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MORPHOMETRIC PARAMETERS OF
PLACENTAL CHORIONIC VILLI IN LABOR
AFTER 40 WEEKS

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Summary.

The placenta is the main organ that ensures the vital activity of the fetus during pregnancy. Studying the morphometric characteristics of chorionic villi allows us to better understand the changes that occur in the placenta in late pregnancy.

Aim. *To find out the percentage of chorionic villi of the placenta at delivery after 40 weeks.*

Materials and methods. *The material for the study was 60 human placental preparations obtained as a result of childbirth: the main group – 30 placental preparations (births after 40 weeks of pregnancy), the comparison group – 30 placental preparations (births that occurred at 37-40 weeks of gestation). A set of classical methods of morphological examination was chosen for the study: morphometry, dissection, and photographic documentation. Digital copies of the image were analyzed using a legal copy of the specialized computer program ImageJ v1.53t. The obtained digital data were processed by statistical methods. The normality of the distribution according to the Shapiro-Wilki criterion did not deviate ($p=0.05$). Approval of the Bioethics Committee of Bukovinian State Medical University (protocol No. 1 of 09.21.2023). The study was performed as part of the research work of the Department of Obstetrics and Gynecology of Bukovinian State Medical University (Chernivtsi, Ukraine).*

Results. *The percentage of such villi as «early» and «late» stem villi, intermediate immature and mature villi, terminal and terminal «specialized villi» was estimated. Morphometric data show that in the main group, compared to the control group, the number of stem «late» choroidal villi is on average 2.8 times higher; the percentage of intermediate immature villi (2.67 times) and the percentage of intermediate mature villi (1.42 times) is significantly lower; the percentage of terminal villi is significantly higher, but the percentage of terminal «specialized» villi, on the contrary, is reduced.*

Conclusions. *In deliveries after 40 weeks, a change in the ratio of chorionic villi of the placenta is noted compared to deliveries at 37-40 weeks of gestation, in particular, the percentage of stem «late» villi and terminal villi increases, but the percentage of intermediate mature and immature villi, as well as terminal «specialized» villi decreases (%).*

Key words: *Labor after 40 weeks; Trophoblast; Chorionic villi; Placenta; Pregnancy.*

Introduction

The placenta serves as the primary organ supporting fetal viability during pregnancy, facilitating essential functions such as nutrient transport, gas exchange, and the removal of metabolic waste. A critical component of the placenta is the chorionic villi, which act as the principal units mediating the exchange of substances between the maternal and fetal circulations [1-3]. These villi are composed of a thin membrane housing blood vessels and are directly exposed to maternal blood [4, 5].

Morphometric features of chorionic villi serve as vital indicators of placental functionality and are known to vary with gestational age [6-8]. Notably, during delivery after 40 weeks of gestation, structural alterations in chorionic villi have been observed, which may influence their functional capacity [9-11]. Such changes in morphometric parameters may signify placental adaptations to prolonged pregnancy and could impact the efficiency of placental circulation [12-14].

Given that placental morphology is a key determinant of its functional capacity, investigating the morphometric characteristics of chorionic villi provides valuable insights into the transformations occurring in the placenta during late pregnancy. This understanding is crucial for evaluating placental health and predicting childbirth outcomes [15-17].

Aim. The aim of the study was to determine the percentage of chorionic villi in placental tissue following delivery after 40 weeks of gestation.

Materials and methods

The study utilized 60 human placental samples collected after childbirth. These were divided into two groups: the

main group consisted of 30 placental samples from deliveries occurring after 40 weeks of pregnancy, while the comparison group included 30 placental samples from deliveries at 37-40 weeks of gestation. Placental tissue samples were collected from the intermediate zone, spanning from the fetal plate to the basal plate. The samples were fixed for 22-24 hours in a neutral buffered 10% aqueous formalin solution (Lilly's solution). Following fixation, the tissue was dehydrated using a graded ethanol series (ranging from 50% to absolute alcohol) and subsequently embedded in paraffin wax at 58 °C.

