Original research

QUANTITATIVE DETERMINATION OF DELAYED MATURATION OF CHORIONIC VILLI IN THE PLACENTA IN PREGNANT WOMEN WITH COVID-19

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Key words: *placenta, COVID-19, SARS-CoV-2, chorionic villi, pathology, delayed villous maturation.*

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Summary*.The structure of the chorion changes according to gestational age, including stem, intermediate, and terminal villi. In the placenta, SARS-CoV-2 affects cells expressing angiotensin-converting enzyme 2 (ACE2), including endothelium and syncytiotrophoblasts. Damage to these cells triggers pyroptosis, inflammation, and arteriolosclerosis. During its maturation, the placenta records and preserves these changes, manifested as impaired chorionic villus formation.*

Objective. *To quantitatively assess delayed maturation of the villous chorion in pregnant women with COVID-19.*

Materials and Methods. *102 placentas from COVID-19-positive pregnancies were examined: Group I (n=90) – placentas from live term births during the acute phase of maternal disease, and Group II (n=12) – placentas from antenatal fetal asphyxia. In Group II, maternal infection occurred at 17–22 weeks of gestation. For comparison, 50 placentas from physiological term deliveries before the COVID-19 pandemic were analyzed. Microscopic, immunohistochemical, and statistical methods were used. Histological slides were photographed with a digital camera through a microscope, and images were analyzed in JPEG format using ONLINE JPG TOOLS to quantify the percentage of pixels corresponding to the structure of interest.*

Results. *In Group II (antenatal fetal asphyxia), there was an increased proportion of stem and intermediate chorionic villi – 36 (25–50) vs. 7 (6–9) in Group I. Stem villi in Group II exhibited qualitative changes, including stromal fibrosis and arteriolosclerosis. The number of terminal villi in Group II was 8 (8–8); p<0.001. In Group I, the number of terminal villi did not differ from the comparison group – 26 (25–27), indicating appropriate villous chorion formation for gestational age. Despite similar numbers of terminal villi in Groups I and the comparison group, the inter-villous space percentage was reduced in Group I – 26 (20–33) vs. 44 (40–49) in the comparison group, indicating edema of terminal villi. A decreased number of villi in Group II led to an increased inter-villous space – 49 (44–55).*

Conclusions. *A quantitative assessment of placental structures was performed in the acute phase of coronavirus disease in cases of live term birth and in cases of antenatal asphyxia following infection at 17–22 weeks of gestation. The basis of placental insufficiency is qualitative and quantitative remodeling of the chorion. Distal villous hypoplasia chorionic villi in cases of antenatal fetal death are a manifestation of SARS-CoV-2–induced injury to stem and semi-stem villi during the second trimester of gestation.*

КІЛЬКІСНЕ ВИЗНАЧЕННЯ ЗАТРИМКИ ДОЗРІВАННЯ ВОРСИНЧАСТОГО ХОРІОНА ПРИ КОРОНАВІРУСНІЙ ХВОРОБІ (COVID-19) У ВАГІТНОЇ

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Ключові слова: *плацента, COVID-19, SARS-CoV-2, ворсини хоріона, патологічна анатомія, затримка дозрівання ворсин хоріона.*

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Резюме. *Структура хоріона змінюється відповідно до терміну вагітності: стовбурові, проміжні та термінальні ворсини. У плаценті SARS-CoV-2 уражає клітини, що експресують ангіотензинперетворювальний фермент 2 (angiotensin-converting enzyme 2 – ACE2), – ендотелій, синцитіотрофобласт. Ураження цих клітин спричиняє піроптоз, запалення та розвиток артеріолосклерозу. Під час свого дозрівання плацента фіксує та зберігає зміни, які проявляються порушенням формування хоріона.*

Оригінальні дослідження

Мета роботи *– кількісне визначення затримки дозрівання ворсинчастого хоріона при коронавірусній хворобі COVID-19 у вагітної.*

Матеріал і методи. *Досліджувались 102 плаценти при COVID-19 у вагітної: І група (n=90) – плацента при народженні живого доношеного плода в гострому періоді хвороби у вагітної, та ІІ група (n=12) – при антенатальній асфіксії. У ІІ групі жінка хворіла на 17-22 тижнях гестації. Для порівняння даних досліджували плаценту (n=50) при фізіологічних пологах до епідемії COVID-19.*

Застосовували мікроскопічний, імуногістохімічний, статистичний методи дослідження. Для визначення кількісного показника гістологічні препарати фотографували через мікроскоп за допомогою цифрової фотокамери, отримані фотографії завантажували у форматі JPEG на сервіс ONLINE JPG TOOLS, за допомогою якого визначали відсоток кольору пікселя у фото, який є відсотком досліджуваної структури.

