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Hofbauer cells and coronavirus disease 2019 (COVID-19) in pregnancy: Molecular pathology analysis of villous macrophages, endothelial cells, and placental findings from 22 placentas infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with and without fetal transmission

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Abstract

Context.– Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can undergo

maternal-fetal transmission, heightening interest in the placental pathology findings from this

infection. Transplacental SARS-CoV-2 transmission is typically accompanied by chronic

histiocytic intervillositis together with necrosis and positivity of syncytiotrophoblast for SARS-

CoV-2. Hofbauer cells are placental macrophages that have been involved in viral diseases

including HIV and Zika virus, but their involvement in SARS-CoV-2 in unknown.

Objective.– To determine whether SARS-CoV-2 can extend beyond the syncytiotrophoblast to

enter Hofbauer cells, endothelium and other villous stromal cells in infected placentas of

liveborn and stillborn infants.

Design.– Case-based retrospective analysis by 29 perinatal and molecular pathology specialists

of placental findings from a preselected cohort of 22 SARS-CoV-2-infected placentas delivered

to pregnant women testing positive for SARS-CoV-2 from 7 countries. Molecular pathology

methods were used to investigate viral involvement of Hofbauer cells, villous capillary

endothelium, syncytiotrophoblast and other fetal-derived cells.

Results.– Chronic histiocytic intervillositis and trophoblast necrosis was present in all 22

placentas (100%). SARS-CoV-2 was identified in Hofbauer cells from 4/22 placentas (18%).

Villous capillary endothelial staining was positive in 2/22 cases (9%), both of which also had

viral positivity in Hofbauer cells. Syncytiotrophoblast staining occurred in 21/22 placentas

(95%). Hofbauer cell hyperplasia was present in 3/22 placentas (14%). In the 7 cases having

documented transplacental infection of the fetus, 2 occurred in placentas with Hofbauer cell

staining positive for SARS-CoV-2.

Conclusions.– SARS-CoV-2 can extend beyond the trophoblast into the villous stroma, involving

Hofbauer cells and capillary endothelial cells, in a small number of infected placentas. Most

cases of SARS-CoV-2 transplacental fetal infection occur without Hofbauer cell involvement.

INTRODUCTION

At the start of the coronavirus disease 2019 (COVID-19) pandemic the medical and public health communities were concerned that the etiologic agent, a novel coronavirus that was eventually termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), would be transmissible from infected mothers to their infants.[1] Previous experience with two other emerging coronaviruses, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), had not identified the existence of vertical (mother-to-infant) transmission, although the numbers of infected pregnant women were limited.[2-4] Analysis of other respiratory RNA viruses revealed that vertical transmission with these agents was either non-existent or, at most, rare.[5] Initial reports from China, where the COVID-19 infection had begun, showed that pregnant women infected with SARS-CoV-2 were not transmitting the virus to their neonates,[6-10] but concern remained for the potential of vertical infection.[11-14] As the COVID-19 pandemic spread to other countries and large numbers of pregnant women became infected, there were reports of neonates having positive testing for SARS-CoV-2 occurring after delivery, raising the specter that vertical transmission might be occurring.[15-18] Placental pathology criteria[19] were developed for the diagnosis of intrauterine transplacental transmission in infected maternal-fetal dyads that utilized molecular pathology methods for identifying SARS-CoV-2 in fetal cells of the placenta.[20] Eventually, it became clear that not only was vertical transmission of SARS-CoV-2 occurring around the time of delivery in a small number of newborns,[21,22] but also that the virus was being transmitted transplacentally from infected mothers to the fetus in some cases.[23-29] Following reports of these and other cases of placental infection with SARS-CoV-2,[30] a study of 11 placentas from infected pregnant

women revealed that there were 3 simultaneously occurring pathology abnormalities that included chronic histiocytic intervillositis, syncytiotrophoblast necrosis, and infection with SARS-CoV-2 detected in tissue by immunohistochemistry and/or in situ hybridization.[16,31,32] Since then, additional similar cases with these findings have been reported.[33-38]

