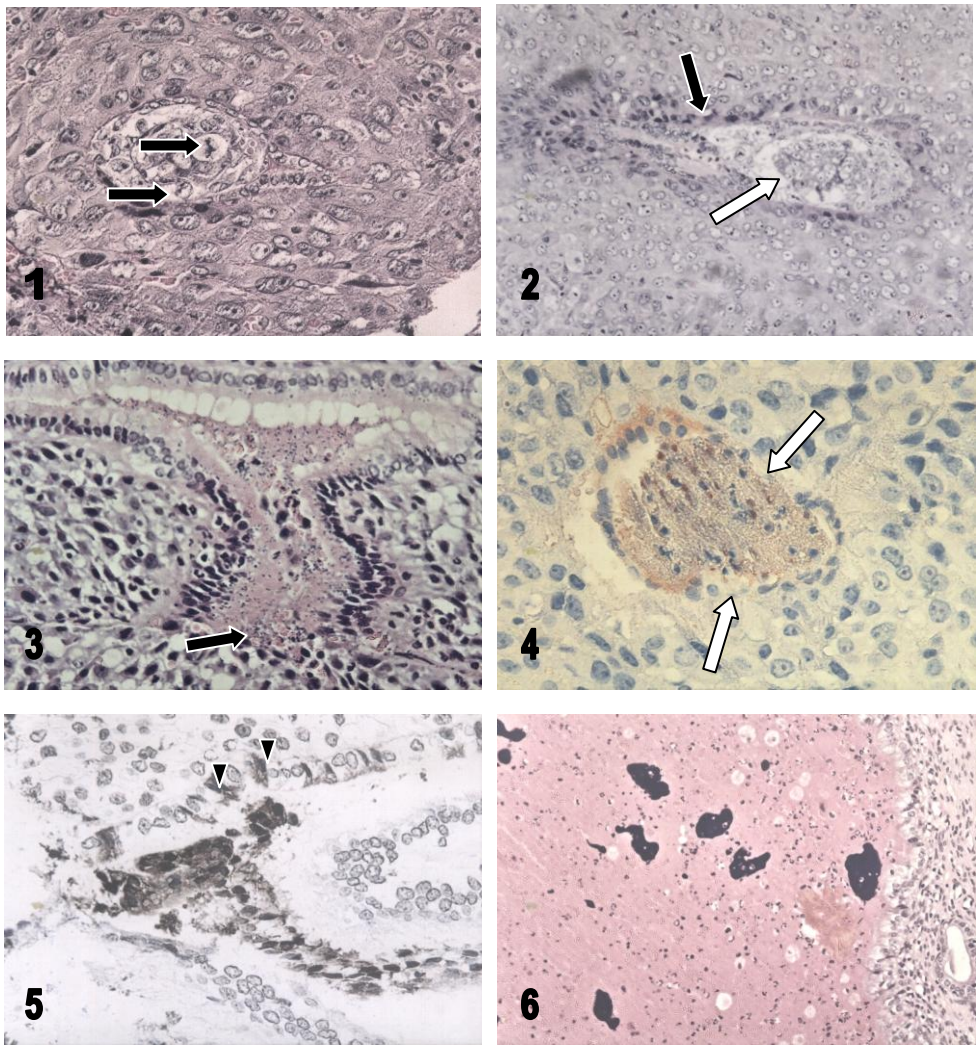


Fig.5.1. Development of apoptosis in rats. **1** - control: normal morula in decidual tissue, serum of a women was not introduced into the fallopian tube in 6 days after fertilization. Following intake at 4 and 8 hours the germinal tissue is free of changes, cell mitoses are seen (black arrows). **2** – following the introduction of women serum, in 2 hours: the nuclei of cells are pyknotic (light arrow) or in apoptosis (dark arrow). **3** - slaughtered after 4 hours: almost all cells of the germ are in apoptosis or disbanded, in some places trophoblast is also apoptosis (dark arrow). **4** - 6 hours later the cells are destroyed, as well as the trophoblast (white arrows). **5** - TUNEL + of most of germinal cells, and some at the mucous epithelium of the rat (▲). **6** - 2 days after the introduction of the serum: Total necrosis of the decidual tissue of rat and sometimes sites of petrification are seen. Magnification in 1, 3, 4, 5 is x400, in 2 is x200 and in 6- x100.

(Lichnovsky V., et al.,1996). In the process of apoptosis several proteins of caspase family: 2, 3, 6, 7, 8, 9, 10 are also involved. Ratio of the complexes of these proteins within embryogenesis, their tissue specificity and phasic functions are of great importance in the formation of organs (Lichnovsky V., et al., 1998). Apoptosis occurs more often in single cells or small groups of them. Cells lose contact with neighboring cells; the nuclei in them shrink and split apart with the organelles of cells, forming apoptotic bodies. They are phagocytized by macrophages or neighboring cells.

The biological role of apoptosis is the maintenance of homeostasis and balance in the body, the replacement of unwanted, old or altered cells. The reasons and conditions for the development of apoptosis vary. These reasons may be physiological, planned in the development of an organism or pathological.

During normal pregnancy, apoptosis is a truly physiological process (Smith SC, Baker PN, Symonds EM 1997; Gao F., et al., 2001) being widely distributed. In decidual tissue, invasive trophoblast prepares the base for implantation of pre-embryo and for lacunae of maternal blood using apoptosis (Garcia-Lloret MJ, et al., 1996; von Rango V., et al., 2003). In the embryonic period of organogenesis, the programmed destruction of cells and replacement of them with others ones occur. This happens in destruction of Rathke's pouch residues with the formation of the anterior pituitary, in the process of transformation of the Muller and Wolff ducts into the female or male genitals, in the destruction of pronephros and mesonephros, of the partitions in the pharynx and the anus, in formation of genital and urinary tracts and in many other cases (Huppertz B., Kingdom JC, 2004).

A large number of publications concerns increased frequency of apoptosis in invasive trophoblast and syncytiotrophoblast with abortion at I trimester (Huppertz B., et al., 2005; de Falco M. et al., 2005). It is indicated the presence of Fas and FasLigand and a significant number of apoptotic cells. The known causes of apoptosis in trophoblast are too diverse to consider them true: an assumption is put forward that apoptosis has regulatory function with excessive development of the embryo or that it is a consequence of placental hypoxia and disturbance of the germinal oxygen supply. It is emphasized the destructive effect of decidual immune cells, in particular - macrophages. Alternatively the apoptotic groups are considered as a protective mechanism against fetal rejection by reducing the activity of NK and T-cells (Streilein JW, 1995; Tafuri A., 1995; Simpson E., 1996; von Rango V., et al., 2003). Still, most of these options represent not physiological but pathological processes which are not included in the genetic development plan of the germ. Sometimes a conflict or a brake in relationship of mother and fetus or the possibility of exogenous stimulation including infection is supposed, but their essence is not explained. It is suggested that apoptosis represents a support for the privileges of germ or that there has been allogeneic conflict. In some cases, the absence of any other changes in the embryo than apoptosis is emphasized. It has been suggested that apoptosis begins to develop in pre-embryonic stage being intensified by spontaneous abortion (3-8 weeks) due to allogeneic conflict of pregnant woman and the embryo (Allison J. et al., 1997; Kang SM, et al., 2000; Frangsmur L., et al., 2005; Vacchio MS, Hoges RI. 2005).

Pathological apoptosis also happens often when the removal of the cells modified by external influence is needed (Levi R., Nelson DM, 2000). The reason for it may be in malignant tumors when apoptosis can destroy malignant cells, in inflammatory processes, in neuro-degenerative diseases, in infections and immune effects in adults and embryos (Bosman FT, et al., 1996; Kokawa K., et al 1998); in the placenta of a diabetic pregnant (Sgarbosa F., et al., 2006), in a case of infection by influenza virus in human fetuses

(Uchide N., et al., 2005). Pathological apoptosis is insufficiently studied, and the authors often do not pay attention to the pathological cause of apoptosis.

Pathological apoptosis differs from the physiological one by number of features. It originates from pathological processes, such as bacterial, viral, hormonal, chemical, immunological effects and, apparently, is not exhausted by this enumeration. A good example of pathological apoptosis is apoptosis in bacterial exposure in group 3B and allogeneic effects in group 4, as well as most of the cases described above. Another feature of pathological apoptosis is a plurality, and sometimes massiveness of changes, the spread into different organs that are affected by the pathogen. Initial changes in the cells, such as the decay of nuclei and other cellular structures are similar to the physiological apoptosis. This stage lasts for several hours (Majno G., Joris I., 1995; Bosman FT, et al., 1996), and then the particles of cells are not phagocytized by macrophages or neighboring cells both in physiological apoptosis but get completely destroyed.

Halperin P. et al. (2008) with our participation conducted studies on rats that on day 6 after fertilization were injected into the fallopian tube with female serum. Slaughtering was performed at 2, 4, 6, 8, 18 hours, 2, 4 and 6 days (Fig. 5.1). This made it possible to trace the development stages of apoptosis in pre-embryos for 2-8 hours, turning them into entire areas of necrosis with small areas of calcification. Thus the following stages of pathological apoptosis under the action of immunoglobulins are composed: 1) - IgG, IgA, IgM, detected in apparently normal cells, 2) - apoptosis: pyknosis and fragmentation of the cell nucleus, immunoglobulins are preserved, 3) - cell disintegration into small granules and destruction of immunoglobulins, 4) - during apoptosis of large groups of cells, this fine-grained substance is not phagocytized while fibrous replacement and (or) petrification are proceeded there after 3-6 days. Coreia-da-Silva G., et al. (2004) called such a conclusion - "cell death by apoptosis and secondary necrosis."

Similar changes are noted in our observations of apoptosis in tissues of embryonic placental barrier during infections (Chapter 4.6, group 3B) and in immune conflict (Chapter 4.7, group 4), when several sites of syncytiotrophoblast, the cytotrophoblast, as well as clusters of invasive trophoblast and trophoblast covering the lacunae undergo apoptosis but the process does not approach fibrosis and petrification because of the coming abortion. In addition, the decidual tissue in groups 3A, 3B, 4, has a large number of macrophages of the pregnant, and they are actively phagocytizing the remains of the invasive trophoblast (Kokawa K., et al., 1998; Ohshima K., et al., 2001; Bulla R., et al., 2003; Ben-Hur H., et al., 2005; Uchide N., et al., 2005; Gurevich P., et al., 2007).

5.3. Transport of immunoglobulins to the embryo

The delivery of immunoglobulin of the pregnant has great importance in the germinal development both in its protection from microorganisms and other pathogens and in the possibility of alloantibodies attacking it. Transport of immunoglobulins through the placental barrier from the pregnant woman to the germinal part of the barrier is supported by a large number of different receptors. These are SC and J-chain, the polyimmunoglobulin receptors. They carry a variety of immunoglobulins (Jauniaux E. et al., 1995; Ben-Hur H. et al., 2001). Receptors Fc gamma RIIIα (CD16), Fc gamma RhII

(CD32) and Fc gamma RI (CD64) specialize in the transport of IgG (Kristoffersen EK et al., 1990; Bright NA et al, 1994; Simister NE et al., 1996; Simister NE 1998, 2003; Han P., Hodge G., 1999). Receptors Fc epsilon RI and RII (CD23) transport IgE in a case of ectopic pregnancy (Sverremark Extrom E. et al., 2002). There are also other receptors. Transport of immunoglobulins from the pregnant woman to the embryo and fetus is sufficiently explored. It was regarded in the manuals "Fundamental immunology" ed. W.E. Paul; "Mucosal immunology" eds. P.L. Ogra et al; "The human placenta" eds. C.W.G. Redman et al; "Patters pathology of the fetus and infants" ed. E.Gilbert-Barness; "Medicine of the fetus and mother" eds. E.A. Reece, J.C. Hobbins; "Immunologic disorders in infants and children" eds. E.R. Stiehm, V.A. Fulgini; "Developmental pathology of the embryo and fetus" eds. J.E. Dimmick, D.K. Kalousek; "Prenatal human development" Ed. А.П. Милованов, С.В.Савельев, as well as in many publications. But several authors (Bright NA, Ockleford CD, Anwar M., 1994; Simister NE et al., 1996; Simister NE 1998 and others) have noted ambiguity of many aspects of transport of immunoglobulins through the placental barrier and their entering into the bloodstream of the fetus. N.E. Simister (2003) has written: "The mechanism of Igs transport across the endothelium of the fetal capillaries is not understood ... However, it is not known whether this receptor transports IgG or prevents transport of immune complexes to the fetus".

According to our data in the transport of immunoglobulins in **the embryo** SIS participates. After transport through syncytiotrophoblast and cytotrophoblast the immunoglobulins approach the stroma of the villi, in which, close to the trophoblast layers, capillaries lay. Their endothelium contains J-chain. Its function is endocytosis, entry of immunoglobulins into the cells (Emansipator SN et al., 1990, sections 1, 2, 3). The function of another receptor, SC is exocytosis, removing immunoglobulins from the cell (Simada S-J. et al., 1999). In the capillary endothelium of villi it is found just occasionally and in a small quantity which means that it is not functioning there. Table 5.1 shows that in the yolk sac where erythroblasts are formed, their quantity, connected with immunoglobulins and J-chain is minor - less than 8%. In the capillaries of the villi the number of erythroblasts with immunoglobulins reaches 18%, and in the cavities of the heart - up to 30% in the cases without pathological processes (group 1). SC, performing exocytosis of immunoglobulins is clearly contained in the vessels of organs - spleen, lung and others. This means that the immunoglobulins are extracted from the blood vessels in the tissue organs.

Thus, one of the ways to transport immunoglobulins into the embryos is as follows: from the blood of pregnant women in the lacunae through the syncytium and cytotrophoblast different receptors carry them into the stroma of chorionic villi. Then the J-chain of endothelial enters them into the blood of capillaries of the villi, where the J-chain and immunoglobulins combining with erythroblasts are transferred with them to the vessels of the embryo. Here SC extrudes them in the tissue of surrounding organs.

Table 5.1. Number of erythroblasts, transporting immunoglobulins to the embryo (% erythroblasts containing J-chain and immunoglobulins)

Location	J-chain and immunoglobulins in erythroblasts (%)	groups			
		1	3A	3B	4
Yolk-sac	J-chain	6.37 ± 0.76	4.83 ± 1.21	6.14 ± 1.17	8.11 ± 0.97
	IgG	5.99 ± 0.88	5.02 ± 0.74	6.72 ± 0.82	6.76 ± 0.75
	IgA	2.52 ± 0.49	3.84 ± 0.72	5.18 ± 0.98 [b]	7.44 ± 0.83 [bc]
	IgM	0	1.32 ± 0.53 [b]	3.31 ± 0.81 [b]	2.41 ± 0.31 [b]
The capillaries of chorionic villi	J-chain	18.36 ± 1.26[a]	14.33±1.36[ab]	22.83±2.57 [ac]	13.7±1.48 [abd]
	IgG	15.44 ± 1.94[a]	9.6 ± 1.92 [a b]	26.5±9.92 [abc]	11.99±1.33[ad]
	IgA	6.99 ± 0.95 [a]	9.67 ± 1.86 [a]	9.0 ± 1.18 [a]	10.83±1.03 [ab]
	IgM	0	1.14 ± 0.82	16.44±1.74[abc]	4.68±0.85 [abcd]
Heart and blood vessels of embryos	J-chain	25.83 ± 2.35[a]	18.32±2.03[b]	49.99±3.47[abc]	35.0±2.43 [abcd]
	IgG	29.93 ± 2.68 [a]	16.15±1.42[ab]	35.23±2.42 [ac]	21.0 ± 2.17 [abd]
	IgA	19.21 ± 2.38 [a]	10.77±1.81 [b]	34.08±2.11[abc]	11.84 ± 1.34[bd]
	IgM	0	3.32 ± 0.92 [b]	33.1± 3.03 [abc]	10.25±1.42[abcd]

[a] - significant ($p < 0.05$-0.001) differences with the indicators arranged above of J-chain, or immunoglobulins; [bsd] - a comparison of the follow-up groups with the previous ones ($p < 0.05$-0.001).
In the yolk sac, where erythroblasts are formed, they contain a minimal amount of immunoglobulins. Their numbers greatly increase on erythroblasts in the capillaries of the villi, where they come through trophoblast barrier. In the absence of pathogenic agents (group 1) transport of IgG and IgA is moderate; IgM does not pass through the barrier. With infections or an allogeneic conflict transport of IgG and IgA is increased as stronger, as greater is pathogenicity and the duration of exposure (group 3B and 4). Transport of IgM is restored. In group 3A transport of immunoglobulins is somewhat lower than in groups 1, 3B and 4.

Transport of immunoglobulins of this type occurs only in embryos, but not with the erythrocytes of fetuses: J-chain in their red blood cells is absent. Table 5.2 shows the dynamics of exchange of erythroblasts by erythrocytes. Accordingly, decrease in the number of blood cells that contain J-chain and immunoglobulins is seen. At week 11, they are already rare disappearing later. Earlier replacement of erythroblast by erythrocytes during infection or allogeneic conflict does not occur: the ratio in groups 1, 3A, 3B and 4 is of the same order. But increase in the number of erythroblasts after 11 weeks is due to compensatory proliferation caused by increased destruction of erythrocytes by pathogens. The transport of immunoglobulins at the J-chain is restored.

Another method of transport of immunoglobulins is used by monocytes. They spread them not only in embryos but also in fetuses. Immunoglobulins passed through syncytiotrophoblast and cytotrophoblast with the participation of SC and J-chain exit towards the stroma of villi. Here they are waited by a significant number of mononuclear phagocytes. They contain no SC, but have receptors for IgG: Fc gamma RI, RII and RIII. With their participation the phagocytes catch immunoglobulins with Fc-fragment, leaving on their own surface free Fab-fragments ready for capturing the antigens (see Fig. 6.1.).

Table 5.2. Dynamics of exchange of erythroblasts by erythrocytes in the blood on I and II trimesters of development (in % of the total number)

The term of development	Number of erythroblasts	P	Number of erythrocytes	P
3.5-7 weeks	94.92 ± 1.29	-	5.08 ± 1.31	-
8-9 weeks	85.61 ± 4.13	<0.05	14.36 ± 2.74	<0.02
10 week	34.43 ± 4.68	<0.001	65.57 ± 6.55	<0.001
11 week	5.92 ± 2.14	<0.001	93.68 ± 3.06	<0.001
12-22 weeks	0.53 ± 0.12	<0.05	99.41 ± 0.31	>0.1

Comparison of the numbers and, consequently, their significance (P) is given in columns: bottom ones versus the neighboring top ones. At the term of 3.5-7 weeks, the number of erythrocytes - 5.08 ± 1.31% may be just an insignificant artifact. The histological section could pass over the nucleus of the erythroblasts or below it, giving an impression of a nucleus-free erythrocyte.

Table 5.3. Transport of immunoglobulins by monocytes in the heart of embryos. Averaged number of immunoglobulins (or their components) on the surface and in the cytoplasm of monocytes

	Location of immunoglobulins	Group 1	Group 3A	Group 3B	Group 4
IgG	**On the cell membrane**	7.28 ± 0.95	8.84 ± 1.37	13.18 ± 1.31$^{a\,b}$	26.88 ± 1.99^{abc}
	In the cytoplasm	6.47 ± 1.88	7.32 ± 0.94	11.23 ± 0.98$^{a\,b}$	42.72 ± 2.78^{abc}
IgA	**On the cell membrane**	6.39 ± 0.45	7.17 ± 1.5	12.38 ± 0.88$^{a\,b}$	19.25 ± 1.34^{abc}
	In the cytoplasm	7.18 ± 0.84	7.34 ± 1.15	13.49 ± 1.72$^{a\,b}$	32.56 ± 2.7^{abc}
IgM	**On the cell membrane**	0.76 ± 0.17	5.43 ± 0.98^{a}	7.57 ± 0.91$^{a\,b}$	10.67 ± 0.93^{abc}
	In the cytoplasm	0.32± 0.11	3.92 ± 0.87^{a}	5.41 ± 0.71^{a}	28.78 ± 1.82^{abc}

Counting the number of immunoglobulins (or their complexes) is produced on a section of monocytes in the blood cavities of embryos heart under magnification of x1100.
^{abc} - significant differences (p <0.05-0.001) with groups 1 (^{a}), 3A (^{b}) and 3B (^{c}).
Increase in the number of IgG, IgA and IgM compared with group 1 is associated with the development of infections, immunologically moderate in the group 3A but more significant and prolonged in group 3B. The dramatic increase in the number of IgG, IgA and IgM in group 4, and especially in the cytoplasm of monocytes reflects the appearance and destruction of allogeneic antibodies of the pregnant against the embryo (see Diagram 4.2. and Table 4.9.)

The involved in endocytosis of J-chain, associated with immunoglobulins and capable to capture the different antigens is also possible. Phagocytes may contain immunoglobulins in their cytoplasm, where they are stored or destroyed, if pathogenic (eg, antibodies with allogeneic conflict (see Fig. 6.1.)). In the lumen of capillaries the monocytes with immunoglobulins are freely circulating in the blood of the embryo if immunoglobulins and monocytes haven't destroyed each other due to allogeneic conflict.

5.4. Objectives, materials and methods

Chapter 4 has reviewed the status of the placental barrier: its parts belonging to the pregnant woman and associated with the embryo, in particular with abortions caused by pathogenic process of immune nature. This is a bacterial infection of the birth canal (groups 3A and 3B) and a possible early allogeneic conflict (group 4). Group 1 - without the inflammatory and immune diseases, birth defects or other pathological conditions - is taken as control.

In Chapter 4 two placental barriers have been discussed. In a part related to the pregnant, the intense humoral immune processes with increasing depletion of IgG, IgA and especially IgM take place. The listed are intensely phagocytized by invasive trophoblast and trophoblast covering lacunae. They are located in parts of the placental barrier, related to the pregnant. At the part of the embryonic barrier, immunoglobulins are phagocytized by two layers of trophoblast and by villous monocytes as well as by capillaries containing erythroblasts. All the components contacting with the immunoglobulins are being destroyed via apoptosis.

Chapter 5 examines the state of the embryos themselves in possibly early allogeneic conflict with pregnant women (group 4), in comparison with bacterial infections (groups 3A and 3B) and in cases with no significant changes (group 1). Thus, this Chapter is a continuation of Chapter 4. For completeness of the comparison it is also viewed the state fetuses at 9-22 weeks of development in groups 1, 3A and 3B. Cases related to group 4 after 8 weeks of development do not occur.

5.4.1. Research materials

This Chapter covers the same cases and applies the same methods that have been used in Chapter 4. As part of the embryos and fetuses during abortions has been completely destroyed, the amount of subjects of the study has decreased: 36 embryos and 42 fetuses at II trimester (see Table 5.4.) All 78 embryos and fetuses are divided into the same groups as in Chapter 4. Group 1 consists of cases essentially unchanged - 27 embryos and fetuses. Group 2 initially included cases with growth retardation (IUGR) and with Hughes antiphospholipid syndrome - 23 cases of embryos and 3 fetuses. But a detailed examination has shown the presence among them the cases of other diseases, and they have been moved into groups 3B and 4. Group 2 is left with two embryonic cases (even the tissue of embryos was not preserved) and three fetal ones (with two fetuses) (see Table 5.4). Because of the small amount of material and the absence of embryos, this group has been withdrawn from consideration in Chapter 5. Group 3A (8 embryos and 20 fetuses) includes cases with inflammation of the decidual tissue and the spread of infection in amniotic fluid and then with the aspiration to the stomach and adjacent organs, bypassing the placental barrier. In group 3B (4 embryo and 2 fetuses), infection from the decidual tissue enters the blood lacunae in the chorionic villi, and, in hematogenous way, spreads to the organs of embryos (or fetuses). Group 4 consists of 15 embryos in which an early allogeneic conflict of the pregnant woman and the embryo is assumed.

Table 5.4. Number of embryos (3.5 - 8 weeks) and early fetuses (9 - 22 weeks) in the groups studied

Periods	Groups	1	2	3A	3B	4	Total
Embryonic	**Examined**	15	2	13	9	43	82
	Including embryos	9	0	8	4	15	36
	%	60.0	0	61.54	44.44	34.88	43.9
Fetal	**Examined**	18	3	22	4	0	47
	Including fetuses	18	2	20	2	0	42
	%	100	66.67	90.91	50.0	0	89.36
Total materials of abortion		33	5	35	13	43	129
Whole embryos and fetuses		27	2	28	6	15	78
%		81.82	40.0	80.0	46.15	34.88	60.46

Number of preserved tissues of aborted germs depends on two factors. First is their age and size: small bodies of embryos (1.5-30 mm) are preserved in group 1 in 60%, and fetuses – in 100%, so according to the summary data, respectively, 43.9% embryos, and 89.36% fetuses. The second factor is the destructive effect of pathogens that caused the deaths of germs in group 3B (haematogenously spreading infection) and in group 4 (allogeneic conflict). Thus, 44.44% and 34.88% embryonic bodies respectively have been preserved. In group 1 (no pathogenic effects) and in group 3A (limited disease), respectively 60.0% and 61.54% have been preserved.

5.4.2. Methods of the study

A wide range of pathological, morphometric and immunohistochemical techniques (see Section 4.2.2) have been used. Morphometric methods included counting of macrophages, monocytes, and promonocytes, cells containing immunoglobulins IgG, IgA, IgM, biologically active cells containing SC, J-chain, Ki67, interleukin 2 (IL2Rα), components of apoptosis Fas, FasLigand, bcl-2, p53 . Counting was carried out at a region of 50,000 μm^2 magnified x400 in 20-50 fields. Number of immune cells: T-lymphocytes CD3, CD4, CD8, B-lymphocyte CD20, CD68 monocytes were counted in the same conditions (see Tables 3.2 and 3.3). Counting the number of phagolysosomes of immunoglobulins in the sections of monocytes, promonocytes and invasive trophoblast cells was performed on 100-150 cells (see Section 4.2.3) under magnification of x1500. Total average number of mononuclear phagocytes was determined by counting the average number of phagocytes per 50000 μm^2 of all embryonic organs, including jaundice sac and villi.

5.5. Group 1, control. Normal state of embryos and early fetuses

Group 1 includes 9 embryos of 5 - 8 weeks and 18 fetuses of 9-22 weeks of intrauterine growth. Stages of development in this and all subsequent groups were determined by morphology of pre-embryos, embryos and early fetuses, developed by the Carnegie Institution (Милованов А.П., Савельев С.В. 2006), as well as by the number of segments of the embryonic chord, by parieto-coccygeal dimensions of the fetus and by the size of their feet (Moore KL 1988; Drews U. 1995; Sadler TW 1995; Robboy SJ et al., 2002). Stage of development in group 1 was considered as normal.

By early embryonic period, the defense mechanisms of the germ are already functioning. One of them is a trophoblast complex and the second is mononuclear phagocytes. During embryonic development the first types of lymphocytes appear; the primary structure of the future immune organs and the overall immune system are based. Biochemical factors regulating immune processes - interleukins, thymosin, thimulin, a group of hormones of T lymphocytes differentiation and several others begin functioning. Along with the formation of organs, a system of individual immune protection of vital organs is developing (Chapter 3).

With the advent of the fetal period, the types and the numbers of immune cells: a variety of T and B lymphocytes, NK diversify and grow. Development of the lymphoid organs of the overall immune system such as thymus, spleen, lymph nodes, and organs of the SIS: the amygdala, Peyer's patches, follicles of the intestine, appendix is continued.

5.5.1. Secretory immune system

At 4-5 days after fertilization, a group of dividing cells - morula becomes a blastocyst (Fig. 2.1). On its surface a layer of cells, strongly interconnected, stands out. This is the future trophoblast. Its functions are: to create in the decidual tissue of the uterus a hole for germinal incubation, providing metabolism and protection for it. It is capable of phagocytosis and contains elements of the SIS - receptors SC and J-chain. They inject into the germ immunoglobulins of the pregnant: antimicrobial and some other, thus providing immune protection of the germ. Receptors in the SIS are also found in other new-formed tissues of pre-embryo - ectodermal, endodermal and partly - mesodermal. The destiny of the SIS in different forming organs is not the same, depending on their future functions. In the organs in which the allocation of immunoglobulins continues going on, receptors SC and J-chain are preserved, and, therefore, remains the SIS itself. Where this function ends, SC and J-chain together and the SIS of a given organ disappear (see Chapter 2.4.).

In embryos SIS performs the following functions: gets immunoglobulins of the pregnant from the blood lacunae, moves them through the syncytio- and cytotrophoblast in the stroma of the villi. Then it is transported into the vessels of the embryo and then - into the tissues (Table 5.3). Along with formation of new organs in accordance with their functions, SIS, (SC and J-chain) provides a lot of immunoglobulin but in addition also other essential substances (Chapters 1.2.3., 1.2.4., 2.4. and 5.3.).

5.5.2. Monocytes of embryos

Mononuclear phagocytes are widely distributed in the organs of embryos (Table 5.5.). Their number in group 1 is approximately the same in different organs, except liver, which has adopted the function of hematopoiesis after the yolk sac. But in the yolk sac they still formed at 5-6 weeks (7.42 ± 1.81per 50000 μm^2). On average in all organs among the mononuclear phagocytes, 96-98% of monocytes and 2-4% of promonocytes are found. Their predecessors (myelomonocytic cells - CD34 +) in group 1 in the blood are not found.