Histological sections of 5 μ m thickness were prepared using an MS-2 sled microtome. After deparaffinization, the sections were stained with hematoxylin and eosin for microscopic examination.

In addition to conventional histopathological analysis, computer morphometry was performed on digital images of the tissue samples. These images were captured using a Delta Optical Evolution 100 microscope equipped with planachromatic objectives and an Olympus SP550UZ digital camera with a proprietary adapter.

The digital images were analyzed using ImageJ v1.53t, a specialized software for histometric studies. A scoring test was conducted on the hematoxylin and eosin-stained sections to quantify the percentage of specific types of chorionic villi. This involved repeated counting to ensure accuracy.

Statistical analysis of the data was performed using PAST v5.02 software. A preliminary Shapiro-Wilk test was applied to assess the normality of the data distribution. Since the hypothesis of normality was not rejected ($p = 0.05$), parametric statistical methods were employed, including the calculation of arithmetic means and standard

errors, as well as the unpaired two-sided Student's t-test. For additional validation, the non-parametric Mann-Whitney test was also applied, although the p-values reported were based on the Student's t-test.

The study adhered to ethical guidelines outlined in the GDPR (1996), the Council of Europe Convention on Human Rights and Biomedicine (04.04.1997), the Declaration of Helsinki (1964-2013), and Order No. 690 of the Ministry of Health of Ukraine (23.09.2009). The research protocol was approved by the Bioethics Commission of Bukovinian State Medical University (Protocol No. 1, dated 21.09.2023).

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

Results and discussion

The primary objective was to evaluate the proportional distribution of different types of chorionic villi in relation to placental aging. Specifically, the study focused on quantifying the percentages of «early» and «late» stem villi, intermediate immature and mature villi, terminal villi, and

terminal «specialized villi.» Notably, immature variants of villi, such as mesenchymal and embryonic villi, were either absent or present in such negligible quantities that their statistical evaluation was deemed irrelevant. Similarly, precursors of chorionic villi, including trophoblastic and villous processes (sometimes referred to as «syncytial buds» due to their role in the formation of new placental villi), were not considered for statistical analysis.

It is important to distinguish these structures from «syncytial nodules,» which are indicative of trophoblast apoptosis. Syncytial nodules are characterized by specific nuclear changes, such as karyopyknosis (nuclear shrinkage), chromatin margination (migration of chromatin to the nuclear periphery), or karyorrhexis (nuclear fragmentation).

The proportional distribution of «early» and «late» stem chorionic villi in deliveries occurring after 40 weeks of gestation, compared to those at 37-40 weeks, is presented in Table 1. The morphometric data reveal a significant difference between the two groups, specifically in the proportion of «late» stem chorionic villi. In deliveries after 40 weeks, these villi were 2.8 times more abundant than in the 37-40 weeks group.

To visually represent these morphometric findings, Figure 1 is provided.

Table 1

Percentage of placental stem «early» and stem «late» chorionic villi in deliveries after 40 weeks compared to deliveries at 37-40 weeks of gestation ($M \pm m$)

Indicator	Childbirth after 40 weeks of pregnancy (n=30)	Delivery at 37-40 weeks of gestation (n=30)
Percentage of stem «early» villi (%)	0,4±0,24	0,3±0,02
Percentage of stem «late» villi (%)	12,6±0,16 *	4,5±0,14

Notes* Difference in mean trends $p < 0.05$ (Student's t-test)

