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## MORPHOLOGY AND PATHOMORPHOLOGY

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# Morphometric Parameters of Placental Villi in Parturient Women with COVID-19

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We performed a comparative morphological analysis of placental villi in parturient women with mild and moderate COVID-19 infection. The area and perimeter of terminal villi, their capillaries, and syncytiotrophoblast were assessed on immunohistochemical preparations with antibodies to CD31 using an image analysis system; the parameters of fetal vascular component in the placental villi were also assessed. Changes in the studied parameters differed in parturient women with mild and moderate COVID-19 infection. The observed increase in the total perimeter with a simultaneous decrease in the total capillary area and the degree of vascularization of the placental villi in parturient women with COVID-19 indicates impairment of circulation in the fetal compartment and the development of placental hypoxia, which can be the cause of unfavorable neonatal outcomes.

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**Key Words:** *novel coronavirus infection (COVID-19); SARS-CoV-2; placenta; knots vascularization; morphometry*

Novel coronavirus infection (COVID-19) first occurred in December 2019; one year later, it was diagnosed in 84,054,366 people and resulted in a fatal outcome in 1,835,018 patients (January 1, 2021) [8]. Pregnancy, which does not depend on other concomitant diseases, is considered to be a risk factor for the development of severe forms of COVID-19 [13]. Pregnant women at late terms gestation (>20 weeks) infected with COVID-19 were more likely to be admitted to the intensive care unit and needed oxygen support more frequently in comparison with non-pregnant women with COVID-19 [7].

According to published reports [6], most pregnant women with COVID-19 have such placental le-

sions as disorders of the maternal and fetal circulatory compartments and the development of inflammatory reactions which directly and indirectly affect the development of the fetus and condition of the newborn.

In light of this, quantitative morphological study of the placental terminal villi as a central component of the mother—placenta—fetus system in pregnant women with COVID-19 is of paramount importance.

Here we carried out a morphometric analysis of placental villi of parturient women with COVID-19 depending on the severity of the disease.

### MATERIALS AND METHODS

The study was based on morphological analysis of 26 full-term placentas that were divided into three groups. The first group consisted of 11 placentas of parturient women aged 24-42 years (mean 29.8±1.6 years), who had a mild form of COVID-19 during childbirth;

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the second group included 7 placentas of parturient women aged 24-42 years (mean  $29.8 \pm 6.1$  years) with a moderate form of COVID-19. COVID-19 was diagnosed on the basis of the detection of SARS-CoV-2 RNA in nasopharyngeal and oropharyngeal swabs when performing PCR in the Laboratory of Molecular Genetics, V. I. Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology. The severity of the disease was determined by the doctors of the Obstetric Department in accordance with Temporary Methodological Recommendations of the Ministry of Health of the Russian Federation for the Prevention, Diagnosis and Treatment of a New Coronavirus Infection (COVID-19) [1]. Such symptoms as body temperature below  $38.5^{\circ}\text{C}$ , cough, weakness, and sore throat referred to the mild course of the disease. Moderate-to-severe COVID-19 was diagnosed in the presence of fever above  $38.5^{\circ}\text{C}$ , respiratory rate more than 22/min, shortness of breath on physical exertion, blood oxygen saturation ( $\text{SpO}_2$ )  $<95\%$ , and the content of C-reactive protein  $>10$  mg/liter in the blood serum. The control group included 8 placentas of parturient women aged 24-42 years (mean age is  $34.2 \pm 2.2$  years) with a normal course of pregnancy and the absence of extragenital and genital pathology.

The placentas were fixed in 10% neutral formalin. After macroscopic examination, tissue samples of the paracentral zone were cut out and paraffin sections were prepared. Villous vessels were detected by immunohistochemical staining with rabbit antibodies against CD31 (SP 38, 231436, 1:50) (Abcam) using an UltraView Universal DAB Detection Kit (Roshe) and a polymer detection system (Spring Bioscience). Preliminary antigen retrieval was carried out by boiling the samples in citrate buffer (pH 6.0), endogenous peroxidase was blocked by incubation of the sections with 0.3%  $\text{H}_2\text{O}_2$  for 15 min. The sections were counterstained with hematoxylin.