Результати. *При антенатальній асфіксії плода (група ІІ) спостерігалося збільшення відсотка стовбурових та напівстовбурових ворсин хоріона – 36 (25; 50) проти 7 (6; 9) у плацентах групи 1. Стовбурові ворсини групи ІІ відрізняли якісні зміни – фіброз строми та артеріолосклероз. Кількість термінальних ворсин у групі ІІ була 8 (8-8); (p<0,001). У плацентах групи І кількість термінальних ворсин не відрізнялася від групи порівняння – 26 (25-27), що є показником адекватного формування ворсинчастого хоріона відповідно до гестаційного віку. При однаковій кількості термінальних ворсин хоріона в групах І та порівняння відсоток міжворсинчастого простору в групі І був зменшений – 26 (20-33) проти групи порівняння – 44 (40-49), що є проявом набряку термінальних ворсин хоріона. Зменшення кількості ворсин у групі ІІ призводило до збільшення відсотка міжворсинчастого простору – 49 (44-55).*

Висновки. *Проведено кількісне визначення структур плаценти в гострому періоді коронавірусної хвороби при народженні живого доношеного плода та при антенатальній асфіксії при інфікуванні на 17-22 тижнях. Основа плацентарної недостатності – якісна та кількісна перебудова хоріона. Гіпоплазія термінальних ворсин при антенатальній загибелі плода – прояв ураження SARS-CoV-2 стовбурових та напівстовбурових ворсин у другому триместрі гестації.*

Introduction. The structure of the chorion changes according to gestational age, consisting of stem, intermediate (immature and mature), and terminal villi [1]. During the first and second trimesters, immature intermediate villi predominate, serving as a source for mature intermediate villi, while in the third trimester, terminal villi predominate [2,3]. Stem villi play a role in regulating blood flow and providing mechanical stability to the villous tree. Intermediate villi serve as a source for the formation of terminal villi, which form the vasculosyncytial membranes [4].

In the placenta, the coronavirus SARS-CoV-2 affects cells expressing angiotensin-converting enzyme 2 (ACE2), including endothelium and syncytiotrophoblasts, leading to pyroptosis, endothelial dysfunction with impaired blood rheology, platelet activation, microcirculatory disturbances, inflammation, and arteriolosclerosis [5–8]. During its maturation, the placenta records and preserves changes that manifest as impaired chorion formation [9], which is reflected by quantitative alterations in chorionic villi and in the intervillous space.

Objective. To quantitatively assess delayed maturation of the villous chorion in pregnant women with COVID-19.

Materials and Methods. To achieve the study objective, 102 placentas from COVID-19-positive pregnancies were examined: Group I (n=90) – placentas from live term births during the acute phase of maternal COVID-19, and Group II (n=12) – placentas from antenatal fetal asphyxia The material was obtained in accordance with a cooperation agreement with the Department of Pathological Anatomy of the National Children's Specialized Hospital Okhmatdyt of the Ministry of Health of Ukraine during the period from 2020 to 2022. In Group I, the post-COVID interval (the time between maternal COVID-19 diagnosis and delivery) ranged from 1 to 3 weeks. In Group II, maternal infection occurred at 17–22 weeks of gestation. COVID-19 in pregnant women was confirmed by a positive polymerase chain reaction (PCR) test for SARS-CoV-2 RNA. For comparison, 50 placentas from physiological term deliveries before the COVID-19 pandemic were analyzed.

Microscopic, histochemical, immunohistochemical, and statistical methods were used. Histochemical staining was performed using the MSB method modified by Zerbino–Lukasevich. Immunohistochemical (IHC) analysis was carried out using monoclonal antibodies against CD34 (Thermo Fisher Scientific, USA) at working dilutions to assess the condition of arteriolar endothelium.

To determine quantitative parameters, histological slides were photographed through a microscope with a digital camera. The obtained images were saved in JPEG format and uploaded to the ONLINE JPG TOOLS service (onlinejpgtools.com/find-dominant-jpg-colors), which allowed the determination of the percentage of pixel color corresponding to the studied structure (Fig. 1; Fig 2A, B) [10].

