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Immunohistological features of the collagen formation in ovaries of fetuses from mothers with a physiological pregnancy on different gestational terms

L. S. Kupriianova, S. A. Ivanchenko

The International Humanitarian University, Odessa Medical Institute, Odessa, Ukraine

Abstract

The comprehensive study on the fetal ovaries allowed us to disclose main features of the collagen synthesis in different organ's components relatively to the gestational term of fetus. We have studied the ovaries' structure in case of fetuses, who died both antenatally and intranatally. The mothers of fetuses, according to the medical documentation, were healthy. As a reason of the fetuses death we can name an acute violation of the utero-placental as well as placental-fetal circulation. According to the specific features of the organs' development, all fetuses were divided into groups: 21-28 weeks, 29-36 weeks, 37-40 weeks. The research methods: macroscopic, morphometric, histological, immunohistochemical, statistical.

We have described a histological structure of the ovaries as well as we have postulated, that this structure corresponds completely with the physiological norm in every term of gestation. By applying the immunohistochemical method we have postulated, that the collagen of the I type prevails in the collagen structure of the connective tissue of fetal organs. It is manifested as an intensive glow. As the same time, the collagen of the III type is manifested as the small foci of moderate intensity. There are two types of collagens in the

walls of blood vessels: IV and III ones. Moreover, we can preferably notice the collagen of the IV type. The collagen of the III type, at the same time, is manifested by foci with a low intensity of glow. All specific features of the collagen formation in the structure of the connective tissue, that were described above, are the same with ones which are corresponding with the physiological norm. The histological and immunohistochemical features of the ovaries' structure, in case of fetuses from the study groups, could be applied as a comparison in a study on the organs' structure in case of fetuses from mothers with a complicated pregnancy.

Key words: fetus; pregnancy; ovary; blood vessel; connective tissue; collagen.

Імуногістохімічні особливості колагеноутворення в яєчниках плодів від матерів з фізіологічною вагітністю у різні періоди гестації

Л. С. Купріянова, С. А. Іванченко

**Міжнародний гуманітарний університет, Одеський медичний інститут, м. Одеса,
Україна**

Комплексне дослідження яєчників плодів дозволило виявити основні особливості синтезу колагенів в різних компонентах органу залежно від строку гестації плода. Вивчали структуру яєчників плодів, що загинули антенатально та інтранатально. Матері плодів, відповідно до медичної документації, були здорові. Причиною загибелі плодів було гостре порушення матково-плацентарного та плацентарно – плодового кровообігу. Відповідно до особливостей будови органів, всі плоди було поділено на групи: 21-28 тижнів, 29-36 тижнів та 37-40 тижнів. Методи дослідження: макроскопічний, морфо метричний, гістологічний, імуногістохімічний та статистичний. Описано гістологічну будову яєчників плодів та показано, що вона повністю відповідає фізіологічній нормі для кожного періоду гестації. Імуногістохімічним методом встановлено, що в структурі колагену сполучної тканини органів плодів превалює колаген I типу. Він виявляється у вигляді інтенсивного світіння. В той час як колаген III типу визначається малими осередками помірної інтенсивності. В стінках судин мають місце два типи колагенів: IV та III. Причому переважно визначається колаген IV типу. Колаген III визначається у вигляді осередків низької інтенсивності світіння. Описані особливості утворення колагенів в структурі сполучної тканини та стінках

судин відповідає таким, що відповідають фізіологічній нормі. Гістологічні та імуногістохімічні особливості будови яєчників плодів досліджуваних груп можуть бути використані в якості порівняння при вивченні будови органів плодів від матерів з ускладненою вагітністю.

Ключові слова: плід; вагітність; яєчник; судина; сполучна тканина; колаген.

Introduction. The ovaries are the leading organs, which are coordinating an activity of the female reproductive system [1, 2]. First of all, it is related to the hormonal activity of organs, which is regulating the development, stabilizations as well as functional activity of the uterus and fallopian tubes [2, 3]. Due to the ovaries' functional activity there is a functioning in the endometrium as well as in the mucous membrane of fallopian tubes. It is well-known, that the implementation of the ovaries begins in the early stages of the fetus development, and is completed up to the period of childbirth [4, 5]. Moreover, the pools of eggs, which are being formed during the fetal period of development, are stable and cannot be renewed in the subsequent ontogenesis of the female organism [6, 7]. Thus, the stabilization of germinal function of ovaries, that determines the main function of the female organism - childbirth, occurs in utero [8, 9, 10]. It will be reasonable to assume, that under the condition of the influence of pregnancy's pathology, some violations in the implementation and formation of the fetal gonads could take place. However, in the aim of postulating changes in the structure of ovaries, we have to provide a clear description of histological and immunohistological features of the ovaries' structure in case of fetuses from mothers with a physiological pregnancy. On the current stage of the medical science' development the physiological structure of fetal gonads on different terms of gestation has not been studied yet. That is why the aforementioned issue became the aim of our current study.

