Histochemical features of the structure of fetal ovaries in different stages of gestation from mothers with physiological pregnancy

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Abstract

In the following article specific histochemical features of the fetal ovaries’ structure from mothers with the physiological pregnancy are considered. All fetals were divided into subgroups by taking into consideration the term of gestation, as well as main stages of fetal gonads’ formation and laying. Namely: 21-28 weeks, 29-36 weeks, 37-40 weeks. All fetuses had died intranatally, as a result of acute uterine-placental circulation. The course of pregnancy in all cases was psychological, according to appropriate medical documentation. In the aim of reaching the scientific goal the following research methods were used: macroscopic, organometric, histological, histochemical, morphometric, statistical. The comprehensive study has revealed the following features of the ovaries’ structure, depending on gestational term: weight, length, thickness, width and volume of the ovaries were reaching their minimum levels in case of fetuses with a gestational age of 21-28 weeks, while the maximum one was reached in case of fetuses on 37-42 weeks of gestation. All gonads are represented by cortical and cerebral matter, with the constant ratio despite of different gestational term. According to the growth of pregnancy term the number of germ cells decreases, while the number of apoptotically altered forms oppositely increases. Primarily it is because of the psychological cells’ death during ovarian formation.
The follicular component at all stages of gestation is represented by primordial and primary follicles; at the 37-40 weeks though the atretic forms and cystically altered specimens are appearing. Moreover, in accordance with the growth of the pregnancy term, the number of primordial follicles decreases, while oppositely, the number of primary ones increases. The aforementioned changes lead to the decrease in the relative volume of follicular tissue and to the increase of the relative volume of the interstitial component. By the following histochemical methods (Felgen-Rossenbeck reaction, Brache reaction, Schiff-reaction) an increase in the functional activity of the fetal ovaries was revealed, by reaching its maximum point during gestation of the 37-40 weeks. The aforementioned features correspond with gestational terms as well as with stages of the ovarian development of the fetus. That is why they could be used as a control group during leading a research on characteristical features of fetal gonads structure of fetuses from mothers with complicated pregnancies.

**Key words:** fetus; physiological pregnancy; ovary; follicle; ontogenesis.

Гістохімічні особливості будови яєчників плодів у різні строки гестації від матерів із фізіологічною вагітністю

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В статті досліджено гістохімічні особливості будови яєчників плодів від матерів із фізіологічною вагітністю. Всі плоди було поділено на підгрупи відповідно до строку гестації та основних етапів закладки і формування fetальних гонад. А саме: 21-28 тижнів, 29-36 тижні та 37-40 тижнів. Всі плоди загинули інтранатально внаслідок гострого порушення матково-плацентарного кровообігу. Перебіг вагітності у всіх випадках був фізіологічним за даними медичної документації. Для досягнення мети було використано наступні методи дослідження: макроскопічний, органометричний, гістологічний, гістохімічний, морфометричний, статистичний. Комплексне дослідження дозволило виявити наступні особливості будови яєчників залежно від строку гестації: показники маси, довжини, товщини, ширини та об’єму яєчників сягали мінімальних значень у плодів зі строком гестації 21-28 тижнів, максимальних – у термін гестації 37-40 тижнів. Всі гонади представлені корковою та мозковою речовиною, співвідношення яких незалежно від терміну гестації сягає постійних
значення. Відповідно до зростання терміну вагітності кількість статевих клітин знижується, а апоптозно змінених форм, навпаки, збільшується. Це обумовлене, насамперед, фізіологічною загибеллю клітин під час становлення яєчників. Фолікулярний компонент у всі терміни гестації представлений примордіальними та первинними фолікулами; на терміні 37-40 тижнів з'являються атретичні форми та кистозно змінені екземпляри. Причому, відповідно до зростання терміну вагітності кількість примордіальних фолікулів зменшується, а первинних – збільшується. Описані зміни призводять до зниження показників відносного об’єму фолікулярної тканини та підвищення відносного об’єму інтерстиціального компоненту. Гістохімічними методами (реакція Фельгена – Росенбека, реакція Браше, ШИК-реакція) встановлено підвищення функціональної активності яєчників плодів, яка сягає максимуму у термін гестації 37-40 тижнів. Описані особливості відповідають строку гестації та етапам розвитку яєчників плодів, тому можуть бути використані в якості групи контролю під час вивчення особливостей будови фетальних гонад плодів від матерів з ускладненою вагітністю.