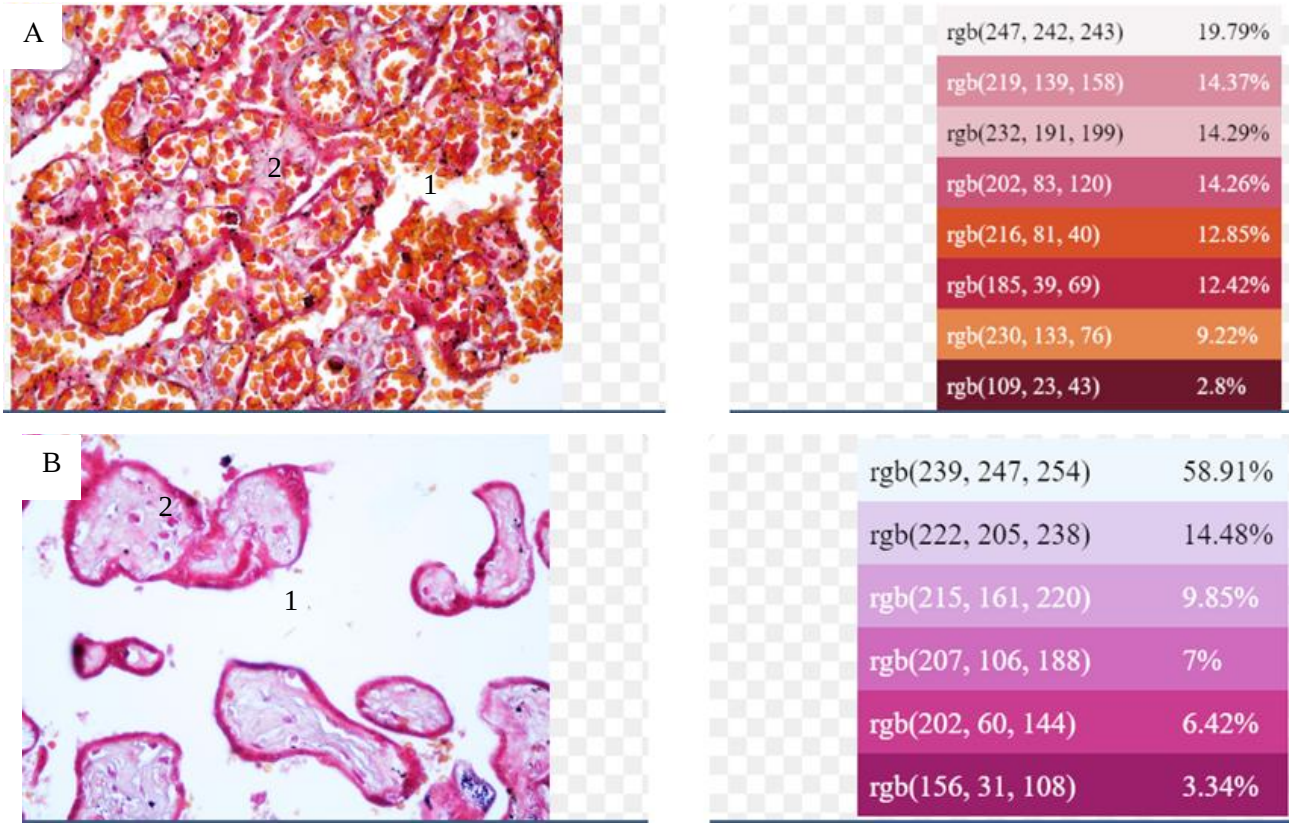

Fig. 1. Screenshots from ONLINE JPG TOOLS: A, B (left) – uploaded JPEG images; right – percentage of colors in the image. 1 – intervillous space (light color); 2 – terminal villi (sum of pink color shades)

To determine the percentage of stem and intermediate (semi-stem) villi, these villi were colored in Microsoft Paint with a color distinct from the other structures in the image (green), followed by percentage calculation using the ONLINE JPG TOOLS service.

Statistical analysis was performed using SPSS IBM v.22 (Armonk, NY, USA), licensed to Bogomolets National Medical University, license No. 128 dated 01.08.2016. Variational statistical methods were applied. For multiple group comparisons of mean values, the non-parametric Kruskal–Wallis test was used. Ranked data are presented as median with lower and upper quartiles (Me [Q1; Q3]). Differences were considered statistically significant at $p < 0.05$.

