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## **Immunohistochemical features of the fallopian tubes' structure in case of fetuses with a gestational term of 21-28 weeks from mothers with a chronic infection of the lower genital tracts**

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### **Abstract**

In the following article we are disclosing immunohistochemical features of the fallopian tubes' structure in case of fetuses with a gestational term of 21-28 weeks. We have studied the structure of organs of 15 fetuses from mothers with a chronic infection of the lower genital tracts (HILGT) (the group of comparison) relatively to one in case of 15 fetuses from mothers with a physiological pregnancy (the main group). All fetuses had a gestational term of 21-28 weeks and had died intranatally as a result of an acute disorder of uterine-placental circulation. Methods of research: organometric, histological, immunohistochemical and statistical. The complex research allowed to reveal a probable decrease of the organometric data as well as indicators of thickness of the main structural components of the organs' wall in case of fetuses from mothers of the group of comparison relatively to ones in case of fetuses from the main group. By applying histological method we had revealed a disorder in the structure of mucous and muscular membranes in the structure of organs of fetuses from mothers with a pathology of pregnancy, which is manifested by decreasing of number of folds, uneven thickness of the mucous membrane as well as disorder in formation

of the muscular membrane' layers. Immunohistochemical method has revealed an increase of apoptotically changed mucosal cells in the fallopian tubes of fetuses from mothers with HILGT. By applying MCAT to endotheline-1 we have revealed an increased glow of endothelial cells in vessels both of arterial and venous types in the structure of the organs' wall in case of fetuses from the group of comparison. During the study on the endocrine activity of organs of fetuses from mothers with complicated pregnancy we have postulated a probable decrease of the cells' glow' intensity towards MCAT of progesterone. At the same time, there is also an inflammatory infiltration in the mucous membrane of the fallopian tubes' wall. The aforementioned changes in the structure as well as in the functional activity of fetuses' organs from mothers' with HILGT are formed under the influence of chronic hypoxia and endocrine insufficiency of the feto-placental complex, which is taking place in case of this pathology. Histological and immunohistochemical features of the fallopian tubes' structure in case of fetuses from mothers with complicated pregnancy are indicating a disorder in formation and maturing of the organ as well as they could lead to development of ectopic pregnancy and tubal infertility in the subsequent ontogenesis.

**Key words: fetus; pregnancy; fallopian tubes; chronic infection; endotheline-1; collagen.**

**The relevance of the following topic** is prescribed by the ever-increasing percent of infertility among women, who were born from mothers with a complicated pregnancy [1, 5]. Moreover, there is a direct connection between existence of the extragenital pathology during pregnancy from the one side and insufficiency of the germinative function of the women's organism in case of offsprings, which was described [2, 4]. According to the data from the modern publications the pregnancy complications, including chronic infection, lead to hypoxia in the mother-placenta-fetus system and stimulate death of the embryonic and fetus' cells later as a result of activation of apoptosis [3, 6, 7]. First of all, it applies to organs and tissues, the formation and implementation of the functional activity of which is taking place during the early periods of intrauterine development [8, 9, 10]. Among these organs we could also point out female genitals of the fetus [11, 12]. As it is known, in case of women, who were born from mothers with complicated pregnancy, more often we could notice a development of the ectopic tubal pregnancy as a result of organ obstruction, as well as a disorder in its peristalsis [13, 14]. The function of fallopian tubes is not restored even after leading appropriate physiotherapy procedures [15]. Despite everything, what was mentioned above, immunohistochemical features of the fallopian tubes' structure in case of fetuses from

mothers with a chronic infection of the lower genital tracts on different stages of the intrauterine development has not been studied yet.

**The main aim of the research** is to reveal immunohistochemical features of the fallopian tubes' structure in case of fetuses with a gestational term of 21-28 weeks from mothers, whose pregnancy was complicated by a chronic infections of the lower genital tracts.

**The material of the research were** fallopian tubes of 15 fetuses from mothers with physiological pregnancy (the main group) as well as organs of 15 fetuses from mothers, whose pregnancy was complicated by a chronic infection of the lower genital tracts (the group of comparison). All fetuses were in their gestational term of 21-28 weeks.