Ключові слова: плід; фізіологічна вагітність; яєчник; фолікул; онтогенез.

The revelance of the topic is prescribed, first of all, by the lack of clear criateria of fetuses’ ovarian maturity on different terms of gestation. Secondly, by the ever-increasing number of pathological states of the gonads, that are developing during in utero fetal existance and lead as a result to formation of primary infertility [1, 2, 3]. More and more often, there is a question postulated about unidirectional endocrine pathology of the mother and fetus, which is manifested by the polygrandular endocrinopathy, regardless of the etiological factors, that had led to it [3, 4, 8]. The variety of the viral infection, chronical diseases, the use of medical treatment in the aim of preserving pregnancy likewise other factors are contributing to the death of the eggs due to apoptosis in the ovaries of fetus [7]. Moreover, the adverse conditions have also a negative influence on development and stability of the fetal uterus and fallopian tubes, what leads to delay in formation of the reproductive system in general, as well as complicates significantly as diagnosisal, likewise therapeutic measures in the future [9, 10]. By not taking into account characteristic features of the female genital organs’ organogenesis in case of fetuses, gynecologists become more and more often powerless towards finding out solutions in the sphere of treatment and restore of the reproductive function [6, 9]. Despite everything, what was mentioned above, currently there is no clear understanding of histochemical features of the fetuses’ ovaries structure in different
stages of the in utero fetus’ development.

**The study materials** were 15 ovaries of fetuses with the gestational age of 21-28 weeks, 15 gonads of fetuses with the gestational term of 29-36 weeks, as well as 20 ovaries from fetuses with the gestational term of 37-40 weeks.

All fetuses had died intranatally during childbirth due to the acute utero-placental circulation (detachment of the normally located placenta, as well as umbilical cord pathology).

The mothers of the study groups were healthy according to the medical documentation.

The fetuses were divided into three groups according to the gestational terms, that are coinciding with main terms of implementing and formation of the fetal ovaries.

**Research methods:** macroscopic, organometric, histological, histochemical, morphometric, statistical.

After removal organs were examined, as well as main sizes of the fetal gonads were measured. From every ovary there were 2-3 pieces cut so, that all layers of the organs could be on a section. The material was fixed in a neutral formalin buffer solution in the aim of reducing the effect on tissues. Afterwards it was performed on alcohols of increasing concentration. In 24-48 hours the material was filled with paraffin [12]. From the manufactured blocks there were 2-3 section made with a thickness of 3-5 μm, as well as it was stained by the histological (by hematoxylin and eosin) as well as histochemical methods (the Brache method, the Folgen-Rossenbeck method, the Schiff reaction).

In the aim of staining by histochemical methods pieces of tissues were fixed in the Carnois fluid (6 parts of an absolute ethanol, 3 parts of the chloroform, 1 part of the glacial acetic acid), which was prepared directly before the ovaries’ fixation. At the end, the specimens were transferred to an absolute alcohol and were filled with paraffin.

In order to determine relative volumes of the main structural components of the fetuses’ ovaries the sections were studied by morphometric methods.

The data was processed statistically on the personal computer by using following statistical packages „Excell for Windows”, „Statistica 7.0. for Windows”, „SigmaStat 3.1. for Windows” 1 [13].

**The results of the research.** The ovaries of fetuses with a gestational age of 21-28 weeks were located in the side wall of the pelvis horizontally. On a gestational term of 29-36 as well 37-40 weeks the ovaries are above, surrounded laterally and medially by the part of the fallopian tube. The tubular end of the ovary was adjacent to the parietal leaf of the
peritoneum, the mecentric end of the gonad was attached to the broad ligament of the uterus. The free end of the ovary hung down to the pelvic cavity.

The shape of the left gonad is oblong-oval, the right one is rounded. Microscopically, the ovaries are whitish with a bumpy surface. The gonad tissue is pink in the section, homogeneous. The right ovary is larger, than the left one. The average organometric parameters of the fetal gonads on different stages of gestation are provided in the Table 1.