Monocytes - the important defenders of the germ – will appear later than invasive trophoblast and later than the final formation of an embryo. But they function in tissues and shells of embryos, while the trophoblast is outside of the embryo in the decidual tissue of the pregnant. Monocytes recognize foreign antigens, phagocytize and destroy

them, take part in eliminating the cells situated in physiological and partly pathological apoptosis. For performing many functions, mononuclear phagocytes have a number of receptors and biological substances. These are the receptors for transport of IgG (Fc gamma RIII-CD16, RII-CD32, RI-CD64). IgA and IgM (5.3 and Chapter 6, Tables 5.3 and 6.1, Fig. 6.1) are also transported. In fetuses of 9-15 weeks, the number of monocytes in the liver rises to 25.79 ± 1.95 per 50000 μm^2.

Table 5.5. Number of mononuclear phagocytes at 5-8 weeks in the organs of embryos (to 50000 μm^2 of the preparate)

Organs	groups			
	1	3A	3B	4
Neural tube	0.96 ± 0.11	2.42 ± 0.51[a]	0.87 ± 0.18 [b]	0.61 ± 0.25[b]
Heart	0.956 ± 0.21	2.2 ± 0.43[a]	0.98 ± 0.28 [b]	0.88 ± 0.31[b]
Liver	18.18 ± 1.85	17.41 ± 1.88	12.38 ± 1.34 [a b]	9.38 ±1.39[ab]
Lungs	1.39 ± 0.53	2.87 ± 0.63	1.88 ± 0.48	0.81 ± 0.35[b]
Mesenchyme	1.84 ± 0.61	6.21 ± 1.09[a]	3.06 ± 0.91[a b]	2.33 ± 0.68[b]
Gonadal ridges	0.8 ± 0.17	1.61 ± 0.48	0.09 ± 0.08 [a b]	1.18 ± 0.38 [c]

[a b c] - significant differences (p <0.05-0.001) of the following groups from groups 1, 3A, 3B. In group 1, the distribution of phagocytes in various organs is roughly the same. A large number of phagocytes in the liver is due to the fact that at the fifth week hematopoiesis and the formation of phagocytes moves from the yolk sac into the liver. In group 3A the number of phagocytes increases indicating the amplification of reactions while the absence of such increase in groups 3B and 4 is a sign of weakening of the reactions.

Table 5.6. Total average number of mononuclear phagocytes of embryos and change in the ratio of monocytes and promonocytes

	groups			
	1	3A	3B	4
The number of mononuclear phagocytes (mean per 50000$\mu м^2$)	4.28 ± 0.61	6.88 ± 0.81[a]	2.34 ± 0.55[a b]	3.94 ± 0.46[bc]
Out of them: monocytes (%)	96.89 ± 6.3	83.57 ± 5.73	68.09 ± 4.15[a b]	48.73 ± 2.78[abc]
Promonocytes (%)	3.11 ± 0.34	16.43 ± 1.85[a]	31.91 ± 2.42[a b]	51.27 ± 3.51[abc]

[a b c] - significant difference (p <0.01 - 0.001) of follow-up groups from groups 1, 3A, 3B. The table reflects the extent of the destruction of mononuclear phagocytes and their compensatory proliferation by the promonocytes in each of the groups studied. Manifestations of compensation in the group 3A and decompensation in groups 3B and 4 is shown.

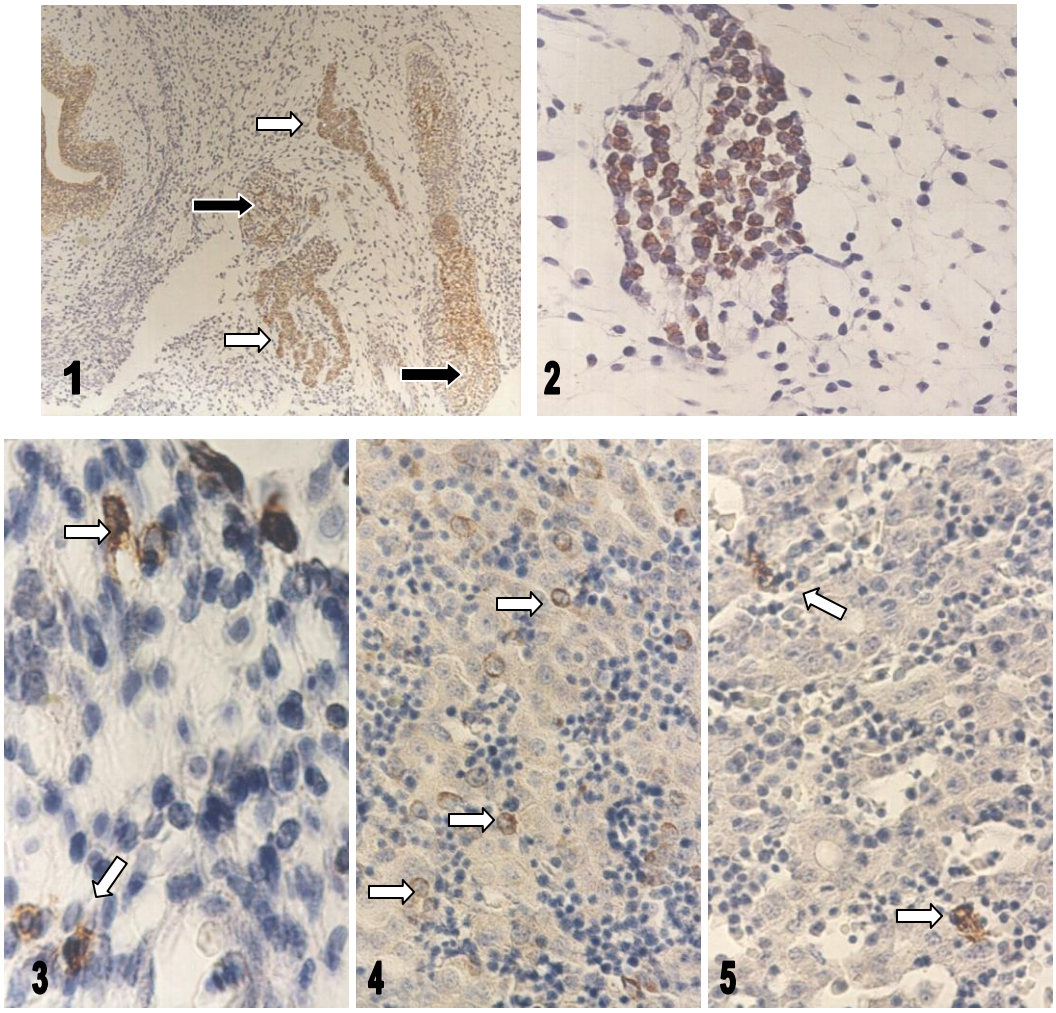


Fig.5.2. State of lymphocytes in the embryos and the laying of the lymphoid organs during normal development. **1** - two parts of the future thymus (black arrows), yet containing no lymphoid cells. Next - two parts of the thyroid (white arrows), SC (+) in it is higher than in the thymus. 5 weeks. x100. **2** - accumulation of CD3 (+) of the prolymphoblasts in the mediastinum around the aorta - the future nodes. 5 weeks. x200. **3** - tab of the spleen between the liver and the pancreas, a few CD20 (+) B-nodes. 8 weeks. x1000. **4** - liver. Multiple hematopoietic cells, including CD3 (+) lymphocytes and CD3 (±) prolymphocytes. **5** - the same thing: some CD20 (+) B lymphocytes in the liver. Both 8 weeks. Magnification is x400.

5.5.3. Overall immune system

At week 4 in the yolk sac one see appearance of lymphoid cells CD3 (+) T-prolymphocytes in the amount of 2.5 ± 0.4/50000 μm^2, and CD20 (+) B-prolymphocytes as 0.18 ± 0.05/50000 μm^2. The main hematogenous organ from the 5-6 weeks becomes liver. Immunocompetent cells: T lymphocytes CD3 (+) in an amount of 0.85 ± 0.54/50000 μm^2 and B-lymphocytes CD20 (+) 0.25 ± 0.21/50000 μm^2 yet containing no immunoglobulins, are formed there. Other lymphocytes (T helper cells, T-suppressors) have not yet formed. Some monocytes and erythroblasts are found. Area of synthesis of all these cells in the liver is much larger than the same area of the yolk sac. Therefore, the number of CD3 (+) and CD20 (+), noted in the liver per 50000 μm^2 should not be regarded as smaller than in the yolk sac. In addition, at 7-8 weeks the number of CD3 (+) increases to 3.58 ± 0.94 / 50 000 μm^2, CD4 (+) 1.73 ± 0.55, CD8 (+) 0.21 ± 0.03, and CD20 (+) B lymphocytes 0.63 ± 0.22 and occasionally they contain little grains of IgM and IgA while IgG is not detected.

At the beginning of 5th week stroma is laid for future major organs of the immune system - thymus forming from the tissue of third and fourth pharyngeal pockets, and spleen situated slightly below the stomach. Immunocompetent cells begin migrating to the thymus from 7.5-8 weeks, but the hormones - thymosin, thymoline, thymopoietin and others (Хлыстова З.С., 2006), regulating cell differentiation, appear already since 6th week. In the thymus and spleen non-specific structures are formed. The first rudiments of the lymph nodes appear at the 5th week below the aortic arch (Fig.5.3.2-6), next to metanephros where small groups of CD3 (+) lymphocytes appear. Then the lymph nodes are formed mainly on the restructuring areas of lymphatic vessels - in the neck, underarm areas, groin, mediastinum and abdomen.

In the spleen by the 14-15 weeks follicles are formed. They contain lymphocytes CD3 (+) - 10.7 ± 1.2, CD4 (+) - 3.9 ± 0.45, CD8 (+) - 5.8 ± 0.6, CD20 (+) -33.7 ± 4.41 / 50 000 μm^2. Part of B-lymphocytes contain immunoglobulins: IgG - 0.88 ± 0.11, IgA - 0.81 ± 0.03 and IgM - 0.71 ± 0.18 / 50 000 μm^2. By the 20-22 weeks of development increase is seen in both: the number of lymphocytes: CD3 (+) - 134.93 ± 32.41, and CD20 (+) 119.0 ± 16.34 / 50 000 μm^2 and the content of immunoglobulins in them: IgG - 6.11 ± 1.03, IgA - 5.02 ± 0.93, IgM - 3.37 ± 0.35 / 50 000 μm^2.

Interleukins (IL), their receptors, including IL2Rα play an important role in the regulation of immune and inflammatory processes in adults. In embryos and fetuses IL2 is detected at 7-19 weeks of development (Compagnoli C. et al., 1967). P. Han and G. Hodge (1999) interpreted the appearance of IL2Rα in umbilical cord blood as an anti-inflammatory factor. In fetuses, interleukins activate the formation of T helper cells, cytotoxic lymphocytes, NK, B lymphocytes and other cells and thus participate in the suppression of infection (Saito S., 2000; Kwak DJ et al., 2000; Hebra A. et al., 2001). According to our findings in embryos of 3.5-8 weeks IL2Rα are contained in large promonocytes of yolk sac (2.47 ± 0.21 / 50 000 μm^2) and liver (1.35 ± 0.12 / 50 000 μm^2), as well as in the epithelium of the stomach, intestines, trachea and bronchi, in gonadal cells rollers, the tubules of mesonephros and metanephros, in the myocardium, epithelium, pancreas tab, in the endothelium of blood vessels and muscles. Such wide distribution IL2Rα CD25 (+) in the cases without inflammation and pathological immune processes in embryos and early fetuses suggests that interleukin 2 is actively involved in the formation of organs. In the spleen and lymph nodes of fetuses at II trimester interleukin IL2Rα is detected in the amount of 0.43 ± 0.06 / 50 000 μm^2.

5.5.4 The process of proliferation in formation of embryonic organs.

The main processes of the embryonic period is the formation and development of its organs with a significant multiplication of cells and changes in their functions. One of these organs is the liver. In the third week of development in the middle part of the primary colon, an area of epithelium is growing, evolving into a tube. The initial part of it is then converted into the gallbladder and the large bile ducts, and in the caudal part the epithelial cords, future hepatocytes are formed. Also blood capillaries outgrow into this area. They are associated with blood vessels that pass from the placenta to the heart of the embryo. As a result, during the fetal development enough blood enriched with everything needed for successful development runs to the growing liver. Beginning from the 4^{th} week and later on the liver is growing rapidly, becoming the largest organ of the body. In good conditions, from the yolk sac come and accumulate there erythroblasts, monocytes and their precursors, as well as lymphoid cells. The number of proliferating Ki67 (+) cells in an average is 9.02 ± 1.03 / 50 000 μm^2, shortly later reaching 32.87 ± 4.19 / 50 000 μm^2.

Intense proliferation is also observed in gonadal cylinders. They are formed at week 4 in the form of wrinkles around mesonephros mesoderm. Mesoderm cells are being introduced under it and actively proliferate, forming large clumps of cells of the gonadal service. Number of proliferating cells Ki67 (+) may reach at this time 70%. Primary sex cells are formed in the yolk sac at 14-15 days of development. They actively proliferate, and 5^{th} week start to move into the gonadal ridges. The total number of Ki67 (+) cells (gonads and their servicing cells) reaches at this time 7.33 ± 0.93 / 50 000 μm^2.

In the neural tube in the process of its development the status of the proliferating cells is changing. Initially, they are all over the tube, while then becoming concentrated in the basal and superficial layers. In the heart, in the mesenchyme of it and in the myocardium the number of proliferating cells is low.

5.5.5. Apoptosis

The physiological apoptosis in group 1 is found occasionally in the form of destruction of single or 2-3 cells. It involves Fas, FasLigand, p53 and other proteins that trigger apoptosis, while bcl2 prevents apoptosis. The appearance of mentioned proteins in the cells is a sign of the coming apoptosis, and the presence of bcl2 - of delays. In the gonadal tubes and monocytes apoptosis involves p53. In the embryos of group 1, Fas is found in hepatocytes in the amount of 0.4 ± 0.01 / 50 000 μm^2 in 15.4% of cases. FasLigand is not identified in all cases. bcl2 is observed in follicular cells of the gonadal ridges at 1.1 ± 0.35 / 50 000 μm^2 and in neuroblast at 2.14 ± 0.82 / 50 000 μm^2 in 30.77% of the cases, p53 is positive in the gonadal cylinders in 1.7 ± 0.5 / 50 000 μm^2. In the heart, lungs, kidneys and other organs apoptosis is very rare in single cells. TUNEL reaction is negative. This state of organs corresponds to the physiological apoptosis.

The same is observed in lymphoid organs at II fetal trimester: in the thymus, spleen and lymph nodes Fas, FasLigand and p53 are not detected, the reaction of TUNEL is negative. Sometimes small groups of bcl2, mainly in the stroma are found.

5.5.6. Conclusion for group 1

One of the objectives of Chapter 5 is to study the pathological changes with bacterial infections and allogeneic conflicts and the possible reaction of protection in human embryos at the early development. To analyze what changes occur under the action of pathogens, it is necessary to know the normal state of possible remedies. This is the main task of the group 1.

The germinal cover tissue, the trophoblast, contains elements of SIS: secretory component (SC) and J-chain as well as immunoglobulins: IgG and IgA, coming from the pregnant woman. Trophoblast is in close contact with the tissues of the pregnant woman. It has, besides many others, the ability to perform phagocytosis and thus may function as first front defender. Along with the development of the embryo, SIS is formed in many organs and tissues, and then, according to changes of their functions, it will remain functional throughout all the human life. In the embryonic period, on the basis of SIS, the system of individual immune defense for cells of the vital organs of embryos and fetuses is separated (Chapter 3).

Mononuclear phagocytes also serve for protection and transport yet before the beginning of the embryonic period. In addition to phagocytosis of dead cells they destroy pathogens, including alloantibodies and bacteria. They also transport immunoglobulins, as carrying receptors Fc gamma RIα, RII and RIII (Chapter 5.3 and Fig. 6.1.).

Immunocompetent cells of the overall immune system: pro-T cells and pro-B cells appear about at 4^{th} week of development. Even before the formation of lymphoid organs, they exhibit some activity and accelerate self-development (Table 3.4). They include interleukins, in particular, the receptor IL2Rα. Lymphocytes are still very few and IL2Rα is located mainly in lots of different parenchymal cells. This suggests that it participates in the creation of new organs.

Formation of internal organs is the main aim of the embryonic period. In this process two functions are most important. One is the elimination of unnecessary organs or tissues, which is produced by physiological apoptosis. Its distinctive feature is the fast processes of destruction and harvesting of dead parts. The second main function is the rapid creation of other cells of the newly forming organ. In these processes cell proliferation is essential when Ki67-positive reaction covers 70 percent or more of the cells.

5.6. Group 3A. Embryos and early fetuses in acute ascending infection of the birth canal with the defeat of the chorion and amnion

The group 3A includes 8 embryos at 5-8 weeks of development, and 20 fetuses at 9-22 weeks (Table 5.4) with acute bacterial infection of the birth canal. Sizes and the progress of development of embryos and fetuses are considered normal.

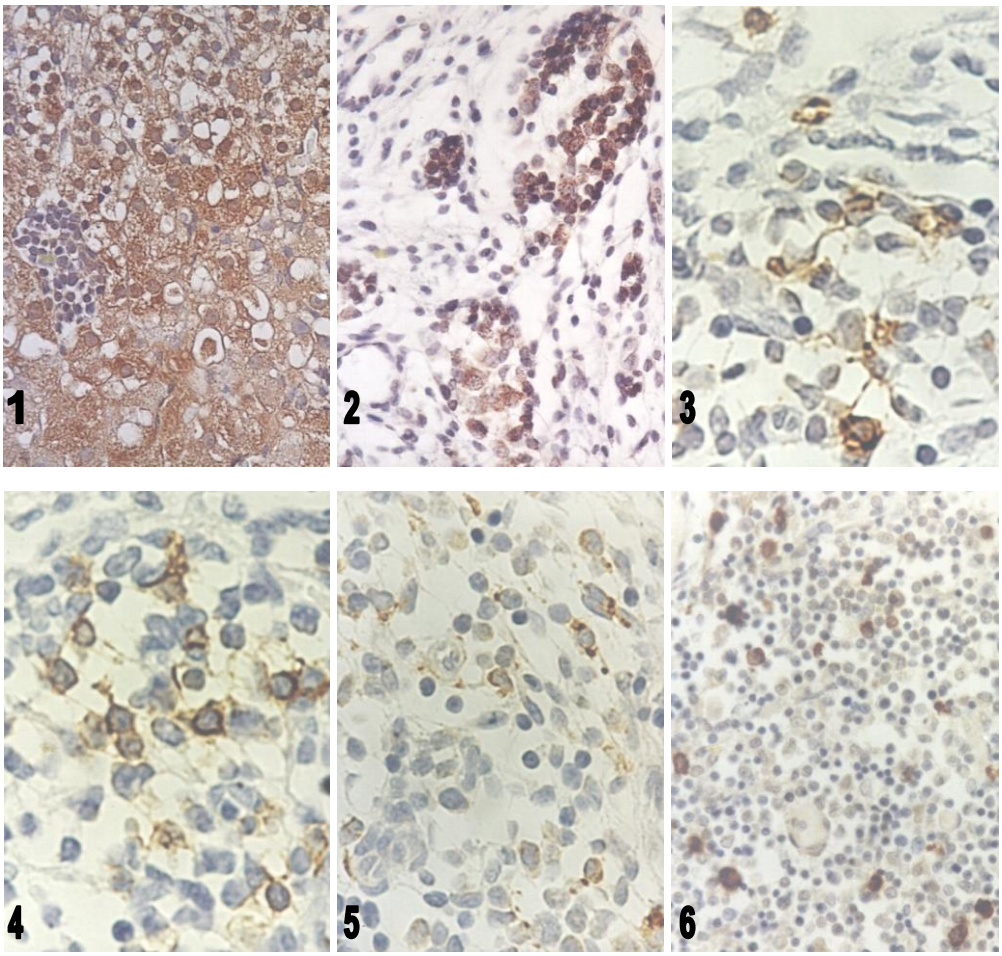


Fig. 5.3. Group 3A. State of lymphocytes at the period of 7 - 12 weeks. **1** - adrenal gland. Group of lymphocytes: J-chain at the definitive and early fetal zones and also the presence of IgG (+), IgA (+) during infection show action of individual immune protection in the adrenal glands (Chapter 3.3.5). **2** - a set of lymphocytes: CD3 (+) in the mediastinum. **3** - ibid lymphocytes CD8 (+). **4** - lymphocytes CD20 (+). **5** - lymphocytes contain IgA (+). **6** - lymphocytes contain IgM, IgG (-). The presence in lymphocytes of CD20 IgM (+) and IgA (+) allows us to assume the possibility of synthesis of immunoglobulins during infection at 12 weeks of development. The magnification in 1, 2 and 6 is x400, in 3, 4, 5 is x1000.

5.6.1. Pathological changes of embryos

Ancestry inflammation of the birth canal of a pregnant is expressed in the endometrium and deciduitis in areas of necrosis, surrounded by some significant areas of leukocytes and macrophages. There microflora, mainly cocci is found. Scattered areas of inflammation may extend to shells of the germ - developing chorioamnionitis. On their surfaces may occur some white blood cells, but they do not penetrate the depth of necrosis of the shells, Microflora of the areas of necrosis may pass into the amniotic fluid. Aspiration and ingestion of the fluid insert the microflora into stomodeum, pharynx, stomach, intestine and trachea. Epithelium of the lining of these internal organs is sometimes necrotizing and peeling. There, as well as in the bronchi, acini and ducts of the pancreas and in liver, SC and J-chain are stained weakly; sometimes they (as well as immunoglobulins) disappear. These changes indicate inhibition of SIS in these areas as an effect of pathogens. In organs and tissues not associated with digestive and respiratory organs: in the skin, mesothelioma, and gonadal cylinders in the epicardium, epithelial tubules of pronephros, mesonephros, metanephros, Muller and Wolff ducts, SC, J-chain and immunoglobulins are preserved. Also, J-chain and immunoglobulins are found in the myocardium, pancreatic islets and some endocrine glands. SC is absent in them (see section 3.3). Transport of IgG and IgA by the erythroblasts towards the embryonic organs is reduced, but their transport by the monocytes is slightly increased and for IgM it increases dramatically (Table 5.1, 5.3). The number of mononuclear phagocytes goes up on account of promonocytes, increased significantly (Tables 4.4. and 5.5.).

The initiation of the overall immune system of the embryo appear in insignificant amount prolymphocytes CD3 and CD20 in yolk sac, and then in the liver. One by one they appear in some other organs (Table 3.3., 3.4.). Moderate effect of pathogens causes the activation of the immune response. In the case of 3A in the endocrine glands of embryos infection causes a slight increase in the number of lymphocytes - a common reaction of CD3 (+), cytotoxic CD8 (+) and B-lymphocytes CD20 (+); helpers CD4 (+) appear (Tables 3.3. and 3.4.) . In the liver, the number of CD20 (+) lymphocytes increases by 7^{th}-8^{th} weeks to 7.12 ± 1.24 / 50 000 μm^2; some of them contain granules in the cytoplasm; the number of lymphocytes containing IgG is 0.98 ± 0.44, IgA 2.02 ± 0.85 and IgM 2.27 ± 0.95 / 50 000 μm^2. The number of mononuclear phagocytes also increases (Table 5.5.), especially due to rise in promonocytes (Table 5.6). All this is due to the strong response to pathogens.

Proliferative response is also increased in response to infection. Increases the number of Ki67 (+) cells in the neural tube, liver, gonadal cylinders and other organs, amounting to 45 - 135 / 50000 μm^2. Very clear is the state of Ki67 (+) cells in the cytotrophoblast and the proliferating trophoblast, which are in close contact with pathogens – there is very little change there. But the invasive trophoblast, which is in the direct contact with microflora, is reduced by 5.8 times (Table 4.4).

Interleukin IL2Rα (CD25) is found in embryos in isolated lymphocytes of the yolk sac, and then in the liver, where the number rises dramatically (3.75 ± 0.95 / 50 000 μm^2). It is widely distributed in the cells of the neural tube, myocardium, liver, adrenal glands, the epithelium of the stomach and intestine, mesonephros and other organs and is also found in fetal organs. This shows a significant involvement of IL2Rα in processes occurring at the early stages of development and the ability to maintain their functions.

Apoptotic cells in the organs of embryos and fetuses of II trimester in the group 3A are rare. Their numbers in different organs does not exceed 2.01 ± 0.67 / 50 000 μm^2 (Group 1 used to have 1.52 ± 0.04, p> 0.1). More commonly p53 (+) cells may be found in the gonadal cylinders. Sometimes in various organs groups of *bcl-2* cells are found.

5.6.2. Pathological changes in fetuses

In fetuses of 9-16 weeks of 3A group pathological processes in the decidual tissue are greater than in embryos. There are various cells, including CD20 (+) B-lymphocytes, which are capable of synthesizing IgM and IgA (see Fig. 5.3).

In the epithelium of the digestive and respiratory organs of fetuses SC, J-chain and immunoglobulin weaken or disappear and the epithelium is sometimes exfoliated (necrosis). Fetuses at 15 weeks in the small bronchi contain few neutrophils, which can be interpreted as pneumonia. In this case a reduction in the number of follicles and the number of lymphoid cells in them was also seen. This is a sign of sub-compensation of immune system. In other cases, there are signs of immune system response: the spleen follicles are enlarged, the number of immunocompetent cells in them is 38.88 ± 4.61 / 50 000 μm^2, of T-lymphocytes (CD3 +) - 16.1 ± 1.21; CD4 + helpers - 5.3 ± 0.72; CD8 suppressors - 5.42 ± 0.43; B-lymphocytes - 25.18 ± 1.9. Cross-sections of some B-lymphocytes in the slice contain granules of IgG 0.88 ± 0.11, IgA 0.81 ± 0.03 and IgM 0.71 ± 0.18. Mononucleate phagocytes are 15.11 ± 1.41 / 50 000 μm^2.

State of fetuses at 17 - 22 weeks is more diverse. Merging microfocal pneumonia has been available in all cases. In various organs and tissues there are small hemorrhages, in epithelium of the stomach and intestines small areas of destruction are seen. In half the cases the spleen and lymph nodes dramatically decrease the number of immune cells. In the spleen the number and size of follicles is decreased. The number of T lymphocytes is: CD3 - 8.1 ± 0.91; CD4 - 1.1 ± 0.6; CD8 - 2.7 ± 0.11; CD20-9.3 ± 1.9; monocytes - 5.9 ± 1.6 / 50 000 μm^2. These signs are lower than in group 1 (p <0.01-0.001). Changes in the lymph nodes are similar. This decompensation of the lymphoid system is a sign of sepsis.

Condition of the lymphoid organs in other cases is close to its state in embryos at 9 - 16 weeks. The number of proliferating Ki67 cells in the follicles of spleen is 3.38 ± 0.19 / 50 000 μm^2, of cells with interleukin IL2Rα (CD25) 5.72 ± 1.0 / 50 000 μm^2. In the various organs single apoptotic cells are occasionally found.

5.6.3. Conclusion for the group 3A

Considered in the group 3A female genital tract disease is caused by the microflora of moderate pathogenicity. Morphological changes are expressed only in small areas of necrosis with a narrow surrounding with leukocytes in decidual tissue of the pregnant. Usually she does not notice it enough, but for the embryos or fetuses at II trimester this infection ends with destruction. Initially the disease is expressed in them without major complications: the microflora does not pass through the placenta and the vessels of the villi, the supply of embryos is well stored. Bacteria pass through the amnion into the stomach, intestine, trachea, which have SIS containing antibodies of the pregnant. This significantly reduces the microflora and it can't provoke apoptosis. But

SIS of some embryos exhausts its antibodies so microflora goes up. Embryo responds by somewhat enhanced proliferation of promonocytes and some other cells of the liver and other organs. But this seems not enough, so at 5-6 weeks 17.86% of the embryos die. Their examination shows poor changes: the collapse of small areas of the epithelium of the stomach and intestine, small hemorrhages and moderate hematogenic proliferation of liver cells, CD20 (+) B cells are few and do not contain immunoglobulins.

At 7-8 weeks there is a significant enhancement of protection systems. It doubles the number of phagocytes due to the proliferation of promonocytes. Number of CD20 (+) B lymphocytes increases dramatically: in the liver in group 1 at 7-8 weeks they are 3.58 ± 0.96 / 50 000 μm^2, and in group 3A - 7.12 ± 1.24 ($p < 0.05$). At the same time in group 1 CD20 (+) B cells are found, some of them contain IgM and IgA; IgG is absent. While group 3A contains lymphocytes with IgM 2.27 ± 0.95 / 50 000 μm^2, IgA 2.02 ± 0.85 and IgG 0.98 ± 0.44, consisting in total 5.27 ± 1.35 lymphocytes synthesizing immunoglobulins per 50000 μm^2 ($p < 0.001$) (Fig. 5.3). It also increases the proliferative activity (Ki67 +) of different cells in the area of placental barrier and in the organs of embryos. Interleukin 2 (IL2Rα) in the liver of group 1 is contained in the 1.35 ± 0.12, and in group 3A - in 3.75 ± 0.95 of cells / 50000 μm^2 ($p < 0.02$). But the advance made looks insufficient: 10.71% of the embryos die. The direct cause of the death is myocardial degeneration, vasospasm. Apoptosis is rare, occasionally villous vessels stay unchanged.