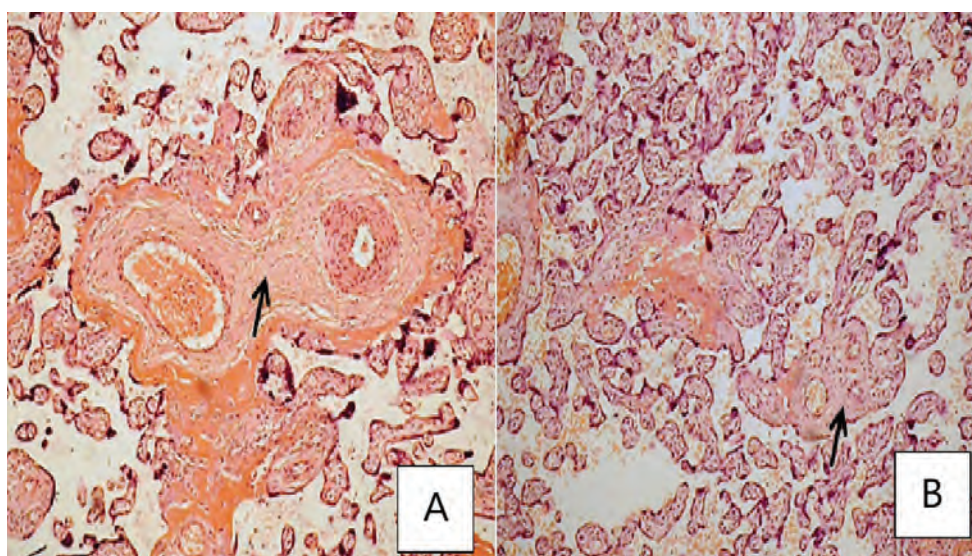


Fig. 1. «Late» placental stem cells (examples are indicated by arrows). Hematoxylin and eosin staining. Objective: 4x, O.10x (optical magnification 40x)

A) Delivery after 40 weeks of gestation

B) Delivery at 37-40 weeks of gestation

The percentage of intermediate chorionic villi in deliveries after 40 weeks compared to deliveries at 37-40 weeks is presented in Table 2. The data indicate that in deliveries after 40 weeks, the percentage of intermediate

immature villi is nearly three times lower, while the percentage of intermediate mature villi is 1.42 times lower compared to the 37-40 weeks group.

Intermediate villi are illustrated in Figure 2.

Table 2

Percentage of intermediate chorionic villi in the placenta at delivery after 40 weeks compared to delivery at 37-40 weeks of gestation ($M \pm m$)

Indicator	Childbirth after 40 weeks of pregnancy (n=30)	Delivery at 37-40 weeks of gestation (n=30)
Percentage of intermediate immature villi (%)	1,2 \pm 0,12 *	3,2 \pm 0,11
Percentage of intermediate mature villi (%)	10,5 \pm 0,15 *	14,9 \pm 0,23

Notes* Difference in mean trends between the study groups $p < 0.05$ (Student's t-test)

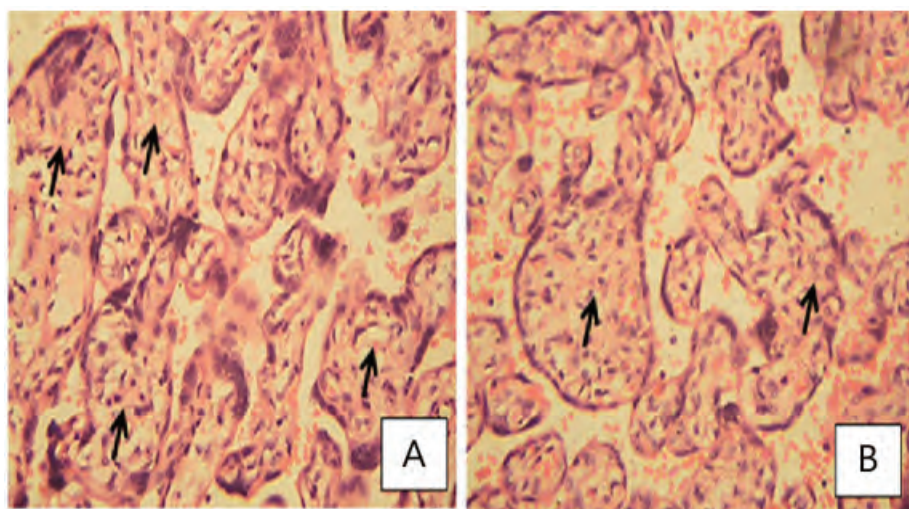


Fig. 2. Intermediate mature placental villi (examples are indicated by arrows). Hematoxylin and eosin staining. Ob.20x, O.10x (optical magnification 200x)