Results and Discussion. In the study of placentas from live term neonates in the acute phase of coronavirus disease (Group I) and from cases of maternal infection at 17–22 weeks of gestation with antenatal fetal asphyxia (Group II), alterations in the proportional distribution of stem, semi-stem, and terminal chorionic villi, as well as the intervillous space, were observed. In cases of antenatal fetal asphyxia (Group II), an increased percentage of stem and intermediate villi of the chorion was observed – 36 (25–50) versus 7 (6–9) in Group I placentas (Table 1; Fig. 2C-F). Stem villi in Group II were characterized by qualitative changes, including stromal fibrosis and arteriolosclerosis (Fig. 2D), as evidenced by blue staining on immunohistochemical analysis.

In some vessels with an obliterated lumen, revascularization was observed as a final attempt to restore vascular patency (Fig. 3D, arrow).

The number of terminal villi in Group II was 8 (8–8), indicating hypoplasia of terminal villi; $p < 0.001$(Fig.2B). In Group I, the number of terminal villi did not differ from the comparison group – 26 (25–27), reflecting adequate formation of the villous chorion for gestational age. Despite a similar number of terminal villi in Groups I and the comparison group, the intervillous space percentage in Group I was reduced – 26 (20–33) versus 44 (40–49) in the comparison group, indicating edema of the terminal villi (Fig.2C; Fig.3).

In Group I, edematous terminal villi were located in close proximity to each other. Due to stromal edema of the terminal villi in the main groups, a reduction in the vascular lumen was observed, with vessels positioned centrally within the villi (Fig. 3.A,B). This led to an increase in the thickness of the vasculosyncytial membranes, resulting in malperfusion [11].