Changes grow to the 15th week. Neutrophils appear and form a picture of a small inflammation, most commonly in the lungs, which get microflora with the amniotic liquid. At 16-22 weeks the inflammation in almost all cases is more prolonged and looks close to the complete picture of inflammation. The development and maturation of the immune system and restoration of the SIS accelerates. However, the impact of pathogens also goes up. It initiates a comprehensive reduction in the number and function of many biological reactions and the immune systems expressed in reduced number and size of follicles, the number of all types of lymphocytes in the spleen and lymph nodes. This is decompensation immune system, accompanied by pneumonia (in all fetuses at 17-22 weeks) which leads to sepsis.

5.7. Group 3B. Ascending infection of the birth canal with hematogenous spread

Group 3B consists of 13 cases. Abortion at 5-8 weeks has occurred in nine of them, including four cases with preserved tissue of embryos. For abortion at 10-16 weeks, two out of 4 fetuses (10 and 11 weeks of development) have retained part of tissue. Dimensions and development of their organs are normal.

5.7.1. Pathological changes in embryos and fetuses

Feature of the infection in group 3B is its spread from a pregnant decidual tissue into the tissue of the embryo (or fetus): through the placental barrier into the villi and then through the hematogenous route into the tissues and organs (Fig. 4.9). In the area of the placental barrier, there is severe tissue damage: villi with functioning capillaries have been preserved only in 18.5 ± 6.81%. In the remaining more than 80% of the villi there are significant areas of necrosis, petrification, fibrosis which are traces of the former sites

of necrosis, thrombosis of capillaries and sometimes grouped apoptosis in capillary endothelium, and, as a consequence of all these processes – appearance of avascular villi (18.9 ± 1.28% of villi, Chapter 4.6). The whole complex of these injuries has been initiated by microflora. They develop during1-2 weeks.

In embryos of 5-8 weeks and in fetuses of 10-11 weeks in different organs there are areas of necrosis: edematous stroma, the shadows of the cell nuclei look structureless, weakly stained. In the myocardium a dramatic swelling is seen, the majority of myocytes have pale nuclei, but the typical striation is preserved (liquefactive necrosis). Part of the myocytes turn into dark spines (coagulative necrosis). This is also observed in some cells of the liver and other organs. In the stomach and intestine the superficial half of the epithelium exfoliates and dissolves. Also separates the epithelium of the trachea and bronchi, partly the epithelium of the skin is exposed to such necrosis too. On the epithelium of organs, which usually contains the SIS, parts of SC and J-chain are often visible, but the immunoglobulins IgG, IgA and IgM are weakly stained or absent. Such a failure or lack, in combination with edema and necrosis of these organs, is a sign of the sharp decline of passage the factors of any kind through the placenta and then to the tissues of embryos and fetuses. In the mesonephros and metanephros liquefactive necrosis is seen: cell nuclei of glomeruli and tubules are pale, have a sign of empty bubble with edematous stroma. In the adrenals all changes are the same. In the neural tube, spinal ganglia and in the stroma there are small hemorrhages.

Embryos and fetuses in their cavities of the hearts and blood vessels get stem cells CD34 (+), precursors of almost all types of hematopoietic cells, including proerythroblasts and myelomonocytic cells. Their appearance is a sign of a deep insufficiency of these cell types. In group 1 they do not occur, in group 3A occur rarely in single cases and in group 3B show up: 1-3 CD34 (+) cells to 50000 μm^2 area of the prep.

Out of the 6 preserved bodies of embryos and fetuses, in three cases a partial apoptosis has appeared in the neural tube (22.7% neuroblasts), in the eye rudiment, mesenchyme (26.2% of cells), less in the liver and stomach. Multiple apoptosis has destroyed erythroblasts in the aorta and major blood vessels and monocytes in the tissues. There also Gram + cocci are found. A small number of cells containing Fas, FasLigand, have appeared in the organs with apoptosis. bcl2 is visible in some places in the cells of gonadal ridges. Proliferation of parenchymal cells is decreased; with heavy damage its calculation is difficult.

Number of monocytes in the organs of 3B group is mostly close to group 1, but in the liver, where they mainly reproduced the number is lower (Table 5.5). In group 3A, where the situation is less severe, mononuclear cells are more frequent than in group 3B. This means the deficit of phagocytes to group 3B. Monocytes contain receptor Fc gamma RIII (CD16) and Fc gamma RII (CD32), as well as Fc gamma RI (CD64). They perform several important functions: phagocytosis, the sorption of genes and immune complexes, antigens; they are involved in cellular cytotoxicity and in other immune reactions (Fig. 6.1.). Promonocytes do not contain these receptors. They phagocytize some substances, such as IgG. In group 1 the number of monocytes is large, 68.78 ± 2.5% of them contain CD16, and 79.0 ± 3.95% - CD32. In group 3B, monocytes level is lower, respectively, CD16 - 34.52 ± 2.79%, and CD32 - 24.36 ± 1.95%. That is why, for example, promonocytes are less phagocytizing hemosiderin, coming from destroyed erythroblasts.

IL2Rα is found in liver in isolated lymphocytes and mononuclear phagocytes in the amount of 6.75 ± 1.22 / 50 000 μm^2, which is significantly greater than in groups 1

and 3A. IL2Rα is also widely presented in tissues and organs mentioned in group 1 and 3A.

5.7.2. Conclusion for the group 3B

In the cases of 3B widespread hematogenous infection causes severe illness, culminating in the death and destruction of embryos and fetuses. This is due to three factors. First: the rapid hematogenous spread of pathogens - microorganisms. Second: the massive damage to the placental barrier (Table 4.2.), which causes severe damage to the bidirectional exchange between the pregnant woman and the germ. And perhaps a third factor - the high toxicity of the microflora.

Effects of many pathological processes lead to multiple outcomes. In the same tissues and cells three types of necrosis are formed: liquefactive (edematous), coagulative (in which the proteins coagulate and the cells are transformed into dense clumps), and abnormal apoptosis. Multiple fractures of chorionic villi (80% are non-functional) causes, among other, the disturbance of water metabolism. It continues to flow through the placental barrier, forming large edematous villi. The excess water in the germ causes swelling of tissues and cells, with the outcome in liquefactive necrosis. The disturbance of proteins metabolism in the cells is ended by coagulative necrosis of them. The action of toxins of microflora induces apoptosis.

In such severe disease some protective processes are involved. In decidual tissue, together with neutrophils, macrophages and various immunocompetent cells of the pregnant also the invasive trophoblast of the embryo and early fetus is acting. At the same time the villous proliferating trophoblast is moving in large groups to the decidual tissue and getting incorporated into it. Strands of it (this is already the invasive trophoblast) are entering the areas of necrosis and phagocytize the particles, but also die then. As a result, the number of groups of proliferating trophoblast in the villi decreases in 6.4 times compared with group 1, while the number of invasive trophoblast cells decreases by 3.8 - 8.4 times. Nevertheless, the number of cells Ki67 (+) in groups of proliferating trophoblast even goes up slightly (Table 4.4). Monocytes phagocytize particles of necrosis of villi, possibly – the microflora, and also die. However, the number of mononuclear phagocytes is not reduced because of division of monocyte there in the villi (5.05 ± 0.95 of Ki67 +), as well as proliferation of promonocytes in the liver. Their number increases in villi: instead of 0.16 ± 0.01 / 50 000 μm^2 in group 1 it reaches 2.15 ± 0.09 / 50 000 μm^2 in group 3B (Table 4.5). By the amount of phagocytosis of necrotic tissue promonocytes do not concede monocytes. Obviously, this is the result of the high activity of interleukin 2 (IL2Rα). It surprises: how in the cases of severe infection in group 3B, in embryos and early fetuses the mechanisms of immune protection are still functional, supporting their livelihoods in a matter of weeks.

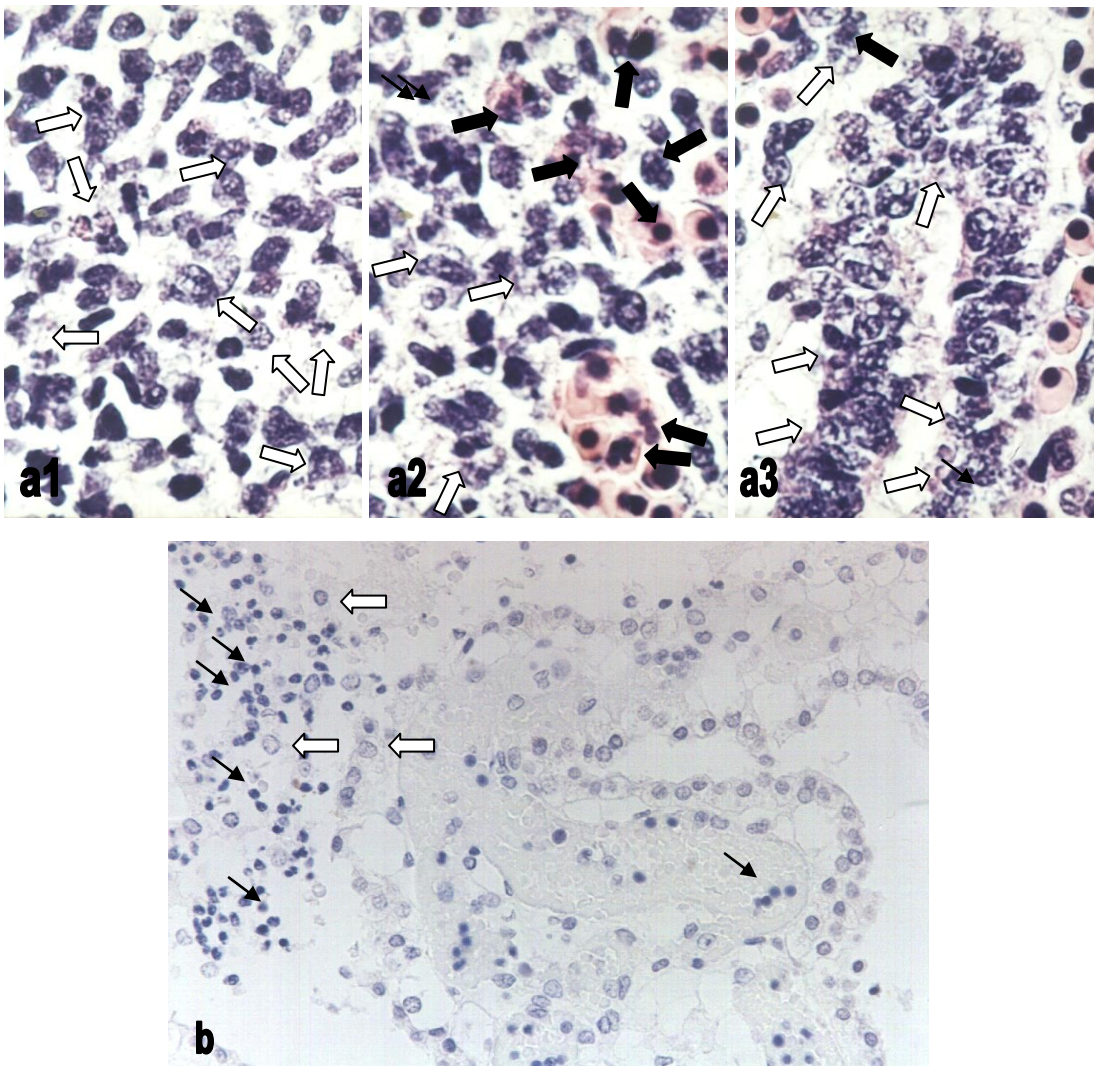


Fig. 5.4. Group 3B. a - embryos of 3.5 weeks. Multiple cell apoptosis in amount of 21-26.2% in hepatocytes (open arrows). **a1** - the neural tube, **a2** - liver: apoptosis covers hepatocytes and erythroblasts (black arrows), **a3**- stomach in formation, epithelium of the mucous layer, small groups of stromal cells, and erythroblasts in the capillary. Apoptosis of epithelial cells is significant, of stromal cells and erythroblasts - moderate. Magnification in a1-a3 is x1000. **b** - the fetus at 21st week. Lateral brain ventricle, choroid plexus. Ependyma is devoid of immunoglobulins, including IgA (-). Apoptosis is not detected. In the ventricular cavity there are cell of lymphocyte-type (black arrows) and leukocyte-type (white arrows). Magnification is x400.

5.8. Group 4. Early allogeneic conflict of the pregnant woman and the fetus

Group 4 consisted of 15 embryos at 3.5-5 weeks of development. Earlier, in Chapter 4.7 studies of 43 abortion tissues have been presented. Clinical diagnosis is: production of conception or missed abortion, or others like that; allogeneic conflict is not mentioned. In this section of Chapter 5 we provides information on the status of the preserved embryos of the same cases as described in Chapter 4.7 and with the same complex of research methods.

5.8.1. Effect of pathogens, apoptosis

Section 4.7 shows that immunocompetent cells of decidual tissue: B lymphocytes and plasma cells, produce immunoglobulins IgG, IgA and especially IgM. T-lymphocytes CD3, CD4 helper cells, CD8 cytotoxic (suppressor) and CD56 NK are not involved in this reaction, their number in group 4, as well as in group 1, 3A and 3B is the same (Table 4.6). Later on IgG, IgA and IgM will be phagocytized by five species of trophoblast of embryos that are in contact with tissue and blood of pregnant women. Mononuclear phagocytes, endothelium of capillaries and erythroblasts in their lumen, located in the tissues of the villi also phagocytize immunoglobulins. The listed cells, phagocytizing immunoglobulins contain receptors involved in apoptosis: Fas, FasLigand, p53 (and positive reaction to TUNEL), which determine the pre-and post-apoptotic states of cells. These cells are destroyed by a multiple (pathologic) apoptosis. Cells in the villi, not phagocytizing immunoglobulins (fibroblasts, smooth muscle cells, villous stroma) do not undergo apoptosis. Thus, apoptosis is the leading pathological process (Table 4.2).

Status of the embryo depends on the extent of damage of the villi, which is not uniform. Not the entire villus is damaged, but only the different parts of the syncytiotrophoblast, at least - cytotrophoblast. Capillaries of the villi are also damaged unequally and not at the same time: in a small amount (3-18% of villi) it happens often (in 83.7% of cases), while much less frequently (in 16.3% of cases) there are more multiple lesions of villi (20-33% at the same time). This is due to unequal appearance of pathogens - immunoglobulins. It is more significant in repeated pregnancies. Apoptosis destroys capillaries but also site by site, but in such a way that this small area becomes destroyed completely and is unable to recover. As a result, the average number of capillaries in the villi drops or they all collapse forming avascular villi. But the damage does not stop by that: very large and swollen villi with no vessels in them are formed. Water gets into them through the trophoblast, and is unable to escape with no capillaries. All of these changes of the villi and all placental barrier lead to metabolic disorders of embryos. But that's not all: apoptosis of embryonic cells induces severe damage.

5.8.2. Status of embryo

Out of 15 embryos with preserved tissues, 8 embryos (53.3%) have been aborted at 3.5 – 4 week of development; at 5, 6, 7 weeks 2 abortions have occurred with the tissues of embryos (13.3% each), and the tissues of the last embryo (6.7%) have been

released at 8 week (Table 4.10). This sequence of destruction of embryos and the abortions has distinct causes.

The sizing of the trunk of embryos is largely hindered by the fragmentation of it in abortion, but still in the embryos of 3.5 - 4 weeks the following dimensions have been defined: from crown to rump, they are 1.8 - 2 mm, being 3 - 5 mm in control (Drews U., 1995; Sadler TW, 1995; Robboy SJ et al., 2002; Milovanov AP, Savel'ev SV, 2006). At the same time, organogenesis of the embryos corresponds to the term of development. Malformations are not detected. In some cases a decidual tissue contained small necrosis with leukocytic environment, microflora was not determined. Distribution of this process onto the chorionic villi and the embryo was absent.

Studies of embryos of group 4 have identified two options for change. In one of them, the leading was specifically multiple apoptosis. In the embryo itself, there is a mass destruction of cells of various organs; capillaries of the villi are slightly damaged. In another option, expression of apoptosis in the embryo is moderate, it is seen in many organs, but quantitatively is lower than in the first option. Circulatory problems are more pronounced, many vessels of the embryos look collapsed, do not contain erythroblasts. There are also severe changes in the chorionic villi, particularly in the vessels (Table 5.7).

5.8.3. Multiple apoptosis of cells in embryonic organs

This part includes tissues of 9 embryos. Abortion in 6 of them took place at 3.5 - 4 week, and at 5, 6, 7 – one for each term. Embryos aborted at 3.5 - 4 weeks contain regions of "apoptosis with secondary necrosis» (Correia-da-Silva G. et al., 2004) in many villi of the syncytiotrophoblast and sometimes in cytotrophoblast. Apoptosis of monocytes, as well as groups of erythroblasts include up to 26.7% and even up to 33.05% of villi. Segments of vessels, where it occurs, are also destroyed and not restored anymore. But in some places proliferation does not stop: distantly in preserved individual capillaries there are single Ki67 + erythroblasts, and in the stroma of the villi - Ki67 + monocytes. As a result, the number of villi with normal vessels is reduced and the number of moderately affected (avascular or obliterated vessels) also increases moderately (Table 5.7).

Embryos, dead and aborted at 3.5 - 4 weeks, are a consequence of multiple apoptosis (Fig. 5.5, 5.6). Particularly the neuroblasts in the outer layer of the neural tube, in the ganglia and the rudiments of eyes, hepatocytes, cells of the mesoderm, mesenchyme are affected. Apoptosis is seen in 20-60% of cells of these structures. In the epithelium of the skin and stomodeum, archenteron, in the mesonephros, erythroblasts in the cavities of the heart and vessels apoptosis covers about 20% of the cells, and in the yolk sac and amnion - about 10%. In part of the cells of mentioned organs J-chain, IgG and IgA, sometimes IgM are found. Cells with immunoglobulins look normal or stay in the early stages of apoptosis. But in the destroyed cells, which remain only as decaying lumps of apoptosis, J-chain and immunoglobulins are also disappearing. In the neural tube IgG (+) neuroblasts at amount of 40.8 ± 10.94 / 50 000 μm^2 are found, and for ones destroyed apoptosis: IgG (-) 13.2 ± 3.19 lumps. In the liver, there are 42.02 ± 7.12 / 50 000 μm^2 IgG (+) hepatocytes and for damaged IgG (-) 18.4 ± 1.81. In the mesenchyme of cells there is IgG (+) 30.01 ± 4.02 while for destroyed by apoptosis IgG (-) 17.0 ± 2.42 / 50,000 μm^2. This means that ones, prepared for apoptosis, and ones already passed it, consist simultaneously not more than 50% of the total.

Table 5.7. State of the villi of 4 and the degree of apoptosis in tissues of embryos and the timing of abortion

Condition of villi	Averaged data		Multiple apoptosis in the tissues of embryos		Moderate apoptosis in the tissues of embryos	
			Terms of abortion (in weeks)			
	Group 1	Group 4	3.5-4	5-7	4	5-8
Normal capillaries (%)	76.37 ± 2.72	28.53 ± 2.97 [a]	59.78 ± 3.92 [ab]	43.24 ± 3.54 [abc]	13.71 ± 1.24 [abcd]	36.32 ± 3.04 [ace]
Obliteration or emptying of the capillaries (%)	18.83 ± 1.63	56.73 ± 3.33 [a]	28.91 ± 2.11 [ab]	35.02 ± 2.27 [ab]	69.48 ± 4.56 [abcd]	39.31 ± 3.18 [abce]
Avascular villi (%)	4.82 ± 0.28	14.74 ± 2.31 [a]	10.62 ± 1.16 [a]	15.67 ± 1.22 [ac]	3.69 ± 0.35 [abcd]	15.83 ± 1.47 [ace]
Average number of vessels in the villi	5.29 ± 0.83	2.07 ± 0.68 [a]	2.56 ± 0.14 [a]	2.15 ± 0.11 [ac]	2.0 ± 0.12 [ac]	1.86 ± 0.15 [ac]
Edematous villi (%)	26.91 ± 3.01	47.12 ± 3.35 [a]	34.19 ± 3.16 [b]	38.07 ± 2.42 [ab]	75.94 ± 4.18 [abcd]	70.02 ± 3.11 [abcd]
Apoptosis of capillary (% of villi)	1.52 ± 0.04	18.53 ± 4.01 [a]	9.72 ± 0.96 [ab]	6.62 ± 0.72 [abc]	16.83 ± 1.33 [acd]	4.41 ± 0.85 [abce]

[a, b, a, d, e] - significant differences ($p < 0.05$-0.001) of the overall indicators of groups 1 and 4 and the rows of multiple and moderate apoptosis with the timing of abortions indicated. The table shows the relationship of timing of abortion with the intensity of apoptosis and destruction of the villi. Effect of destruction of the placental barrier on the possible causes of embryonic death is discussed in the text.

And this is only the action of IgG, while IgA and IgM inward flow enhances the action of the pathogen and increases the number dying cells. The neural tube contains IgA (+) 2.11 ± 0.43 neuroblasts, while for IgA (-) - 51.5 ± 3.28 / 50 000 μm^2 shattered remnants. Similarly, in the liver: IgA (+) 5.25 ± 0.37 hepatocytes and IgA (-) 37.08 ± 2.98 / 50 000 μm^2 residues. This suggests that with continued significant entrance of pathogens whole organ and whole body are destroyed very quickly. According to the experiments (Halperin R., et al., 2008), full gastrula cell apoptosis in rats injected with the serum of women in the fallopian tube, occurs within 2 - 6 hours.

Villus blood circulation in cases of abortion at 3.5 - 4 week in almost all cases is satisfactory. This is reflected in preservation of 59.78 ± 3.92% of villi looking normal, including their capillaries (Table 5.7). In the embryo, blood vessels of organs and cavities of the heart are filled with erythroblasts, some with immunoglobulins and J-chain. Only in one case and in the neural tube only, most vessels did not contain erythroblasts; they were fallen, or filled with plasma. Necroses of neuroblasts are not expressed, the stroma of the neural tube is somewhat swollen. Number of neuroblasts in apoptosis is not different from that observed in other cases. In this case, shortly before the abortion disruption of vessels in the neural tube occurred, similar to that observed in the chorionic villi with allogeneic conflict and in some other processes (infection, group 3B).

Components of apoptosis: Fas, FasLigand and p53 are sometimes missing or observed during apoptosis of each organ cells, monocytes and erythroblasts in blood vessels. They are also found in the yolk sac. bcl2 in the tissues of the embryos are rarely found in monocytes. TUNEL reaction initiates apoptosis in cell nuclei, and then in their cytoplasm, which reflects the propagation of apoptosis.

SC, as a receptor of SIS, is found in the epidermis of the skin, notochord, archenteron, the epicardium and pericardium, the epithelium of tubules of mesonephros, yolk sac, etc. This means that SIS is preserved. Interleukin 2 and its receptor IL2Rα (CD25 +) is one of the growth factors. It is widely distributed in the organs, even in such difficult cases as allogeneic conflict in early embryos. IL2Rα is contained in those parts of organs, where apoptosis is less expressed - in parts of the neural tube, ganglia cell groups, in myocardium and other forming organs, in the capillaries, actively growing during this period, and in others. In the same condition is the proliferation of embryonic cells: it weakens, but does not stop. Scattered Ki67 (+) cells are found even in organs that are undergoing apoptosis. The proliferation is concentrated in yolk sac, where 69.4 ± 10.11% Ki67 (+) different types of cells may be found.

Mononuclear phagocytes consist of 3.4 ± 0.21% CD68 (+) monocytes and of 96.6 ± 18.2% CD68 (-) promonocytes. They are large, with a large nucleus and phagosomes clearly distinguishable from monocytes. The total number of mononuclear phagocytes in the zone of the placenta barrier and the embryo does not decrease (Table 4.5). But the lack of any increase in their number (as noted in the group 3A - Table 5.5) indicates failure in functions of mononuclear system, which is greater than in severe infection in group 3B. In the yolk sac at 3.5 - 4 weeks, apart erythroblasts, also CD34 + large, round precursors of them - BFU-E are generated (Fig. 4.16.a1). Under normal circumstances, they do not enter the bloodstream. But in allogeneic conflict, severe infections and other processes they can be seen in the cavities of the heart and even in the capillaries of the villi. They are free and by this differ from the vascular endothelium, which is also

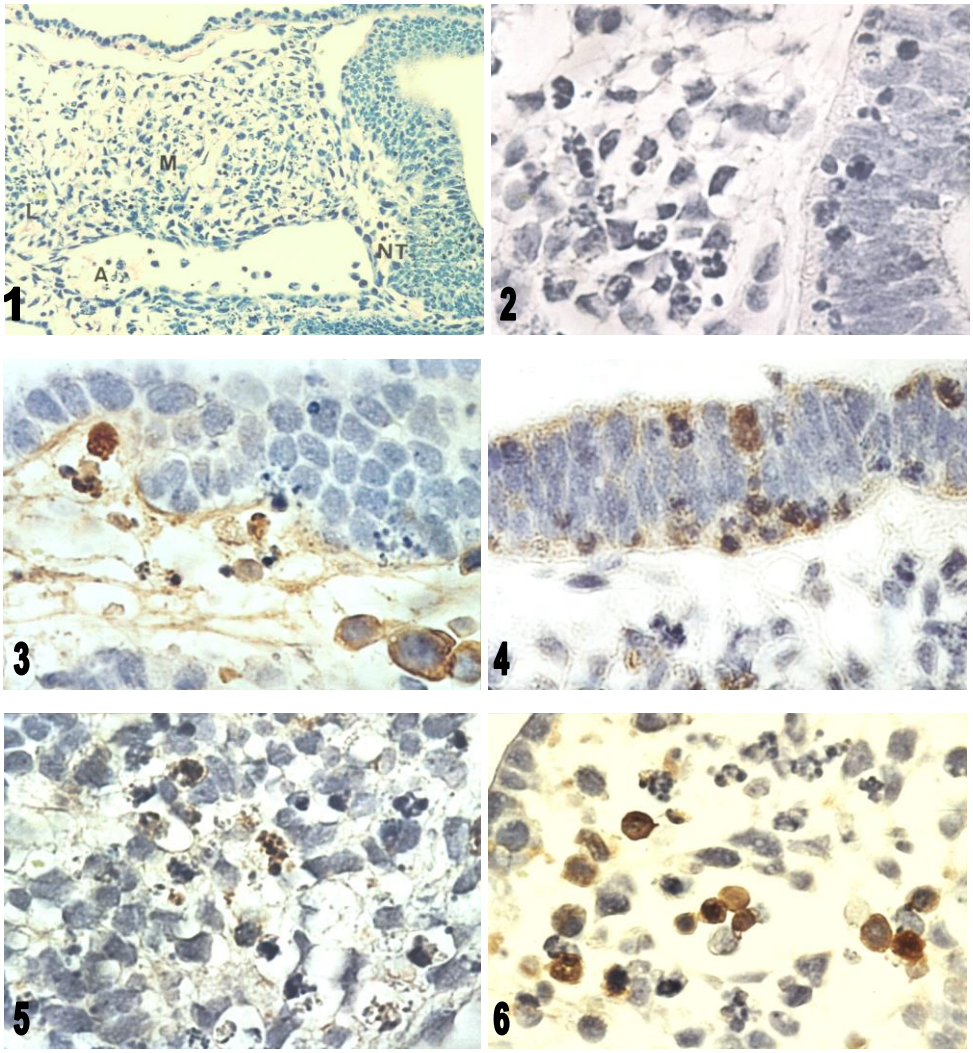


Fig.5.5. Group 4. Multiple apoptosis of embryonic organ cells. All cases of 3.5 weeks of development. **1** - total upper body: the neural tube (NT), mesenchyme (M), aorta (A), liver (L), flora is absent (staining by Giemsa). **2** - in the mesenchyme (left), neural tube (right) FcRIIIA (CD16-). **3** and **4** - the neural tube (above), mesenchyme (below): IgA (+) and IgG (+) are contained in the part of cells, including phagocytes. Destroyed cells lose immunoglobulins. **5** and **6** - the mesenchyme, same as in 3 and 4: some of the cells, including phagocytes contain immunoglobulins, which also get destroyed in apoptosis. Magnification of 1 is x200, of 2-6 is x1000.

