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HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL FEATURES OF DIFFERENTIATED TROPHOBLAST IN CHORIONIC VILLI OF THE PLACENTA IN PRETERM LABOR

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Summary

The importance of studying the histochemical and immunohistochemical features of the differentiated trophoblast in chorionic villi of the placenta in preterm labor is determined by the threatening nature of this condition for both mother and fetus, as well as its high prevalence and serious consequences for the health of both. The understanding of the damage to the trophoblast of chorionic villi in the placenta can be expanded by histochemical and immunohistochemical methods, which allow the assessment of the concentration of specific marker molecules in one way or another.

Objective. *To determine certain histochemical and immunohistochemical characteristics of proteins in the trophoblast of the intermediate and terminal chorionic villi of the placenta in preterm labor.*

Material and Methods. *The obtained material (30 placentas from preterm deliveries and 30 placentas from normal pregnancies) was fixed for 20-24 hours in 10 % neutral formalin solution buffered in Lilly's phosphate buffer. After tissue removal, the placental tissue was dehydrated in an ascending ethanol series and embedded in paraffin at a temperature of approximately 58 °C. Serial histologic sections were cut at 5.0 μm thickness using an MS-2 sliding microtome. After deparaffinization, histological sections were stained with hematoxylin and eosin, histochemical methods for total protein with bromophenol blue according to Bonhéme, and immunohistochemical techniques according to the manufacturer's protocols (Dako, Denmark). In particular, immunohistochemical reactions were performed with monoclonal antibodies against trophoblast hormone-placental lactogen and placental alkaline phosphatase. Visualization of primary antibodies was performed using the Dako polymer visualization system with diaminobenzidine as chromogen (resulting in brown staining of the sites of studied antigens).*

In addition to the descriptive method of histopathologic research, computer morphometry of digital microphotographs of histologic sections was performed using a Delta Optical Evolution 100 microscope and an Olympus SP550UZ digital camera. Digital copies of the images were processed using a legitimate copy of the ImageJ v1.52f computer program developed for histometric studies (National Institutes of Health, USA). Specifically, the evaluation of staining intensity (optical density) was performed on digital microphotographs using the method of computer microdensitometry. For this purpose, a microprobe method was used to obtain a computer brightness value in an 8-bit analysis system with 256 gray levels – from black (0) to white (255). The obtained values were then transformed into relative optical density values (r.OD) by logarithmic transformation (natural logarithm method). The relative optical density value ranges from 0 (absolute transparency of the object) to 1 (absolute opacity of the object).

The obtained digital data were processed using statistical analysis methods. A legitimate copy of the statistical analysis computer program PAST v4.14 was used, with a preliminary check for normal distribution using the Shapiro-Wilk test. Since, according to this test, the hypothesis of normal distribution was not rejected for the statistical samples studied (at p=0.05), parametric methods of statistical analysis were applied: calculation of the mean and its standard error, Student's t-test (two-tailed, unpaired). In addition, the non-parametric Mann-Whitney test was used for the reliability of the conclusions.

The research was conducted as part of the scientific research project «Preservation and Restoration of Reproductive Health in Women and Girls with Obstetric and Gynecological Pathology» at the Department of Obstetrics and Gynecology of Bukovinian State Medical University (state registration number 0121U110020, duration 2021-2025).

Results. *Microscopic examination of histological sections stained with hematoxylin and eosin did not reveal any differences in the structure of chorionic villus trophoblast in the placenta of preterm labor compared to normal pregnancy. However, histochemical and immunohistochemical methods of investigation revealed a number of features in the trophoblast of chorionic villi, where fundamental events related to substance exchange occur – intermediate immature, intermediate mature, terminal villi, including terminal «specialized villi».*

Conclusions. *According to the obtained histochemical and immunohistochemical data, in preterm labor, compared to normal pregnancy, no changes are observed in the trophoblast of intermediate immature villi, while in intermediate mature and terminal villi, there is a decrease in histochemical staining for total protein and immunohistochemical staining for specific trophoblast proteins – placental lactogen hormone and placental alkaline phosphatase enzyme.*

Key words: *Preterm Labor; Chorionic Villi of the Placenta; Differentiated Trophoblast.*

Introduction

The importance of studying the histochemical and immunohistochemical features of chorionic villus trophoblast in the placenta during preterm labor is determined by the threatening nature of this condition for both mother and fetus, as well as its high prevalence and serious consequences for the health of both. Worldwide statistics indicate a steady increase in the number of preterm births, highlighting the need for an in-depth understanding of the molecular and cellular processes underlying this pathological condition.