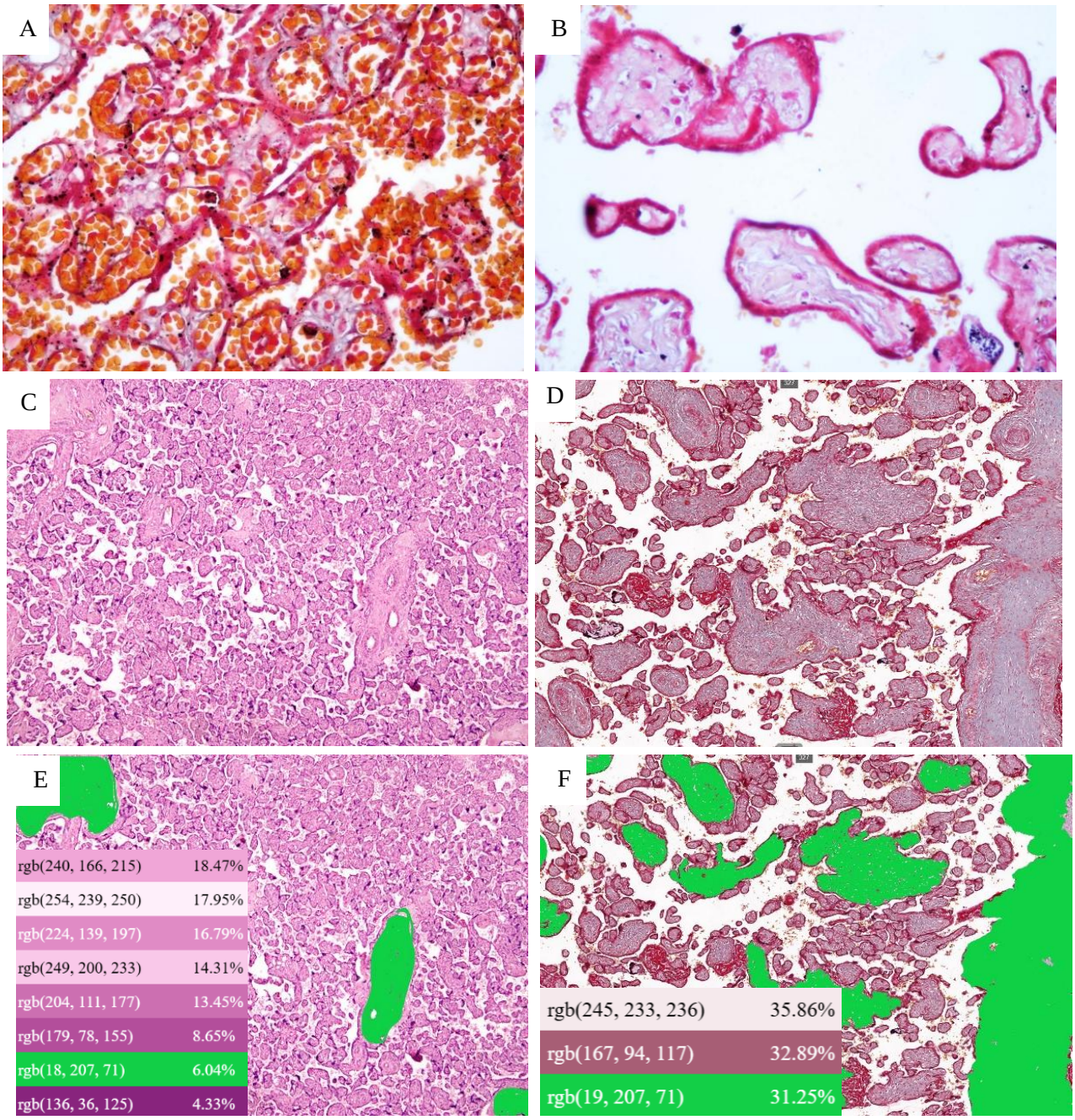

Fig. 2. Structural changes of the placenta in pregnant women with COVID-19.A,C,E – placenta from a term pregnancy with COVID-19 at 37 weeks of gestation. B,D,F – placenta from antenatal fetal demise at 34 weeks of gestation, COVID-19 at 19–20 weeks of gestation. MSB staining modified by D.D. Zerbino and L.L. Lukasevich; A, B ×400; C–F ×40

Table 1

Pathomorphological changes in the placenta in pregnant woman with COVID-19

Parameters	COVID-19 (N=102)			p
	Live births (Group I) (N=90)	Antenatal asphyxia (Group II) (N=12)	Comparison group (N=50)	
Percentage of stem and semi-stem chorionic villi; %	7 (6; 9)	36 (25; 50)*	7 (7; 9)	p<0,001
Number of terminal villi[1]	26 (25-27)	8 (8-8)*	26 (25-27)	p<0,001
Intervillous space, %	26 (20-33)*	49 (44-55)	44 (40-49)	p<0,001

*Note: *p<0.001 (Kruskal–Wallis test); [1] – number of terminal villi in a single field of view at 400× magnification*