**Table 1 - The organometric indexes of fetal ovaries in case of fetuses on different gestational terms**

| Gestational term | The ovary weight, kg x 10⁻³ | The ovary length, m x 10⁻² | The ovary width, m x 10⁻² | The ovary thickness, m x 10⁻² | The relative volume of the ovary, m³ |
|------------------|-----------------------------|----------------------------|---------------------------|-------------------------------|----------------------------------|
| 21-28 weeks      | 1.89±0.02                   | 1.85±0.03                  | 1.78±0.06                 | 1.31±0.02                     | 0.039±0.001                      |
| 29-36 weeks      | 2.85±0.07                   | 2.34±0.08                  | 2.17±0.07                 | 1.34±0.04                     | 0.067±0.002                      |
| 37-40 weeks      | 3.06±0.11                   | 2.59±0.09                  | 2.45±0.08                 | 1.48±0.05                     | 0.090±0.003                      |

Note. p≤0.05

The data in the Table 1 are showing a moderate increase of the organometric indexes of fetal gonads, that were provided, according to the gestational term. Moreover, the minimum indexes are corresponding with fetuses’ ovaries with a gestational term of 21-28 weeks, while the maximum ones are corresponding with fetuses’ organs with a gestational term of 37-40 weeks.

All organs are covered with a protein shell, the thickness indexes of which are generally reaching: in case of fetuses with a gestational age of 21-28 weeks – 24.67±0.26x10⁻³ m, in a gestational term of 29-36 weeks – 26.73±0.32x10⁻³ m, on the 37-40 weeks of the in utero development – 22.68±0.29x10⁻³ m. The structure of the protein shell is presented mostly by collagen fibers, which are staining in red-pink color by picrofuxin by van Gieson.

The germ cells in a form of clusters and islets are located under the protein shell. A part of cells are with degeneration phenomenon or in a condition of apoptosis. The average values of a general number of the germ cells, as well as apoptotically altered forms in case of fetuses in different gestational terms are presented in a Table 2.
Table 2 - The indexes of number of germ cells, as well as its apototically altered forms in case of fetuses’ ovaries in different gestational terms

| Gestational term | The number of germ cells, specimens in sight | The number of apototically altered forms, % |
|------------------|--------------------------------------------|------------------------------------------|
| 21-28 weeks      | 44.89±1.06                                 | 38.8±1.36                                |
| 29-36 weeks      | 18.94±0.67                                 | 31.64±1.12                               |
| 37-40 weeks      | 15.29±0.54                                 | 35.30±1.23                               |

Note. p≤0.05

According to the Table 2, the number of germ cells decreases by a gestational term, while the number of apototically altered forms increases. Moreover, the maximum number of germ cells could be noticed in case of fetuses’ gonads with a gestational term of 21-28 weeks, while the minimum indexes are observed in case of fetuses’ ovaries with a gestational age of 37-40 weeks. From the point of view of indexes of the apototically altered forms, there is another tendency. Namely: the indexes reach their maximum in a gestational term of 21-28 weeks, while the minimum one is reached in case of fetuses with a gestational term of 29-40 weeks.

All organs are represented by the cortical and cerebral substance, the border between which is unclear in case of fetuses with a gestational term of 21-28 weeks; is clear in case of fetuses with a gestational term from 29 to 40 weeks. The relative volumes of the cortical and cerebral substances reach on average: in case of fetuses with a gestational term of 21-28 weeks: 61.2±2.14 % and 38.8±2.25 %; in a gestational term of 29-36 weeks: 61.57±2.12 % and 38.43±1.38 %; in a period of 37-40 weeks of the in utero development: 61.12±2.10 % and 38.88±1.41 % relatively.

In a cortical layer of all fetuses we could recognize primordial and primary follicles, that are surrounded by connective tissue. Primordial follicles are represented by an oocyte, which is surrounded by squamous epitheliocytes and connective tissue membrane. Primary follicles consist of the oocyte and the cubic follicular epithelial cells.

On the gestational term of 29-40 weeks primordial follicles contain one oocyte, the nucleus of which is situated in the center and is filled with euchromatin. Flattened folliculocytes with eosinophilic cytoplasm are located around the oocyte. Primary follicles are represented by a growing oocyte, around of which there is a transparent zone of several layers of cubic folliculocytes and a basement membrane.
In ovaries of fetuses with a gestational term of 37-40 weeks primary follicles are represented by a rounded oocyte, which is surrounded by a cubic follicular epithelium. The nuclei of oocytes are larger, that in case primordial follicles, they are also saturated by euchromatin and contain a large number of nucleoli; the karyolemma is clearly contoured. There are also follicles on different stages of development, even to the cavity ones, that could be noticed. In the cells of the follicular epithelium of such forms there are mitoses, as well as vacuolation and lysis of the cytoplasm, that are taking place. The final stage of these forms of follicles’ development is atresia or cystic degeneration.