The aim of the research: to disclose main features of the collagen formation in ovaries of fetuses from healthy mothers on different stages of the fetal development.

The study material: as the study material we have taken fetal ovaries. During the observative histological research it was postulated, that depending on structure features, all cases are supposed to be divided according to the gestational term: 21-28 weeks, 29-36 weeks, 37-40 weeks. According to those terms, we have provided divisions towards the study material. The total number of cases was 54. All fetuses have died intranatally and antenatally, as a result of an acute disorders in utero-placental and placental-fetal circulation. The mothers of fetuses were healthy, according to the medical documentation.

The research methods: organometric, morphometric, histological, immunohistochemical and statistical. After the microscopic research as well as measuring the main organs' parameters, we have cut 3 slices from each ovary for histological and immunohistochemical research so that all components could be put into the specimen. The material has been treated by an alcohol of increasing concentration and then had been put into the paraffin. We made 5-7 sections from blocks 3-5 μm thick, as well as it has been stained by histological (hematoxylin and eosin) and immunohistochemical methods (by applying MCAT to collagens of the I, III and IV types).

The study on specimens has been delivered by applying Olympus BX-41 microscope with the use of Olympus Db-soft software (Version 3:1) [11, 12]. The immunohistochemical study has been carried with an application of the direct Koons method in the M.Brosman modification (1979) with an application of MCAT to collagens of the I, III and IV types (Chemicon International Inc., Temecula California), as well as with peroxidase method [13,14].

The statistical data processing was performed on a personal computer using statistical packages „Excell for Windows”, „Statistica 7.0. for Windows”, „SigmaStat 3.1. for Windows” [15]. The verification of the distribution for compliance with the Gauss' law was performed using the Shapiro-Wilk or χ^2 Pearson criteria [15].

Research results and its discussion: According to the gestational term of the ovaries their location and shape were different. Thus, in case of the gestational term of 21-28 weeks, ovaries were located near the side wall of the pelvis horizontally, their shape of the right one was triangular, flattened, while the left one was oblong and oval. In case of fetuses with a gestational term of 29-36 weeks, the location of the organs was similar to the ones on the gestational term of 21-28 weeks, while the gonads' shape was oval or ribbon-like. In case of the gestational term of 37-40 weeks the ovaries were located near the lateral wall of the pelvis longitudinally, the shape of gonads was oval, rounded or triangular-prismatic. Macroscopically, all gonads were bluish-white with a smooth surface. The tissue on a section was grayish-pink, with homogeneous structure. The size of the right ovary prevailed in all cases comparing to the size of the left one. Main organometric indexes of gonads on different gestational terms are provided in the Table 1.

The data from the Table 1 reveals an increase of organometric indexes of fetal organs with an increase of the gestational term.

Table 1**Organometric indexes of the fetal ovaries on different gestational terms**

Gestational term	The ovary's weight, kg	The ovary's length, m	The ovary's width, m	The ovary's thickness, m	The ovary's volume, m ³
21-28 weeks	1,89±0,02 x10 ⁻³	1,85±0,03 x10 ⁻²	1,78±0,06 x10 ⁻²	1,31±0,02x 10 ⁻²	0,039±0,001
29-36 weeks	2,85±0,07 x10 ⁻³ *	2,34±0,08 x10 ⁻² *	2,17±0,07 x10 ⁻² *	1,34±0,04x 10 ⁻² *	0,067±0,002*
37-40 weeks	3,06±0,11 x10 ⁻³ *	2,59±0,09 x10 ⁻² *	2,45±0,08 x10 ⁻² *	1,48±0,05x 10 ⁻² *	0,090±0,003*

* Note $p \leq 0,05$

All gonads were covered with a protein shell, with a structure represented by thin collagen fibers. Under the protein shell there are germ cells, collected into the lusters, forming islets. Some germ cells are apoptotically altered.