Scientific research on the histochemical and immunohistochemical aspects of the differentiated trophoblast in this context is important to uncover new perspectives in the diagnosis and treatment of preterm labor. The understanding of the damage to the trophoblast of the chorionic villi in the placenta can be extended by histochemical and immunohistochemical methods,

which allow the evaluation of the concentration of specific marker molecules. In particular, the study of the interaction between cells of the differentiated trophoblast and the placental microenvironment may contribute to the discovery of new molecular markers and therapeutic approaches. This methodology has the potential to improve the prognosis of the pathology, reduce the risks for both mother and fetus, and influence the strategy of pregnancy management in the case of preterm labor.

Objective. To determine certain histochemical and immunohistochemical features of proteins in the differentiated trophoblast of the intermediate and terminal chorionic villi of the placenta in preterm labor.

Material and Methods. The collected material (30 placentas from preterm deliveries and 30 placentas from normal pregnancies) was fixed in 10 % neutral formalin solution buffered with Lillie's phosphate buffer for 20-24 hours. After tissue removal, the placental tissue was dehydrated in an ascending ethanol series and embedded in paraffin at a temperature of approximately 58 °C. Serial histologic sections were cut at 5.0 μm thickness using an MS-2 sliding microtome. After deparaffinization, histological sections were stained with hematoxylin and eosin. In addition, a histochemical technique for total protein with bromophenol blue according to Bonhéme was applied, as well as immunohistochemical methods according to the protocols provided by the manufacturer (Dako, Denmark). In particular, immunohistochemical reactions were performed with monoclonal antibodies against trophoblast hormone-placental lactogen and placental alkaline phosphatase. Visualization of primary antibodies was performed using the Dako polymer visualization system with diaminobenzidine as the chromogen, resulting in brown staining of the sites of the studied antigens.

In addition to the descriptive method of histopathologic research, computer morphometry of digital microphotographs of histologic sections was performed using a Delta Optical Evolution 100 microscope and an Olympus SP550UZ digital camera. Digital copies of the images were processed using a legitimate copy of the ImageJ v1.52f computer program developed for histometric studies by the National Institutes of Health in the USA. Specifically, the assessment of staining intensity (optical density) was performed on digital microphotographs using the method of computer microdensitometry. For this purpose, a microprobe method was used to obtain a computer brightness value in an 8-bit analysis system with 256 gray levels – from black (0) to white (255). The obtained values were then transformed into relative optical density values (r.OD) by logarithmic transformation (natural logarithm method). The relative optical density value ranges from 0 (absolute transparency of the object) to 1 (absolute opacity of the object).

The digital data obtained were processed using statistical analysis methods. Using a legitimate copy of the statistical software PAST v4.14, a preliminary normality check was performed using the Shapiro-Wilk test. Since, according to this test, the hypothesis of normal distribution was not rejected for the statistical samples studied (at p=0.05), parametric statistical analysis methods were applied, including the calculation of the mean and its standard error, as well as the Student's t-test (two-tailed, unpaired). In addition, the non-parametric Mann-Whitney test was used to ensure the robustness of the conclusions.

The research was conducted in accordance with the plan of the scientific research work of the Department of Obstetrics and Gynecology at Bukovinian State Medical University, titled «Preservation and Restoration of Reproductive Health in Women and Girls with Obstetric and Gynecological Pathology» (January 2021 – December 2025), with the state registration number 0121U110020.

Results and Discussion. Microscopic examination of histologic sections stained with hematoxylin and eosin did not reveal any differences in the structure of chorionic villus trophoblasts in the placenta between preterm and normal pregnancies. However, histochemical and immunohistochemical methods of investigation revealed a number of features in the trophoblast of chorionic villi in the placenta, where key events in substance exchange take place – intermediate immature, intermediate mature, terminal villi, including terminal «exchange-specialized villi». For convenience, the latter villi will be referred to as «exchange» villi.

In the study of histochemical preparations stained for total protein by the Bonhé bromophenol blue method, a quantitative evaluation was performed by computer morphometry, obtaining the parameter «optical density of staining for total protein» in relative units of optical density (r.OD). These data are shown in Table 1.

Specifically, the data in Table 1 show that in trophoblasts from intermediate immature villi during preterm labor, the optical density of the staining for total protein remains unchanged, whereas in trophoblasts from intermediate mature and terminal villi, it decreases ($p<0.05$ according to Student's and Mann-Whitney statistical criteria).