The indexes of follicles’ number in ovaries of fetuses on different gestational terms are provided in the Table 3.

Table 3 - The indexes of follicles’ number in the ovaries of fetuses on different gestational terms, specimens in sight

| Gestational term | Primordial follicles | Primary follicles |
|------------------|----------------------|-------------------|
| 21-28 weeks      | 31,63±1,11           | 35,70±0,02        |
| 29-36 weeks      | 28,07±0,98           | 36,01±0,21        |
| 37-40 weeks      | 24,25±0,49           | 38,17±0,36        |

Note.  p≤0,05

Thus, we could come up with a solution, that by increasing of a pregnancy term the number of primordial follicles decreases, while the number of primary ones, oppositely, increases. What is more, in the ovaries of fetuses with a gestational term of 37-40 weeks, the atretic and cystically atered follicles are appearing.

The gonads of all fetuses are represented by an interstitial and follicular tissue. The average indexes of relative volumes of main strucutral components of ovaries are presented in the Table 4.

The indexes in the Table 4 are disclosing the fact of increasing of relative volume of an interstitial tissue, as well as decreasing the relative volume of the follicular tissue in gonads of fetuses on terms from the 21st to the 40th weeks of in utero fetus’ development.

The intensity of reaction towards DNP and RNP, in case of germ cells of fetal ovaries, was postulated by the histochemical methods. The average results are presented in Tables 5, 6 and 7.
Table 4 - The indexes of relative volumes of the main structural components of fetuses’ ovaries on different gestational terms, %

| Gestational term | Interstitial tissue | Follicular tissue |
|------------------|---------------------|------------------|
| 21-28 weeks      | 26,4±0,61           | 73,6±2,54        |
| 29-36 weeks      | 29,60±1,03          | 70,41±2,46       |
| 37-40 weeks      | 32,82±1,15          | 67,28±2,35       |

Note. p≤0,05

Table 5 - The indexes of an optical density of DNP and RNP in structural elements of fetuses’ ovaries in gestational term of 21-28 weeks

| The structure of ovary | The optical density of DNP in the cells’ nuclei | The optical density of RNP in the cells’ cytoplasm |
|------------------------|-----------------------------------------------|--------------------------------------------------|
| Germ cells             | 0,193±0,007                                   | 0,171±0,006                                      |
| Primordial follicles   | 0,201±0,007                                   | 0,194±0,007                                      |
| Primary follicles      | 0,179±0,006                                   | 0,157±0,005                                      |
| Stroma cells           | 0,185±0,006                                   | 0,127±0,005                                      |

Note. p≤0,05

Таблиця 6 - The indexes of an optical density of DNP and RNP in structural elements of the fetuses’ ovaries in gestational term of 29-36 weeks

| The structure of ovary | The optical density of DNP in cells’ nuclei | The optical density of RNP in cytoplasm |
|------------------------|---------------------------------------------|----------------------------------------|
| Germ cells             | 0,198±0,007                                 | 0,171±0,006                            |
| Genital strands        | 0,186±0,006                                 | 0,171±0,006                            |
| Primordial follicles   | 0,202±0,007                                 | 0,194±0,007                            |
| Primary follicles      | 0,204±0,007                                 | 0,179±0,006                            |
| Stroma cells           | 0,185±0,006                                 | 0,127±0,005                            |

Note. p≤0,05
Table 7 - The indexes of an optical density of DNP and RNP in structural elements of the fetuses’ ovaries in gestational term of 37-40 weeks

| The structure of ovary         | The optical density of DNP in the cells’ nuclei | The optical density of RNP in cytoplasm |
|--------------------------------|-----------------------------------------------|----------------------------------------|
| Germ cells                    | 0,167±0,006                                    | 0,191±0,007                            |
| Genital strands               | 0,164±0,006                                    | 0,158±0,005                            |
| Primordial follicles          | 0,184±0,007                                    | 0,164±0,007                            |
| Primary follicles             | 0,212±0,008                                    | 0,181±0,007                            |
| Growing follicles             | 0,251±0,009                                    | 0,199±0,007                            |
| Stroma cells                  | 0,166±0,006                                    | 0,145±0,005                            |

Note. p≤0,05

The Felgen-Rossenbeck staining revealed an intensive reaction in the integumentary epithelium’ cells’ nuclei of fetal gonads, in cells of the genital strands, as well as in the nuclei of cells of the follicular epithelium of primordial and primary follicles. Moreover, the intensity is growing relatively to the gestational term. Namely: it reaches its minimum in case of fetuses’ ovaries with a gestational term of 21-28 weeks, while the maximum is reached in case of fetuses’ gonads with a term of 37-40 weeks.