Hofbauer cells are fetal macrophages that are normally present within chorionic villi from as early as 18 days gestation up to delivery. Located within the chorionic villous stroma, in close proximity to both the trophoblast layer as well as the villous capillaries, Hofbauer cells are in an optimal position to respond to pathogenic organisms potentially crossing the maternal-fetal interface. These immunocompetent cells possess an M2 anti-inflammatory, regulatory phenotype, and have been previously implicated in placental viral infections including human immunodeficiency virus (HIV) and Zika virus.[39,40] Because SARS-CoV-2 has now been found to result in transplacental fetal infections in a small number of infected mothers,[31,32] we examined 22 placentas infected with this virus originating from 7 countries to determine whether Hofbauer cell involvement was present. This study also investigated the relationship of Hofbauer cell involvement with SARS-CoV-2 and placental pathology findings to determine if it was a common accompaniment to viral involvement of other fetal cells, including endothelial cells, in the chorionic villi. This case series represents the largest cohort of placentas infected with SARS-CoV-2 to be reported.

MATERIALS AND METHODS

This case-based retrospective study enrolled maternal-fetal pairs consisting of women having had a positive test result for SARS-CoV-2 during pregnancy using reverse transcriptase polymerase chain reaction (RT-PCR) occurring prior to delivery in which placenta was submitted

for pathology examination and found to be infected with SARS-CoV-2 by direct visualization of fetal-derived placental cells, either using immunohistochemistry for SARS-CoV-2 antigens, RNA in situ hybridization for SARS-CoV-2 nucleic acid, or both. These cases were contributed to the investigation by a collaborative group of 29 perinatal physicians and molecular pathologists and biologists working with the disease.

Hofbauer cells were identified using immunohistochemistry to a variety of macrophage antigens including CD14, CD68, and CD163. In some cases, double staining was performed using immunohistochemical staining for Hofbauer cells and either immunohistochemistry for SARS-CoV-2 antigens or RNA in situ hybridization for SARS-CoV-2 nucleic acid. The placentas from Cases 10 and 15 were immunohistochemically evaluated for Hofbauer cell proliferation by staining with antibody to the Ki67 nuclear proliferation marker. In each case, pertinent maternal and neonatal clinical data and the results of testing for SARS-CoV-2 including maternal-fetal transmission status (when available) were obtained, and the presence of significant placental pathology abnormalities was recorded.

In those cases that previously had some aspect of the case published, the references are provided. For all 22 cases occurring in 7 countries that comprised this study group, the 29 pathologists, clinicians, or others involved with these patients were personally contacted by one of the authors (DAS) requesting reexamination of the placenta to evaluate Hofbauer cell involvement and confirmation of the clinical, laboratory, and pathology findings. In some cases, this led to additional molecular testing of placental tissues. For all cases there was either approval received from the local institutional review boards or institutional waiver and parental

permission obtained, and there was compliance with the Declaration of Helsinki for Human

Research.

RESULTS

Hofbauer Cell Staining for SARS-CoV-2

A total of 22 placentas were examined for the presence of SARS-CoV-2 within Hofbauer

cells using immunohistochemistry, RNA in situ hybridization, or both (Tables 1, 2 and 3). With

one exception (Case 20, discussed below), all placentas had strong positive staining of the

syncytiotrophoblast for SARS-CoV-2 using immunohistochemistry, RNA in situ hybridization, or

both methods.

Nineteen of 22 placentas (86%) did not reveal staining of any villous stromal cells,

including Hofbauer cells, for SARS-CoV-2 (Figure 1A,B, Figure 2A,B, Figure 3A,B, and Figure 4). In

some of these placentas, double staining immunohistochemistry was performed using

antibodies to macrophages and to SARS-CoV-2 nucleocapsid protein, which were also negative

for Hofbauer cell involvement. In some cases, Hofbauer cells were identified that closely

approached, and in some cases abutted upon, the trophoblast basement membrane zone

(Figure 4), but these macrophages were also negative for viral staining.

Among these 22 placentas there were 4 placentas (18%) that demonstrated positive

staining of Hofbauer cells for SARS-CoV-2. These included Cases 15 (Figure 5, 6A,B,C), Case 19

(Figure 7 A,B), Case 20 (Figure 8, 9B,C,D) and Case 21 (Figure 10A,B). In the 7 cases with

documented transplacental infection of the fetus (Cases 10,11,12,14,15,19,22), 2 occurred in

placentas with Hofbauer cell staining positive for SARS-CoV-2.