According to the results of clinic and laboratory research of the lobes of the internal organs of fetuses and placenta (bacteriological and biochemical examination, polymerase chain reaction), the most common infections were caused by herpes virus, cytomegalovirus infection, enterococcus, chlamidial infection, and a combination of cytomegalovirus and chlamidial infections; chlamidial infections and excherichia coli; herpes virus and cytomegalovirus infection.

All fetuses had died intranatally and antenatally as a result of acute uterine-placental circulatory disorders (premature detachment of the normally located placenta), as well as placental-fetal blood circulation (umbilical cord pathology).

Examinations of the fetuses from the main group revealed signs of the photometric disproportion, subcutaneous lymphadema and ascites.

Antropometric indicators of the fetuses from the group of comparison reached the following values: body weight  $0,538\pm 0,019$  kg, body length  $0,26\pm 0,04$  m, the coefficient of harmony  $19,84\pm 3,61$  kg/m<sup>3</sup>. In the main group the body weight of fetuses reached the following data:  $0,548\pm 0,195$  kg, body length –  $0,24\pm 0,02$  m, the coefficient of harmony –  $18,07\pm 0,63$  kg/m<sup>3</sup>.

By analyzing antropometric indicators of fetuses from the studied groups, we can come up with the following conclusion: the body weight of fetuses from mothers with a complicated pregnancy is clearly bigger, while the body length and the coefficient of harmony are clearly lower, that the same indicators in case of fetuses from healthy mothers. Moreover, the increase of the body weight as well as the decrease of the body length are confirmations of the photometric disproportion in the main group.

**The research methods:** macroscopic, organometric, histological, immunohistochemical, morphometric, statistical.

Histological methods included staining by hemotoxylin and eosin, as well as staining by the method of van Gieson [16, 17].

The study on specimens, that were stained by histological methods, as well as morphometric research had been performed on the Olympus BX-41 microscope using Olympus Db-soft (Version 3:1) software [18, 19, 20].

The immunohistochemical study was held by applying a direct Koons method with a modification of M. Brosman (1979) with using MCAT to collagens of the I, III and IV types, to endothelin-1 and CD 95 (Chemicon International Inc., Temecula California), as well as by peroxidase method [11, 12]. The specimens that had been treated by a direct Koons method were studied on fluorescent “Axioskor 40” (Carl Zeiss, Germany) microscope. The optical density of immunofluorescence of collagens of the I, III and IV types, endothelin-1, as well as a number of apoptotically altered eggs, were identified by applying a method of G.I. Gubina-Vakulik and co-authors (G.I. Gubina-Vakulik, I.V. Sorokina, V.D. Markovskiy, L.S. Kupriianova, R.V. Sydorenko. Method of quantitative determination of antigen content in biological tissues. Patent for utility model № 46489; CO 1 № 33/00 from 25.12.2009, Bulletin № 4). The researches were held on “Axioskor 40” microscope using Biostat.exe. software.

The evaluation of immunohistochemical reactions with treatment of specimens by MCAT to estrogen and progesterone were held by taking into account both the intensity of staining, as well as establishing a percentage of immunopositive cells relatively to a general number of cells [21]. As MCAT an estrogen-receptor alfa (ER), (DAKOcloneEP1) and progesterone-receptor (PR), (DAKOclonePgR 636) had been used.

The statistical processing of an obtained data was held on the personal computer using statistical packages „Excell for Windows”, „Statistica 7.0. for Windows”, „SigmaStat 3.1. for Windows”<sup>1</sup> [20, 21].

### **The results, that were obtained and discussion**

In all cases we have identified two fallopian tubes, that were situated in the pelvic cavity in vertical or oblique direction on a gestational term of 21-23 weeks as well as were situated in an almost horizontal direction on a gestational term of 24-28 weeks.

The shape of fallopian tubes was close to straight and curved. Each tube, despite of being right or left, had from 2 to 4 constrictions. The most clear constriction could be noticed between the ampoule and isthmus of the organ.

In all organs we could clearly separate the following components: the ampullary part, the isthmus and the uterine part. In most of observations the length of the right fallopian tube was clearly longer, than one in case of the left organ. The average indexes of weight, length

and thickness of the fallopian tubes' wall of organs from the study groups are provided in the Table 1.