Table 1

Optical density of histochemical staining for total protein in the trophoblast of «exchange» villi of the placenta during preterm labor and normal pregnancy (M±m)

Indicator	Normal pregnancy (n=30)	Preterm labor (n=30)
Optical density of staining for total protein in the trophoblast of intermediate immature villi (r.OD)	0.26 ± 0.010	0.25 ± 0.011
Optical density of staining for total protein in the trophoblast of intermediate mature villi (r.OD)	0.31 ± 0.012	0.26 ± 0.011
Optical density of staining for total protein in the trophoblast of terminal and terminal «specialized» villi (r.OD)	0.34 ± 0.012	0.28 ± 0.012

The described patterns regarding the results of the histochemical method for total protein with bromophenol blue according to Bonheg are illustrated using Figures 1 and 2.

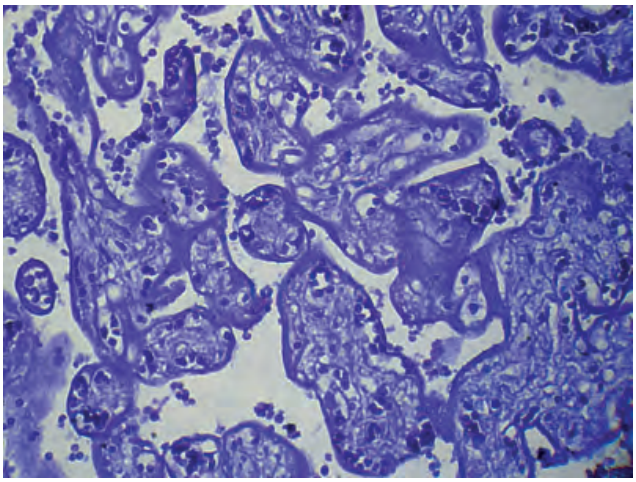

Fig. 1. Physiological pregnancy. Intermediate mature and terminal villi in the field of view. Histochemical method for total protein with bromophenol blue according to Bonheg. Obj. 20x, Ocular 10x (optical magnification 200x)

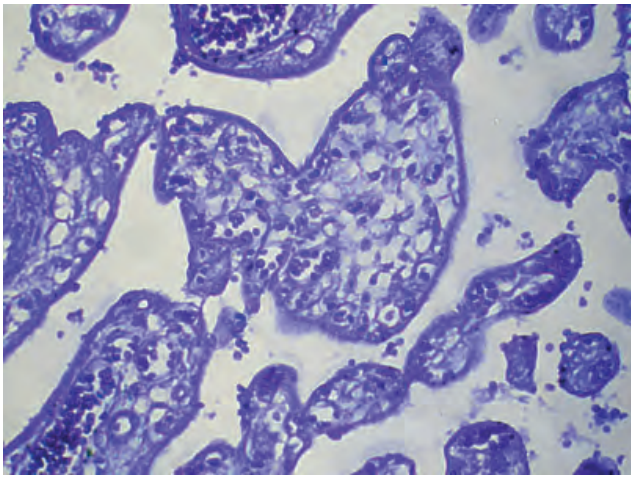

Fig. 2. Preterm labor. Intermediate mature and terminal villi in the field of view. Histochemical method for total protein with bromophenol blue according to Bonheg. Obj. 20x, Ocular 10x (optical magnification 200x)

A similar study using the immunohistochemical method was conducted for a specific protein produced by the trophoblast of the placenta during pregnancy – placental lactogen hormone. The results of the objective evaluation based on the immunohistochemical determination of placental lactogen in the trophoblast of «exchange» villi are presented in Table 2 and illustrated with microphotographs in Figures 3 and 4.

Specifically, from the data in Table 2, it can be seen that while there are no changes in placental lactogen production in the trophoblast of intermediate immature villi during preterm labor, a decrease in the production of this hormone is observed in intermediate mature villi and terminal «specialized» villi based on the indicator «optical density of staining for placental lactogen in the trophoblast».