In Case 15, immunohistochemistry revealed cells with strong intracytoplasmic staining for SARS-CoV-2 nucleocapsid protein present within the stroma of multiple chorionic villi. These cells had the morphological features of macrophages, consistent with Hofbauer cells (Figure 5). Double staining using antibodies to macrophage markers CD68, CD163 and CD14 together with antibody to SARS-CoV-2 nucleocapsid protein demonstrated that Hofbauer cells were positive for intracytoplasmic SARS-CoV-2 staining (Figure 6A, B, C). In addition, there was intense staining for SARS-CoV-2 of the syncytiotrophoblast. No viral staining of the villous capillary endothelium was present. Hofbauer cell hyperplasia was present, and double staining using antibody to Ki67 nuclear proliferation antigen and SARS-CoV-2 nucleocapsid protein revealed that occasional Hofbauer cells were in the proliferation phase of the cell cycle.

Case 19 was a preterm placenta in which there was positive intracytoplasmic staining of villous stromal cells morphologically consistent with Hofbauer cells as well as intense positive staining of the syncytiotrophoblast using antibody to SARS-CoV-2 nucleocapsid protein (Figure 7A,B).

Case 20 had both a unique clinical history as well as array of placenta pathology findings. The mother had initially been hospitalized for symptomatic SARS-CoV-2 infection at 26 weeks gestation. She delivered her infant 11 weeks later, at 37 weeks gestation, at which time both mother and neonate had negative nasopharyngeal swabs for SARS-CoV-2 using polymerase chain reaction (PCR) testing. The placenta had extensive fibrin deposition (Figure 8), syncytiotrophoblast necrosis and mild chronic histiocytic intervillositis. Immunohistochemistry using antibody to SARS-CoV-2 nucleocapsid protein demonstrated numerous villous stromal cells having the morphological features of Hofbauer cells with

positive intracytoplasmic staining (Figure 9). Capillary villous endothelial cells also stained

positively for the virus, and in some vessels, it appeared that more than one endothelial cell

stained positively for SARS-CoV-2 (Figure 9C). The syncytiotrophoblast did not stain for SARS-

CoV-2 – the only placenta in this cohort to show negative staining in these cells.

Case 21 was a placenta from a stillborn infant at 35 4/7 weeks gestation. The placenta

showed extensive intervillous fibrin deposition suggesting massive perivillous fibrin deposition,

syncytiotrophoblast necrosis and mild chronic histiocytic intervillositis. Immunohistochemical

staining with antibody to SARS-CoV-2 nucleocapsid protein showed intense positive staining of

syncytiotrophoblast. There were numerous villous stromal cells having the morphological

features of Hofbauer cells that stained positively for SARS-CoV-2 (Figure 10A,B). Positive

staining for SARS-CoV-2 was also present in occasional villous capillary endothelial cells (Figure

10C). The stillborn infant was not tested for SARS-CoV-2.

Hofbauer Cell Hyperplasia and Proliferation

Three of 22 placentas (14%) (Cases 10, 15 and 20) demonstrated Hofbauer cell

hyperplasia (Figure 3A,B and 9A). Double immunohistochemical staining of a placenta (Case 15)

from a fetus with transplacental SARS-CoV-2 infection using the proliferation marker Ki67 and

antibody to SARS-CoV-2 nucleocapsid protein showed numerous cytotrophoblast cells in the

proliferative phase of the cell cycle, but relatively few Hofbauer cells (Figure 11A). In the

placenta of another fetus having acquired infection through transplacental transmission (Case

10), immunohistochemistry with antibody to Ki67 showed that there were many

cytotrophoblast but very few Hofbauer cells in the proliferative phase of the cell cycle (Figure

11B). The lack of prominent Hofbauer cell proliferation is in marked contrast to that seen in placentas of fetuses having congenital Zika syndrome (Figure 11C).

Villous Capillary Staining for SARS-CoV-2

In 2 placentas (Cases 20 and 21) there was positivity of villous capillary endothelial cells identified for SARS-CoV-2 (Figures 9, 10C). These 2 placentas also had staining of Hofbauer cells present. Both Cases 20 and 21 appeared to show that some villous vessels had greater than one endothelial cell staining positive per lumen for SARS-CoV-2. No villous capillary endothelial staining was present in the 19 placentas in which Hofbauer cell staining was absent.