Table 1. The average organometric indexes of the fallopian tubes of fetuses from the study groups

Group	Weight, kg	Length of the right fallopian tube, m	Length of the left fallopian tube, m	Thickness of the wall, m
Main group	$1,28 \pm 0,04 \times 10^{-3}$	$23,14 \pm 0,81 \times 10^{-2}$	$22,09 \pm 0,77 \times 10^{-2}$	$1,75 \pm 0,06 \times 10^{-3}$
Group of comparison	$1,17 \pm 0,04 \times 10^{-3*}$	$22,07 \pm 0,78 \times 10^{-2*}$	$21,14 \pm 0,74 \times 10^{-2*}$	$1,59 \pm 0,06 \times 10^{-3*}$

Note.  $p \leq 0,05$

By analyzing the data from the Table 1 we could come up with the following conclusion: the organometric indexes of the fallopian tubes in case of fetuses from mothers with HILGT are exactly decreased relatively to ones in case of fetuses from the healthy mothers. The indexes of length of the right fallopian tube are higher, than ones in case of the left organ in all cases.

The review of preparations, that had been stained by hematoxylin and eosin, revealed the following facts: namely, the main structural components of the fallopian tubes' wall are mucous, muscular and serous membranes, and the boundary between them could be clearly defined. The average indexes of components are provided in the Table 2.

Table 2. The indexes of thickness of structural components of the fallopian tubes' wall in the study groups

Group	Thickness of the mucous membrane, m	Thickness of the muscular membrane, m	Thickness of the serous membrane, m
Main group	$0,55 \pm 0,02 \times 10^{-3}$	$0,85 \pm 0,03 \times 10^{-3}$	$0,35 \pm 0,01 \times 10^{-3}$
Group of comparison	$0,47 \pm 0,02 \times 10^{-3*}$	$0,80 \pm 0,03 \times 10^{-3*}$	$0,32 \pm 0,01 \times 10^{-3*}$

Note.  $p \leq 0,05$

The indexes, that are presented in the Table 2 are indicating a clear decrease of thickness of all structural components of the fallopian tubes' wall in case of fetuses from the group of comparison relatively to ones in case of the main group. Moreover, there is a noticed

predominance of the muscular component in the fetuses' fallopian tubes' wall in all observations.

By comparing data from the Tables 1 and 2 we could say, that the thinning of the fallopian tubes' wall in case of fetuses from mothers with a complicated pregnancy explains a decrease of indexes of the main structural components relatively to ones in case of fetuses from healthy mothers.

Our attention was attracted by changes in thickness of the mucous membrane in organs of fetuses from the main group relatively to the group of comparison. Thus, if the thickness of the musocus membrane in fallopian tubes in case of fetuses from healthy mothers is similar throughout, then in case of organs of fetuses from mothers with a complicated pregnancy, in the isthmus area this thickness is thin, while in the funnel and ampoule area is expanded.

By applying MCAT we were able to reveal a predominance of the collagen of the I type in structure of connective tissue of submucosal layer in organs of fetuses from the main group, as well as an intensive luminiscence of the collagen of the III type in the structure of mucous membrane of the fallopian tubes in case of fetuses from the group of comparison.

The folds of the mucous membrane in organs of fetuses from mothers with a physiological pregnancy are well expressed with an average number of 20-30. Every primary fold has features of formation of the secondary folds, what is corresponding with this stage of the fetal development. The mucous membrane of the fallopian tubes in case of fetuses from mothers with HILGT is forming from 18 to 27 folds without clear features of formation of the secondary folds. As in the structure of the submucous layer, as well as in the structure of folds of the mucous membrane in case of fetuses fom the group of comparison we could notice a predominance of the collagen of the III type relatively to the structure of fetuses' organs in the main group.

By applying immunohistochemical method with with using MCAT to CD95 we had revealed, that the average index of the apoptotic index in the fallopian tubes of fetuses from the main group reaches  $10,07 \pm 0,35$  %, while in case of fetuses' organs from the group of comparison -  $14,48 \pm 0,51$  %. Moreover, it is noteworthy, that the cells of secretory type are prevailing among apoptotically altered forms.

In the mucous membrane of fallopian tubes of fetuses from mothers with HILGT we could reveal a diffuse cellular infiltration of lymphocytes, plasma cells and single leukocytes, by which a presence of features of the chronic inflammation in the fetal organs is indicated.