The treatment of specimens by the Brache method has revealed an intensive red color in the nuclei of folliculocytes of primary and primordial follicles, as well as a moderate staining in the cytoplasm of germ cells and stroma cells. Moreover, there is the same tendency of increasing intensity of staining, like one, which was revealed by staining according to Felgen-Rossenbeck method: there is an increase of the reaction intensity relatively to increase of the gestational term.

The intensive Schiff-reaction was observed in basement membranes of the follicular epithelium of primordial and primary follicles, as well as in the stroma of the organ’s cortical layer. The maximum reaction takes place in case of fetuses’ ovaries with the gestational term of 37-40 weeks, while the minimum one takes place in case of fetuses’ organs with the gestational term of 21-28 weeks.

The morpho-functional and histochemical features of the fetuses’ ovaries structure from mother with a physiological pregnancy in different gestational terms are corresponding with physiological norm [5, 11]. Depending on change of the main indexes, all fetuses were divided into groups relatively to the gestational terms, as well as stabilization of functional
activity of the fetal gonads [14, 15]. Namely: 21-28 weeks, 29-36 weeks and 37-40 weeks. 

Despite of gestational terms, all ovaries were represented by cortical and cerebral substance, 
the ratio of which is approximately the same in all terms of in utero development, but the 
relative volume of the cortical substance prevails towards one of the cerebral substance. The 
main organometric indexes of fetal gonads are increasing relatively to the gestational term, 
which is determined by the fetus’ growth and increase of relative volumes of the organs’ 
functional elements [7, 9]. The follicular element is represented mostly by primordial and 
primary follicles in gestational terms of 21-36 weeks. In 37-40 weeks the atretic and 
cystically altered forms are appearing, what becomes a manifestation of a functional 
maturation of fetal gonads [3, 10].

Moreover, the index of maturation and stabilization of the fetuses’ ovaries as 
endocrine organ is decrease of a relative volume of follicular tissue and increase of the index 
of relative volume of interstitial tissue [7, 8]. These changes are determined also by increase 
of number of apoptotically altered forms on the background of decrease of general percent of 
the germ cells [12, 15]. The indexes of ovaries’ functional activity were assessed by 
histochemical methods: Felgen-Rossenbeck, Brache and Schiff-reaction. By evaluation of 
these reaction the same reaction of their change takes place: the minimum indexes are 
corresponding with fetuses with a gestational term of 21-28 weeks, while the maximum ones 
are corresponding with 37-40 weeks of in utero development.

Thus, the histochemical features of the fetuses’ ovaries development from mothers 
with physiological pregnancy, which were described in the current article, are corresponding to 
main stages of fetal gonads’ stabilization, as well as are indicating a harmonious development 
of the fetuses’ development in study groups.

**Conclusions**

1. The organometric indexes of ovaries, as well as thicknesses of the protein shell 
of fetuses’ gonads from mothers with physiological pregnancy are changing in proportion to 
the gestational term as well as reaching its maximum in a term of 37-40 weeks.

2. The number of germ cells is decreasing by the pregnancy term’ growth, which 
is determined by increasing index of apoptotically altered forms.

3. In the follicular apparatus a prevalence of primary and primordial follicles in 
all gestational terms could be noticed, as well as an appearance of tertiary and cystically 
altered forms in the fetuses’ ovaries with a gestational term of 37-40 weeks.

4. The decrease number of germ cells and individual forms of follicles is
contributing to decrease of the relative volume of follicular tissue in a period of 37-40 weeks on the background of increase of relative volume of an interstitial component.

5. The functional activity of fetal gonads with its minimum indexes relatively to gestational term of 21-28 weeks, as well as its maximum indexes relatively to gestational term of 37-40 weeks were assessed by the histochemical methods.

6. The histochemical features of the fetuses’ ovaries structure in different gestational terms, that were described in a current article, could be applied during leading a study on such features towards fetuses from mothers with complicated pregnancy.

The perspective of future research: the postulation of immunohistochemical features of the fetuses’ ovaries structure from healthy mothers in different gestational terms; study on histochemical and immunohistochemical features of the fetuses’ ovaries structure from mothers, whose pregnancy was complicated by preeclampsia of different stage of severity, as well as chronic infection of lower genital tract.

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