Trophoblast Staining for SARS-CoV-2

The syncytiotrophoblast stained positive for SARS-CoV-2 in 21 of the 22 (95%) placentas. The only placenta in which the syncytiotrophoblast did not stain was Case 20, in which there was an 11-week interval between maternal illness and test positivity for SARS-CoV-2 and the delivery.

In one case (Case 15), staining of both syncytiotrophoblast and cytotrophoblast for SARS-CoV-2 was present.

Chronic Histiocytic Intervillositis

Chronic histiocytic intervillositis was present in all placentas (100%). In all placentas the characteristic accumulation of intervillous histiocytes was identified by both routine histologic staining as well as immunohistochemical staining for macrophage antigens. The degree of intervillous inflammation varied between the different cases; in some cases where there was extensive fibrin deposition or massive perivillous fibrin deposition, the extent of chronic

histiocytic intervillositis appeared to be less severe than in cases where the fibrin deposition was not as prominent.

Additional Findings

Although it was not a focus of our study, it was observed in 5 placentas having chronic histiocytic intervillositis that histiocytes within the inflammatory infiltrate stained positive for SARS-CoV-2 nucleocapsid antibody.

DISCUSSION

The COVID-19 pandemic has been remarkable for the wide range, spectrum and duration of disease that it produces, affecting persons of all ages and ethnicities. In particular, infection of pregnant women and vertical transmission of SARS-CoV-2 has remained a special public health problem due to the susceptibility of mothers, fetuses and neonates to viral infections.[41-43] Following the demonstration that SARS-CoV-2 can be transmitted from a pregnant woman to her fetus through the placenta,[19,31,32] this coronavirus can now be considered a TORCH (Toxoplasma, Other, Rubella, Cytomegalovirus, Herpes) infection.

Examination of placentas in cases of maternal-fetal infection due to TORCH agents has been greatly beneficial in understanding not only the specific placental cell types that are susceptible to infection, but also in evaluating potential mechanisms of transplacental transmission. In studying transplacental viral infections, among the most important cells to be examined are the resident population of immunologically active placental macrophages, the Hofbauer cells. Hofbauer cells are large, pleomorphic fetal-derived macrophages that constitute an important cellular component of the maternal-fetal interface and the placental immune system. They are located in an advantageous position to perform this function, within the

chorionic villous stroma adjacent to both the fetal villous capillaries and the overlying

trophoblast layer, where potential transfer of substances and microbial agents across the

maternal-placental interface can occur.[44] Hofbauer cells are present throughout all trimesters

of pregnancy and have features which most closely resemble alternatively activated

macrophages that have been termed M2a, M2b, M2c, and M2d polarity subtypes that have an

anti-inflammatory or regulatory phenotype.[45] Hofbauer cells serve many functions –

angiogenesis and vasculogenesis, host defense, villous development and stromal maturation,

fluid homeostasis, clearance of apoptotic cells.[45-47] Evidence suggests that Hofbauer cells may

also be involved in the vasoregulation of placental blood vessels, as *in vitro* studies have found

that they produce both prostaglandin E_2 and thromboxane.[46] It has been experimentally

demonstrated that when exposed to an infectious agent or inflammatory stimulus, Hofbauer

cells may express an M1 or pro-inflammatory phenotype that can release cytokines, damage

the villus, and result in a fibrotic response.[45,48-50]

Similar to other types of macrophages, Hofbauer cells have plasticity in their functions,

and in addition to their role in placental development and homeostasis they have been

implicated in the pathogenesis of a number of TORCH agents.[51] In particular, they can be the

targets for several viruses including HIV and Zika virus.[39, 51-53] Hofbauer cells are susceptible to

HIV infection – they express not only the CD4 receptor, which is bound by the viral *env*

receptor, but also HIV coreceptors including DC-SIGN (dendritic cell specific intercellular

adhesion molecule-3-grabbing non integrin), CCR5 (C-C chemokine receptor type 5) and CXCR4

(C-X-C chemokine receptor type 4).[39,54] Prior to the onset of the COVID-19 pandemic, the most

recent example of a newly emergent virus causing transplacental infection of the fetus was a

flavivirus – Zika virus. Similar to HIV, placentas infected with Zika virus from neonates with congenital infection are not typically associated with an inflammatory process of either maternal or fetal origin. Although the mechanism(s) by which Zika virus penetrates the trophoblastic barrier remains unclear, the virus can undergo productive infection of Hofbauer cells in vitro.[55-57] In placentas from fetuses with congenital Zika syndrome following intrauterine infection, Hofbauer cells have been found to contain the Zika virus and to undergo proliferation in response to placental infection.[40,58]