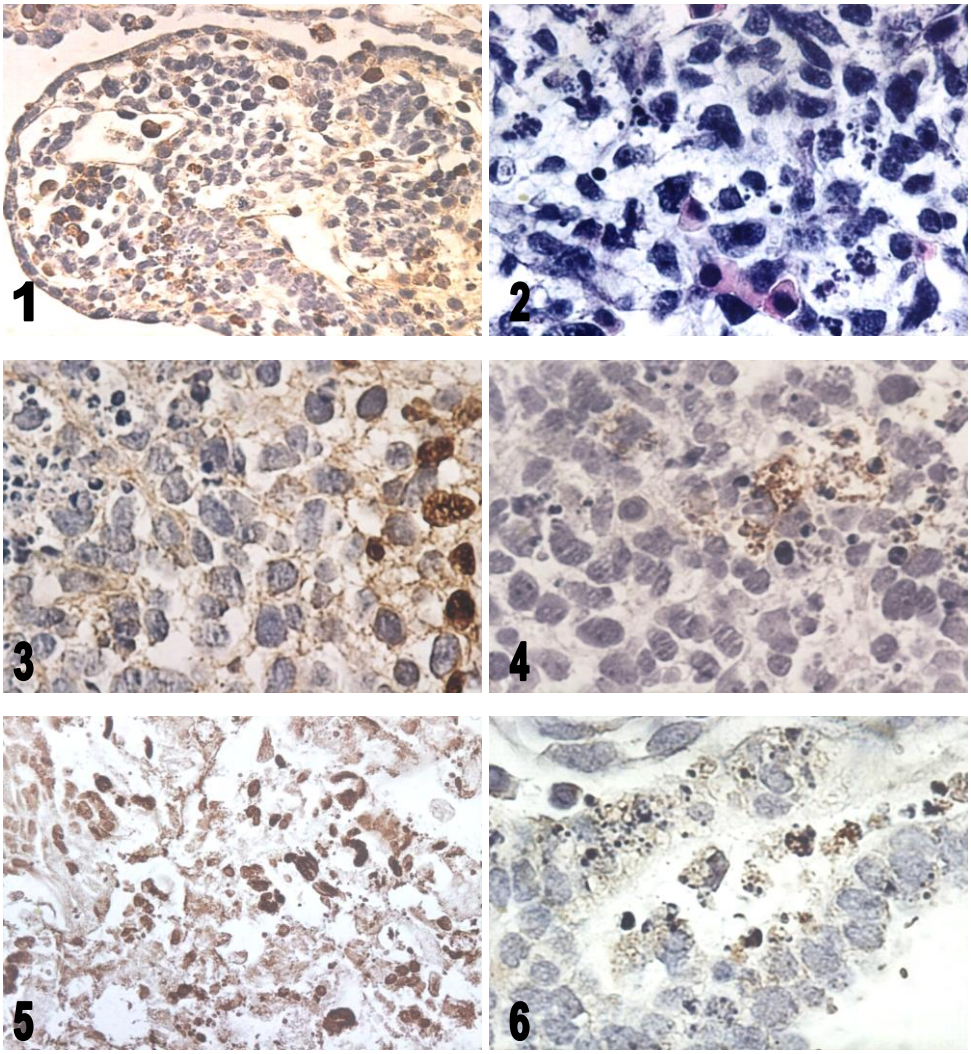


Fig.5.6. Group 4. Multiple apoptosis. Development of 3.5-4 weeks. **1-5** - liver. IgG (+) hepatocytes and phagocytes (many in the vessels) look preserved, IgG (-) a lot of grains of cells damaged by apoptosis. **2, 3** - consecutive morphological stages of apoptosis: the capture of immunoglobulins, pyknosis of nuclei, the decay of the nucleus and the whole cell into small lumps, the destruction of immunoglobulins, the further decay into dust and disappearance. **4** - CD68 (+) is preserved even on small granules. **5** - reaction of TUNEL is positive at the whole process of apoptosis - before pyknosis of the nucleus and up to the formation of fine dust. **6** – the stomach under formation: the number of apoptotic cells is higher than in 50%. Magnification of 1 is x100, of 5 - x400, 2, 3, 4, 6 - x1000.

CD34(+), but is located on the wall of capillaries being flat.

Cell proliferation continues in other places too (Fig. 5.7). In amnion Ki67 (+) proliferating cells ale of lower amount than in yolk sac - in the amnion 19.0 ± 1.2 / 50 000 μm^2. The proliferation of trophoblast does not stop as well. Invasive trophoblast cells are situated in decidual tissue of pregnant, next to the B-lymphocytes. They actively phagocytize allogeneic antibodies, but dying themselves because of it. This loss is tried to be compensated by some groups of proliferating trophoblast cells: the number of groups decreases sharply (Table 4.4.), their cells are moved to the decidual tissue and become invasive trophoblast. But this is not enough: the amount of invasive trophoblast is dramatically reduced. It opens the way for pathogens. The following system of protection is operating on the territory of the villi and the embryo itself: the mononuclear phagocytes. It was noted above their intense proliferation with the formation of a large number of promonocytes. But even this system is insufficient (Table 4.5).

There were 3 cases of embryos with multiple apoptosis in which abortion occurred at 5, 6 and 7 weeks (Table 5.7). They are different from embryos in which the abortion occurred earlier - at 3.5-4 weeks. It includes: decrease in the number of villi with normal capillaries, the average number of capillaries in the villi as well as villi with apoptotic capillaries, but along with increased number of avascular villi or exhausted capillaries. This shows that in cases of abortion at 5, 6, and 7 weeks the action of the pathogen was weaker, but longer than that of embryos in which apoptosis happened at 3.5 - 4 weeks. The remaining manifestations of the pathogen are similar: some areas of syncytiotrophoblast, and sometimes cytotrophoblast develop apoptosis, turning into necrosis, decrease the number of proliferating Ki67 (+) trophoblast cells (except the syncytiotrophoblast, which isn't dividing generally) (Table 4.4). Invasive trophoblast and mononuclear phagocytes actively phagocytize immunoglobulins of the pregnant being then destroyed themselves (Table 4.9). The number of mononuclear phagocytes of villi is preserved (6.15 ± 0.83 / 50 000 μm^2, Table 4.5).

Embryos are presented by small pieces of skin, liver, eye rudiments, muscle tissue, several ribs and by other mesenchyme. They contain significant accumulations of cells in a state of apoptosis at various stages of destruction. Their condition and the changes are similar to those observed in embryos, aborted by 3.5 - 4 weeks. The number of apoptotic cells is somewhat smaller in the liver - 28.3 ± 4.9, in the mesenchyme - 13.1 ± 0.92, in the cartilages - 10.62 ± 0.17 and in the muscles of 1.8 ± 0.03 / 50 000 μm^2. In the amnion and chorion apoptotic cells consist 12.25 ± 2.11 / 50 000 μm^2. Blood supply of embryos with abortion at 5 - 7 weeks is satisfactory: in the tissue capillaries containing erythroblasts may be found. This confirms the presence of considerable number (43.24 ± 3.54%, Table 5.7.) of normal villi. This entire means that the pathogenic immunoglobulins could pass freely through the placenta barrier to the embryo.

5.8.4. Moderate apoptosis of embryos' organs cells

This section covers tissues of six embryos, in which apoptosis is moderate: not more than 20 / 50000 μm^2 cells of certain organs are affected. In two cases, abortion was at the fourth week of development, while in the remaining four - at 5, 6, 7, and 8 weeks. In early abortion (fourth week) only 13.71 ± 1.24% villi had functional capillaries (Table

5.7) while the rest were avascular, swelling, or obliterated. Embryos aborted at the 4th and 5 – 8th weeks were found in the form of small pieces of tissue: neural tube, skin, intestine, liver, mesonephros, and mesenchyme. Cells at the status of different stages of apoptosis were distributed in small groups of 3 - 8 / 50000 μm^2 in the skin, intestine, mesonephros, and 15 - 20 / 50000 μm^2 in the neural tube, liver and mesenchyme. Some of the cells in apoptosis contained IgG, IgA and IgM, while ones decayed in apoptosis did not contain any of them. Necrosis, hemorrhage and other changes were absent.

Number of mononuclear phagocytes in the villi in abortion at the 4th week was significantly reduced (3.26 ± 1.0 / 50 000 μm^2), total amount of monocytes was only 11.94 ± 1.28%, along with many promonocytes - 88.06 ± 1.78% (Table 5.8). In cases of abortion at 5 - 8 weeks, the number of monocyte-derived phagocytes, including monocytes increased, probably due to increased time for ripening of promonocytes. Their formation occurred mainly in the yolk sac (21.08 ± 1.08% of phagocytes Ki67 +), but also in the villi. Cytotrophoblast (Ki67 + 29.64 ± 2.34%) and invasive trophoblast (Ki67 + 23.53 ± 2.05%) were also proliferating. Phagocytes contained interleukin 2 receptor (IL2Rα Ki67 + 17.34 ± 0.97%).

Table 5.8. Effect of long-lasting pathological process (3.5-4 and 5-8 weeks) on the formation of mononuclear phagocytes in the villi with allogeneic conflict (group 4)

	Multiple apoptosis in the tissues of embryos		Moderate apoptosis in the tissues of embryos	
	Terms of abortion (weeks)			
	3.5 - 4	5 - 8	3.5 - 4	5 - 8
The number of mononuclear phagocytes /50000 μм^2	5.16 ± 1.33	11.28 ± 2.17 [a]	3.26 ± 1.0	7.85 ± 1.57 [a]
out of them - monocytes (%)	42.06 ± 6.65	72.3 ± 7.54 [a]	11.94 ± 1 23 [b]	32.64 ± 4.15 [ab]
Out of them - promonocytes (%)	57.43 ± 6.41	27.72 ± 6.52 [a]	88.06 ± 1.78 [b]	67.36 ± 6.83 [ab]

[a] - significant differences (p <0.05-0.001) of the terms of abortion 3.5 - 4 and 5 - 8 weeks with the same state of apoptosis (number of phagocytes and the ratio of monocytes and promonocytes). [b] - significant differences (p <0.02-0.001) of multiple and moderate apoptosis in terms of abortion at 3.5 - 4 versus 5 - 8 weeks in monocytes and promonocytes. Explanation is given in the text.

Thus, between cases of early (4 weeks) and late (5 - 8 weeks) abortion there are significant differences. At an early abortion, apoptosis of capillaries and obliteration of them happens often, while normal villous capillaries are rarely preserved (Table 5.7). In contrast, with abortion at 5 - 8 weeks the number of apoptotic capillary is small but normal capillaries and avascular villi are often found. This means that in cases of early abortion the amount of allogeneic immunoglobulins was great, and for late abortions the dose of them was small, but acting for longer time.

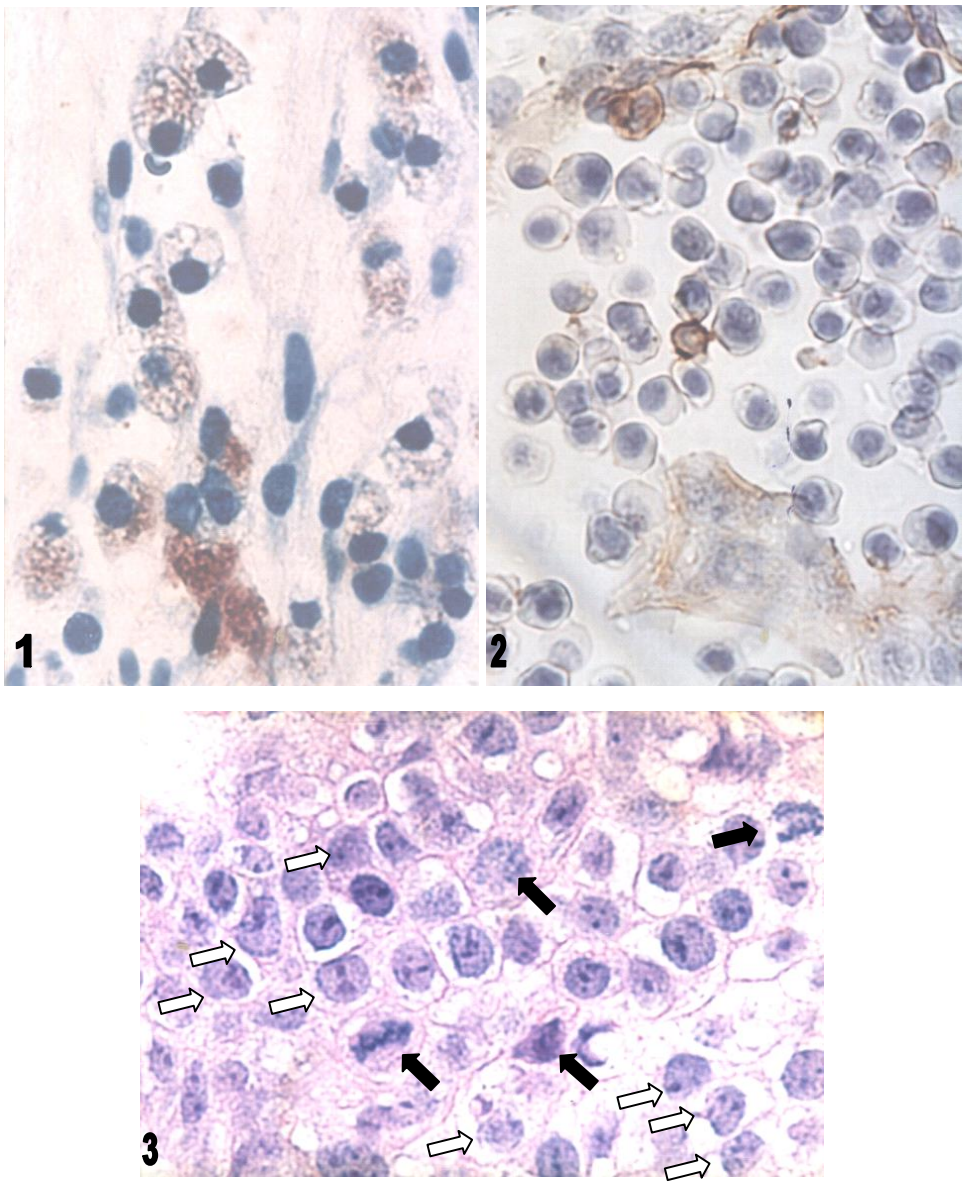


Fig.5.7. Group 4. Multiple apoptosis does not preclude further proliferation of surviving cells. **1** - 3.5 weeks of development. In the mesenchyme the number of CD68 (+) monocytes is 15%, and promonocytes CD68 (±) or (-) rises to 85%. **2** - 3.5 weeks of development. In the blood of heart and in other vessels CD34 (+) BFU-E precursors of erythroblasts appear. **3** - 8 weeks of development. In the area of proliferation of trophoblast apoptosis (white arrows) occurs in 42.6% of the cells, and nearby there is 7.4% of cells in mitosis (black arrows). Similar processes are observed in the neural tube, liver, mesenchyme, etc. Magnification is x1000.

5.8.5. Conclusion for the Group 4

Multiple pathological apoptosis: the effect of allogeneic antibodies. Our studies (Chapters 4.7 and 5.8) showed that the major factor in tissue damage of the embryo and its shells are alloantibodies. Other pathological factors, in particular, various microorganisms (groups 3A and 3B) are causing many other pathologic processes (necrosis, leukocyte infiltration, and others) which are absent in the tissues of the embryos of group 4 (Table 4.2). In group 4, they are sometimes found in the decidual tissue and intervillous lacunae without spreading to the placenta and fetal tissue as a concomitant disease. In the tissues of embryos, shells and villi of group 4 immunocompetent cells of the pregnant are completely missing: natural killer (NK), all types of lymphocytes, macrophages and neutrophils. Only in some cases a little bit of neutrophils and macrophages are detected in fibrin clots on the surface of the damaged section of syncytiotrophoblast (Table 4.8.) but the fibrin is from the blood from lacunae of the pregnant women. Moreover, in the decidual tissue and blood lacunae of the pregnant in cases of group 4 the number of lymphocytes CD3, helper CD4, CD8 CTL and NK, CD56 does not change (Table 4.6. and 4.7.); they are not involved in this process. All this allows us to exclude the possibility of cellular immune responses from the site of the pregnant woman in an early allogeneic conflict.

In decidual tissue and blood lacunae of the pregnant an increase in the number of B-lymphocytes and plasma cells is noted (Table 4.6 and 4.7). This means an increase in the activity of humoral immune responses. They intensively synthesize IgG, IgA and IgM. These immunoglobulins are phagocytized by cells of invasive trophoblast, which is located nearby, in the decidual tissue, while in the blood of pregnant women, by the trophoblast cells that cover the lacunae, and by the syncytiotrophoblast. Penetrating into the stroma of villi immunoglobulins are captured by mononuclear phagocytes. In groups 1, 3A and 3B, small amounts of immunoglobulins are located on the cell membrane or in their cytoplasm (Fig. 6.1.) whereas in group 4 many of them are located on the membranes, but even more - in the cytoplasm of phagocytes (Table 5.3), in erythroblasts and capillary endothelium. Here, all these cells are destroyed by apoptosis. Characteristically, the same phagocytes of groups 3A and 3B, as well as macrophages of the decidual tissue of pregnant in group 4 phagocytize immunoglobulin in much smaller quantities, and they themselves do not undergo apoptosis (Table 4.9. and Fig. 4.2). This means that the antibodies of the pregnant in groups 3A and 3B are not pathogenic for themselves and also for self-macrophages in group 4. But they are very pathogenic for mononuclear phagocytes, for villous trophoblast and other tissues of germs of group 4.

Cells in the organs and tissues of the embryo also undergo apoptosis. They first uptake the immunoglobulins, then apoptosis occurs, and with the decaying cells, the immunoglobulins get destroyed. Physiological apoptosis occurs in all the embryos during the formation of organs, but it is limited unlike the multiple pathological apoptosis. All marked indicates that immunoglobulins of the pregnant under conditions of group 4 are pathological for the embryo. They initiate the allogeneic conflict.

The physiological apoptosis has its own characteristics. Its aim is to destroy the target tissue to create new cells for the new organ or tissue. An example of that is the restructuring of the neural tube in the brain, when replacing neuroblasts, a mass of neurons of different structures and functions, as well as various glial cells are formed.

Another example is the transformation of sacculus hypophysialis of throat into the front of pituitary, and many others. Apparently, the purpose of pathological apoptosis in general and, in early allogeneic conflict in particular is to completely destroy unwanted cells and organs, which then should not recover. It is clearly seen how apoptosis eliminates villous capillaries so they becomes avascular. Apoptosis does not destroy in a row all cells and tissues of the body, but only those that are intended for fracture.

Allogeneic immunization of pregnant women. Allogeneic antigen that causes early conflict of the pregnancy and the germ is a complex problem. In 1944, P. Medavar proposed the concept of "fetus as allograft". It was assumed that the death of the germ occurs by the cellular immune reaction involving T lymphocyte suppressors and NK. This view was first accepted. It puts forward the major histocompatibility complexes MHC I (HLA-A, B, C) MHC II (HLA-DR,-DQ,-DP). But they do not exist in those tissues of the embryos that have to be close to the decidual tissues of the pregnant. Searches are very active *. More and more trophoblast antigens corresponding to these properties are found. These are antigens MHC II (HLA-G, HLA-G1, HLA-G1A and-G1B, HLA-G2, HLA-E, HLA-L). They are found in invasive trophoblast, in groups of proliferating cells of the villi, in the trophoblast covering the lacunae and the spiral arteries, in villi: in monocytes, endothelia and erythroblasts of capillaries in the cells of the amnion. These antigens are found throughout fetal development, but being weakened in the III trimester . Extensive HLA-G variants were found in healthy Japanese (Tamaki J. et al., 1993; Yamashita T. et al., 1996), the African American and Caucasian peoples (Europoid) race (Hviid TV et al., 1997).

In general, which allogeneic antigens cause the conflict is not yet known. We shall leave this question and better consider the stages in the development of allogeneic immunization of pregnant women and the subsequent stages of the conflict.

Stages of allogeneic conflict of the pregnant woman and the fetus. Dimensions of embryos at 3.5 - 4 weeks are half the normal (Chapter 5.8.2.), while their organogenesis corresponds to that age. Such a delay in grow indicates that the duration of the pathological process prior to abortion is about 1.5 - 2 weeks.

In the first week of normal development morula is surrounded by fertilization shell (fertilization membrane), and around it there are the cells of the pregnant forming radiate crown (corona radiata) (Fig. 2.1). They nourish and protect the morula. At the end of the first and the beginning of the second week this protection is destroyed, and on the surface of blastocysts cells of the embryo (the future trophoblast) are formed. Since that time, it is becoming possible to reveal the incompatibility of the germinal antigens by macrophages (antigen- presenting cells of the pregnant women). Since that time the

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*- Thomas ML, et al., 1985; Kovats S., et al., 1990; Chumbley G., et al., 1993; Tamaki J., et al., 1993; Bright NA, et al., 1994; McMaster M. et al., 1995; Hara N. et al, 1996; Jamashita T. et al., 1996; Jurisicova A. et al., 1996; Hviid T.V. et al., 1997; Loke Y., King A., 1997; Rouas-Freiss N., et al., 1997; LeBouteiller PL et al., 1999; Navarro F. et al., 1999; Goldman-Wohl DS, et al, 2000, 2007; Kapasi K. et al., 2000; Contini P., et al., 2003; Landenberg P.van, et al., 2003; Laird SM et al., 2003; Hunt J.S. et al., 2005; Milchev N. et al., 2006; Shoenfeld Y. et al., 2006; and others.

immune response formation and the response itself begin. All the processes of early allogeneic conflict of the pregnant woman and the embryo consist of six stages.

The first step - recognition of incompatible antigen in the germ carry macrophages of the pregnant found in the decidual tissue and in the lacunae. They reveal allogeneic antigens in trophoblast at the end of the second week of development.

The second stage - the transfer of information received about the presence of allogeneic gene to immunocompetent cells of the pregnant - CD4 helpers and CD20 B-lymphocytes. All of them are located close to each other in the decidual tissue. With blood of lacunae macrophages spread the information in the entire body of the pregnant.

The third stage - the synthesis of allogeneic antibodies. It takes about two weeks. With repeated allogeneic conflict, if a woman is immunized during the former failed pregnancies and alloantibody are found in her blood in sufficient quantity, the first three phases are reduced by approximately one week.

The fourth stage - the transport of immunoglobulins to tissues of the embryo, mainly to the surfaces of cells of different types of trophoblast. This stage passes quickly, as the trophoblast and B-lymphocytes are located nearby. The first four stages of the conflict take place at the early periods of development of morula, blastocyst and early embryo. During this period of the second and third weeks of development, the erms are still very small (2-5 mm) and stimulation of allogeneic antibodies formation is moderate.

The fifth stage – allogeneic conflict at the zone of placental barrier begins with phagocytosis of immunoglobulin by invasive trophoblast and by trophoblast covering the lacunae. Number of phagocytized antibodies is high: in the section of each phagocyte IgG, IgA and IgM together comprise about 150 phagolysosomes. It's much more than invasive trophoblast phagocytize with infections (groups 3A and 3B) and macrophages of the pregnant in group 4 (Table 4.9, Diagram 4.2). These two types of trophoblast are destroyed along with the antibodies inside of them. But the struggle continues. Groups of proliferating trophoblast at villi move close to the decidual tissue, forming a so-called anchoring villi. Their trophoblast using strands move deeper into the decidual tissue becoming an invasive trophoblast and the trophoblast that covers the lacunae. They continue to englobe antibodies being also killed. As a result, the number of groups of proliferating trophoblast is reduced almost five times and same of invasive trophoblast - more than 6 times (Table 4.4). In that way the first line of protection against exposure to alloantibodies in the early embryonic period phagocytize.

Alloantibodies reach some villi. The picture is the same: covering the villi syncytiotrophoblast phagocytizes them by its several sites. Apoptosis, which turns into necrosis, happens on them. Initially, these places are not covered by fibrin (Table 4.10) but then at 5-7 weeks the fibrin builds up and covers the areas of 30-37% of villi. Part of alloantibodies is transferred by syncytio- and cytotrophoblast towards the villous stroma. Here they are met by monocytes - the next line of defense. They also phagocytize allogeneic antibodies, some of them get destroyed by apoptosis, and some move the antibodies to villous capillaries. Here, the endothelium of capillaries and erythroblasts undergo apoptosis and disappear. As a result, the number of allogeneic antibodies goes down, only a portion of the capillaries, erythroblasts and monocytes is destroyed: in 83.7% of cases, only 3-18% villi are damaged, while more serious damage - 20-33% is found only in 16.3% of cases. A small number of allogeneic antibodies may be due to two reasons. The first perhaps is a small formation of antibodies in the small-sized - 2-5 mm - embryos at 3.5-4 weeks of pregnancy. The second reason seems more reasonable:

the massive destruction of allogeneic antibodies in many kinds of phagocytes. Phagocytes are also destructed massively, but their number recovers (Table 4.4., 4.5., 4.6.).

Results of the fifth stage of the alloantibody activity are significant. The destruction of small sections of the capillaries leads to the cessation of blood circulation due to obliteration or thrombosis. Completely destroyed capillaries are not restored, so that avascular villi are formed. Syncytio- and cytotrophoblast, partially destroyed, permit the water flow from the lacunae in the villi being accumulated there but due to a decrease or absence of capillary; villi become large and swollen. This disrupts the embryonic metabolism, including significant reduction of allogeneic antibodies penetrating into it. All these numerous complications such as the destruction of invasive and other forms of trophoblast, monocytes, erythroblasts, and blood vessels, the deposition of fibrin and other changes are a consequence of activity of allogeneic antibody via apoptosis.

Sixth stage - spread the allogeneic conflict in the tissues of the embryo. It can happen in two ways. The first is the penetration of a large number of allogeneic antibodies into the embryo through the slightly damaged vascular system of the villi, when 40-60% of normal capillaries are still functional. Multiple apoptosis in the majority of forming organs occurs: in the neural tube, liver, mesenchyme and others. Simultaneously, apoptosis affects up to 40-50% of the cells. The destruction of embryos typically happens at 3.5-4 weeks of development and only a few isolated, separated into small pieces of tissue embryos are aborted at 5-7 weeks. This variant accounts for 60% of those detected during abortion (Fig.5.5, 5.6).

The second option is seen in 40% of cases. It occurs with the penetration of a moderate amount of allogeneic antibodies through the significantly damaged villous capillaries with only 13-36% of them functioning normally (Table 5.7). Embryos are presented in the form of small pieces of tissue. But allergenic antibodies continue to operate; apoptosis destroys cells in small groups of 13-18. Cells that are not in apoptosis are preserved. Sometimes even proliferating, Ki67 (+) cells may be found among them (Fig. 5.7). There are mononuclear phagocytes (2-4 / 50000 μm^2) mainly promonocytes (80%). Part of them contains interleukin 2 receptor (IL2Rα). In epithelial organs typical for SIS, cells containing the SC and J-chain are sometimes found. All this shows a partial preservation of cell function have not undergone apoptosis. Any pathological processes such as necrosis, fibrosis, calcification, and others do not show up. The main reason for the death of the embryo is the destruction of leading organs by apoptosis: the neural tube, liver, heart, and others. It can be either massive with cell deaths at 3.5-4 weeks of development or less massive with cell death at some later stages when parts of the embryo can be divided into small pieces or decay completely. Thus, abortion can be delayed. Woman's age does not affect the term of abortion: it could happen at 3.5-4 week in women aged 17-25 and in 44-year-old.

5.9. Conclusion for Chapter 5

Already before the beginning of the embryonic period in the shells, and then in the embryo itself various biologically active systems that act to maintain the normal development are well represented.

1. Secretory immune system (SIS) and its main receptors - SC and J-chain are present in trophoblast, and then in its variations. They are part of the placental barrier and exercise metabolism of the pregnant and the germ, including supplying the latter with immunoglobulins. With the formation of certain organs, SIS enters into them in the form of cellular barriers in the neural tube, mesoderm, epithelium, and in many other structures. On the basis of SIS the individual immune defense of vital organs and cells is formed (Chapter 3.7.).
2. Phagocytic defenses are also formed on the basis of the trophoblast: this is an invasive trophoblast and trophoblast that covers the lacunae with the blood of pregnant women. In addition to place prepared for the blastocyst in the uterine tissue, they actively phagocytize and destroy pathogens in the germ, even outside of it, in the decidual tissue.
3. The system of mononuclear phagocytes (monocytes and promonocytes) is located in the tissues of the embryo, especially in the chorionic villi and to a lesser extent, in the embryo itself. These cells are capable of phagocytosis and destruction of some pathogens and necrotic cells. All of the above systems transport also immunoglobulins of the pregnant to the embryo. They play a significant role in protecting it from microorganisms and other pathogens, but may cause early allogeneic conflict.
4. The precursors of immune cells - lymphocytes - already appear in yolk sac and later in the liver. They are slightly scattered in other organs. In bacterial infections, already at 7-8 weeks the number of lymphocytes CD3, CD4, CD20 is getting increased in the liver (Chapter 5.6.1.) and endocrine glands (Tables 3.3. and 3.4.). The number of neutrophils in CD20, containing IgM, and IgA and, to lesser extent, IgG increases. In the middle of the second trimester the main organs of immune system: thymus, spleen, lymph nodes are formed and the synthesis of immunoglobulins of the fetus begins. Obviously, unlike an adult, a fetus is not capable of immune recognition of multiple bacterial and other foreign antigens. But it is a sign of inexperience of the immune system rather than its handicap.
5. Interleukin 2 (IL2Rα CD25 +) - growth factor and its receptor is found in normally developing embryos as early as at 3.5-4 week and as well as in pro-lymphocytes and promonocytes (CD34 +) of yolk sac, and later in the liver in an amount of 0.1 - 2 / 50000 μm^2. IL2Rα are also found in many cells of developing organs: in the parts of the neural tube, in the epithelial cells of the stomach, intestines, trachea and bronchi, cells of the gonadal ridges, mesonephros and metanephros, initial pancreas and other organs. In cases of bacterial infections (groups 3A and 3B) and in allogeneic conflict (group 4) the amount of IL2Rα in the liver increases to 6.75 ± 1.22 / 50 000 μm^2, more significantly in group 3B (severe hematogenous process). In group 4 IL2Rα is contained in regions of organs, which are less involved in multiple apoptosis - in parts of the neural tube, the myocardium, the capillaries and other emerging

organs. Availability of IL2Rα not only in prolymphocytes and promonocytes, but mainly in the parenchymal cells of forming organs points to the importance of his participation in this process.