The morphometric analysis of immunohistochemical preparations was performed using the image analysis system based on Nikon Eclipse 80i microscope with DS-Fi1 digital color camera (Nikon) and NIS-Elements AR 4.11. software. The cross-sections of the villi were measured: the area and perimeter of the terminal villi, their capillaries and syncytiotrophoblast were determined. Damaged villi were excluded from the study. After obtaining morphometric parameters, we calculated the percentage of syncytiotrophoblast as well as indicators of fetal vascular component in the placental villi: the degree of villous vascularization as the ratio of the sum of the area of the capillaries in the villus to its cross-sectional area, the ratio of the perimeter of the villus to its cross-sectional area, the ratio of the sum of the perimeters of capillaries to the sum of the areas of these capillaries in the villus, the ratio of

the perimeter of the villus to the sum of the perimeters of its capillaries, the ratio of the perimeter of the villus to the sum of the areas of its capillaries [5].

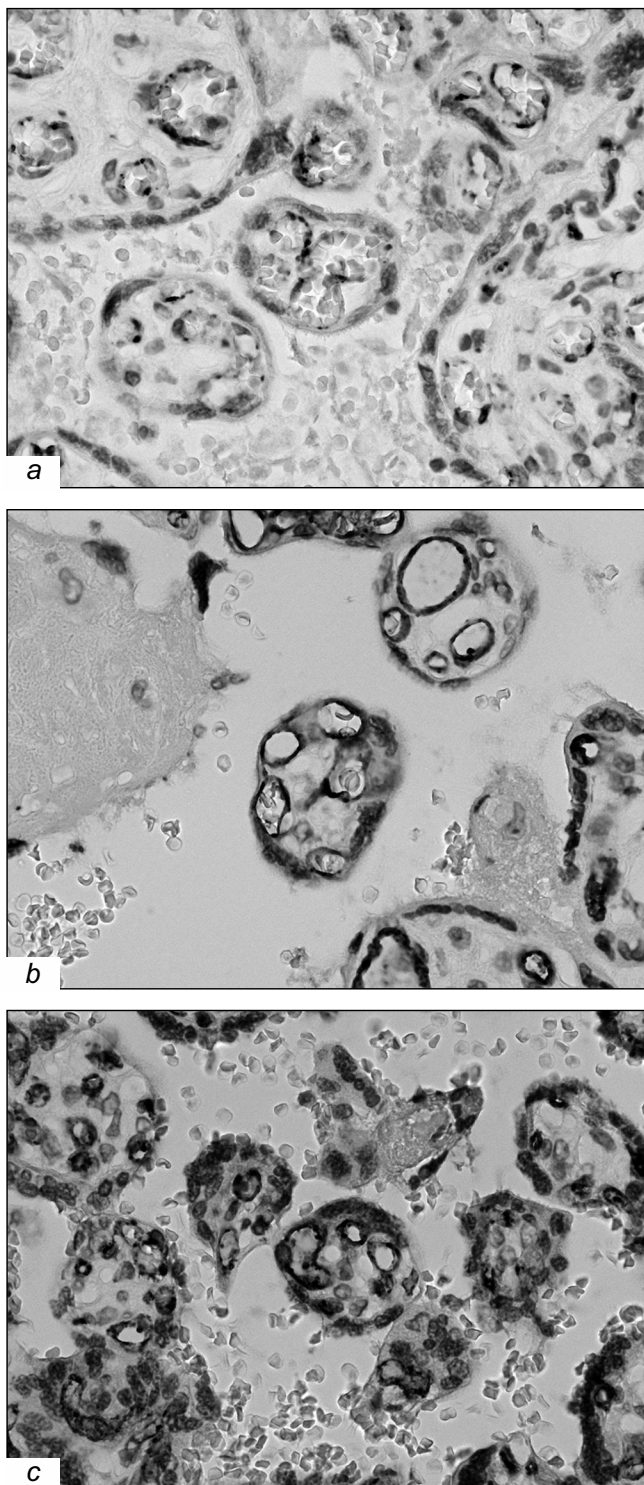
The obtained data were statistically processed using Statistica 10.0 software (StatSoft, Inc.); Me (Q1; Q3) were calculated. Intergroup comparisons were performed using the Mann—Whitney  $U$  test. The differences were considered significant at  $p < 0.05$ .

## RESULTS

The quantitative analysis of immunohistochemical preparations of the placental tissue in the control group (Fig. 1, *a*) showed that the medians of the specific area of the syncytiotrophoblast and the degree of villous vascularization were 24 and 40.4%, respectively (Table 1). The ratio of the sum of the perimeters of the capillaries in the villus to the sum of their areas, which characterize the possibilities of metabolic processes, was 0.29 (0.24-0.32).