Table 2

The optical density of immunohistochemical staining for placental lactogen in the trophoblast of «exchange» villi in preterm labor and physiological pregnancy (M±m)

Indicator	Normal pregnancy (n=30)	Preterm labor (n=30)
Optical density of staining for placental lactogen in the trophoblast of intermediate immature villi (r.OD)	0.22 ± 0.005	0.21 ± 0.008
Optical density of staining for placental lactogen in the trophoblast of intermediate mature villi (r.OD)	0.32 ± 0.009	0.24 ± 0.010
Optical density of staining for placental lactogen in the trophoblast of terminal and terminal «specialized» villi (r.OD)	0.37 ± 0.009	0.26 ± 0.008

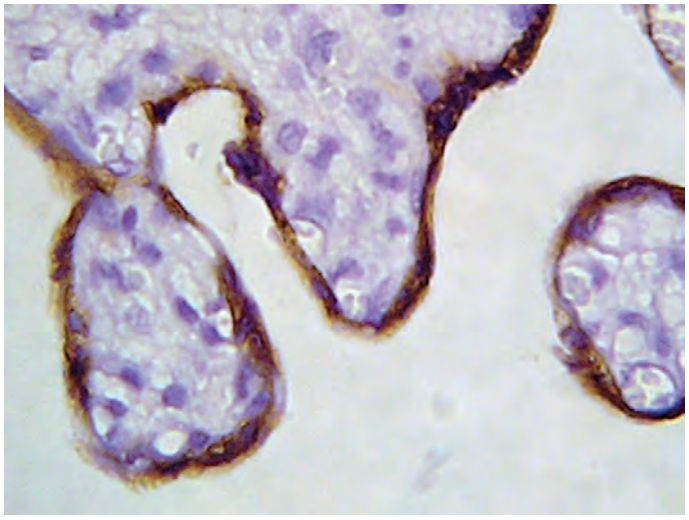

Fig.3. Physiological pregnancy. In the field of view, intermediate mature and terminal villi. Immunohistochemical method with primary antibodies against placental lactogen and a polymer detection system (visualization with diaminobenzidine – indicated by arrows) with counterstaining by Groat's hematoxylin. Obj.40x, Ocular 10x (optical magnification 400x)

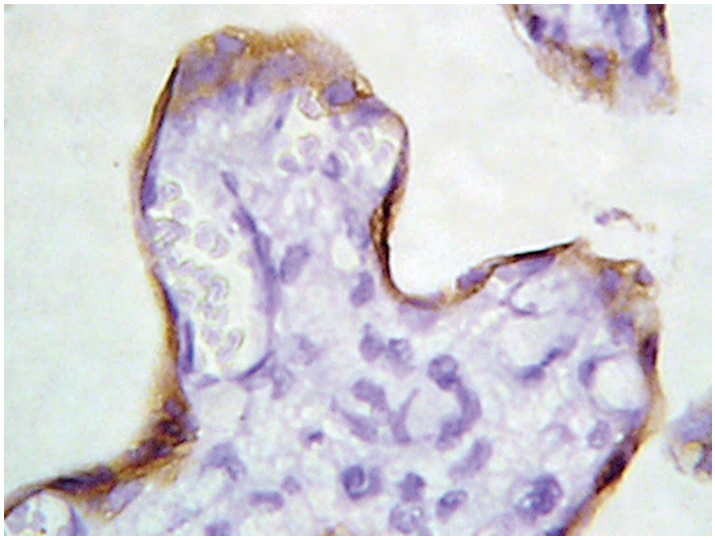

Fig.4. Preterm labor. In the field of view, intermediate mature and terminal villi. Immunohistochemical method with primary antibodies against placental lactogen and a polymer detection system (visualization with diaminobenzidine – indicated by arrows) with counterstaining by Groat's hematoxylin. Obj.40x, Ocular 10x (optical magnification 400x)

Similar to placental lactogen, immunohistochemical studies were performed for another specific trophoblast protein, placental alkaline phosphatase. Quantitative methods (Table 3) showed the same patterns as for placental lactogen.

Table 3

The optical density of immunohistochemical staining for placental alkaline phosphatase in the trophoblast of «exchange» villi in preterm labor and physiological pregnancy (M±m)

Indicator	Normal pregnancy (n=30)	Preterm labor (n=30)
The optical density of staining for placental alkaline phosphatase in the trophoblast of intermediate immature villi (r.OD)	0.21 ± 0.007	0.20 ± 0.007
The optical density of staining for placental alkaline phosphatase in the trophoblast of intermediate mature villi (r.OD)	0.31 ± 0.010	0.23 ± 0.008
The optical density of staining for placental alkaline phosphatase in the trophoblast of terminal and terminal «specialized» villi (r.OD)	0.36 ± 0.009	0.24 ± 0.009

Immunohistochemical staining for placental alkaline phosphatase in the trophoblast of placental villi in preterm labor and physiological pregnancy is shown in Figures 5 and 6.