6. Cell proliferation is determined by the reaction of Ki67. It identifies the active stage of division (G1, S, M, G2, but not G0). In normal condition (group 1), most of the cell Ki67 (+) are in the yolk sac while after 4 weeks of development - in the liver, as well as in gonadal cylinders. The number of Ki67 (+) cells ranged in them from 7.33 ± 0.93 to 32.87 ± 4.19 / 50 000 μm^2. During pathological processes the proliferative ability of embryonic cells varies in different ways. In group 3A Ki67 (+) increases in the three organs noted. In group 3B it is reduced by several times in the areas of inflammation, while in other areas it is associated with the long duration of the process, as in group 1. In group 4, in "the zone of highest action of allogeneic immunoglobulins - in the placenta", the number of proliferating cells at the 3.5 - 6 weeks decreases in 6 - 9 times, and at 7 - 8 weeks - only twice compared with group 1. In the yolk sac the number of Ki67 (+) cells goes op.
7. The physiological apoptosis plays an important role in the formation of the embryo. Apoptosis produces fast (several hours-lasting) destruction of the former structures, new site preparation and construction of future organs. Therefore, cells in a state of apoptosis are seen singly or in small groups (1.52 ± 0.04 / 50 000 μm^2). In the case of 3A, in which the microflora is distributed in the tissues of the embryo, there are components of SIS - SC, J-chain and immunoglobulins that weaken microflora. Joint action of the SIS, mononuclear phagocytes and positive actions of other regulatory systems (Chapter 5.6.1) do not allow the development of multiple apoptosis and prevent the pathological changes. However, participatory processes, significant in the decidual tissue and minimal in the embryo, cause an abortion.

   In the cases of 3B microflora penetrates to the embryo hematogenous route through the main pathways to bypass the SIS. The suppression of biological systems, including mononuclear phagocytes, the invasive trophoblast, and others, cause severe destruction of villi and fetal tissue necrosis of several species, including significant apoptosis. This ends the development of the embryo.
8. Changes caused by early allogeneic conflict in the tissues of the embryo, in its shell, and in decidual tissue of pregnant women (group 4), compared to the normal condition (group 1) and to bacterial processes (groups 3A and 3B), are significantly differ in the nature of pathological processes and their consequences. In decidual tissue in all experimental groups, there is a significant number of lymphocytes involved in cellular immune responses. These are T-lymphocytes (CD3), T-suppressor (cytotoxic CD8) and natural killers (NK CD56). In all experimental groups, their indicators are not significantly different (Table 4.6., 4.7.) and, consequently, the cellular immune response is not happening in them. The humoral immune response involves B cells (CD20) and plasma cells. In groups 3A, 3B and 4 in decidual tissue, there are significant differences from group 1: they are moderate in group 3A and significant in groups 3B and 4 (Tables 4.6, 4.7, 5.6), indicating

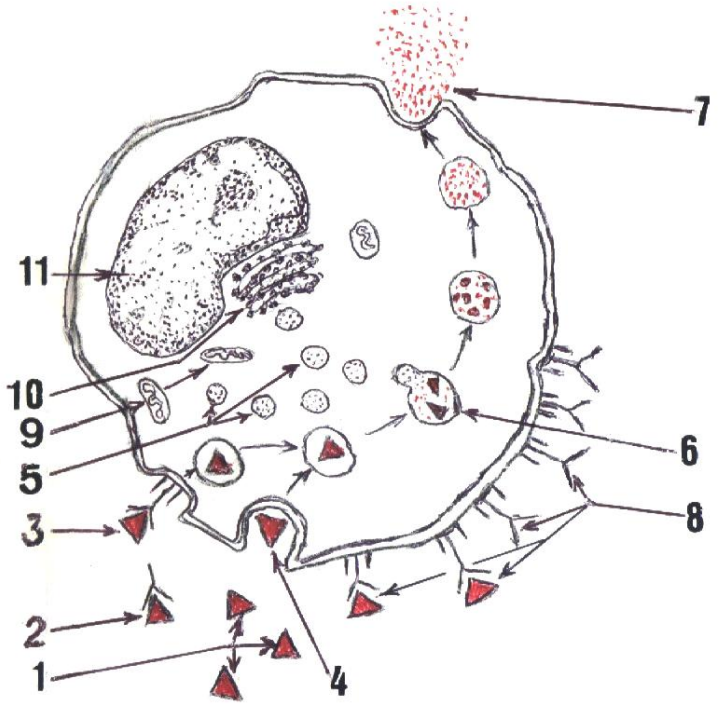
a severe pathogenic effects on the pregnant. Similar state is seen for mononuclear phagocytes of embryonic villi (Table 4.5): in group 3A the number doubles, which indicates a significant immune response. In groups 3B and 4 the number of phagocytes tends to decrease, despite the manifold increase in promonocyte level. These are signs of insufficient reaction of embryonic phagocytes in groups 3B and 4 to dramatic effects of pathogens. These conclusions are supported by significant drop in the number of proliferating (Ki67 +) phagocytes and by content of interleukin 2 (IL2Rα) in them.

Thus, immunohistochemical, morphometric and pathomorphological studies provide a basis for analyzing the course of pregnancy, embryo development and for the appearance of irregularities in them.

Chapter 6. Mechanisms of immune protection at pre-embryonic and embryonic periods

Little attention is given to immune defense of the new organism in the first two months of its development. Meanwhile, pre-embryos and embryos in the organism of the pregnant are exposed to various hazards - bacterial and viral infections, intoxication, and allogeneic conflicts. During these periods, five mechanisms are sequentially activated to ensure the continuous germinal protection.

Fig.6.1. Stages of the functions of mononuclear phagocytes



1 - antigens, **2** - antigen gets in touch with immunoglobulin (first way of absorption of an antigen), **3** - fixation of the antigen with immunoglobulin within receptor (Fc gamma RIIIα, RII, RI, or other), **4** - phagocytosis of the antigen without immunoglobulin (second way of absorption of an antigen) **5** - lysosomes, **6** – a phagolysosome containing an antigen, the destruction of the antigen, **7** - rejection of the destroyed contents phagolysosomes, **8** - receptors and immunoglobulins with antigens without their destruction, **9** - mitochondria (power supply of the phagocyte), **10** - Golgi complex (formation of lysosomes, etc.) , **11** - the nucleus of the phagocyte. (В.Г.Галактионов, 1986, as amended).

Protecting of the forming man begins with his creation. In the ovary, the egg cell is surrounded by small follicular cells, forming a radiant wreath (corona radiata). These cells originate from the mesothelium of gonadal ridges and (as the mesothelium itself) contain components of SIS: SC, J-chain and immunoglobulins. With their protrusions, they reach the egg cells and provide them with the metabolism, whereas the elements of

the SIS provide them with immunoglobulins, realizing their immune protection. When a woman's ovum reaches puberty and leaves the ovary, it is accompanied by some follicular cells (Pereda et.al., 1985, 1995; Metta et al., 1995; Greenenbaum E, 1998; Hargreave TB, Mills JA 1998). Their SIS provides the ovum cytoplasm with J-chain and immunoglobulins from the mother. In the case of fertilization, the zygote gets ready to immune defense against bacteria and other pathogens, provided if the pregnant woman has previously had contact with them, and if she has got the appropriate antibodies for the rapid response (Fig. 6.1). If this pathogen is new, the germ may die before the pregnant woman will have time to prepare the necessary protection. There is also another danger: the allogeneic conflict. After all, half the genes of the germ come from the father, and if some of them are incompatible with the antigens of the mother, it the repeated pregnancies, the death of the germ will occur even before its implantation it in decidual tissue, already during the first week (Wilcox AJ et al., 1988; Kutteh WH, 1999; Кулаков В.И. et. al., 2005; Adolfsson A, Larrson PG, 2006).

This mechanism is **the first stage of protection**. It comes entirely from the pregnant with her follicular cells with her own SIS and immunoglobulins, thus it ceases to function with the rejection of follicular cells after 4-6 days of fertilization.

The second stage of protection comes with the termination of the first one. When the morula becomes a blastocyst, the follicular cells and fertilization membrane (fertilization membrane) are destroyed. Their place is occupied by densely interconnected trophoblast cells which are the part of the germ. The main aim of the trophoblast at this stage is to create a bed in the decidual tissue for pre-embryo and lacunae for the blood of pregnant women. Therefore it is considerably capable of phagocytosis and destruction of the decidual tissue. Trophoblast contains SC and J-chain, thus being a part of both types (mucosal and barrier) of SIS, and able to capture and transport immunoglobulins of the pregnant to the cells of blastocyst. Thus the own defense mechanism of pre-embryo begins functioning, but with the participation of pregnant's immunoglobulins called "passive immunity» (E. Jauniaux et.al., 1995). According to our data (Chapter 4.7) the trophoblast is more active than monocytes in the search of friend or foe. It is able to capture and destruct the pregnant's allogeneic antibodies, especially during early allogeneic conflict when it phagocytize the allogeneic antibodies 4 times more active than in the group 1, or than the pregnant's macrophages in the group 4 (by 8-64%) (Diagram 4.2, Table 4.9). In the embryonic period the most active in this respect are: the invasive trophoblast, penetrating the decidual tissue of pregnant, the trophoblast covering the lacunae and the villous syncytiotrophoblast. All they are in contact with tissue and blood of pregnant women and die themselves during the fracturing of phagocytized allogeneic immunoglobulins. This death conceals the special phagocytic activity of the invasive trophoblast. In cases of bacterial infection of group 3B, its cells penetrate deeply into the necrotic mass and create an additional shaft (Fig. 6.2.). This creates the impression of a greater phagocytic activity of the invasive trophoblast, rather than macrophages of decidual tissue and phagocytes of the embryo. Viability and activity of the invasive and proliferative forms of trophoblast is seen in the intensity of their reproduction, when 71-76% of cells are Ki67 positive (see Table 4.4). We do not dwell on such important functions of trophoblast as formation of germinal slot and lacunae for the activity of the

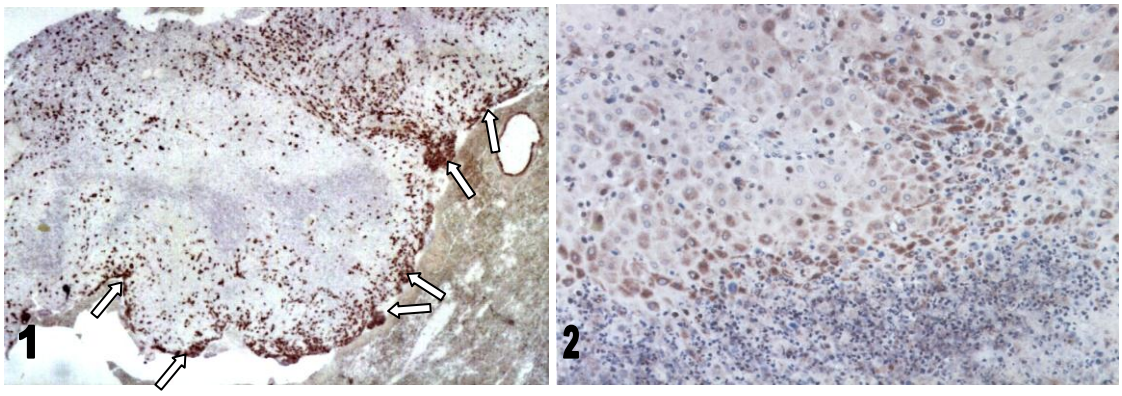


Fig.6.2. 5 weeks of development. Group 3B. **1** - decidual tissue with an area of necrosis, surrounded by a rampart of various leukocytes. Particularly noticeable is the invasive trophoblast (reaction AE1 - AE3+). It does not only surround the necrosis, but penetrates into it. The increased amount of proliferative trophoblast comes from the "anchoring villi" (arrows), which are embedded into necrotic tissue. Magnification is x40. **2** - the same place. Reaction of trophoblast is SC + showing the penetration of invasive trophoblast deep into necrotic zone. Magnification is x200.

placental barrier. But the supply of the germ with pregnant's immunoglobulins against infections and other pathogens as well as phagocytosis and destruction of allogeneic antibodies are no less important functions of the trophoblast. Thus the secretory immune system is laid, which turns out to be so important in development, immune protection and the very existence of not only a pre-embryo, embryo and a fetus, but for a person throughout his life.

The third stage of protection is implemented by mononuclear phagocytes. They perform their duties right in pre-embryos, embryos, fetuses and on their shells. They do not go beyond the borders, as the types of trophoblast do: particularly the invasive type, located between the pregnant's decidual cells, the trophoblast lining the lacunae with blood in them and syncytiotrophoblast. A significant portion of mononuclear phagocytes is located in the chorionic villi, at the embryonic (and fetal) side of the placental barrier. In organs and tissues of embryos they are fewer (Table 5.5). The complex of these cells consists of myelomonocytic stem cells, promonocytes and monocytes. Monocytes are sometimes referred to as Кащенко-Hofbauer macrophages, but they are younger than macrophages. Distinguishing between monocytes and macrophages is not difficult. On the preparations of placenta treated with CD68 and CD14 in decidual tissue the large, intensely stained (with one or the other) CD cells can be seen; these are pregnant's macrophages. In chorionic villi CD68 (+) and CD14 (+) but weaker stained cells are seen; these are monocytes. And at the same location the CD68 (-) and CD14 (+) cells are promonocytes (Fig. 5.7.1). Myelomonocytic stem cells can be seen in the yolk sac. In severe injuries with massive destruction of monocytes (infection of the group 3B and allogeneic conflict of group 4), they appear as single cells in the blood of heart among the erythroblasts under the treatment with CD34.

Monocytes and promonocytes have many abilities. They phagocytize and destroy pathogens (Table 4.9, Fig. 6.1). Promonocytes and monocytes proliferate not only in the yolk sac and then in the liver, but also in tissues, especially in the placental barrier zone (Table 4.5) They transport of immunoglobulins in blood vessels of the embryos (Table

5.3). They contain a number of active substances and receptors that perform different functions: IgG - Fc gamma RIIIα (CD16), RII (CD32) and RI (CD64), interleukin 2 (IL2Rα - CD25)), proliferating cells (Ki67 +), J-chain and others. Monocytes and promonocytes actively adapt to changes in the situation, including - the emergence of pathological processes (Table 5.8).

When transporting the immunoglobulins, there are two options to capture them or other proteins (see Fig. 6.1). The first option involves the receptor Fc gamma RIIIα, RII and RI. They capture immunoglobulins using Fc fragment, and both Fab-fragments remain free. Such a complex on monocytes or promonocytes spreads through the embryo, neutralizing the corresponding pathological antigens. The second variant of capture happens without the participation of immunoglobulins. This is phagocytosis with formation of phagolysosomes in the cytoplasm in which pathogens are destroyed. In the cases of allogeneic conflict when a huge amount of IgG, IgA and IgM is captured and destroyed (see Table 4.9), this type of phagocytosis is especially effective.

Under intense exposure to pathogens (microflora or immunoglobulin in allogeneic conflict; see section 4.6 and 4.7) and the death of a significant portion of monocytes the production promonocytes is dramatically increased. Their number increases from 2% to 80-100% of the number of monocytic phagocytes, being 54.17 ± 3.61% in average. Number of phagocytes in group 1 (control) and in groups 3B and 4 (in the severe pathogenic effects) stays the same while in group 3A even doubles (Tabl.4.5). This is a

Table 6.1. Biologically active substances and receptors contained in monocytes and promonocytes of the embryos (percentage of total number of monocytes or promonocytes)

	CD68	CD14	CD16	CD32	CD25	Ki67
Monocytes	+	+	70.09±4.35	82.48±1.35	57.54±2.78	13.16±1.67
Promonocytes	-	+	12.06±2.11[a]	10.55±2.15[a]	12.45±1.57[a]	7.48±1.21[b]

[a] - in promonocytes content of CD16, CD32, CD25 was significantly lower than in monocytes ($p < 0{,}001$)
[b] - in promonocytes Ki67 was significantly lower than in monocytes ($p < 0.02$)

sign of the powerful defense even in the early embryonic period, when the dimension of the germ is 1-5 mm, and the overall immune system is still missing. By changes in the amount and composition of cells the processes can be assessed even in embryos at 3.5 - 5 weeks. For example, a constant number of monocytes and promonocytes (related to 50,000 μm^2 of the slide surface or to a percentage of their ratio: Table 4.5, groups 1 and 2) means the normal state. Increased number of monocytes and promonocytes (Group 3A) is typical for the compensated inflammatory or immune processes. Whereas the increased promonocytes with equal (group 3B) or, moreover, reduced number of monocytes (group 4) show the insufficiency or even decompensation of the processes. The latter situation can be considered as an embryonic analogue of sepsis.

The fourth stage of the immune defense is formed along with the organogenesis in embryos and then fetuses. This individual immune system of the vital cells and organs, such as developing brain neurons and ganglia, parenchymal cells of the main endocrine glands, myocardium, hepatocytes, male and female gametes (Chapter 3). Feature of these

cells and organs is the lack or very little regeneration: after damage and destruction they do not recover or slightly recover. The key point of this mechanism is in the accumulation of large amounts of immunoglobulins in the cytoplasm. The role of receptor here is played by J chain. When pathogens surround the cells mentioned, their immunoglobulins are not spent but used for self-defense of this parenchymal cells, while the cells around are protected by SIS and the overall immune system when it is formed. First immunoglobulins come from a pregnant woman, and later, when the own synthesis of immunoglobulins is established, they are added as well. With the development of early allogeneic conflict at the embryonic period, when the individual immune system has not yet been formed, the action of alloantibodies causes extensive destruction of neuroblasts.

Fifth stage: the overall immune defense. It is the leading system and it integrates already existing mechanisms of immune protection and complements them with some important new structures: with cellular and humoral immune systems, with immunocompetent cells and immune organs. First news are the immunocompetent cells pro-T-lymphocytes (CD3 +) and pro-B-lymphocytes (CD20 +) which appear in the yolk sac, and at 5 - 6 weeks in the liver. The latter gradually becomes a leading hematopoietic and immune organ, while yolk sac ceases to function. Lymphocytes are gradually spreading throughout the organs. At 7 - 8 weeks for bacterial infections their number in the liver is considerably increased, and in the cytoplasm of B-lymphocytes immunoglobulins appear.

The thymus is formed from the epithelium of 3^{d} and 4^{th} pairs of gill-channels (see Fig. 5.2.1). By 8^{th} week, the cortical epithelium layer is formed. Its cells contain SC, J-chain and immunoglobulins. Then, this layer becomes Hassall's corpuscles which have endocrine functions, synthesize thymosin and Thymopoietin (Хлыстова З.С., 2006). At 8^{th} week the brain area is populated by T-lymphocytes and by a small number of B cells. In the thymus, T cells differentiate into CD4 + helper cells and into CD8 + cytotoxic lymphocytes, which then spread throughout the body.

The spleen is laid at 4^{th} week; at 11-12 weeks lymphocytes colonize it, and from 13-14 weeks of development lymphoid follicles are formed. B-lymphocytes predominate in the spleen.

Lymph nodes begin to develop in the late first trimester; in the second trimester their number is increasing. They are located mainly in areas of lymphoid branching vessels: in the neck, armpit and groin areas in the mediastinum and abdomen.

Clusters of lymphocytes in the form of lymphoid follicles are located along the gastrointestinal, airway and urinary ways. They can form clusters in the form of the tonsils in the oral cavity and pharynx, Payer’s patches in the ileum and appendix. These clusters, as well as part of small follicles, remain in the SIS. They provide the synthesis of immunoglobulins, mainly IgA, for releasing them in the cavities of the mentioned organs, as well as of the lacrimal glands of the eyes, breasts, etc. All of them are beginning to form at 8-9 weeks, then in the second and third trimesters they develop rapidly, and after birth operate and restructure according to the needs.

Since the second half of fetal period red bone marrow becomes an important immune organ whereas the liver loses these functions completely (Рябчиков О.П. at al., 2006). In the bone marrow red blood cells, granulocytes (neutrophils, eosinophils, and basophils), megakaryocytes and platelets, mononuclear phagocytes and lymphocytes are produces. Hence, they are spread throughout the body.

Conclusion

From the moment of fertilization, when the life of a new body begins, yet in the form of one or more cells, the body's immune defense within 4-6 days execute the components of the SIS from his future mother: the follicular cells, protecting the egg, and immunoglobulins from the same source. This protection is designed against external pathogens: microflora and other pathogens. But with a possible early allogeneic conflict the pre-embryo is defenseless. This is the first stage of protection of the germ.

The second stage of protection beginning from 4-5 days is formed with the cells of the pre-embryo, the blastocyst. This is the trophoblast, which is located outside it. Besides its main aim - the implantation of germ into the decidual tissue, the trophoblast is an active phagocyte; it has SC and J-chain and represents a part of the future SIS, using pregnant's immunoglobulins. Thus, already at the early stages of development, germs form their own system of protection: the trophoblast, and then mononuclear phagocytes. In a case of early allogeneic conflict, trophoblast phagocytizes intensively and destroys the immunoglobulins and other pathogens directed against pre-embryo (Chapter 4.7, Diagram 4.2, and Table 4.9).

The third stage of protection is represented by mononuclear phagocytes: monocytes and promonocytes. They are formed in the yolk sac from the second to the seventh week of development, and from the fifth week - mainly in the liver, but also in the area of the placental barrier. Their main task is to protect the growing embryo, being inside of it, by phagocytosis and destruction of pathogens. By all mentioned they complement the protective functions of the trophoblast. Following allogeneic conflict, mononuclear phagocytes, as well as the trophoblast, phagocytize and destroy the pregnant's immunoglobulins directed against the embryo.

The fourth stage of protection is formed with the process of organogenesis in the embryo and fetus during the first and second trimesters. This is the individual protection of vital cells and tissues that do not regenerate or possess just a small degree of it. Among them are neurons of the brain nuclei and ganglia, parenchymal cells of major endocrine glands, myocardium, hepatocytes, and gametes. The essence of the protection is in the accumulation of immunoglobulins in the cytoplasm of these cells.

The fifth stage includes formation of immune defense which is laid in the embryonic and early fetal period by the formation of special cells and organs. Mononuclear phagocytes, in addition to the function of phagocytosis become antigen-presenting cells. They recognize pathogen-associated antigens, alert and using the participation of biologically active substances stimulate actions to neutralize them. Immunocompetent cells of different types execute cellular or humoral immune responses in accordance with the information received. Cellular ones are operated by several types of T-lymphocytes and NK; the humoral responses are realized by cells that synthesize immunoglobulins: B cells, while after the birth plasma cells join them too. Precursors of immunocompetent cells colonize previously established frameworks: bone marrow, thymus, spleen, they form lymph nodes, and the other antigen-presenting and immunocompetent cell complexes. The own synthesis of immunoglobulins begins, but

the fetus continues use the pregnant's immunoglobulins as well, and only after the birth, a child gradually gets rid of maternal antibodies, coming with her milk.

Sometimes one can face an idea of low self-defense ability of the fetus. This is a misperception. Protection mechanisms have consistently become more and more complex from the zygote. But the pathogenic influences on the fetus and the more on the pre-embryo and embryo are novel and quick. In our studies, the development of the overall immune system and, in particular, of its humoral part, is observed in cases with no pathological effects (group 1), under inflammatory processes (light and heavy) (group 3A and 3B) and with immune response in early allogeneic conflict (several versions of group 4). In each case, the immune responses, consistent with the nature of the disease were clearly realized. Also consistent were the action of mononuclear phagocytes, various types of trophoblast, the reactions IL2Rα, cell proliferation, intense degree of apoptosis, etc. All this is described in Chapter 5.9. In Chapter 7.7 the edematous form of hemolytic disease of the fetus is observed. It lasts several months of utero conflict. Nevertheless, the adaptation for the life preservation under these conditions sometimes looks amazing. But life in certain conditions after the birth is no longer possible, and the newborn dies.

After the birth, the overall immune system protects the entire body of the child and the adult in tight contact with other systems that have been functioning already during the intrauterine development, in pre-embryonic, embryonic and fetal periods. Began to form in the early days of germinal development, entire immune system and its parts undergo great improvement throughout the life of man.

Chapter 7. Late allogeneic conflict – hemolytic disease of the fetus and the newborn

Hemolytic disease of the fetus and newborn (HDFN) is a manifestation of immune conflict between the pregnant woman and her fetus in the second half of her pregnancy. Cause of conflict is the incompatibility antigens inherited from the father and contained in red blood cells and cells of some fetal organs. Pregnant woman's immune system produces antibodies against these antigens. With the penetration of antibodies into the body of the fetus it destroys the red blood cells and tissues of several organs, a hemolytic anemia is therefore generated along with various complications.

7.1. A brief history of HDFN research

Neonatal jaundice has been known since antiquity. The first serious attempts to find its cause had been performed in the second half of XVIII century, when the Royal Surgical Academy in Paris, invited researchers "to describe newborn jaundice and determine in which cases various medical means of treatment should be taken against it, and when it should be left to the forces of nature" (cited by С.Б.Вермель, 1898). The first cases of edematous preterm newborns have been described by F. Platter (1614), Darstenius (1684), and F. Osiander (1796). H. Schridde (1910) has described in detail the edematous form. Brain damage (kernicterus) was described by J. Orht (1875), M. Runge (1888), and G. Schmorl (1904). L. Diamond, K. Blackfan, J. Baty (1932) showed that severe jaundice, edema and anemia are three versions of the same disease. The fourth form - the birth of macerated child without edema and jaundice – was described by Ph.Levine et al (1941).

Causes of HDFN long remained unclear. С.Б.Вермель (1898) notes, that every author who has studied this issue proposes a new theory. The first rational ideas were put forward by L. Hirszfeld and H. Zborovsky (1926). They suggested that following different blood groups in the erythrocytes of the fetus and the mother she should be producing antibodies against the fetal red blood cells.

Ph.Levine and E. Stetson (1939) described the following clinical case: a 25-year-old mother, which has lost a newborn, developed complications that required blood transfusions. She got the blood of her husband but during the transfusion an atypical reaction occurred which was clearly related to the incompatibility of their blood. However, the incompatibility of the known back then factors: A, B, O, M, N, P was not found. The authors of the article speculated that the woman was immunized by unknown factor of the fetus, inherited from his father. In 1940 K. Landsteiner and A. Wiener discovered this factor: first in the erythrocytes of monkey *macaccus rhesus*, and then in the erythrocytes of the Europeans. The previously mentioned woman and her husband were re-examined: her red blood cells did not contain the Rh factor, and in the husband it was present. Thus HDFN was described in the article of Ph.Levine et al in 1941. The term "hemolytic disease of the newborn" (HDN) was first used by A. Wiener (1946).

7.2. Epidemiology

Later the variants of Rh factor: C, D, E, c, d, e were identified. It turned out that the conflict in 90-93% of cases of HDFN is initiated by factor D, while the factors C and E rarely cause the conflict, and c, d, e - very rarely (Mc Adams RM, et al., 2008). The name "Rh-positive antigen" is preserved for Rh^oD; blood which does not contain it is called Rh-negative, although it may contain the other Rh-factors. The incompatibility between the pregnant and fetal blood by the other blood antigens (A, B, O) is responsible for HDFN, mainly with the group O (I) in a pregnant woman and A (II) in the fetus. The course of the icteric HDFN in these cases is much lighter than with Rh^oD conflict: while the A, B, O incompatibility is observed in 20-25% of women and their husbands, but there are laboratory signs in only 2-2.5% and medical treatment is required in only 0.1% of such pregnant women. Edematous and other forms of HDFN with these antigens are not found, and the conflicts with other blood factors in fetuses are casuistic. More than 14 systems of antigens other than Rh-Hr and ABO are known: MNSs, Lutheran (Lu), Kell (K), Lewis (Le), Daffy (Dy), Kidd (Jk), Diego (Di), Sutter (Js) and others. Because of the incompatibility by gene KEL1 and presence of antibodies K, in Poland there was detected HDFN in 6 cases and one of them was fatal (Zupanska B., et al., 2008). Rare cases of HDFN are associated with the presence of antigen Gerbich-3 (Ga-3) on the membranes of red blood cell. It causes hyperbilirubinemia and severe anemia (Blackall DP et al., 2008).