A) Delivery after 40 weeks of gestation

B) Delivery at 37-40 weeks of gestation

The average percentages of terminal villi and terminal «specialized» villi were also calculated, as shown in Table 3.

The data presented in Table 3 reveal that while the percentage of terminal villi is significantly higher in deliveries after 40 weeks, the percentage of terminal «specialized» villi is notably lower compared to deliveries at 37-40 weeks. This finding is critical, as these villi are the primary sites of metabolic exchange between maternal and fetal blood. At first glance, the total percentage of terminal and terminal «specialized» villi appears similar between the two groups (75.3% vs. 74.1%). However, the most intensive metabolic exchange occurs in terminal

«specialized» villi, where «syncytiotrophoblastic membranes» are present. These membranes are characterized by the close proximity of fetal capillaries to the trophoblast, with thinning of the trophoblast layer, facilitating optimal diffusion of substances.

Thus, it must be emphasized that the observed decrease in the percentage of terminal «specialized» villi necessarily implies a reduction in metabolic efficiency in deliveries after 40 weeks compared to those at 37-40 weeks of gestation.

A representative morphological depiction of terminal «specialized» villi in deliveries after 40 weeks and at 37-40 weeks of gestation is illustrated in Figure 3.

Table 3

Percentage of terminal placental villi in deliveries after 40 weeks compared to deliveries at 37-40 weeks of gestation ($M \pm m$)

Indicator	Childbirth after 40 weeks of pregnancy (n=30)	Delivery at 37-40 weeks of gestation (n=30)
Percentage of terminal villi (%)	42,3 \pm 0,12 *	36,2 \pm 0,24
Percentage of terminal «specialized» villi (%)	33,0 \pm 0,21 *	37,9 \pm 0,22

Notes* Difference in mean trends between the study groups $p < 0.05$ (Student's test)

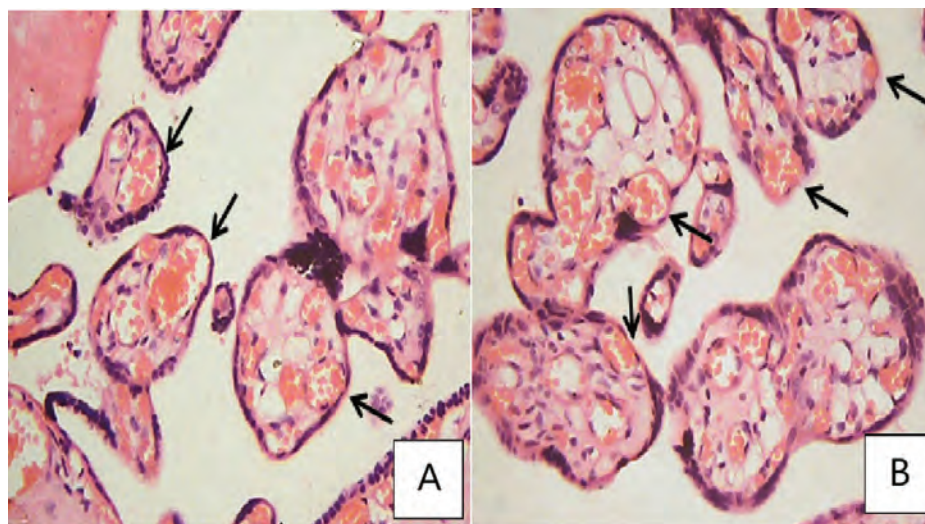


Fig. 3. Terminal «specialized villi» of the placenta (examples of «syncytiocapillary membranes» are indicated by arrows). Hematoxylin and eosin staining. Ob.20x, O.10x (optical magnification 200x)