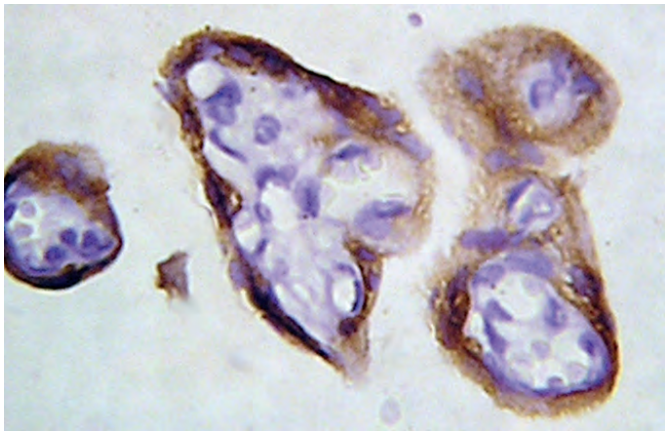

Fig. 5. Physiological pregnancy. In the field of view are intermediate mature and terminal villi. Immunohistochemical method with primary antibodies against placental alkaline phosphatase and a polymer detection system (visualization with diaminobenzidine – indicated by arrows) with counterstaining with Groat's hematoxylin. Obj.40x, Ocular 10x (optical magnification 400x)

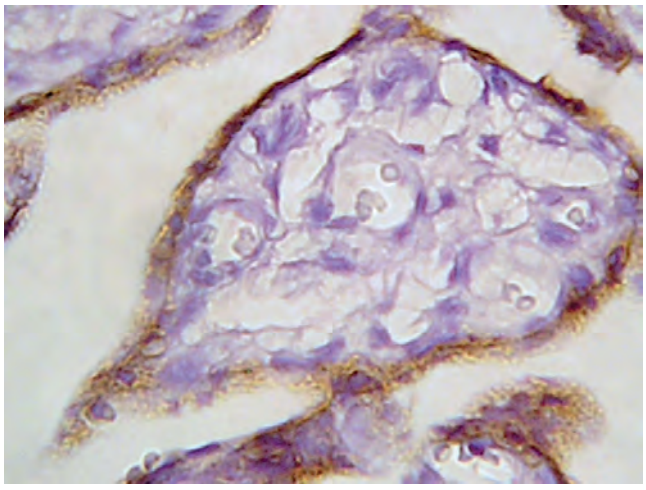

Fig. 6. Preterm labor. In the field of view are intermediate mature and terminal villi. Immunohistochemical method with primary antibodies against placental alkaline phosphatase and a polymer detection system (visualization with diaminobenzidine – indicated by arrows) with counterstaining with Groat's hematoxylin. Obj.40x, Ocular 10x (optical magnification 400x)

Conclusions

According to the obtained histochemical and immunohistochemical data, in preterm labor compared to normal pregnancy, no changes are observed in the trophoblast of intermediate immature villi, while in intermediate mature and terminal villi, there is a decrease in histochemical staining for total protein and immunohistochemical staining for specific proteins of the trophoblast – placental lactogen hormone and placental alkaline phosphatase enzyme.

Prospects for further research. The study of the preterm placenta, which did not reveal any structural differences in the trophoblast of the chorionic villi compared to normal pregnancy, raises new questions regarding the functional aspects of these structures. Histochemical and immunohistochemical analyses, identifying «specialized villi» with reduced optical density of protein in intermediate, mature and terminal villi, emphasize the need for more detailed investigations of their role and impact on substance exchange in the context of preterm labor. Additional statistical and molecular studies may elucidate the mechanisms underlying these features and determine their clinical significance.

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ГІСТОХІМІЧНІ ТА ІМУНОГІСТОХІМІЧНІ ОСОБЛИВОСТІ ДИФЕРЕНЦІЙОВАНОГО ТРОФОБЛАСТА ХОРІАЛЬНИХ ВОРСИНОК ПЛАЦЕНТИ ПРИ ПЕРЕДЧАСНИХ ПОЛОГАХ

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Резюме.

Актуальність вивчення гістохімічних та імуногістохімічних особливостей дифенційованого трофобласта хоріальних ворсинок плаценти при передчасних пологах визначається загрозливістю цього стану для матері та плоду, а також його високою поширеністю та серйозними наслідками для здоров'я обох. Уявлення про ушкодження трофобласта хоріальних ворсинок

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плаценти можуть розширити гістохімічні та імуногістохімічні методи, які дозволяють так чи інакше оцінити концентрацію певних маркерних молекул.