Dissemination of positive Rh factor among residents of various continents is different. In Europeans, including Russian and European Americans, 85% are Rh-positive 15% - negative. Therefore, the frequency of HDFN for them is higher, for example, in Russia 1.5% of all births end of Rhesus-conflict. In China, Japan, the American Indians Rh-positives are 99%, in African Americans - 93% and, therefore, Rhesus-conflict in the deliveries among them is very rare. In Africa (Zimbabwe) out of 23,493 children born HDFN was detected in 191 children (0.85%). The most common cause of the disease was associated with antigens AB, and much rarer – with D antigen and just single cases - with antigen Kell (Mandisodza AR et al., 2008). It should be noted that the authors did not consider cases of stillbirth – the early ones and on time ones. In Africa, the anti-hrB and anti-HrB antibodies that cause HDFN and reactions at blood transfusions have been discovered (Win N., Needs M., Tillyer L. 2007). In Korea, a severe case of HDN with a combination of Di (a + b +) in the child, and Di (a + b-) in the mother (Oh EJ et al., 2008) have been observed. In the Netherlands, it was investigated the case of HDN due to antigen Vel with severe jaundice, requiring phototherapy (von Gammern AJ et al., 2008).

In addition to red blood cells, Rh is contained in the liver, kidney, spleen, pancreas, adrenal and some other organs (П.Н.Косяков, 1975). These locations have special significance for surgery: before the organ transplant it is required to check the possibility of allogeneic conflict. In the trophoblast, and in particular, in invasive trophoblast of the germinal Rh-antigen is not contained.

7.3. Rh-antigenic conflict: common problems

To date, the methods of diagnosis and treatment of HDFN are developed, so the mortality reduces. However, new variants of allogeneic conflict appear, including early allogeneic conflicts. Many aspects of their pathogenesis, diagnosis, prevention and treatment remain unclear. The most important at present is the early allogeneic conflict, which begins in pre-embryonic period and develops in the embryos at 3.5 - 8 weeks of development at the full scale. With re-pregnancy, when the corresponding antibodies are already existing in the pregnant, allogeneic conflict may develop at the end of the first or at the second week after fertilization. The early conflict is quite frequent: it consists about 50% of early spontaneous abortions.

The purpose of this Chapter is to describe our studies of different clinical forms of late allogeneic conflict and compare them with the conflict in the early embryonic period. We deliberately consider the materials obtained some forty years ago: they are, so to say, still in the natural form, without any changes brought about by modern successful treatment interventions. These materials are supplemented by new data and methods that are included in the following sections of Chapter 7.

7.3.1. Anamnesis and course of pregnancy

98 women were observed (Table 7.1). 92 of them had a conflict by Rh^oD-antigen (93.88%), in two cases (2.04%) the conflict was by the other options of the Rh factor, which at that time we were unable to identify, and 4 cases (4.08%) with the conflict by the ABO blood groups. In these 4 cases, the blood of three women was O (I) group and of one - B (III) group. Blood of two children were B (III) group, and of other two - A (II) group. These children developed icteric HDN. Among women with Rh-conflict, 20 were immunized by blood transfusion (21.74%) and 72 (78.26%) - during pregnancy.

Table 7.1. Age of immunized women

Up to 20 years/o.	21-30 years/o.	31-40 years/o.	41-44 years/o.	Total
1	48	44	5	98

Most of the women were observed for a long time and explored during every pregnancy and every birth. Most of the women were healthy: more or less significant previous illnesses have been recorded only in 8 women: rheumatic heart disease – in 3; and hypertension, pyelonephritis, epilepsy, previously found hepatitis and toxoplasmosis – each in one case. Thus, any connection of Rh^oD immunization with the other diseases of these women was not found.

In total these women had 314 pregnancies, not counting the 184 induced abortions and 73 cases in which the result of pregnancy was unknown (Table 7.2). 12 women had more than 10 pregnancies: two - 20 and 26. 84 children (26.75%) were born healthy, without signs of HDFN, including 55 of them following the first pregnancy. In the subsequent pregnancies the number of healthy births had decreased and after the fifth pregnancy, they were no longer observed (Diagram 7.1). Out of 230 immunologic havoc

pregnancies, 108 (46.95%) were born dead, 112 (48.7%) of newborns died after the birth from HDN, 10 (4.35%) of them stayed alive while suffering from HDN.

With Rh-conflict, the blood groups of mother and child were identical in 81.25%, including O (I), A (II), B (III) groups – in 21.7 - 30.4%, and AB (IV) group - in 4.35%.

Table 7.2. Results of pregnancy in immunized women related to the number of fetuses

		Sequential number of pregnancy										Total	% HDFN	% of total
		1	2	3	4	5	6	7	8	9	10-26			
Fatalities from HDFN	Icteric form	12	15	18	9	18	8	8	5	2	6	101	43.91	32.17
	Edematous form	2	3	3	10	11	8	5	5	4	8	59	25.65	18.73
	Anemic form	1	2	3	2	3	1	1	-	-	-	13	5.65	4.14
	Fetophaty with maceration	2	8	12	6	4	6	3	4	-	2	47	20.44	14.97
Living children with a history of HDFN		-	2	2	3	1	1	1	-	-	-	10	4.35	3.18
Total HDFN		17	30	38	30	37	24	18	14	6	16	230	100	73.25
Living and healthy children		55	16	9	3	1	-	-	-	-	-	84	-	26.75
Total		72	46	47	33	38	24	18	14	6	16	314	-	100

7.3.2. Stages of HDFN development

Although, in general, the development of early allogeneic conflict of the embryo and the pregnant is similar to the stages of the late allogeneic conflict of the fetus or newborn with his mother (HDFN), they differ significantly.

Stage 1: immunization. Erythrocyte antigens of the fetus, causing HDFN, are not on its surface and are not in contact with maternal antigen presenting macrophages. Contacts are possible during the birth of the fetus, during operations, blood transfusions or tissue transplantations without proper survey and in other emergencies. Therefore, immunization of the mother often does not occur at the first (up to five) pregnancies.

2 and 3 stages: the transfer of antigen by macrophages to B lymphocytes (CD20) and helpers (CD4), and the synthesis of antibodies is typically happens at 2 - 2.5 weeks or less following the repeated pregnancies.

4 and 5 stages: transport of allogeneic antibodies to the fetus from the pregnant woman and their effects. With early allogeneic conflict that transport passes just a small distance from the decidual tissue through the placenta to the embryo. However, its efficacy is reduced by the phagocytes: invasive trophoblast in the decidual tissue and monocytes in the villi. They die at that time and the passage of allogeneic tissue does not stop. Transport across the placental barrier of anti-microbial and other antibodies is carried out in embryos rather freely by receptors: SC, J-chain, FcRI (CD64), FcRII (CD32), FcRIII (CD16) and others. During HDFN - the late allogeneic conflict - the transfer of allogeneic antibodies is more complicated. It does not occur at all before 22 weeks of development, when the regular such as anti-microbial antibodies pass freely through the placental barrier. In subsequent stages of pregnancy with HDFN the transfer of allogeneic antibodies occurs only in rare cases, often only upon the date of the

sequential birth. Two factors remain unclear in HDFN: why alloantibodies do not pass freely to the fetus during the first 22 weeks of pregnancy, and what factors cause the passage of alloantibodies at the later terms, sometimes suddenly and shortly or for a long time such as months or even only during the birth.

At late allogeneic conflicts antigens are in the depths of the fetus, on erythrocytes, cells of liver or other organs. Therefore, the recognition of them is not an easy process. At birth, when chorionic villi and their vessels are being destroyed, the blood of the fetus may mix in some degree with the mother's blood, sometimes in sufficient quantity, even perhaps too low for immunization. Such cases are shown in Diagram 7.1, when healthy children are born at the first, second, third, fourth, fifth successive pregnancies. The number of such situations is gradually declining, from 76.3% during the first pregnancy to 2.7% - in the fifth one. This may be due to the lack of re-immunization. But another reason for re-birth of healthy fetuses is possible: the gradual attenuation of immunization. This happens especially if the previous pregnancy ended with a minor immunization and the next appeared only in few years. During this period the titer of alloantibodies can greatly decrease or disappear. But later, according to our observations, from the 6^{th} and to the 26^{th} pregnancy, healthy children are not anymore born. The most common become swollen forms, somewhat rare – icteric ones and occasionally - fetopathy with maceration, which are caused by a significant amount of antibodies. While the anemic form, caused by a small amount of antibodies, disappears. All these changes mean that repeated pregnancies cause increased immunization and increased numbers of allogeneic antibodies. In mentioned one can see an analogy with repeated vaccinations to strengthen the immunization for the prevention of infections.

We have seen cases of edematous forms of HDFN at the first pregnancy. This shows the possibility of penetration of Rh^oD antigens and sequential immunization quite early, at the $6\text{-}7^{th}$ months of pregnancy. Immunization of women can also occur during therapeutic blood transfusions or transplantation of organs without proper laboratory test. It is also mentioned the possibility of future woman immunization at her own fetal stage, with Rh^oD negative, while her pregnant mother is Rh^oD positive.

7.3.3. Forms of HDFN

4 forms of HDFN are specified: icteric (in two options: the congenital icteric and postnatal icteric), edematous, anemic and hemolytic fetopathy with maceration. According to the periods of initiation and development, all forms differ considerably (Fig. 7.2). Each form is dominant at a particular period of pregnancy. Any sequence of these forms is possible; some women deliver only icteric or edematous children. The titer value of agglutinating antibodies in the blood is more significant with icteric and edematous forms, while anemic form and fetopathy with maceration reveal mainly low and just few moderate alloantibody titers (Table 7.3).

Diagram 7.1. Various forms of GBPN in subsequent pregnancies in 98 women, 314 pregnancies (in%)

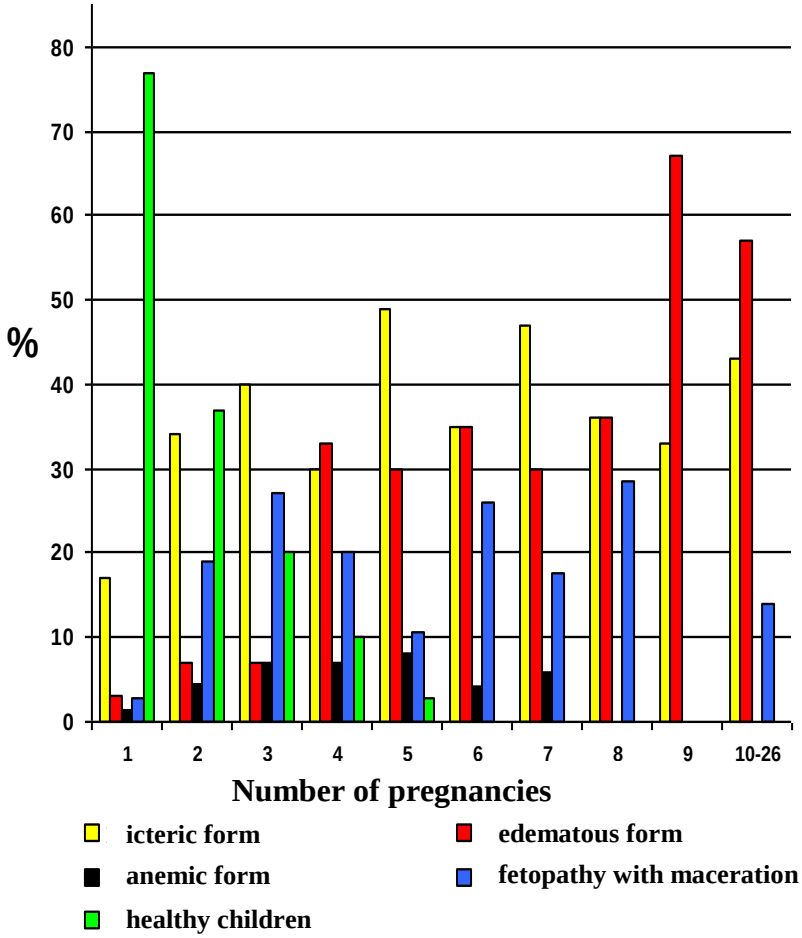


Diagram 7.2. Correlation between forms of HDSN and the degree of intrauterine growth (in% for each period of development)

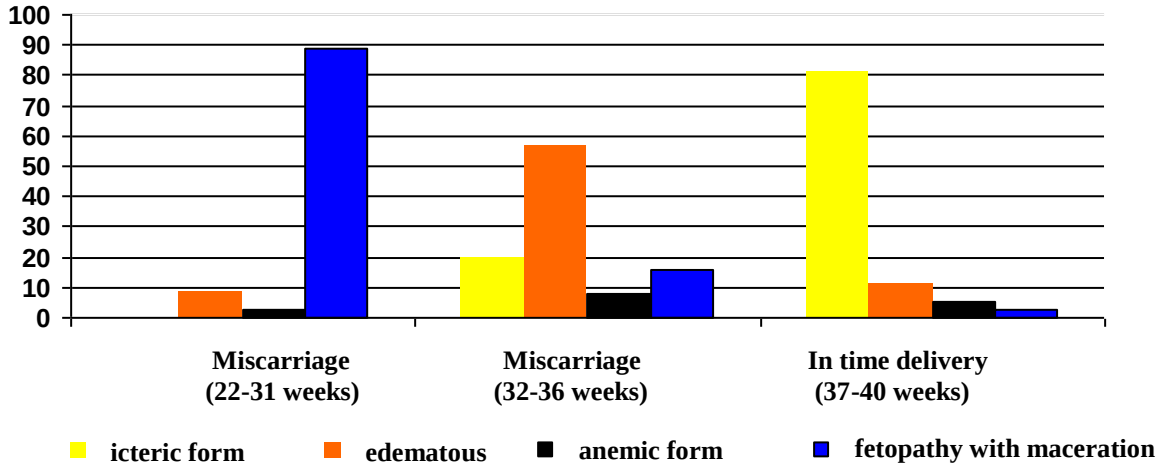


Table 7.3. Titer of agglutinating antibodies of the pregnant with various forms of HDFN (number of cases)

Titer	small				middle		high			Total
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	
Icteric form	6	4	16	3	10	11	2	3	2	57
Edematous form	2	5	8	7	7	5	3	1	-	38
Fetopathy with maceration	1	1	4	1	1	2	-	-	-	10
Anemic form	3	1	3	3	1	2	-	-	-	13

Number of agglutinating antibodies according to degree of their titer (in% of cases):

Titer	small	middle	high
Icteric form	50.88	36.84	12.28
Edematous form	57.89	31.6	10.52
Fetopathy with maceration	70.0	30.0	0
Anemic form	76.92	23.08	0

Table 7.4. Results of pregnancies in immunized women (early abortion, before 12 weeks, is not included)

		Delivery in time (37-40 weeks)	Miscarriage		Total	% of HDFN	% of total fertility
			(32-36 weeks)	(22 – 31 weeks)			
HDFN	**Icteric form**	96	15	-	111	48.26	35.35
	Edematous form	13	43	3	59	25.65	18.79
	Fetopathy with maceration	3	12	32	47	20.44	14.97
	Anemic form	6	6	1	13	5.65	4.14
Total HDFN		118	76	36	230	100	73.25
Alive and healthy children		73	11	-	84	-	26.75
Total		191	87	36	314	-	100

The cases of icteric form include both: died after birth (101), and remained alive (10) children (Table7.2). This explains the difference between the number and % of icteric forms in Tables 7.2 and 7.4.

Pregnancies in women who gave birth to children with icteric HDFN are usually proceed without complications in full-term cases or with minor complications in preterms (15 cases, 13.5%, Table 7.4.). Single cases of nephropathy, anemia, polyhydramnios, or increments of the placenta were found.

7.4. Pathogenic agents

Development of various forms of late allogeneic conflicts, HDFN, is associated with the pathogenic action of antibodies directed at various antigens of erythrocyte membranes. These are groups of Rhesus-antigens, ABO and other groups of antigens. Damaging effect of passing through the placenta antibodies depends on the intensity and duration of exposure. Damaging properties of antibodies and functional significance of antigens being damaged will also matter: for example, the destruction of the Rh antigens is of much greater impact than the effect of antibodies on antigens of ABO group and on some others. Just that action of alloantibodies and not the pregnant's immunocompetent cells will trigger the process of HDFN.

During the progress of conflict, another pathogenic agents appear, besides the allogeneic antibodies, This switch on receptor Fas, FasLigand, bcl-2, p53, caspases and some others causing apoptosis not only in erythrocytes but also in cells of some fetal organs. Then anoxia is developed resulting from hemolysis of erythrocytes as well as indirect bilirubin and other toxic products produced during hemolysis, disruption of general metabolism as a result of the deterioration of the liver and other organs, increase of vascular permeability, fetal growth retardation and several others. Their overall effect significantly complicates the pathogenesis of HDFN.

At the same time the fetus is not staying passive in the conflict. In develops a variety of reactive changes, depending on the duration and severity and on the stage of intrauterine fetal life. It includes proliferation of erythroblasts as an attempt to compensate for the destruction of erythrocytes. In a case of long lasting process of conflict, it causes a huge increase in the volumes of liver, spleen and other organs. In disruption of blood circulation, for example in its edematous form, a significant hyperplasia of the heart is developed. Proliferative processes in the immune system are expressed in an increasing number of monocytes and other changes in the lymphoid organs. At the final stages of the conflict a decompensation of whole lymphoid system occurs. It is expressed in thymic atrophy (involution) and disappearance of lymphoid elements in spleen and lymph nodes.

The above processes are not equally valid for different forms of HDFN. For example, in edematous and anemic forms there is no effects of bilirubin which is removed through the placenta towards the pregnant's body. But if the child remains alive, it can develop hyperbilirubinemia and jaundice. Following the fetopathy with maceration, no organ weight change and no other manifestations of pathological processes at the early stages of development (22 - 31 weeks) are detected, except for a quick and very massive destruction of Rh-antibodies. This is manifested in mass apoptosis and necrosis of tissues, turning into maceration.

But how such a variety and different options of symptoms are developed under the action of the same pathogens? In fact in the early allogeneic conflicts in embryos the picture more or less of the same type. While in later conflicts, such diversity is created, to be even considered as different diseases. Also at present time they are named differently: hydropic, anemic, with maceration, and the icteric being divided into two versions - icteric congenital or postnatal. These specifications are made according to most prominent manifestation which concludes the pathological process. A confluence of

different processes significantly complicates pathogenesis of HDFN compared with early allogeneic conflict. But the leading factors of the early and late conflicts are the same: apoptosis and disruption of organs.

7.5. Hemolytic fetopathy with maceration

This form of HDF is mostly observed at 22-31 weeks of development (Table 7.2, 7.3, 7.4, Diagrams 7.1, 7.2). All fetuses die before the birth. The most significant change is the maceration of all tissues and organs. The skin exfoliates in rags, the periosteum exfoliates of the skull bones and the meninges, and the skull subsides. Brain is ointment-like or attenuated. Dietary fiber and body muscles are pale, moist. Internal organs are bloodless, flabby, macerated, their weight has not changed. Maceration is less intense in the organs, which maintain the shadows of cells and of their nuclei. In the blood vessels and heart cavities the outlines of individual red blood cells with signs of apoptosis are sometimes see. They are also visible in some organs: liver, kidneys and pancreas. Such massive apoptosis occurs in the tissues of embryos and their shells in early allogeneic conflict under the influence of pregnant's alloantibody, but at the embryo stage tissue maceration is not observed: its dimensions are 3-30 mm and it is completely dissolved. In liver cells, and less in the spleen, hemosiderin is found. Weight of the fetus and its organs is normal. Rh^oD-antibodies act quickly: it takes a few hours to destroy the cells via apoptosis, and then the whole tissue collapses. Clinically, in women with Rh-conflict a very fast destruction of fetuses is seen: 14-20 hours before birth there were still a heartbeat and other signs of life, but the fetuses were born with severe maceration. All this suggests that at fetopathy with maceration a single, massive dose of Rh^oD-antibodies, covering the entire fetus and acting for less than one, maximum two days, arrives.

In cases of fetopathy with maceration in the fetuses, a small agglutinating antibody titer in its mothers dominates (titer 1:2 - 1:8 = 60%, titers 1:16 - 1:64 = 40%, Table 7.3), larger titers have not been noted. But one should take into account the small size of the fetus (22-31 weeks of development). Fetopathy with maceration is an example of a sudden and brief break through of alloantibodies, when the entire fetus in a short time (less than one day) becomes macerated mass. Traces of other pathogens are absent.

7.6. Anemic form of HDFN

Anemic form includes the smallest number of cases - 13 (5.65%). It occurs in all periods beginning from 26 weeks before delivery in time (Table 7.4, Diagram 7.2) only the first seven pregnancies; in later ones it is no longer found (Fig. 7.1). Titer of maternal agglutinating antibodies is low (Table 7.3). 11 children were born alive and died within the first day after birth; one lived for 2 and another – for 5 days. The most significant changes were seen in lungs: hyaline membrane, atelectasis, pneumonia. In fetuses born at 32-39 weeks or in time, the spleen was increased by 50%, and the liver, lymph nodes and adrenal glands were increased just slightly. Jaundice and edema were absent, but the amount of bilirubin in the blood increased, which indicated the destruction of hemoglobin (Table 7.8). Microscopic changes were as follows: occasional apoptosis of erythrocytes and erythroblasts while necrosis and apoptosis in the liver and other organs was absent.

Small groups of erythroblasts are seen the blood vessels, somewhat larger ones - in the spleen and liver, which causes slight increase in their volume. Enlargement of the spleen and lymph nodes is a manifestation of large groups of lymphocytes and less - erythroblasts. In hepatocytes it is indicated a little bit of hemosiderin as a consequence of destruction of erythrocytes.

The above changes indicate that the anemic form of HDFN occurs with small in its intensity and duration exposure to pathogens. The low intensity is supported by little destruction of erythrocytes (apoptosis) and by minor changes in main organs, a slight increase of bilirubin in the blood plasma and of hemosiderin in the liver. Some defensive responses are occurring: mild proliferation of erythroblasts in the liver and spleen and some manifestations of the immune response by appearance of groups of lymphocytes in the spleen and lymph nodes. The duration of the process is about two weeks. The immediate causes of death are in the delayed lung and their blood vessels development, the alveolar hyaline membranes, their atelectasis and the pneumonia.

7.7. Edematous form of HDF

Second by the frequency of cases is the edematous form, containing 25.65% of the total number of HDFN. Most often it falls on the birth at 32-36 weeks (Table 7.4 and Fig. 7.2). During the first three pregnancies it is rare: 3-5%, during the fourth to eighth - 30-35%, and in all subsequent 9 to 26 pregnancies, it reaches 57-68% of total (Diagram 7.1). Children with edematous form of HDF are stillborn or die within minutes or hours after birth.

Edematous form begins with the mild effects of allogeneic antibodies that destroy cells by apoptosis, containing antigen Rh^oD: erythrocytes, erythroblasts, less hepatocytes and some other types of cells. Other organs, as heart, lungs, kidneys, endocrine glands, the brain, the first time stay unaffected. In the future, action of antibodies is not terminated, but remains in the background. The compensatory processes come next. Instead of dead erythrocytes, the proliferation of erythroblasts is deployed in the bone marrow, liver, spleen, and less in other organs. But as erythroblasts, in turn, are destroyed by the antibodies, their synthesis in the liver and spleen produces a manifold enlargement of these organs, accompanied by mass creation of small vessels. Therefore, heart undergoes hyperplasia and functions satisfactorily. But the organs of the lymphoid system and the endocrine glands are suppressed, and the lungs are not functioning and gradually atrophy. In the liver dying hepatocytes are replaced by interlobular fibrosis, which leads to severe metabolic disorders. Immaturity of newly formed capillary walls and their damage by alloantibodies dramatically increase their permeability which creates general edema and hydrocephalus.

Newborn children look painful with dramatic swelling of the face, head and torso, and a huge belly. Limbs are usually not swollen. Skin is pasty, tense, pale or bluish. Subcutaneous tissue is jelly. Due to facial edema the eyes are not opened, and the bridge sinks. All the internal organs are swollen except for liver, heart and kidneys. Hydrops of cavities is dramatically expressed; abdominal cavity contains 170-800 ml of clear yellowish fluid, and the pleural cavities - 5-100 ml in each; sometimes the diaphragm protrudes into the abdominal cavity; the pericardium contains 2-40 ml of liquid. Multiple small hemorrhages appear in the skin, subcutaneous tissue, the muscles, mucous membranes. The spleen is enlarged very much, its weight reaches 20 to 130 g (the weight

of an adult human spleen in average is 150 g). There were cases of its rupture during the birth. The increase in spleen weight is due to proliferation of erythroblasts and lymphocytes, but mostly due to edema. The liver (230% of normal) and heart (153%) are also enlarged (Fig. 7.3.1,2,3). Weight of the lungs is reduced (88% of normal) (Table 7.5). Placenta is hydropic, reaching 880-2450 g; the villous vessels contain erythroblasts.

Microscopically, in the liver, spleen, lymph nodes, lungs, pancreas and thyroid glands and other organs there is apoptosis of erythrocytes and erythroblasts, some of them are phagocytized by monocytes (Fig. 7.1). In the liver there are: necrosis of hepatocytes, significant fibrosis, multiple large groups of erythroblasts; monocytes are few. The plasma permeates the walls of capillaries and small vessels of various organs, up to necrosis, and in several places the thrombosis is formed (see Fig. 7.2.1, 4). In the spleen, liver, lungs and other organs some groups of erythroblasts surround the lakes of plasma containing free hemoglobin and hemosiderin as the traces of destruction of erythroblasts. Vascular permeability is highly increased, as well as the swelling of surrounding tissues and cavities along with small hemorrhages. Development of the lungs (especially the alveoli) and the glomeruli of the kidneys are delayed; male testes are located in the genital tubules, or even in the abdominal cavity.

But there are also compensatory changes. In response to the destruction of erythrocytes the proliferation of erythroblasts is deployed not only in the bone marrow, but also in liver, spleen, in the stroma of various organs. And this is despite the fact that in the normal course the termination of synthesis of erythroblasts appears already at 11 weeks of development (Table 5.2). But the newly formed erythroblasts are also destroyed, and anemia increases. Along with damage to blood vessels it causes an overload of the heart by 1.5 times or more. It increases not due to edema, sclerosis or hypertrophy but due to hyperplasia – the increase in the number of cardiomyocytes as a real compensation for increased workload of the heart because of dilution anemia: the increased blood thinning. The whole complex of these changes: adaptive, replacing and leading to atrophy under the continued receipt of a moderate amount of alloantibodies the fetus can withstand from a few weeks to 2-4 months. Compared with other organs changed, the brain less suffers. This negates the action of Rh^oD antibodies in the brain. The immediate cause of fetal death is a total disruption of the metabolism and organ functions occurring before, during or shortly after birth. This completes the edematous form. Its slow course is due to a relatively small, but continuous and long-acting Rh^oD antibodies and powerful compensatory, adaptive processes during intrauterine fetal life.

The immune system of a normal fetus at 32-40 weeks is already well developed: it is capable to some degree for life after birth. Some manifestations of the immune response to the impact are also seen with the edematous forms as increased number of lymphoblasts in the spleen and lymph nodes. However, although moderate, but long-term pathogenic effects are resulting immune system to decompensation by reducing the number of B lymphocytes that synthesize IgM + immunoglobulins and by dramatically decreasing the levels of monocytes, total lymphocytes and lymphoblasts as well as all other parameters (Table 7.6.) The thymus is halved. Pathogens expand, and Rh-antibodies are not only affecting the erythrocytes but also some other factors and the different tissues and organs. Blood vessels are damaged, hematopoietic and endocrine systems are inhibited, anemia, necrosis and edema are progressing (Fig. 7.2.4), development of organs is delayed along with fibrosis, particularly in the liver (Fig. 7.3.5). All this makes the fetus unviable.

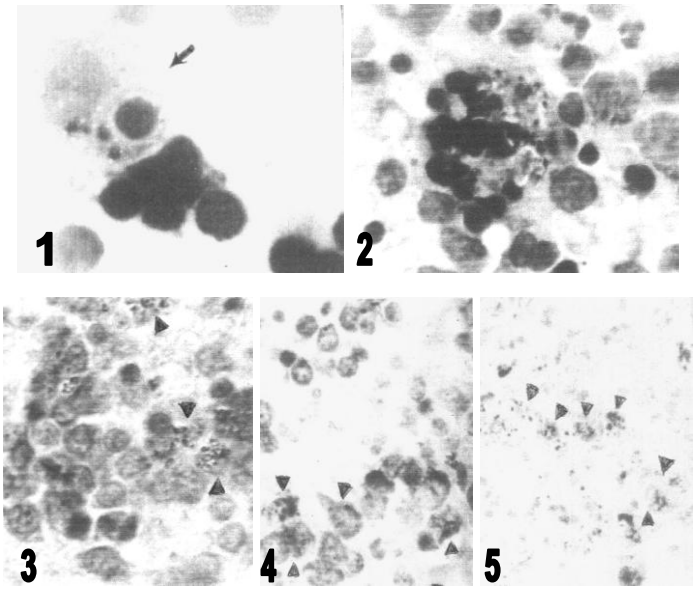


Fig. 7.1. HDFN results from the effect of pregnant's antibodies to Rh^{o}D or other antigens that are absent from her, but exist in a fetus, mostly in the erythrocytes. Therefore, the initial pathogenic factor in HDFN is the destruction of antibody-bound erythrocytes. It is produced by monocytes of the fetus or by apoptosis. **1** - spleen. Monocyte (arrow) fixes, phagocytize (see.Fig.6.1) erythroblasts and destroys them, x1300. **2** - bone marrow, the same, x1100. 1 and 2 - reaction of Romanowsky-Giems. **3** – liver. Apoptosis of erythrocytes and erythroblasts (▲), x800. **4** - spleen, the same (▲), x500. **5** - adrenal gland, the same, x400. Panels 3, 4, 5 represent the staining by Brachet.