In the early stages of the COVID-19 pandemic, pathology investigations of placentas from pregnant women having SARS-CoV-2 infection revealed a range of findings that included fetal vascular malperfusion,[59] maternal vascular malperfusion,[60] and even no specific abnormalities.[61] In the majority of these cases, the placentas and neonates were not found to be infected with SARS-CoV-2. However, the placental pathology findings from fetuses infected with SARS-CoV-2 following transplacental transmission are very different from those of neonates with congenital Zika virus infection. Intrauterine transplacental transmission of SARS-CoV-2 has been found to be associated in most cases with 3 significant pathology findings – chronic histiocytic intervillositis, necrosis of the syncytiotrophoblast, and identification of the virus in syncytiotrophoblast using immunohistochemistry or RNA in situ hybridization.[31,32] In addition to these 3 findings, increased fibrin deposition within the intervillous space, which in some placentas may be so severe that it reaches the criteria for massive perivillous fibrin deposition, appears to be accompany placental infection with SARS-CoV-2.[31,32] In contrast to placental Zika virus infection in which virus is most frequently identified in Hofbauer cells, in placentas infected with SARS-CoV-2 the syncytiotrophoblast appears to be the most frequently

involved cell type.[62] However, with the demonstration that SARS-CoV-2 can be identified in the syncytiotrophoblast from infected placentas as well as from fetuses having intrauterine infection, the question arises as to whether the virus can pass beyond the trophoblast and into cells in the underlying villous stroma.

There have been previous individual case reports of both positivity and negativity of Hofbauer cells for SARS-CoV-2 staining,[26,38,63] but this present article describes the first systematic investigation of SARS-CoV-2 involving Hofbauer cells and other cell types in a large cohort of placentas infected with the virus. Evaluation of 22 placentas infected with SARS-CoV-2 revealed that Hofbauer cells were immunohistochemically positive for viral staining in 4 cases (18%) (Cases 19, 15, 20, 21). In some cases, Hofbauer cells that were adjacent to and even in intimate contact with, the trophoblast basement membrane zone stained negative for SARS-CoV-2. While we were unable to determine if positive staining represented intact virions, it indicates that, at the least, SARS-CoV-2 material can penetrate through the trophoblast layer into the core of the chorionic villus. It should be emphasized that in this article we have referred to staining positivity of Hofbauer cells for SARS-CoV-2 as involvement, not infection, because positive staining of Hofbauer cells is only indicative of viral material within the cytoplasm of macrophages. Whether productive SARS-CoV-2 replication is occurring within these Hofbauer cells cannot be determined but would be important to know. Hofbauer cell involvement was also not a requirement for transplacental viral transmission in this series. Among the 22 infected placentas, there were 7 cases of transplacental SARS-CoV-2 transmission (Cases 10, 11, 12, 14, 15, 19, 22). In only 2 of these transmitting cases, Cases 15 and 19, were Hofbauer cells found to stain positive for SARS-CoV-2.

Because Hofbauer cell proliferation and hyperplasia were significant features in some placentas infected with Zika virus, we looked for these features in our placental cohort. Although Hofbauer cell hyperplasia was present in 3 placentas, Case 10 (Figure 3A,B), Case 15, and Case 20 (Figure 9A), none demonstrated the extent of hyperplasia seen in Zika virus-infected placentas. Immunohistochemical staining of 2 placentas (Cases 10 and 15) infected with SARS-CoV-2 using the nuclear proliferation marker Ki67 showed some proliferative activity of Hofbauer cells, but the extent of Hofbauer cell proliferation was not as high as present in placentas infected with Zika virus (Figure 11C).