Мета дослідження. Встановити деякі гістохімічні та імуногістохімічні особливості білків трофобласта проміжних та термінальних хоріальних ворсинок плаценти при передчасних пологах.

Матеріал і методи дослідження. Отриманий матеріал (30 плацент при передчасних пологах та 30 плацент при фізіологічній вагітності) фіксували протягом 20-24 годин у 10 %-му нейтральному розчині формаліну, забуференому в фосфатному буфері за Ліллі. Після вирізки тканину плаценти зневоднювали у висхідній батареї етанолу та заливали в парафін-віск при температурі близько 58 °С. На санному мікротомі МС-2 отримували серіями гістологічні зрізи 5,0 мкм завтовшки. Після депарафінізації гістологічні зрізи забарвлювали гематоксиліном і еозином, застосовували гістохімічну методику на загальний білок з бромфеноловим синім за Бонхегом, а також імуногістохімічні методики у відповідності до протоколів, наданих виробником (Dako, Данія). Зокрема, провели імуногістохімічні реакції з моноклональними антитілами до гормону трофобласта – плацентарного лактогену та плацентарної лужної фосфатази. Візуалізацію первинних антитіл здійснювали полімерною системою візуалізації Dako з барвником діамінобензидином (дає коричневе забарвлення місць розташування досліджуваних антигенів).

Окрім описового методу гістопатологічного дослідження виконана комп'ютерна морфометрія цифрових мікрофотографій гістологічних зрізів (мікроскоп Delta Optical Evolution 100 та цифрова камера Olympus SP550UZ). Цифрові копії зображення обробляли за допомогою легітимної копії комп'ютерної програми ImageJ v1.52f, яка розроблена для гістометричних досліджень (National Institutes of Health, США). Зокрема, на цифрових мікрофотографіях оцінку інтенсивності забарвлення (оптичної густини) здійснювали за допомогою методу комп'ютерної мікроденситометрії. Для цього мікрозондовим методом отримували комп'ютерну величину яскравості забарвлення у 8-бітній системі аналізу з 256 градацій сірого – від чорного (0) до білого (255), а потім отримані величини шляхом логарифмічного перетворення (метод натурального логарифму) переводили у величину відносної оптичної густини (в.од.опт.густини). Величина відносної оптичної густини коливається від 0 (абсолютна прозорість об'єкта) до 1 (абсолютна непрозорість об'єкта).

Отримані цифрові дані оброблено методами статистичного аналізу. За допомогою легітимної копії комп'ютерної програми для статистичних обрахунків PAST v4.14 застосовували попередню перевірку на нормальність розподілу за критерієм Shapiro-Wilk. Оскільки щодо вивчених статистичних вибірок згідно з названим критерієм, гіпотеза про нормальність розподілу не відхилялася (при p=0,05), то застосовували параметричні методи статистичного аналізу: обрахування величини середньої арифметичної та її похибки, критерій Стьюдента (двобічний непарний). Також для надійності висновків використали й непараметричний критерій Mann-Whitney.

Дослідження виконано в межах науково-дослідної роботи «Збереження та відновлення репродуктивного здоров'я жінок та дівчат при акушерській і гінекологічній патології» кафедри акушерства та гінекології Буковинського державного медичного університету (державний реєстраційний номер 0121U110020, термін виконання 2021-2025).

Отримані результати та їх обговорення. Під час мікроскопії гістологічних зрізів, забарвлених гематоксиліном і еозином не виявлено жодних відмінностей у будові трофобласта хоріальних ворсинок плаценти при передчасних пологах у порівнянні з фізіологічною вагітністю. Однак, гістохімічний та імуногістохімічний методи дослідження показали низку особливостей трофобласта хоріальних ворсинок плаценти, на території яких відбуваються основні події щодо обміну речовин – проміжні незрілі, проміжні зрілі, термінальні ворсинки, у тому числі термінальні «спеціалізовані ворсинки».

Висновки. Згідно отриманих гістохімічних та імуногістохімічних даних, при передчасних пологах, у порівнянні з фізіологічною вагітністю, в трофобласті проміжних незрілих ворсинок змін не відмічається, тоді як у проміжних зрілих та термінальних ворсинках спостерігається зниження гістохімічного забарвлення на загальний білок та імуногістохімічного забарвлення на специфічні білки трофобласта – гормон плацентарний лактоген та фермент плацентарну лужну фосфатазу.

Ключові слова: передчасні пологи; хоріальні ворсинки плаценти; диференційований трофобласт.

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