The muscular membrane of the uterine wall in case of fetuses from mothers with a physiological pregnancy is represented by the inner circular and outer longitudinal layers, that consist of smooth muscle tissue. The thickness of the muscular membrane varies depending on the part of organ: thus, it increases from the funnel of tube to the isthmus. In the thickness of the muscular layer we could identify a great number of thin-walled vessels with a moderate blood supply. We have to notice, that between main layers of the muscular fibers there is a plenty of strands of myocytes, that have an oblique direction. It means, that this is an implementation of the third layer, that exists in organs of adult women.

The structure of the muscular membrane of organs' walls in case of fetuses from mothers with a complicated pregnancy differs on different gestational terms. Thus, on the term of 21-23 weeks there is only one layer of muscular fibers: circular one. These myocytes form specific bundles, that are closely intertwined between each other, and are directed in oblique and longitudinal directions. On the gestational term of 24-28 weeks muscular component is represented by two layers of muscular cells in circular and longitudinal directions, with an existence of some cells directed in oblique direction as well. Oppositely to the muscular layer of the organs' wall in case of fetuses from the main group, there is a massive growth of the connective tissue in the structure of the fallopian tubes' component in case of fetuses from the group of comparison.

In all cases we have studied an endothelin-producing activity of the vascular component of fetuses' organs. The indicators of glow of endotheliocytes in vessels both of arterial and venous types are provided in the Table 3.

Table 3. The indicators of glow of the endothelin-1 in vessels both venous and arterial types in fallopian tubes of the study groups (conv.un.opt.dens.)

Group	Vessels of arterial type	Vessels of venous type
Main group	0,33±0,01	0,48±0,02
Group of comparison	0,38±0,01*	0,53±0,02*

Note.  $p \leq 0,05$

According to the data from Table 3, in fetuses' organs from the study groups we could emphasize an increasing of glow of the endothelin-1 in vessels of venous type comparing to the same indexes in case of vessels of arterial type. In the vascular component of fallopian

tubes in case of fetuses from mothers with HILGT there is a probable increase of glow of endotheliocytes comparing to organs' vessels in case of fetuses from healthy mothers.

The serous membrane of fetuses' fallopian tubes from all study groups is represented by fibrous connective tissue. By applying immunohistochemical method we have revealed, that the structure of connective tissue in fetuses' organs in case of healthy mothers is represented mainly by the collagen of the I type, while in case of fallopian tubes of fetuses from mothers with a complicated pregnancy - it is represented as by the collagen of the I type, as well as by the collagen of the III type.

By applying MCAT to estrogen and progesterone we were able to postulate, that there are the following features of the hormone-producing function in the fetuses' fallopian tubes. Namely: in all cases we could notice a negative reaction while treating preparations by MCAT to estrogen. In organs of fetuses from the main group there is a moderate reaction to progesterone (+++, up to 50% of cells of the mucous membrane of the fallopian tube). In the fallopian tubes of fetuses from the group of comparison the reaction to progesterone was decreased - ++, up to 40% of cells of the mucous membrane.

Thus, we had studied immunohistochemical features of the fallopian tubes' structure in case of fetuses with a gestational term of 21-28 weeks. Specific features of the organs' structure in case of fetuses from mothers with a physiological pregnancy, that were postulated above, are corresponding with physiological norm [20, 21]. In organs of fetuses from mothers, whose pregnancy was complicated by HILGT, we have revealed a probable decrease of organometric indexes, thickness of the main structural components of the fallopian tubes' wall, which is determined by the chronic hypoxia under the influence of which fetal organs have been forming [22, 23]. Moreover, the increase of apoptotic index among secretory cell of the mucous membrane also takes place, what is stimulated both by hypoxia and infectious agents [22, 24]. Among structural features of the mucous membrane structure in fallopian tubes of fetuses from mothers with a complicated pregnancy we could name an uneven thickness as well as a decreased number of folds, and in case of muscular one - a disorder of muscular fibers' layers formation. The changes towards mucous and muscular membranes, that were described, are indicating immaturity of the organ as well as inconsistency between its structure and gestational term [10, 13]. By applying immunohistochemical method we have revealed a predominance of the young collagen of the III type as a component of the connective tissue, what could be a manifestation of disorder in maturing of main types of collagen under the influence of the chronic hypoxia [20, 24]. By applying MCAT to endotheline-1 we have revealed an increase of endotheliocytes' glow in