The average number of germ cells as well as their apoptotically altered forms is provided in the Table 2.

Table 2**An average number of germ cells as well as their apoptotically altered forms in the fetal ovaries on different gestational terms**

Gestational term	Number of germ cells (specimens in the sight)	Number of apoptotically altered forms/ %
21-28 weeks	44,89±1,06	22,72±0,80
29-36 weeks	21,48±0,77 *	27,65±0,80 *
37-40 weeks	18,94±0,67 *	31,64±1,12 *

*Note $p \leq 0,05$

By analyzing the data from the Table 2, we can come up with a conclusion, that the number of germ cells is decreasing relatively to the growth of the gestational term, while the number of apoptotically altered forms is growing under the same conditions.

All ovaries are represented by cortical and cerebral substance, while the border between them: in case of the gestational term of 21-28 weeks is conditional, and on the subsequent gestational terms is clear. In all cases the cortical substance prevails relatively to the cerebral one. On the gestational term of 21-28 weeks the cortical layer is represented by primordial and primary follicles. Primordial follicles contain an oocyte, which is surrounded

by squamous epithelial cells and connective tissue. Primary follicles are represented by an oocyte, which is surrounded by the follicle epithelium cells. The nuclei in primary oocytes are stained more intensively comparing to ones in case of primordial follicles.

In case of fetuses with a gestational term of 29-36 weeks, primordial follicles are represented by an oocyte, with a nucleus containing euchromatin and which is surrounded by folliculocytes. Primary follicles contain a growing oocyte, with a transparent zone of couple of layers of cubic folliculocytes and a basal membrane all around.

In case of gonads of fetuses with a gestational term of 37-40 weeks the primordial follicles with a normal structure as well as on various stages of atresia are appearing. Nuclei in the altered follicles contain oocyte with a low euchromatin content, surrounded by flattened folliculocytes. The oocytes in the primary follicles are surrounded by a follicle epithelium and contain a large number of nucleoli. There are also few primary follicles on different stage of development, single of them have a cavity, atresia phenomena or cystic degeneration.

By applying the immunohistochemical method with MCAT to collagens of main types we have postulated that in the structure of basal membrane of primordial follicles the collagen of the IV type prevailed as a linear glow of a moderate intensity. In the meanwhile, the collagen of the III type is represented by separate small centers with a decreased glow intensity. The average indexes of glow of the collagen of the IV and III types in the fetal ovaries on different stages of pregnancy are represented in the Table 3.

Table 3

The indexes of glow intensity of the collagen of the IV and III types in basal membranes of ovaries' follicles in case of fetuses with different gestational terms (conv.un.opt.dens.)

Gestational term	Glow intensity of the collagen of the IV type	Glow intensity of the collagen of the III type
21-28 weeks	1,57±0,05	0,42±0,01
29-36 weeks	1,63±0,06 *	0,48±0,02 *
37-40 weeks	1,67±0,06	0,52±0,02

* Note $p \leq 0,05$

The data, which was provided in the Table 3 is revealing a prevalence of the collagen of the IV type in the structure of basal membranes of primordial follicles. Moreover, there is direct clear connection between glow intensity of this collagen's type from one side, and the gestational term of fetus from another one.

By applying the peroxidase method we have presented the following reaction's intensity in the basal membranes of follicles: on the gestational term of 21-28 weeks - the collagen of the IV type - overreaction (+++); the collagen of the III type - moderate reaction (++) on the gestational term of 29-36 weeks as well as 37-40 weeks: the collagen of the IV type - overreaction (+++), the collagen of the III type - weak reaction (+). By taking into account the aforementioned data we could come up with a following conclusion: from 21st to the 40th weeks of pregnancy, in case of fetuses, there is a maturing of the main collagens' types with a subsequent prevalence of the mature collagen of the IV type.

The cerebral layer is narrow, concentrated in the area of organ's gates in all cases. The cellular elements have chaotic orientation and are represented by elastic fibers, a small number of muscular elements as well as by the connective tissue. The arteries and veins of gonads are located in the cerebral substance.

In the structure of connective tissue of main structural components of ovaries we could define the collagens of the I and III types. Moreover, if the collagen of the I type is presented a linear intensive glow, the collagen of the III type is represented by separate areas of moderate intensity though. The indexes of glow of the collagens of the I and III type in a structure of connective tissue are provided in the Table 4.

Table 4

The indexes of glow intensity of the collagens of the I and III types in the structure of connective tissue of fetal ovaries on different terms of gestation (conv.un.opt.dens.)