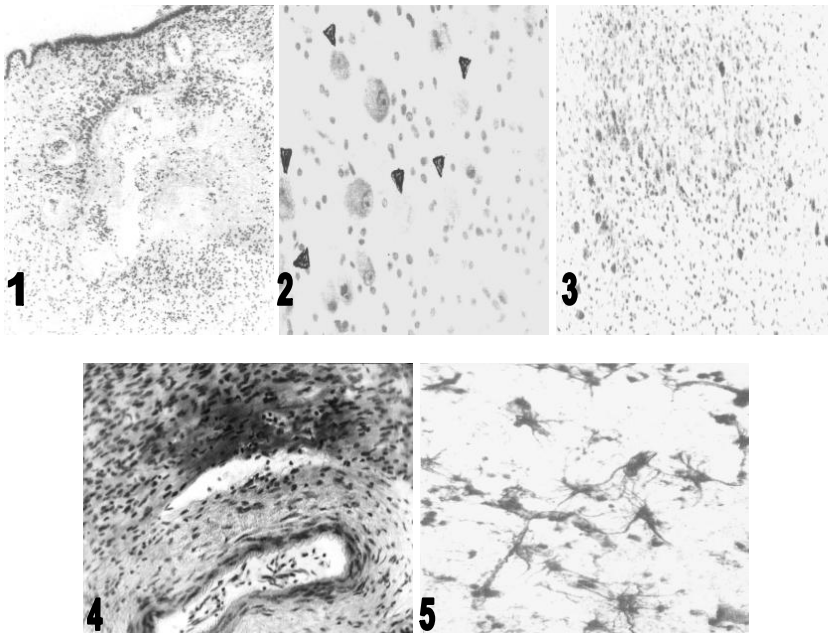


Fig. 7.2. Further development of pathological processes. **1** - brain cord. Necrosis with the formation of cysts, hyperplasia of glial and white blood cells, x80, hematoxylin-eosin. **2** - cerebellum. Neuronal death - shadows (▲), x400, staining by Nissl. **3** - the same: a significant reduction in the number of neurons, glial proliferation, x150, Nissl staining. **4** - heart. Myocardial necrosis, edema, little leukocyte infiltration, x200, hematoxylin-eosin. **5** - cerebellum. Hypertrophy and hyperplasia of astrocytes, x600, staining by Ramón y Cajal.

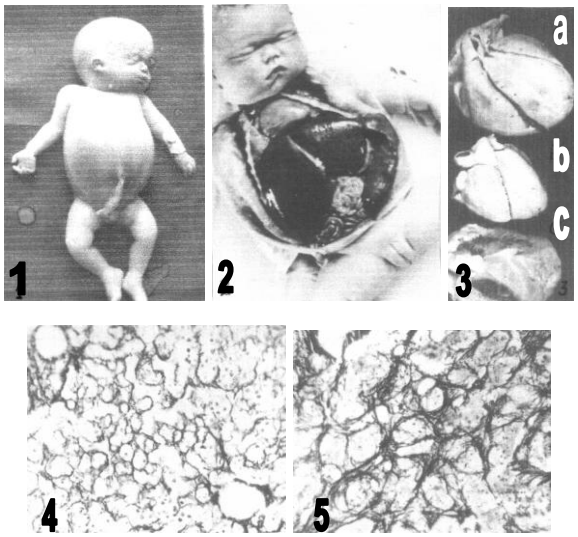


Fig.7.3. The longest-lasting form of HDFN is the edematous form. **1** - with a moderate course (up to 6 - 8 months): dramatic swelling, especially of the head and torso and ascites. **2** - a longer-lasting course (up to 8 - 9 months): a dramatic increase of the liver (the weight is 250 g, with control being 156 ± 4 g), the spleen (101 g, and control is 11.9 ± 1.0 g), moderate swelling. **3** - hypertrophy of the heart: **a** - 30.0 g, **b** - control, normal fetus 9 months - 17.9 ± 0.7 g, **c** - 37.2 g. **4** - liver, normal collagen content in full-term fetus. **5** - the liver in the edematous form of HDF contains a very large amount of collagen, clear fibrosis. 4&5 are stained by Foot, x200.

Table 7.5. Weight of the fetal organs with lethal HDFN (% to control)

Organs (in % of weight)	Control n=28	Anemic form n=6	Icteric form, postpartum n=32	Icteric form, congenital n =38	Edematous form n=52
Body weight	100±6	100±12	100±17	106±10	158±20[a,b,c,d]
Spleen	100±11	155±15[a]	182±23[a]	272±36[a b c]	476±64[a,b,c,d]
Thymus	100±7	90±7	71±12[a]	61±6[a b]	56±8[a b]
Heart	100±6	93±12	89±11	100±15	150±14[a,b,c,d]
Lungs	100±4	100±13	103±9	117±19	81±8[a]
Liver	100±4	109±9	102±12	139±9[a b c]	198±15[a,b,c,d]
Kidneys	100±5	105±16	87±7	92±10	97±21
Thyroid gland	100±5	100±19	98±7	100±9	127±12[a c]
Adrenals	100±9	86±10	83±8	114±12[c]	138±14[a b c]

Significance of differences ($p<0.05$-0.001): [a]-to control; [b]-to anemic form; [c]-to icteric postpartum from; [d] - to icteric innate.

The table reflects the pathological changes in the forms of HDFN caused by the influence of pathogens (intensity and duration of exposure), by the development of compensatory reactions and the manifestation of decompensation. In the forms the severity of changes increases from the left to the right (Table 7.6 and 7.7).

These severe processes can cause reactions in the body of the pregnant. There are evidences on rejection of the complex pregnant - fetus (O. Genbacev et al., 1999; F. Reister et al., 1999, 2001; R. Austgulen, 2004) with the participation of HLA-DR antigens (MRBurk et al., 2001). There are reasons to believe that not only the

trophoblast and the fetus serve the targeted for immune influence of the pregnancy (R. Bulla et al, 2003; JGGross, 2003), but also the fetus can respond to her allogeneic influences (S.I. Fisher, 2004; I.L. Surgent et al., 2006).

Table 7.6. Morphometric parameters of the spleen in HDFN

Parameters	Control n=18	Icteric form, postpartum n=25	Icteric form, congenital n=27	Edematous form n=18
Mean weight of spleen of newborns (%)	100 ± 11	182 ± 23[a]	272 ± 36[a b]	476 ± 64[a b c]
Maximum weight, g	12.4	22.4	64.5	130.2
The number of follicles per 1 мм^2 of prep	5.2 ± 0.9	4.5 ± 0.8	2.1 ± 0.4[a b]	0.2 ±0.1[a b c]
Surface of follicles (% of prep surface)	22.1 ± 2.4	24.4 ± 2.3	12.8±1.9[a b]	2.1 ± 1.4[a b c]
Number of cells in follicle (per 10000 μм^2)	150.2±14.3	142.8 ± 9.4	114.8±8.1[a b]	85.8±11.6[a b c]
Lymphocytes in follicles (% of the number)	80.1 ± 6.7	52.6 ± 3.1[a]	46.4 ±2.7[a]	33.0 ±2.9[a b c]
Lymphoblasts in follicles (% of number)	3.2 ± 0.9	18.2 ±2.2[a]	24.3 ±1.9[a b]	18.1 ±2.1[a c]
Monocytes in follicles (% of number)	2.2 ±0.3	4.4 ± 0.7[a]	5.2 ± 0.7[a]	1.3 ±0.2[a b c]
The number of cells in the red pulp (per 10000 μм^2)	88.7 ± 5.5	84.1 ± 3.6	66.7 ±5.1[a b]	98.5 ±6.1[b c]
Lymphocytes in the red pulp (% of number in follicles)	39.8 ±5.2	25.6 ±4.8	23.1 ±5.6[a]	13.3 ±1.2[a b c]
Lymphoblasts in the red pulp (% of number in follicles)	2.0 ±0.8	10.6 ±3.4[a]	10.9 ±4.1[a]	4.9 ±0.8[a b c]
Monocytes in the red pulp (% of number in follicles)	4.6 ±0.2	7.4 ±1.1[a]	12.9 ±2.1[a b]	1.1 ±0.8[a b c]
Erythroblasts in the red pulp (% of number in follicles)	0.1 ± 0.1	0.1 ± 0.1	23.6 ±5.1[a b]	63.2 ±8.8[a b c]
Number of IgM(+) monocytes (per 10000 μм^2)	0.1 ± 0.1	1.3 ± 0.2[a]	4.3 ± 1.5[a]	0.1 ± 0.1[b c]

Significance of differences ($p < 0.05$-0.001): [a] - to control; [b] - to icteric postpartum; [c] - to icteric innate.

Spleen reflects immune response in the forms of HDFN. In the icteric postpartum form moderately positive immune reactions occur: increase of spleen weight, increased number of lymphoblasts and monocytes in the follicles and red pulp, the amount of IgM (+) B-lymphocytes (increased synthesis of IgM). Reduction in the number of lymphocytes is compensated by the growing number of lymphoblasts. In icteric congenital form the changes are the same, but more significant. In the edematous form there are signs of severe deficiency of immune responses: a rapid, tenfold reduction in the number and area of the follicles, the number of cells in them, including monocytes. Number of IgM (+) B-lymphocytes decreased up to control level. The huge proliferation of erythroblasts in the red pulp was the result of the mass destruction of red blood cells and, along with edema, served the cause for undue increase in the spleen volume.

7.8. Icteric form of HDN

Icteric HDN is the most common among other forms of allogeneic conflicts, including 48.26% in our observations (Table 7.4). Its frequency increases from first to third pregnancy, and then remains, together with the edematous form, the most frequent (Diagram 7.1). Children with this form of HDN are usually born at term and only occasionally at 32-36 weeks (Diagram 7.2). All the icteric children born alive, their condition looks satisfactory or even good.

There are two variants of icteric HDFN: icteric congenital (or with anemia), and icteric postpartum (or without anemia).

7.8.1. Icteric congenital HDN (or with anemia)

Throughout the pregnancy, fetal development occurs normally. Shortly before the birth certain amounts of alloantibodies reach the fetus. Moderate apoptosis is seen in such fetuses, mainly of the red blood cells in the liver and spleen, the organs are moderately increased (a manifestation of the proliferation of erythroblasts). All this is happening just shortly. This form includes 20.72% of icteric HDN. Children are born alive with a slight increase of weight (Table 7.5) and mild jaundice. Their condition state is deteriorating hour by hour with increasing jaundice. Spitting of icteric masses begin, sometimes with blood inside. Minor bleedings are also possible from the navel, nose and skin. On the second or third day the bilirubin level in the blood rises to a state of emergency (Table 7.8.). Overall condition is very grave. Infectious complications may be developed: significant pneumonia can appear already in the second half of the first day, and sepsis - at 2-4 days. Cause of infection is the vulgar strains of Escherichia coli. The average life expectancy is 1.7 days, with maximum of 4 days.

At the autopsy, the children are with no signs of prematurity; color of skin and tissues is saffron-yellow. Spleen is significantly (2.6 times) enlarged (Table 7.6 and 7.7). In the lymph nodes the follicles are missing, sinuses are dilated. Liver is enlarged by 1.4 times, gets red-brown color; hemosiderin is not high; there are areas of necrotic lobules. Heart is of normal size. Lungs are enlarged by pneumonia, sometimes in the abscessing form (Table 7.5), hyaline membranes in the alveoli are sometimes yellowish. Kidneys are somewhat reduced, sometimes with amber-yellow papillae of the pyramids; in the epithelium of tortuous tubules of the kidneys some hemosiderin is found. Stomach and intestine contain small hemorrhages. The thymus is reduced by half, being at the stage IV of accidental involution. Apoptosis of red blood cells is seen in the liver, lungs, destroyed erythroblasts are phagocytized by monocytes.

Thus, in the congenital version of icteric HDN the allogeneic conflict begins shortly before birth. This is evidenced by a moderate, but quite a clear change of organs and, in particular, the spleen and lymph nodes. A compensatory proliferation of lymphoblasts and monocytes is held there, and the synthesis of IgM against the pregnant's antibodies begins.

Table 7.7 Amount of immunoglobulins in the serum of newborns with Rh^{0}D hemolytic disease (mg/100 ml)

Types of immunoglobulins	Healthy newborns (control)	Icteric form, postpartum	Icteric form, congenital	Edematous form
IgG	252 ± 156	1395 ± 115 [a]	1241 ± 92 [a]	578 ± 142 [b]
IgA	12.2 ± 3.3	30.6 ± 10.1 [a]	43.4 ± 11.4 [a]	3.1 ± 2.4 [a b]
IgM	22.3 ± 2.1	42.5 ± 2.9 [a]	51.4 ± 4.2 [a]	5.2 ± 4.1 [a b]

[a] - significant (p <0.02-0.001) differences from the control group; [b] - significant (p <0.01-0.001) distinctions from both icteric forms.

In both forms of icteric HDN fetuses demonstrate dramatic (fivefold) increase in IgG, and IgA-antibodies, mainly of pregnant's origin. This indicates the heavy action of pregnant woman against the fetus. But the amount of fetal IgM production against the pregnant's antibodies is also increased. These changes reflect the recent development of allogeneic conflict between pregnant - fetus.

In the edematous form of HDFN the amount of IgG of pregnant's production is twice higher than in control, but smaller than their amount in the icteric form. This may be a consequence of prolonged moderate income of pregnant's antibodies to the fetus. Number of IgM in fetuses is four times lower than in the control and 8-10 times lower than that in both icteric forms. This is a consequence of decompensation of the immune system of the fetus in the edematous form.

7.8.2. Icteric HDN postpartum (or without anemia)

In our material, this option includes 79.28% of icteric cases. Prenatal development occurs normally, without any complications. Only during delivery maternal blood containing alloantibody, penetrates into the body of newborn. At birth, there are no signs of illness, the good condition of the newborn is indicated, he demonstrates a loud cry, active mobility, absence of jaundice and pallor. The newborn takes well the mother's breast. Increased organs and anemia are absent (amount of erythrocyte is at least 5 million/ml), bilirubin is nearly normal (Table 7.8). This condition persists during the first 12-24 hours. State changes with the advent of jaundice, deteriorating in proportion to its increase. However, anemia does not reach the grave state. Small hemorrhages in the skin and mucous membranes are noted in half of the newborns.

Morphological changes are small. Spleen is increased by 1.5-2 times (Table 7.6), mainly due to congestion of red pulp, where the destruction of red blood cells by apoptosis goes on; monocytes contain a lot of hemosiderin (remnants of erythrocytes). The destruction of red blood cells and monocytes, intima of small vessels and sometimes necrosis occur in the liver lobules. In intralobular bile capillaries and interlobular bile ducts bilirubin is accumulated, sometimes in the form of clots. Rupture of bile capillaries form some lakes of bile, communicating with the lymphatic system. This facilitates the spread of indirect bilirubin in the body. It accumulates primarily in the brain, as well as in fatty-tissue-rich organs. In the myocardium sometimes small focuses of necrosis are seen.

Table 7.8. Amounts of bilirubin in the blood of children with HDFN (in mg%)

	Detection time	Number of detections	Bilirubin (mg%)
Icteric form	**0-12 hours**	16	3.75 ± 1.51
	12-36 hours	12	14.44 ± 6.3
	36 hours – 3 days	8	35.7 ± 5.82 [a b]
	4-5 days	2	31.2 ± 3.91 [a b]
Edematous form		13	1.04 ± 0.43
Anemic form		9	4.0 ± 3.1

[a b] - significant (p <0.05-0.001) differences from the first and second rows.

In the edematous form the fetuses die in utero, the level of bilirubin in their blood is normal. In anemic form the newborns die after the birth during the first day, the bilirubin level is moderately elevated. In newborns with icteric form in the first 12 hours after birth the bilirubin level also increased moderately, but then it dramatically rises with each passing hour. These comparisons show that the removal of bilirubin through the placenta is more rapid than the ability to release it by the liver and kidneys of newborns.

Analysis of the data shows that post-partum variant of icteric HDN develops as a result of short-term, moderate income of alloantibody during the birth. The effect of these antibodies is concentrated mainly in the red blood cells in the liver and spleen, as well as in parenchyma and the heart. The destruction of erythrocytes leads to the formation of indirect bilirubin and high toxic levels of it appear in the blood. The effect of indirect bilirubin causes severe damages to the brain, which are the immediate cause of death in newborns: bilirubin encephalopathy. Infectious complications are noted in half of the newborns. Ten children (9.1% of 111 newborns with both variants of icteric form, to which they belong), endured the HDN and recovered without any specific treatment.

7.8.3. Icteric form of HDN in a conflict by the ABO system

According to our data and the literature, some forms of HDFN: edematous, anemic and fetopathy with maceration do not occur with allogeneic ABO-conflict, there is only postpartum icteric form. After the birth, during the first 1-2 days the newborns are apparently healthy with anemia absent. The condition worsens with the emergence and increasing of jaundice. The average life expectancy is 5.4 days. No evidences of long-natal injuries exist. The main manifestation of allogeneic conflict is the destruction of red blood cells by their apoptosis. These damages of tissues and organs are small. Changes in the brain generally correspond to bilirubin encephalopathy in a case of rhesus conflict. Inflammatory changes are similar.

Thus, the HDN by ABO antigens is basically the same as icteric form of postpartum HDN with its bilirubin encephalopathy. In icteric HDN with Rh^oD and ABO antigens, alloantibodies do not penetrate through the placenta into the fetus during pregnancy. Penetration of antibodies occurs only once: just before the birth (prenatal option) or during birth and is manifested after it (post-partum variant). Their specific action, the destruction of cells by apoptosis, is limited to a few organs, mainly damaging

the red blood cells in the liver and spleen with a small cell lesion. This means that the amount of alloantibodies infiltrated into the fetus is small. With the destruction of red blood cells a lot of indirect bilirubin is released: it reaches 14-35 mg% in the fetal blood at the end of the first and the second day. Feature of icteric HDN is jaundice when bilirubin impregnates tissues, especially the fat-rich ones. In all other forms of HDFN in which Rh^oD antibodies of pregnant also destroy red blood cells and cells of organs, jaundice is not formed. The reason for this is that the bilirubin, along its appearance, is removed through the placenta to the pregnant woman and her big liver removes it quickly and without complications. But in icteric variants (prenatal and postnatal), the fetus receives allogeneic antibody during the birth, when the connection with the mother is ended. And when alloantibodies destroy red blood cells and rapidly (within a few hours) overload the blood with more and more bilirubin, the liver of newborn does not have time to neutralize and remove it. Blood carries bilirubin throughout the body and it becomes a major pathogen. Damaging action of alloantibodies ends; relationship with the mother ends too. Anemia is low or absent, the lymphoid system (spleen, lymph nodes, and thymus) is only slightly damaged, blood flow is preserved. At 4-5 days after the birth, bilirubin in the blood is already beginning to decline. But the majority of newborns with icteric HDN die. And that's because the fetus with edematous form of SBP, at the earlier stage of the intrauterine development, with severe changes in all body systems, but without jaundice, maintains fetal life even up to 2-4 months. The cause of death in neonates with icteric HDN is the effect of bilirubin on the brain - bilirubin encephalopathy.

7.8.4. Conclusion for sections 7.4 - 7.8. Features of pathology in different forms of HDFN

Penetration of allogeneic antibodies in the fetal organism remains largely unclear. Before 22 weeks of pregnancy, allogeneic antibodies do not pass through the placental barrier. But in subsequent periods antibodies do pass in different ways and at different times (Table 7.4). Sometimes alloantibodies burst in a form of a violent attack, often at 22-31 weeks. This form is fetopathy with maceration: the fetuses die and macerate completely within 14-20 hours. In the next 32-36 weeks, the penetration of allogeneic antibodies usually goes slowly, gradually and takes long time which produces the edematous form. The fetus is forced to perform reactions of adapt to these extreme conditions. It's highly disturbs the activity of liver, spleen, heart: all the organs, making the fetus ugly. In the last 37-40 weeks of pregnancy the most frequent is icteric form, appearing shortly before the birth or during it. Termination of the release of hemoglobin breakdown products through the maternal placenta brings an additional severe pathogen: indirect bilirubin.

One can distinguish two variants of delivery of allogeneic antibodies to the fetus during Rh^oD: the acute one with fetopathy and maceration and the post-partum version of icteric form, when during a short time the fetus receives a considerable amount of antibodies. Another variant is subchronic edematous form and partly antenatal variant of icteric form. Anemic form is located between the two options with its low speed of

delivery of antibodies, the amount of them and the low titer. In the late conflict with HDFN, only the icteric form of allogeneic conflict appears.

Transportation of allogeneic antibodies with HDFN, especially at Rh^oD conflict, remains unclear. An opinion on their unimpeded passage through the placenta to the fetus is excluded. The presence of barriers for the penetration of alloantibodies is based on several facts. It is undeniable impermeability during the first 22 weeks of pregnancy. The formation of the four forms of HDFN happens following the explicit break down of some barriers for the allogeneic antibodies. It may be a one-off and a massive penetration (at fetopathy with maceration and partly with postpartum icteric version), a prolonged and less intense one (edematous form and partly the antenatal icteric form), or short and weak one (anemic form). Often, the placenta remains impermeable to alloantibodies during the pregnancy, despite the fact that immunoglobulins of non-allogeneic nature quite freely pass through the placental barrier. Penetration of pregnant's blood with alloantibodies is only possible when the birth is approaching and during it, with a contraction of uterus and the rupture of placental villi. The mechanism of passage of antibodies looks possible (at least, theoretically). But how the alloantibodies pass through during the pregnancy, repeatedly or protractedly, or never pass, leaving the fetus intact, remains unclear.

The immune system of the fetuses after 22 weeks of development is capable of many actions, including the production of immunoglobulins against all types of proteins. But their production against allogeneic antibodies is unlikely for two reasons. With the rapid development of the conflict the fetus does not have time to prepare itself for the production of antibodies (2-2.5 weeks), because for the fetus the conflict is new, being for a pregnant woman even the fifth or tenth one. And a prolonged conflict by itself suppresses the entire immune system by alloantibodies.

However, excluding the immune system of the fetus with the late allogeneic conflict is too prematurely. Among women who gave birth to children with edematous form of Rh^oD HDN, pre-eclampsia, toxaemia of the pregnant, anemia of unknown origin are observed (YWLoke, A. King, 1997; DSGoldman-Wohl et al., 2000; F. Reister et al., 2001; R. Austgulen, 2004; В.И.Кулаков and others, 2005). It appears that these processes may be a consequence of immune and other responses of the fetus, but studies in this area are insufficient. Immunoglobulins G and A, of anti-microbial or other species, are quite freely transported across the placental barrier by lots of different receptors. But IgM in normal conditions of pregnant woman is not able to be transported through the placental barrier. Only under infections, immune and other effects, the transfer of IgM through the barrier is realized by receptors: SC and J-chain: those ones, which transport of IgG and IgA. These receptors are located in the trophoblast included in SIS. Alloantibodies in early conflict also phagocytize various types of trophoblast in the most active way. Apparently, the delay of alloantibodies in the late conflict is realized by the same mechanisms, by which IgM are stopped.

7.9. Bilirubin-induced encephalopathy in jaundice of newborns and in HDN

Bilirubin encephalopathy sometimes begins on the background of the normal state of the newborn. At the end of the first - to the second or third day after birth, jaundice appears - and the general condition is rapidly deteriorating. In blood serum of the newborn the levels of immunoglobulins, especially IgG (maternal) is increased 5.5 times compared to the healthy newborns, the amount of bilirubin in the blood increases in 15-40 times (Table 7.8). The following signs of brain damage appear: convulsions, strabismus, tension and bending back. The brain changes: in a white or slightly yellowish brain matter it becomes clearly visible the bright orange-yellow color of certain areas (Figs 7.4-7.9). The consequences of bilirubin encephalopathy are severe: if the child does not die in the acute period, then after a light period (from several months to 10 years) post-icteric encephalopathy may develop. It is expressed in muscle hypertonicity, ataxia, athetosis, mental retardation up to the idiocy.

For the first time the jaundice newborns with a bright yellow coloration of brain has been described by J. Orth (1875). G. Schmorl (1904) gave the name of nuclear jaundice (kernicterus), now the term bilirubin encephalopathy is widely used. It was found that kernicterus, besides HDN may complicate other pathological processes associated with neonatal jaundice. It includes sepsis, massive hemorrhage, anoxia, Crigler-Najjar syndrome and others. It has been found that the indirect bilirubin is a heavy cellular toxin, which blocks the enzymes. As a result, it is not clear: is the damage of the brain nuclei is a consequence of bilirubin action and whether the severity of brain damage depends on the level of indirect bilirubin in the blood? Perhaps the nuclei of the brain are damaged by any other pathogens, and bilirubin only stains the broken parts of the brain. The answers to these questions are given in section 7.10.

7.9.1. State of the brain in neonatal jaundice not associated with HDN

Out of the 2,379 autopsies on the perinatal death, on which we base our study, the overall jaundice has been found in 122 cases (5.1%). Kernicterus was found in 54 of them (2.3% of all autopsies and 44.3% of overall jaundice). Overall neonatal jaundice, besides HDN, was seen with jaundice of prematurities, with sepsis and other infections, with jaundice which follows massive hemorrhage and with biliary atresia. 20.4% of all mentioned consisted of the brain kernicterus cases and 79.6% were children with HDN.

Overall jaundice of preterms -31 cases. Birth weight was 920-2100 g. Overall jaundice appeared at 1-2 days, sometimes at 3-4. Pneumonia (21) or pneumonia with birth trauma (10) was the cause of death. By autopsy the brain had signs of immaturity, with diffuse poorly articulated icteric staining of 29 patients' brain, and kernicterus in 2.

Overall septic jaundice - 16 cases with umbilical sepsis (10 cases), lung (4), cutaneous on the basis of bullous epidermolysis and infected meningocele (one of each) were observed. Weight of children was 2200-4400 g. The overall jaundice appeared at the 3-6 day after birth, the life duration was 3-30 days. Sepsis was caused by Escherichia coli, streptococcus, or S. aureus. Kernicterus of brain (7 cases) was less intense and less

widespread than in HDN, in three of them also the diffuse staining of the brain was seen. One of these children developed purulent meningitis.

Other infections - 4 cases, congenital generalized cytomegalic inclusion disease (2), toxoplasmosis (1) listeria (1): all full-term. Overall jaundice was poorly expressed, the brain staining was missing.

Overall jaundices with significant hemorrhage – includes 8 children, including 7 cases of cephalhematoma, 5 of them - in combination with intracranial hemorrhage, one - with hemorrhage into the adrenal glands, in the retroperitoneal tissue and with hemoperitoneal bleeding. Weight of newborns was 2400-4400 g, the jaundice appeared on the first day in 3 cases, on the second - in 4, on the third day – in one. Kernicterus was found in one, suffered a Down's syndrome in addition to birth trauma.

Table 7.9. Causes and frequency of overall and nuclear jaundice

	Jaundice								
	of prematu -res	Sepsis	Other infections	Major hemo- rrhages	Biliary atresia	Autopathic	Total without HDN	HDN	Total
General icterus	31	16	4	8	2	3	64	58	122
Kernicterus of brain	2	7	-	1	-	1	11	43	54
% of Kernicterus	6.4	43.7	-	12.5	-	-	17.2	74.1	44.3

The data shows: bilirubin encephalopathy in the neonatal period occurs in jaundice of prematurity, in sepsis and major hemorrhages: the general ones or restricted to the brain. Accession of purulent meningitis, brain hemorrhage, or congenital malformations in the background of general jaundice does not affect the development of kernicterus. Effect of bilirubin is shown in Table 7.10. All cases of bilirubin encephalopathy without HDN amount to 0.46% of all perinatal deaths and 17.2% of total neonatal jaundice. Icteric staining of nuclei of the brain stem in a listed forms of jaundice are largely consistent with kernicterus of HDN: parts of nuclei of the medulla oblongata, the bridge, the cerebellar dentate nucleus, ammonium horn, thalamus, nucleus lentiformis and nucleus caudatus. Icteric staining with bilirubin encephalopathy is less spread and less intense than in HDN. Neuronal imaging shows an acute swelling of neurons and the absence of severe changes: necrosis and apoptosis. Changes in glial cells and blood vessels are also expressed much weaker than in icteric HDN.

7.9.2. Embryonic brain damage during early allogeneic conflict

These damages can get two directions. One of them is the direct effect of allogeneic antibodies. It is expressed in mass apoptosis of most organs. At the same time it covers more than 50% of liver cells, neural tube, somewhat less - in the kidneys, heart and other organs and little part of stromal cells. In this case, cells are TUNEL-positive; contain IgG, IgA and IgM. Some of these cells are still intact. Others have already started to disintegrate, yet containing the immunoglobulins, which confirm their pathogenic action. Cells, broken into small granules, are already losing immunoglobulins. Such a

massive destruction of vital cells is completed by a quick death and destruction of the embryo (Chapter 5.8).