Villous capillary endothelial staining for SARS-CoV-2 was identified in 2 of 22 placentas (9%) (Cases 20 and 21). Although these endothelial cells lining the villous capillaries are in direct contact with fetal blood circulating in the chorionic circulation, there was no identifiable association with viral staining of these cells and maternal-fetal SARS-CoV-2 transmission. An interesting observation is that in both placentas that had positive staining of endothelial cells for SARS-CoV-2, Hofbauer cells also stained positively for the virus. Staining of endothelial cells for SARS-CoV-2 was not observed in placentas that did not also demonstrate Hofbauer cell staining for the virus. Although these numbers are too small for statistical analysis, it seems not only biologically feasible but almost beyond coincidence that these findings are unassociated and suggests that when SARS-CoV-2 penetrates through the protective trophoblastic layer and gains access to the chorionic villous stroma, viral material (or virions) can enter or be sequestered within such stromal cells as Hofbauer cells and endothelial cells. Endothelial cells that line the blood vessels have many roles that include limiting access of infectious agents, toxins, and other materials between the bloodstream and surrounding tissues. Although not

phagocytic, endothelial cells have been reported to internalize, or sequester, micro-size objects that include blood clots, senescent cells and even bacteria such as *Listeria monocytogenes*.[64-66] This phenomenon may explain the staining positivity of endothelial cells in these 2 placentas.

Chronic histiocytic intervillositis has been found to occur together with placental infections with SARS-CoV-2 as well and transplacental transmission and stillbirths.[31,32,62] All 22 placentas (100%) that were infected with SARS-CoV-2 in this cohort had the finding of chronic histiocytic intervillositis in varying degrees of intensity. Another published finding associated with placental infection with SARS-CoV-2, trophoblast necrosis, was also present in all 22 cases.

One case needs to be discussed in detail due to its atypical features. Case 20 was unusual because of the 11-week interval between the diagnosis of SARS-CoV-2 at 26 weeks gestation in a symptomatic pregnant mother and the delivery of her uninfected infant at 37 weeks.[38,67] The placenta demonstrated chronic histiocytic intervillositis, trophoblast necrosis and increased fibrin, with immunohistochemical positivity for SARS-CoV-2 in Hofbauer cells and villous capillary endothelial cells but not syncytiotrophoblast. Although speculative, given the 11-week interval between symptomatic maternal infection and delivery of the placenta (and infant), we suggest the following explanation. An initial placental infection around the time of maternal SARS-CoV-2 infection at 26 weeks gestation, accompanied by infection and necrosis of the syncytiotrophoblast with chronic histiocytic intervillositis and fibrin deposition. During the subsequent weeks, extension of SARS-CoV-2 into cells in the villous stroma as well as residual inflammation and fibrin deposition, but with resolution and clearing of virus from necrotic trophoblast and regeneration of uninfected syncytiotrophoblast. It has been previously

observed that neonatal test positivity for SARS-CoV-2 is frequently transient, becoming negative in many newborn infants a short time following a positive result.[15,68]

This investigation of 22 placentas infected with SARS-CoV-2 has reinforced previous research that showed chronic histiocytic intervillositis and trophoblast necrosis are associated with placental infection.[31,32,62] It has also demonstrated that varying degrees of increased fibrin deposition, up to the level of massive perivillous fibrin deposition, are also associated with placental infection with SARS-CoV-2. Significantly, we have demonstrated that in a small number of infected placentas, SARS-CoV-2 is not limited to the trophoblast layer but can extend beyond it and into the chorionic villous stroma to involve Hofbauer cells and villous capillary endothelial cells, and that positive staining of both villous cell types can occur together. Unlike Zika virus infection, in most placentas infected with SARS-CoV-2 the Hofbauer cells did not demonstrate any excessive hyperplastic or proliferative activity. Although our series of placentas is small, there was no evidence that viral staining of either Hofbauer cells or capillary endothelial cells was associated with a greater probability of maternal-fetal transmission or poor outcome. It seems probable that most cases of transplacental infection of the fetus with SARS-CoV-2 occur in the absence of Hofbauer cell and endothelial involvement.

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FIGURE LEGENDS

Figure 1. Case 5. A. Immunohistochemistry with antibody to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleocapsid protein shows strong staining of syncytiotrophoblast lining the villi and occasional staining of monocytes within the maternal (intervillous) vascular spaces; however, villous stroma, including Hofbauer cells, is negative for the virus. Antibody to SARS-CoV-2 nucleocapsid protein (Sino Biological, Wayne, PA). Original magnification x400. B. Double staining immunohistochemistry highlights syncytiotrophoblast positive for SARS-CoV-2 anti-nucleocapsid antibody (brown chromogen) and intravillous Hofbauer cells positive for histiocytic marker CD68 (red chromogen). No co-localization of SARS-CoV-2 and histiocytic signals was observed. Double staining with antibody to SARS-CoV-2 nucleocapsid protein (Sino Biological, Wayne, PA) and CD68 antibody. Original magnification x600.