vessels both of arterial and venous types in organs of fetuses from the group of comparison. Endotheline-producing activity on the background of the chronic infection could be determined by changes in the vascular component of placenta as well as it could be stimulated by viral agents [22, 24]. The hormone-producing activity in organs of fetuses from mothers with HILGT is probably clearly decreased relatively to one in fallopian tubes of fetuses from mothers with a physiological pregnancy, that could be explained by decrease in number of secretory cells of the mucous membrane on one hand, as well as by endocrine disorders in fetoplacental complex from on the other hand [22, 25].

### **Conclusions**

1. The location of fallopian tubes as well as their separation into main structural components were typical in all cases. However, the organometric indexes of weight, length and thickness of the organs' wall in case of fetuses from mothers with HILGT were clearly decreased comparing to the same indexes in case of fetuses from healthy mothers.

2. In all observations the wall of fallopian tubes was represented by three main structural components, when as the predominant one we could name - muscular component. Moreover, in case of organs of fetuses from mothers with HILGT we could point out a decrease of thickness' indexes towards all components of wall comparing to ones in case of fetuses from healthy mothers.

3. The thickness of mucous membrane of the fallopian tubes in case of fetuses from mothers with a complicated pregnancy is different throughout, that could further contribute to ectopic pregnancy' development.

4. The average index of numbers of primary folds in mucous membrane in case of fallopian tubes of fetuses from mothers with HILGT is clearly decreased comparing to one in case of organs of fetuses from mothers with a physiological pregnancy. Moreover, in case of organs of fetuses from the group of comparison there is no clear features of the secondary folds' formation in the mucous membrane. These postulated changes could further interfere passaging of the egg through fallopian tubes.

5. By applying immunohistochemical method we have postulated an increase of apoptotic index in mucous membrane of fallopian tubes of fetuses from mothers with HILGT comparing to one in case of organs of fetuses from healthy mothers due to secretory cells.

6. In the mucous membrane of fallopian tubes of fetuses from mothers with a complicated pregnancy there is a diffuse lymphocytic-plasmacytic infiltration, that is a feature of the chronic inflammatory process in fetal organs.

7. The structure of muscular layer of the fallopian tubes' wall in case of fetuses from the group of comparison differs on different gestational terms. Namely: on the 21st-23d weeks there features of disorder in the layer's maturing, when on the 24th-28th weeks it begins to form all necessary components. In addition, in the muscular layer the massive growth of the connective tissue between bundles of muscles' fibers takes place.

8. In vessels both of arterial and venous types of organs of fetuses from the group of comparison we could notice a probable increase of specimens' glow, that had been treated by MCAT to endotheline-1 towards one in fallopian tubes of fetuses from the main group. It could be a manifestation of chronic hypoxia, which takes place under the conditions of HILGT as well as could further lead to disorder in organ's blood supply.

9. In the structure of connective tissue of the serous membrane of fallopian tubes of fetuses from mothers with a complicated pregnancy we have postulated an existence of disorder of the collagen maturing as well as a predominance of the collagen of the III type comparing to the layer's structure in case of fetuses from healthy mothers.

10. By applying MCAT to estrogen and progesterone we have revealed, that in all case there are a negative reaction to estrogen as well as a probable decreased reaction to progesterone in organs of fetuses from mothers with a complicated pregnancy.

11. All structural and functional changes in fallopian tubes of fetuses from mothers with HILGT, that had been revealed, are determined, first of all, by a chronic hypoxia, which is stimulated by the infectious agent and a chronic placental insufficiency, that take place in case of this pregnancy's pathology.

12. All specific features of the mucous and muscular membranes' structure of fallopian tubes of fetuses from mothers with a complicated pregnancy, that had been revealed, could further lead to development of the ectopic pregnancy as well as could stimulate forming of the tubal infertility.

**Perspectives of the further research:** to postulate immunohistochemical features of the fallopian tubes' structure in case of fetuses with a gestational term of 29-36 weeks and 37-40 weeks from mothers with a chronic infection of the lower genital tracts comparing to ones in case of fetuses from healthy mothers.

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