Gestational term	Intensity of glow of the collagen of the I type	Intensity of glow of the collagen of the III type
21-28 weeks	3,78±0,13	2,64±0,09
29-36 weeks	4,12±0,17 *	2,14±0,08 *
37-40 weeks	4,48±0,16 *	1,92±0,07 *

* Note $p \leq 0,05$

The data from the Table 4 reveals a clear increase of the glow intensity in case of the collagen of the I type as well as its decrease in case of the collagen of the III type relatively to the growth of the gestational term.

By applying the peroxidase method in all cases we have postulated the intensive reaction towards the collagen of the I type (+++) as well as moderate one in case of the collagen of the III type (+) in the structure of connective tissue.

The vascular component both of cortical and cerebral substances is represented by thin-walled vessels with a moderate blood supply. The indexes of glow intensity of the collagens in vessels' walls of fetal ovaries relatively to the gestational term are provided in the Table 5.

Table 5

The indexes of glow intensity of the collagens in vessels' walls of fetal ovaries on different gestational terms (conv.un.opt.dens.)

Gestational term	Intensity of glow of the collagen of the IV type	Intensity of glow of the collagen pf the III type
21-28 weeks	1,25±0,04	0,48±0,02
29-36 weeks	1,37±0,05 *	0,44±0,02 *
37-40 weeks	1,41±0,05 *	0,40±0,05 *

* Note $p \leq 0,05$

The indexes from the Table 5 fact are revealing a fact of increase of glow intensity in case of the collagen of the IV type as well as decrease of one in case of the collagen of the III type relatively to the growth of gestational term.

By applying peroxidase method we have postulated that there is an intensive reaction of the collagen of the IV type (+++) in all cases. On the gestational term of 21-28 as well as 29-36 weeks there was a moderate reaction of the collagen of the III type (++); while in case of fetuses with a gestational term of 37-40 weeks - the reaction of the collagen of the III type was weak (+).

Thus, by analyzing specific features of the structure of ovaries in case of fetuses from mothers with a physiological pregnancy, that were presented above, we can come up with a following conclusion: the gonads are formed in accordance with anatomical and physiological norm [16, 17]. The main indexes of the structural maturation of ovaries are: a prevalence of the collagen of the I type in the structure of basal membranes of the primordial follicles and connective tissue of main structural components of fetal gonads, as well as the collagen of the IV type in the vessels' walls [18, 19, 20]. All features that were postulated above are indicating physiological maturation of the collagens, what will subsequently play the leading role in a functional activity of the girl's ovaries as well as germinative function of the female organism in the further ontogenesis [21].

Conclusions

1. The organometric research allowed us to postulate a proportional increase of main indexes of fetal organs relatively to the gestational term.

2. The observative histological research has revealed that all organs regardless of the gestational term are formed correctly and are represented by the cortical and cerebral substances.

3. The number of germ cells is decreasing relatively to the growth of the gestational term, while the number of their apoptotically altered forms is increasing, what is revealing a physiological death of eggs until the girl's birth.

4. By applying the immunohistochemical method with MCAT to the main types of the collagens as well as by the peroxidase method we have postulated the prevalence of the collagen of the IV type in the structure of basal membranes of primordial follicles, which glow intensity is increasing relatively to the gestational term.

5. In the structure of connective tissue of fetal organs on different gestational terms we have revealed the collagens of the I and III types. Moreover, we could notice an increased glow of the collagen of the I type with a simultaneous decrease of the glow intensity in case of the collagen of the III type as a gestational term increases.

6. In vessels' walls we have revealed the collagens of the IV and III types. Moreover, we can notice a relatively increased glow intensity of the collagen of the IV type as well as a clear decrease of one in case of the collagen of the III type, speaking about fetuses with a gestational term of 37-40 weeks relatively to the terms of 21-28 and 29-36 weeks.

7. All aforementioned features of the collagen formation in fetal ovaries on different gestational terms are corresponding with physiological ones.

8. All revealed features of formation of main types of the collagens in ovaries of fetuses from mothers with a physiological pregnancy could be applied as a comparison during leading a study on the gonads' structure in case of fetuses from mothers with a complicated pregnancy.

The perspectives of the subsequent research: study on immunohistochemical features of the collagen formation in the structure of connective tissue as well as vessels' walls of ovaries of fetuses on different gestational terms from mothers with a complicated pregnancy.

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