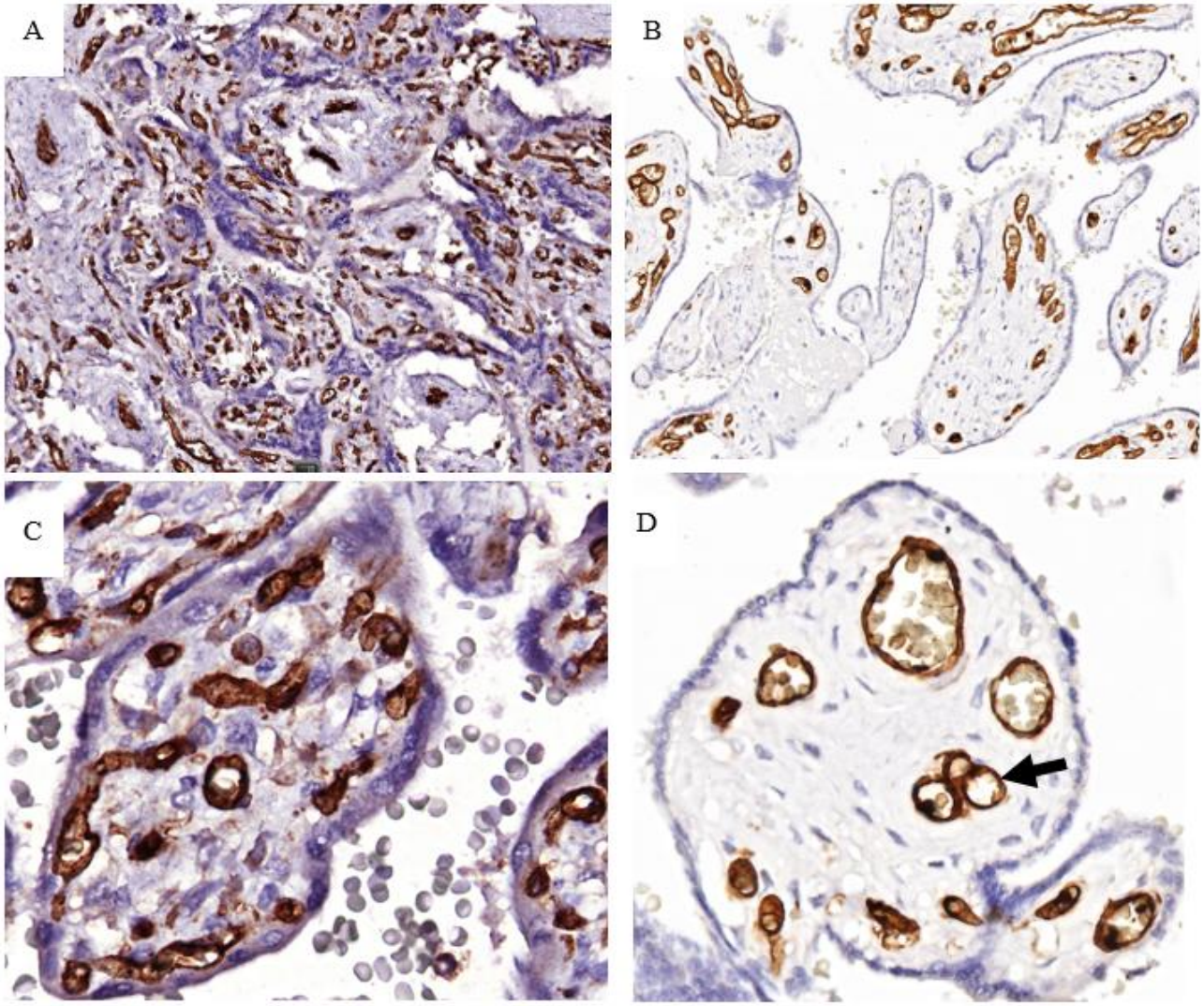

Fig. 3. Structural changes of terminal chorionic villi in placentas from pregnancies complicated by COVID-19. A,C – placenta from a term pregnancy with coronavirus disease at 39 weeks of gestation. B,D – placenta from antenatal fetal demise at 34 weeks of gestation; maternal COVID-19 at 19–20 weeks of gestation. Immunohistochemical study showing expression of monoclonal antibodies against CD34 in the vascular endothelium of chorionic villi. A, B ×200. C,D×400

A large number of syncytial knots were identified between the terminal chorionic villi; their presence is considered a compensatory mechanism aimed at increasing the intervillous space (Fig. 3A). According to other researchers, an increased number of syncytial knots reflects telocyte damage and loss of their regulatory control over syncytiotrophoblast apoptosis [12].

In Group II, despite a reduced proportion of terminal villi, an increase in the percentage of the intervillous space was observed –49 (44–55). The latter is described as a three-dimensional system of channels between chorionic villi, the unobstructed patency of which ensures adequate maternal perfusion.

On the one hand, edema of terminal chorionic villi (Group I), and on the other, the surrounding villous environment – characterized by the accumulation of fibrinoid material or inflammatory cells – intervillositis (Group II) – lead to a reduction in the size of the intervillous channels, ultimately resulting in the development of placental insufficiency.

The study of placentas from pregnant women with coronavirus disease at different gestational ages revealed both qualitative and quantitative changes in the formation of the chorionic villous tree. Quantitative differences were manifested by alterations in the proportional distribution of chorionic villi across the main study groups, which in turn were associated with qualitative changes in stem (and semi-stem) villi following SARS-CoV-2 – induced injury at the corresponding stage of pregnancy.

In Group II, SARS-CoV-2 infection resulted in stromal fibrosis and arteriolosclerosis of stem villi. Since these structures serve as a growth source for terminal villi, their number within the cotyledon was reduced, resulting in distal villous hypoplasia chorionic villi. Thus, placental insufficiency in Group II is based on structural remodeling of the chorion.

Conclusions. A quantitative assessment of placental

structures was performed in the acute phase of coronavirus disease in cases of live term birth and in cases of antenatal asphyxia following infection at 17–22 weeks of gestation. The basis of placental insufficiency is qualitative and quantitative remodeling of the chorion. Distal villous hypoplasia chorionic villi in cases of antenatal fetal death are a manifestation of SARS-CoV-2 – induced injury to stem and semi-stem villi during the second trimester of gestation.

Future Directions. A prospective direction for further research is the study of villous chorion formation in pregnant women with COVID-19 during early gestational stages.

Conflict of Interest. The authors declare no conflict of interest, including financial, personal, or other relationships that could have influenced the presented study and its results.

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