The second direction of the death of the embryo during early allogeneic conflict is a dramatic breach of its metabolism and the amount of apoptosis of allogeneic antibodies. The pre-embryo and the embryo are too small to cause significant immunization of the pregnant and therefore the production of antibodies is low (Chapter 5.8). The intense phagocytic activity of the invasive trophoblast, lacunae trophoblast, villous syncytiotrophoblast and monocytes also participate in reducing the number and the activity of antibodies. The destruction of villous capillaries and erythroblasts also plays a role. As a result, the average number of vessels at one villus decreases from 5.29 ± 0.83 to 2.07 ± 0.68, the number of avascular villi increases from 4.82 ± 0.28 to 14.74 ± 2.31, so, the number of non-functioning villi exceeds 70% (Table 4.2). All this leads to disruption of metabolism of the embryo and its destruction.

Out of 43 cases of early conflict investigated, embryonic tissues were available in 15 (34.88%) cases. Out of these, 7 fetuses (16.28%) died from the direct action of alloantibodies (apoptosis), and 8 - from metabolic disorders (anemia, degenerative changes, and minor necrosis). Jaundice of skin, tissues, organs and brain in the embryo was absent at all since the synthesis of bilirubin could begin in the liver and appear in its ducts only at 4 months of fetal development (Бархина Т.Г., 2006).

7.9.3. State of the brain in $Rh^{o}D$ HDFN

Out of these noted 2379 cases of perinatal death, we investigated the brain and peripheral nervous system in 132 cases (5.55%) of various forms of HDFN, including fetopathy with maceration, anemic, edematous and icteric forms. Following tissues were examined: spinal cord and medulla oblongata, pons, pedunculi cerebri, the cerebellum and cerebral hemispheres (Figs 7.4 - 7.9). Besides the survey staining, several specific ones were used: by Nissl, Perls; visualization of Sudan, myelinated fibers, oligodendroglia, astrocytic glia, and others: 16 techniques in total.

As a control we conducted a study of brains of 5 fetuses at 32-40 weeks of development, who died of acute-fledged intrauterine asphyxia. It was clarified the state of neurons. Changed neurons were rare. Hemosiderin and sudanophilic substances were not detected. Pigment in the neurons of the substantia nigra was absent as it would appear only after the birth. Oligodendroglial cells, astrocytes and blood vessels looked intact.

Hemolytic fetopathy with maceration of HDF – includes 22 cases. The brain looked as a white and pink structureless wet mass without icteric coloration, sulci of the brain were flat, and hemorrhages were not detected. Microscopically it could be seen: varicose veins containing some erythroblasts, partly in apoptosis as well as perivascular and pericellular edema. Nuclei and cytoplasm of neurons stained weakly with Nissl staining not defined, and apoptotic neurons not detected (Fig. 7.2.2). Hemosiderin was absent. Neurons of spinal cord were somewhat better preserved. Our studies detected that the brain was less autolyzed than the other organs. Liver, spleen, pancreas and other organs looked only as a structureless mass while in the brain cellular elements and their nuclei could be seen.

Anemic form of HDF - 13 cases. The brain was not changed significantly besides swelling of the membranes and occasional small hemorrhages. Microscopically it could

be seen: pronounced vascular changes such as spasms of the arteries, veins, perivascular and pericellular edema, fresh hemorrhages, multiple thrombi; in neurons: more modest changes such as rare central chromatolysis. The number of neurons was not reduced and apoptosis in them was not determined. Jaundice was absent with overall moderate violation of the circulation in blood vessels and hypoxic encephalopathy.

Edematous form of HDF is presented by 38 cases. Smaller and larger hemorrhages were found. Brains had signs of immaturity, colored white or pale pink. Microscopic changes: small groups of hypochromic neurons in many parts of the brain stem, cerebellum and hippocampus; contours of nuclei and cytoplasm – hyperchromic; some neurons - with chromatolysis, moderately swollen or pyknotic, with uneven contours and reduced size as well as hyperchromic nuclei and cytoplasm. Any inclusions in the cells were absent. Neuronal apoptosis was very rare. Oligodendroglial cells and astrocytes could form a small chain as a result of moderate proliferation (Fig. 7.2.3). Vascular dystonia, swollen intima, perivascular edema, blood clots, small areas of necrosis were found. Peripheral nervous system stayed without pathological changes. In general we elicited the moderately severe hypoxia due to general disruption of the circulation. The prolonged and significant effect of allogeneic antibodies, which caused severe and varied changes of the whole body organs, had just little impact on the state of the brain.

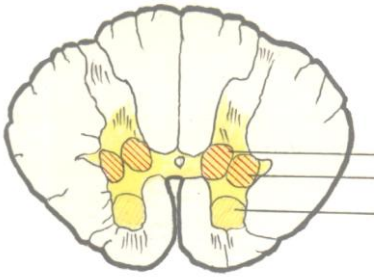
Icteric form of HDF has 58 cases. The changes varied depending on the duration of life after birth and rising levels of bilirubin in the blood.

In the first 12 hours the amount of bilirubin in the blood was 3.75 ± 1.51 mg%. Newborns died from accidental causes such as birth trauma, aspiration pneumonia, malformation, and others, at the background of vascular dystonia, venostasis, perivascular edema, blood clots in the veins. Meninges in some cases got light yellow color. Ischemic changes of neurons, large numbers of hyperchromic ones as a sign of hypoxic encephalopathy were developed (Fig.7.2.5).

After 13-36 hours bilirubin in the blood reached 14.44 ± 6.3 mg%, and no more than 21.5mg%. Nature of the damages had changed. Brain became wet, white, with faintly yellowish diffuse color in the deceased at the beginning of the second day. In the cerebellum, the nuclei of the brain stem and spinal cord, neurons could swell and die, converting into cell-shade without signs of significant neuronal apoptosis. Causes of death were: pneumonia, birth trauma or complications during blood transfusions.

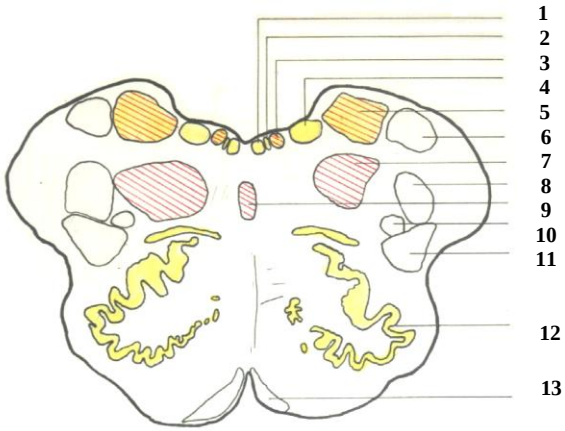
At the terms exceeding 36 hours and up to 3 days the level of bilirubin in the blood could reach 35.7 ± 5.8 mg%. Death occurred from uncomplicated bilirubin encephalopathy, and, in half the cases - in conjunction with pneumonia, umbilical sepsis, birth trauma and others causes. Brain weight was normal in children up to 36 hours, increasing to 100 - 165 g in those who died during 2 - 5 days. Total jaundice was intense. Kernicterus of brain could be observed in 91.11% of newborns who died after 36 hours. Lesions of neurons mentioned above, were more significant and acute swelling progressed. Proliferating glia in these areas could be seen.

Fig. 7.4. Spinal cord



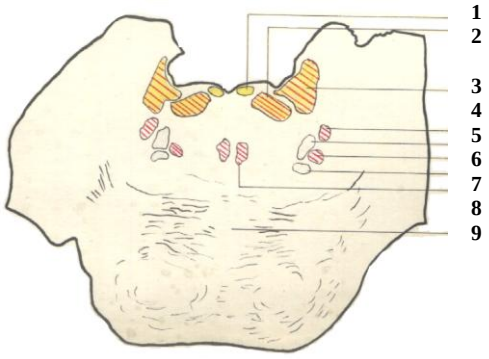
1 - nucleus thoracicus
2 - lateral horn
3 - anterior horn

Fig. 7.5. Medulla oblongata



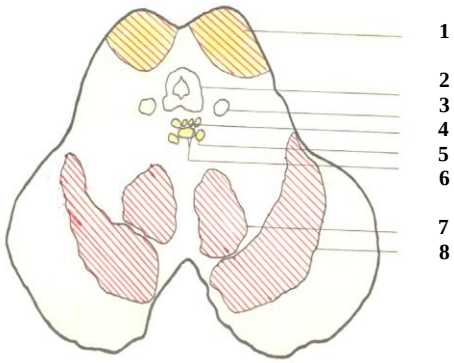
Nuclei: **1** - hypoglossal. **2** - interstitialis, **3** - eminentia medialis, **4** -dorsalis nervi vagi, **5** - vestibularis, **6** - cuneatus, **7** - centralis superior, **8** – spinalis inferior, **9** – longitudinalis medialis, **10** - ambiquus, **11** – olivocerebellaris, **12** - inferior olivaris, **13** - arcuatus

Fig.7.6. Pons



Nuclei: **1** - eminentia medialis, **2** – of VI nerve
3 - vestibularis,
4 – of V nerve (gelatinous substance),
5 – of VII nerve,
6 – reticularis lateralis,
7 - superior olivary,
8 – tegmentum reticularis,
9 - pons

Fig.7.7. Crus cerebri

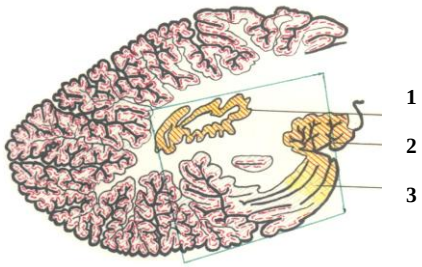


Nuclei: **1** - corpora quadrigemina (superior)
2 - central gray substance
3 - tegmentum dorsali,
4 - Edinger-Westphal
5 – of IV nerve, **6** – of III nerve
7 - red
8 - substantia nigra

Color codes of the drawings

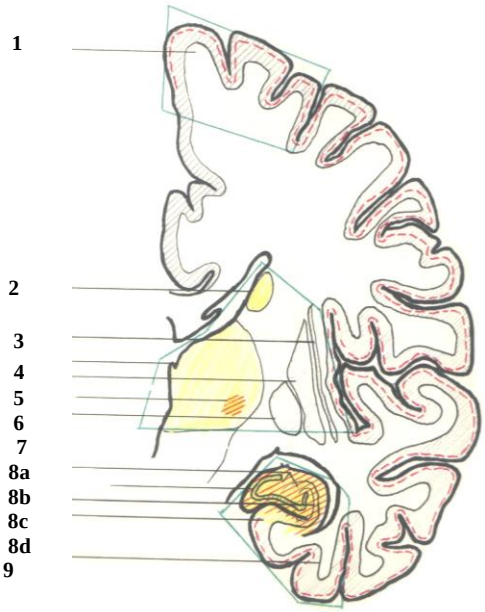
White: neurons are not stained by bilirubin, without any changes.
Yellow: bilirubin stains neurons there, but no significant pathological changes found.
Yellow with red dashes: bilirubin stains neurons with significant pathological changes up to necrosis.
White with red dashes: neurons are not stained by bilirubin, but there are significant pathological changes.
Green line limits the sites studied.

Fig.7.8 Cerebellum.



1 – nucleus dentatus, **2** - folliculus
3 - amygdala

Fig.7.9. Hemisphere



1-parietal lobe, **2** – nucleus caudatus, **3** - claustum, **4** - thalamus, **5** - putamen, **6** - nucleus subthalamicus, corpus luysi, **7** - globus pallidus, **8** - hippocampus (a – areas H5-H4, b - dentate gyrus, c - H2-H1 area, d - subiculum), 9 - temporal lobe

At 4-5 days the level of bilirubin in the blood reached 31.2 ± 3.91 mg%. Causes of death remained the same: uncomplicated bilirubin encephalopathy in half the cases, and in the second half - the same but in combination with pneumonia, omphalitis, and umbilical sepsis. Changes in the brain were progressing. Some parts of the brain stem nuclei and the central ganglia were drastically damaged, many neurons died. Type of lesion looked the same: an acute swelling and disintegration of neurons. Typical was the absence of apoptosis of neurons and glial cells along with a significant proliferation of glial cells, even if some of them died. Out of 46 who died in this and subsequent periods, 3 newborns died at days 4-5, without yellow staining of the nuclei of the brain. The level of bilirubin in the blood reached 18.4 - 20.5 mg%. Changes in the brain were similar to those if died during first 13-36 hours. Cause of death was macrofocal pneumonia and bilirubin encephalopathy.

At days 6-7 bilirubin concentration in the blood decreased to 26.8 ± 2.3 mg%. In died at this background, the acute processes in the brain decreased slightly, but considerable loss of neurons in brain stem nuclei continued along with massive pulmonary pneumonia.

7.9.4. Brain state during HDN caused by ABO incompatibility

Newborns died by 3-9 days. Amounts of bilirubin in the blood reached 29.55 ± 4.31 mg%, slightly lower than in children with Rh^{o}D HDN (see Table 7.8). In a child with only diffuse brain icterus the amount of bilirubin in blood equals 3.18 mg%. Changes in the brain are similar to changes in Rh^{o}D HDN, but somewhat less spread and

less severe at the same periods of time. Significant proliferation of oligodendrocytes and astrocytes can be seen. Neuronal apoptosis is negligible.

Table 7.10. Limits of bilirubin (mg%) in the blood of newborns with overall jaundice and bilirubin encephalopathy

	Sepsis, other infections, major hemorrhages, biliary atresia, prematurity	HDFN Rh^oD			HDN ABO
		Anemic form	Edematous form	Icteric form	Icteric form
Without overall icterus	0.8-1.3	0.8-5.2	0.9-1.6	2.2-14.5	-
Diffuse brain icterus	7.7-15.1	-	-	17.8-20.5	18.3
Kernicterus of the brain	15.0-25.7	-	-	27.1-41.9	25.7-33.4

7.10 Conclusion

Our studies have shown the absence in icteric HDN any signs of direct action of alloantibodies such as apoptosis of neurons. Also these features have not been found in fetopathy with maceration, edematous and anemic forms of HDN. This confirms the absence of a direct action of alloantibodies on neurons in HDN. But with the early allogeneic conflict, where the allogeneic antibodies not to the Rh^oD or ABO, but to another, yet unknown antigen operate, multiple direct damages of the cells of embryonic neural tube are definitely available. Both options of icteric HDN: ABO and Rh^oD, major damage to occur from the effect of indirect bilirubin. Diffuse yellow staining of the meninges may appear already in 9 hours after birth.

In neurons of different brain regions sensitivity to indirect bilirubin and other pathogens varies considerably. In jaundice bilirubin differently stains different organs of the body too, but the situation in the brain is more complicated. As shown in Figs 7.4-7.9, as well as in Diagram 7.1, the staining of the nuclei by bilirubin and the pathological changes of neurons are of four different types:

1) – nuclei are stained by bilirubin, their neurons are pathologically altered and dying partly;
2) – nuclei are stained by bilirubin, but only a small part of neurons looks weakly pathologically altered;
3) - bilirubin staining is missing, but significant part of the neurons is pathologically altered.
4) - unstained nuclei contain well preserved neurons.

This shows that neurons in the brain of newborn are not only influenced by indirect bilirubin, but other factors. One of them is alloantibodies. Even without creating apoptosis, they can alter the reactivity of neurons to indirect bilirubin or other pathogens.

Such a response is caused by toxins of microflora in sepsis, combined with HDN or not. Neonatal sepsis causes bilirubin encephalopathy at 15 mg% of indirect bilirubin in the blood, whereas allogeneic conflict – at more than 25 mg%. Another factor may be found in biochemical features of neurons in different nuclei of the brain that may be unable to accept indirect bilirubin, for example, because of small amount of lipids or possessing a sufficient resistance to it.

* * *

In early allogeneic conflict of preembryonic and embryonic periods the protection against allogeneic antibodies is executed at certain degree by phagocytic system: invasive trophoblast in the decidual tissue, trophoblast that covers the lacunae, and monocytes in the placental barrier villi. In some cases they protect the embryos from the direct action of antibodies, which causes death within a few hours. However, protection of placental barrier in the area of the villi is relative: it only delays the destruction of the capillaries and other tissues of the villi for 2 - 4 weeks, and eventually eliminates up to 70% of the villi of the total metabolism. It results in destruction of cells forming the brain and other organs and erythroblasts which can happen in two ways: by direct effect of allogeneic immune antibodies producing apoptosis, or by indirect effects via the destruction of the placental barrier. Jaundice and nuclear encephalopathy are absent here.

Late allogeneic conflict is possible with HDF in fetuses from 22 weeks and older, when their immune system is already formed, but its function is not yet perfect. In cases of fetopathy with maceration, the attack of alloantibodies is so massive and rapid (taking less than one day) that no immune effect has enough time to evolve and the fetus dies without developing any jaundice.

In newborns the bilirubin encephalopathy is found not only under allogeneic conflicts. Icteric staining and destruction of neurons in the brain stem is sometimes found in sepsis, major hemorrhage and in preterms. Still, no jaundice evolves. The level of bilirubin in the blood ranges from 0.8 to 1.3 mg% (Tables 7.8, 7.9 and 7.10).

With HDFN in its anemic form and edematous form, following the long-lasting and significant action of Rh^oD antibodies, the yellow staining is missing. This is due to the fact that the fetuses in these forms stay in utero, where the indirect bilirubin is processed by the liver and excreted through the placenta barrier to the pregnant. The blood of fetuses with edematous and anemic forms contains just a small amount of bilirubin (0.8-5.2 mg%), and therefore jaundice is absent (Tables 7.8 and 7.10). A similar situation is created in the icteric Rh^oD and ABO conflict: some newborns die within the first day, when the bilirubin is just beginning to accumulate in the blood ranging around 2.2-14.5 mg%, but the critical levels for patients with HDN are higher, so that below 15-17 mg% icteric staining does not arise and diffuse jaundice appears only at 17.8-20.5 mg%, and brain kernicterus – at 25.7-41.9 mg% (Table 7.10).

Icteric coloration of neurons (brain kernicterus) does not appear in all nuclei, even within the brain stem (Figs. 7.4-7.9). These options depend on certain factors. One of them is **the sensitivity of cells to pathogens**. For example, with Rh^oD allogeneic conflict, antibodies act on those cells that contain the RhD antigen. These are red blood cells, hepatocytes and cells of certain other organs (П.Н.Косяков, 1975). In a case of ABO conflict, the same cells and erythrocytes contain the antigen. But in the case of edematous form of HDF, in which RhD antibodies for a long time (weeks or even 2-4 months) and more or less intensively effects on the fetus, while changes in brain cells are

moderate, it is likely that these cells do not contain the RhD antigen. In addition to the action of pathogens, for the perception of toxic indirect bilirubin an **important factor is level of lipids in the cells.** Reason for such changes is in **the toxicity of pathological effects** of ABO or RhD antibodies, toxicity of microflora that causes sepsis, or some other pathological agents. For example, for the development of diffuse icteric form or brain kernicterus under the action of alloantibodies it requires significantly more bilirubin (respectively, 18-20 mg% for diffuse jaundice and 27-42 mg% for kernicterus) than under the action of toxins of microflora in sepsis (respectively, 7.7 -15 mg%, and 15-25 mg%). This means that for the occurrence of both types of jaundice of the brain (diffuse and nuclear) not only bilirubin is required, but also the action of pathogens. This **combined effect of pathogens and bilirubin** is another condition for the development of bilirubin encephalopathy.

* * *

Rh^{o}D late allogeneic conflict, the most common among people of European descent, has a number of features. In contrast to early allogeneic conflict in the embryo and the majority of late conflicts, Rh^{o}D conflict appears in four forms that differ one from another in the course of the disease, complications and mechanisms of death. These are: icteric form with two options - pre-natal (or with anemia) and postpartum (or without anemia), edema, anemic form and fetopathy with maceration. For clinical and pathological designation of various late conflicts allogeneic (Rh^{o}D other options of Rho, ABO and many other groups of allogeneic conflicts) it is adopted the common term: hemolytic disease of the fetus and newborn (HDFN).

Development of that or another form of HDFN is determined by a large spectrum of conditions and factors from the sides of the pregnant woman and the fetus. From the site of pregnant woman it includes features of alloantibodies. For example, Rh^{o}D antibodies are more pathogenic, cause the development of four forms of HDFN at different stages of fetal development; while ABO antibodies - only icteric form of HDN in the maternity period. The titer of alloantibodies also makes some sense: the higher in is in the pregnant woman, the more possible is the development of severe HDFN forms during her pregnancy (Table 7.3).

At the same women, the frequency of various forms of HDFN varies from one to another pregnancy (Diagram 7.1). For example, for Rh^{o}D immunized women, delivery of perfectly healthy children after the first pregnancy is reduced, and after the fifth pregnancy is no more possible. Number of children with icteric form increases from 16.7% during the first pregnancy to 47.1% at the fifth pregnancy, and then lasts around 32-42%. Fetopathy with maceration increases from 2.8% to 14-28.6%. Anemic form after the seventh pregnancy has not been observed. Number of edematous forms increases from 2.8% during the first pregnancy to 66.7-69.3% at the ninth and subsequent pregnancies. These changes clearly reflect the degree of immunization and increased allogeneic conflict: with each successive pregnancy the number of severe HDFN (edematous, icteric and fetopathy with maceration) increases and the less severe form of disease (anemic) disappears.

Each form is more common at a certain period of pregnancy (Diagram 7.2). Basically at 22-31 weeks of development the fetopathy with maceration is typical, at 32-

36 weeks the edematous form predominates, and at 37-40 weeks two versions of icteric form happen more often: prenatal begins before the birth, and postpartum begins at birth and continues after it. Anemic form, the rarest one in HDFN, is equally represented in the last two periods, beginning from 32 weeks before birth. Minor morphological changes of the internal organs in anemic form and a small degree of anemia suggest that it is a consequence of the minimal impact of allogeneic antibodies for a limited time.

The development of each form of HDFN is the result of several factors. Among them is the number of allogeneic antibodies penetrated into the fetus. It consists of the concentration of antibodies, duration of their income and their re-entry through the placenta. The reliability of related results is increased with the use of complex morphological, immunohistochemical and clinical data. An important factor is the degree of development of the fetus, its age and the ability to proliferative and its compensatory reactions. For example, fetopathy with maceration is most common in the period of 22-31 weeks, when these mechanisms are still insufficient and a large one-time dose of allogeneic antibodies arrives. It causes a rapid apoptosis, and maceration of all organs. Fetal death occurs in less than one day.

The action of pathogens during HDFN is a set of complex processes. Only with fetopathy with maceration a sole pathogen (set of alloantibodies) acts. They are mainly of low titer, but act quickly, by a large dose. In the other three forms of HDFN alloantibodies trigger other pathogens, creating a complex of pathological processes with different clinical manifestations. An example of such an effect is edematous form. The total titer of penetrating alloantibodies is slightly higher, sometimes a high titer appears (Table 7.3), but their penetration and action is long - from several weeks to 2-4 months. During this time, there is intense development of compensatory processes in different organs, such as formation of erythroblasts to replace the destroyed erythrocytes and previously produced erythroblasts. It happens not only in bone marrow; the production of erythroblasts, stopped at the end of I trimester restores in the liver and spleen. Sizes of both organs go up dramatically, so that spleen in some cases reaches 100-130 g - the weight which is almost typical for an adult. Liver and kidneys process and strongly neutralize indirect bilirubin released from damaged erythrocytes and erythroblasts and secrete the remains into the bloodstream of pregnant women. Hyperplasia of the myocardium is also significant. Yet the development of a very large swelling indicates a depletion of self-defense capabilities of the body of the fetus. Strong inhibition of lymphoid system leads to the fact that even such representatives of microflora as saprophytes can cause inflammation: pneumonia, omphalitis, meningitis or sepsis. Nonfunctioning lungs atrophy, so that at birth they are unable to provide the beginning of breath. A huge liver and spleen, combined with the same-sized swelling may delay the process of birth, or cause the rupture of self-tissues.

Anemic form of HDFN arises under the influence of a low dose of allogeneic antibodies, having for the most part just a small titer (1:2 - 1:16) - 76.9%. Therefore, the duration of their activity is moderately long. Inhibition lymphoid system occurs as well as the atrophy of the lungs. Pneumonia and sepsis are consequence of the mentioned above.

Icteric HDN occurs at the birth of the fetus. This is the time of breakdown of fetal and pregnant's direct relations and the termination of bilirubin extrusion into her body. Major pathogen in this scenario is the toxic effect of indirect bilirubin in the brain, liver and kidneys. Brain is most severely damaged, developing bilirubin encephalopathy. Several pathogens influence the brain. The leading role is played by indirect bilirubin, it creates kernicterus: destruction and coloring of neurons in many nuclei of the brain stem, cerebellum, and some sections of the cerebral hemispheres. Themselves alloantibodies with Rh^oD HDN do not cause destruction of neurons: in other forms of HDFN, even with the edematous form when antibodies are many and their effect is long-term, significant damage to neurons does not arise. But in the development of kernicterus two more factors play a major role. One of them is toxic pathogens that accompany the indirect bilirubin: alloantibodies with HDN, bacterial toxins in sepsis, in which bilirubin encephalopathy also happens, and some others. Smaller toxicity of alloantibodies compared to the high toxicity of bacteria causing sepsis, is expressed in the fact that kernicterus with HDN occurs at the level of indirect bilirubin in the blood of just over 25 mg%, while for sepsis the level of 15 mg% is sufficient. Another factor is in the biochemical characteristics of neurons of some nuclei and their unequal sensitivity to the toxic effects of indirect bilirubin. As a result, some nuclei with bilirubin unstained and not significantly altered neurons are located near the nuclei, where the neurons, which have bright orange-yellow color, are heavily modified (Figs 7.4-7.9).

Recognition of two variants of icteric form, often with favorable prognosis, has clinical significance. Especially, with the birth of a child revealing post-natal HDN, the pronounced pathological changes are still poorly expressed; still there is enough time for the corresponding therapeutic treatment. State of newborns with both options, including the state of their organs of immune defense, liver and heart are not difficult to establish (Table 7.7-7.8). It may give clear guidance for the application of appropriate therapeutic measures.

Abbreviation

B cells, T-cells - B lymphocytes, T lymphocytes
BBB - blood brain barrier
BFU - burst forming unit; precursors of blood cells
BFU-E - burst forming unit elytroid; precursors of erythroblasts
CD - cluster of differentiation; group of receptors' differentiation
DAF - decay acceleration factor; factors, accelerating the destruction
DNA - deoxyribonucleic acid, the components of the cell nucleus
Fab - immunoglobulin molecules that interact with antigen in the cytoplasm
Fc - immunoglobulin molecules that interact with antigens on the cell membrane
Fas, FasLigand, TNF, p53, bcl2 - components involved in apoptosis
Fc gamma RII, RIII, (CD32, CD16) – phagocytes, receptors for immunoglobulins G
HDFN - hemolytic disease of the fetus and newborn
HDF - hemolytic disease of fetus
HDN - Hemolytic disease of newborn
HIV - human immunodeficiency virus
HLA-D, HLA-G - major histocompatibility complex of class 2, see MHC
Igs - immunoglobulins
IgG, IgA, IgM - immunoglobulin G, A, or M
Interleukins IL - factors that carry out the interaction between cells in the immune or inflammatory processes
IL2Rα (CD25) - receptor of interleukin 2
IUGR - intrauterine growth retardation
J-joining chain, J-chain - the receptor, which injects substances into cell, especially immunoglobulins; endocytosis
Ki67 – detects proliferation; cell preparing to divide
MCP - membrane cofactor protein; a protein that disrupts membrane
MHC - major histocompatibility complex; a complex of genes that are unique to each organism. There are two classes: MHC-I (HLA-A, HLA-B, HLA-C) and MHC-II (HLA-D and HLA-G – several options)
NK - natural killers; group of lymphocytes that execute immune cell responses
PR - pregnancy rests; termination of pregnancy
Rh^oD - one option of antigens, most often causing HDFN
RNA - ribonucleic acid
RPL - recurrent pregnancy loss
SC - secretory component, part of polyimmunoglobulin receptor executing exocytosis - an extraction of immunoglobulins from the cell
SIS - secretory immune system
TOP - termination of pregnancy; abortion
VEGF - vascular endothelial growth factor
VPF - vascular permeability factor

CD marker antigens

CD3 - T lymphocytes
CD4 - T helper cells
CD8 - T cytotoxic lymphocytes, killer cells
CD14, CD33, CD68, CD111 - various macrophages, monocytes, promonocytes, mielomonocyte cells
CD16, CD32 - receptors Fc gamma RIII, RII
CD20 - B lymphocytes, but not plasma cells
CD25 - interleukin IL2Rα, growth factors of T lymphocytes
CD31 - endothelium, macrophages, killer cells
CD34 - myelomonocytic, lymphoid stem cells, capillary endothelium
CD45LCA - common antigen of leukocytes, monocytes of adults, but negative in embryos
CD56 - marker of natural killers cells and partially CD3 lymphocytes
CD79A - B lymphocytes and plasma cells

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