A) Delivery after 40 weeks of gestation
B) Delivery at 37-40 weeks of gestation

Conclusion

In deliveries after 40 weeks of gestation, a shift in the proportional distribution of placental chorionic villi is observed compared to deliveries at 37-40 weeks. Specifically, the percentage of stem «late» villi and terminal villi increases, while the proportion of intermediate mature and immature villi, as well as terminal «specialized» villi, decreases.

Prospects for further research

The findings from the morphometric analysis of placental chorionic villi in deliveries after 40 weeks highlight the need for further investigation into the correlation between these morphometric changes and clinical outcomes. In particular, future studies should explore the relationship between these structural alterations and the development of complications associated with delayed delivery.

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МОРФОМЕТРИЧНІ ПАРАМЕТРИ ХОРІАЛЬНИХ ВОРСИНОК ПЛАЦЕНТИ ПРИ ПОЛОГАХ ПІСЛЯ 40 ТИЖНІВ

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Резюме.

Плацента є основним органом, що забезпечує життєдіяльність плода під час вагітності. Вивчення морфометричних характеристик ворсин хоріона дозволяє краще зрозуміти зміни, що відбуваються в плаценті на пізніх термінах вагітності.

Мета. З'ясувати відсоткове співвідношення хоріальних ворсинок плаценти при пологах після 40 тижнів.

Матеріали і методи. Матеріалом для дослідження послужили 60 препаратів плаценти людини, отримані в результаті пологів: основна група – 30 препаратів плаценти (пологи після 40 тижнів вагітності), група порівняння – 30 препаратів плаценти (пологи, які відбулись у терміні 37-40 тижнів гестації). Для дослідження було вибрано комплекс класичних методів морфологічного дослідження: морфометрія, препарування, фотодокументування. Для аналізу цифрових копій зображень використовували легальну копію спеціалізованої комп'ютерної програми ImageJ v1.53t. Отримані цифрові дані обробляли статистичними методами. Нормальність розподілу за критерієм Шапіро-Уїлкі не відхилялася ($p=0,05$). Схвалення Комісії з питань біоетики Буковинського державного медичного університету (протокол № 1 від 21.09.2023 року). Дослідження виконувалися у межах виконання НДР кафедри акушерства та гінекології Буковинського державного медичного університету (м. Чернівці, Україна).

Результати дослідження. Було оцінено відсоткове співвідношення таких ворсин, як «ранні» та «пізні» стовбурові ворсинки, проміжні незрілі та зрілі ворсинки, термінальні та термінальні «спеціалізовані ворсинки». Морфометричні дані показують, що в основній групі порівняно з групою контролю кількість стовбурових «пізніх» хоріальних ворсинок в середньому у 2,8 разів вища, відсоток проміжних незрілих ворсинок (у 2,67 разів) і відсоток проміжних зрілих ворсинок (в 1,42 рази) достовірно нижчий, відсоток термінальних ворсинок достовірно вищий, але відсоток термінальних «спеціалізованих» ворсин, навпаки, знижений.

Висновки. При пологах після 40 тижнів відмічається зміна співвідношення хоріальних ворсинок плаценти у порівнянні з пологами в терміні 37-40 тижнів вагітності, зокрема збільшується відсоток стовбурових «пізніх» ворсинок та термінальних ворсинок, але зменшується відсоток проміжних зрілих і незрілих ворсинок, а також термінальних «спеціалізованих» ворсинок (%).

Ключові слова: пологи після 40 тижнів; трофобласт; ворсинки хоріона; плацента; вагітність.

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