The morphometric analysis of the placental preparations of parturient women suffering from COVID-19 (Fig. 1, *b, c*) allowed us to identify deviations of certain parameters from the indicators of the control group (Table 1). Thus, the medians of the cross-sectional area of the villi in patients with mild and moderate COVID-19 were higher than in the control group by 24.2% ( $p < 0.05$ ) and 8.2%, respectively; the perimeter of the villi was higher by 13.8% ( $p < 0.05$ ) and 7.3%, respectively. The area of syncytiotrophoblast in the villi of patients with COVID-19 had almost the same values and exceeded the values of the control group by 18.5-18.4%; the specific area of syncytiotrophoblast in women with mild course of COVID-19 was lower by 4.6% and in patients with a moderate course it was higher by 9.2% ( $p > 0.05$ ) than in the control group.

Changes in the morphometric parameters of capillaries of the villi that reflect the state of the fetal component of blood circulation in the placenta are also worthy of note (Table 1). The number of capillaries in the villi of the placentas obtained from parturient women with mild and moderate COVID-19 exceeded the values of the control group by 25 and 37.5% ( $p < 0.05$ ). At the same time, the medians of the area and perimeter of one capillary in parturient women with a mild course of COVID-19 were lower than in the control group by 15.4 and 4.9%, respectively; in patients with moderate course of COVID-19, these parameters were lower by 29.4 and 15.3%, respectively ( $p < 0.05$ ). The sum of the areas and perimeters of the capillaries in the villus had different values: the sum of areas of the capillaries in patients with COVID-19 was smaller than that in the control group, and the sum of their perimeters was higher.



**Fig. 1.** Placental villi of parturient women of the control group (a) and patients with mild (b) and moderate (c) forms of COVID-19. Immunoperoxidase method, antibodies to CD31,  $\times 400$ .

The calculated degree of villous vascularization in patients with mild and moderate COVID-19 was below the control by 10 and 15%, respectively. The ratio of the sum of the perimeters of the capillaries to the sum of the areas of the capillaries in the villus and

the ratio of the perimeter of the villus to the sum of the areas of its capillaries were higher than the corresponding indicators of the control group, especially in women with moderate COVID-19 (Table 1).

Thus, the results of morphometric analysis of placental immunohistochemical preparations revealed changes in the size of the villous capillaries in parturient women with mild and moderate forms of COVID-19. First of all, these changes reflect circulatory disorders in the fetal compartment of the placenta (fetal vascular malperfusion) [3].

According to published reports [6], different types of placental damage in pregnant women with COVID-19 were revealed. Detailed analysis of the results of 20 studies on placental morphology in the third trimester [15] revealed the presence of signs of circulatory disorders in the maternal compartment of the placenta in 46% cases, circulatory disorders in the fetal compartment in 35.3%, and signs of inflammation in 8.7% cases.

The authors of the study [11] also indicate more frequent detection of signs of circulatory disorders in the fetal compartment of the placenta in pregnant women with SARS-CoV-2: chorangiosis (RR=2.41 (1.51-3.84),  $p=0.001$ ), fibrin deposits in the vascular wall ( $p=0.023$ ), and vasodilation (RR=2.16 (1.38-3.38),  $p=0.004$ ). Other signs of circulatory disorders in the fetal compartment of the placenta (avascular villi, thrombosis in the fetal chorionic plate and villous stromal-vascular karyorrhexis) were also more often detected in pregnant women with SARS-CoV-2, but they did not significantly differ from the control values [11].

Our study was based on the quantitative assessment of the size of functioning vessels in the villi that provide blood supply to the fetus. At the same time, we revealed an increase in the specific area of the syncytiotrophoblast that reflects the state of the maternal compartment of the placental blood circulation. This increase in the syncytiotrophoblast area, along with the increase in the number of syncytial knots and bridges in the terminal villi of the placenta in parturient women with COVID-19 [4], indicates the presence of preplacental hypoxia. In our opinion, the latter is the main cause of circulatory disorders of the fetal compartment of the placenta, namely, the increase in the number of capillaries in the placental villi which was revealed in the patients with COVID-19 participating in our study. More frequent detection of signs of chorangiosis in the placentas of pregnant women with COVID-19 also confirms this hypothesis [6,11]. Chorangiosis is known to be characterized by increased number of capillaries in the terminal villi of the placenta and it is indicative of chronic placental hypoxia. Chorangiosis is diagnosed when microscopic examination of histological preparations of the placenta reveals at least 10

**TABLE 1.** Morphometric Characteristics of Placental Villi in Parturient Women with Mild and Moderate Course of COVID-19 (Me (Q1; Q3))

Parameter	Control	Patients with COVID-19	
		mild course	moderate course
Area of the villus, $\mu\text{m}^2$	1831.2 (1506.3; 2129.8)	2275.0 (1796.8; 2777.9)*	1981.6 (1707.7; 2828.5)
Perimeter of the villus, $\mu\text{m}$	161.8 (148.7; 174.8)	184.1 (165.2; 200.3)*	173.6 (160.9; 206.8)
Area of the syncytiotrophoblast in the villus, $\mu\text{m}^2$	439.1 (367.2; 565.7)	520.4 (445.3; 682.9)*	520.1 (439.8; 630.3)*
Number of capillaries in the villus	4 (3; 4)	5 (4; 6)*	5.5 (4.5; 6.0)*
Area of the capillary, $\mu\text{m}^2$	192.2 (154.4; 251.8)	162.4 (118.7; 231.3)	135.7 (108.0; 163.1)*
Perimeter of the capillary, $\mu\text{m}$	55.6 (48.0; 64.2)	52.9 (40.0; 62.6)	47.1 (39.2; 49.9)*
Sum of the areas of the capillaries in the villi, $\mu\text{m}^2$	741.1 (598.9; 896.4)	718.2 (612.2; 942.6)	646.7 (545.0; 915.6)
Sum of the perimeters of the capillaries in the villi, $\mu\text{m}$	201.9 (187.7; 243.4)	228.4 (190.0; 269.1)	224.0 (196.6; 268.0)
Degree of villous vascularization, %	0.40 (0.33; 0.51)	0.36 (0.28; 0.40)*	0.34 (0.30; 0.35)*
Ratio of the perimeter of the villus to its cross-sectional area	0.09 (0.08; 0.10)	0.08 (0.07; 0.09)*	0.09 (0.07; 0.09)
Ratio of the sum of the perimeters to the sum of the areas of the capillaries of the terminal villi	0.29 (0.24; 0.32)	0.32 (0.26; 0.36)*	0.34 (0.31; 0.37)*
Ratio of the perimeter of the villi to the sum of the perimeters of its capillaries	1.29 (1.19; 1.43)	1.24 (1.14-1.41)	1.29 (1.23; 1.35)
Ratio of the perimeter of the villi to the sum of the cross-sectional areas of its capillaries	0.22 (0.19; 0.25)	0.23 (0.20; 0.30)	0.28 (0.22; 0.30)

**Note.** \* $p < 0.05$  in comparison with control.

villi containing at least 10 capillaries per high power field [2].

In this study, we also found a decrease in the sum of the areas and an increase in the sum of the perimeters of the capillaries in the placental villi in patients with COVID-19 in comparison with the corresponding indicators of the control group. These changes in the area of blood vessels indicate a decrease in the volume of fetal blood flow with a simultaneous compensatory increase in the surface of exchange between fetal blood and villus.

It should be noted that the degree of villous vascularization of the placenta in parturient women with COVID-19 was lower than in the control group. That is, placental hypoxia that develops in patients with COVID-19 is caused by maternal pathology, as well as by vascularization disorders in the terminal villi of the placenta. These changes are likely to have an impact on the development of the fetus and condition of the newborn.

It should also be noted that the main thing when a child is born to a mother suffering from COVID-19 is the diagnosis of vertical (transplacental) transmission

of SARS-CoV-2. It is noteworthy that transplacental infection is rare (1-4% cases) [9,10]. Moreover, comprehensive examination of 242 parturient women with COVID-19 during pregnancy or childbirth and their 248 newborns [12] did not detect a fact of COVID-19 transmission during childbirth and the first month of life, including cases of breastfeeding. Similarly, the study [14] did not reveal the fact of vertical or perinatal transmission of COVID-19 during examination of mother—newborn dyads (116 mothers with COVID-19 during childbirth and 120 newborns during the first month of life).

Thus, the results of the morphometric study of placental villi in parturient women with COVID-19 revealed changes in the obtained parameters which were different in patients with mild and moderate course of the disease. The study showed an increase in the sum of the perimeters of the capillaries, a decrease in the sum of the areas of the capillaries and decrease in the degree of villous vascularization. These results can indicate impaired circulation in the fetal compartment and development of placental hypoxia which may result in adverse neonatal outcomes.

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