Figure 2. Case 3. A, B. Double staining using antibody to CD68 (magenta) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleocapsid protein (brown) shows infection of the syncytiotrophoblast, and absence of staining of Hofbauer cells in the chorionic villi. Double staining with CD68 antibody and antibody to SARS-CoV-2 nucleocapsid protein (Genetex, Irvine, CA). x40.

Figure 3. Case 10. A, B. Placenta from a case of maternal severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection that was transmitted transplacentally to the fetus prior to delivery. Double-staining with CD-163 antibody to macrophages (red) and RNA in situ hybridization (RNAscope) to SARS-CoV-2 (brown) demonstrates intense positivity of syncytiotrophoblast for SARS-CoV-2 and Hofbauer cell hyperplasia. No viral staining of Hofbauer

cells is present. Double staining with antibody to CD163 and RNAscope for SARS-CoV-2. A, x10; B, x20.

Figure 4. Case 10. High magnification of an infected placenta double-stained with CD-163 antibody to macrophages (red) and RNA in situ hybridization (RNAscope) to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (brown) demonstrating stromal Hofbauer cells lying just subjacent to the basement membrane zone of the infected trophoblast layer. Despite their intimate association with the trophoblast layer, the Hofbauer cells do not stain positively for SARS-CoV-2. Double staining with antibody to CD163 and RNAscope for SARS-CoV-2, x100.

Figure 5. Case 15. Chorionic villi from a placenta that transmitted severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a pregnant mother to the fetus. In addition to intense staining of the syncytiotrophoblast for SARS-CoV-2 nucleocapsid protein, a cell in the center of the image demonstrates intense cytoplasmic staining that spares the nucleus. Its shape, size and location are consistent with it being a Hofbauer cell. Antibody to SARS-CoV-2 nucleocapsid protein (Sino Biological, Beijing, China). x40.

Figure 6. Case 15. A. Chorionic villus containing syncytiotrophoblast and a centrally located Hofbauer cell (arrow) with intracytoplasmic positivity for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Antibody to SARS-CoV-2 nucleocapsid protein, x40. B. Immunohistochemical double staining for SARS-CoV-2 nucleocapsid protein and CD163 demonstrates a Hofbauer cell (blue) that stains positively (brown) for the virus in the center villus (arrow). The syncytiotrophoblast also stains positive for SARS-CoV-2. Antibodies to SARS-CoV-2 nucleocapsid and CD68, x40. C. Immunohistochemical double staining for SARS-CoV-2

nucleocapsid protein and CD163 shows 3 Hofbauer cells (blue) in a single chorionic villus that are positive for the virus (brown) (arrows). Double staining with antibodies to SARS-CoV-2 nucleocapsid and CD163, x40.

Figure 7. Case 19. A, B. Placenta from a 34 4/7-week gestation liveborn neonate having had intrauterine infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Immunohistochemistry demonstrates diffuse and intense positivity for SARS-CoV-2 nucleocapsid staining in syncytiotrophoblast. Some cells in the villous stroma having the morphology of Hofbauer cells also show positive intracytoplasmic staining for SARS-Cov-2. Antibody to SARS-CoV-2 nucleocapsid protein (Sino Biological 40143-T62, Beijing, China). A, x20 B, x40.

Figure 8. Case 20. Placenta from a neonate delivered 11 weeks following hospitalization of the mother for symptomatic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. There was chronic histiocytic intervillositis and trophoblast necrosis. As illustrated in this image, there was also markedly increased intravillous fibrin deposition. H&E. x22

Figure 9. Case 20. A. Immunohistochemistry with antibody to CD163 reveals Hofbauer cell hyperplasia. Antibody to CD163. B.C,D. Immunohistochemistry reveals that there is positive staining of both Hofbauer cells (B) as well as villous capillary endothelial cells (C,D) present using antibody to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleocapsid protein. Interestingly, this is the only case in the cohort to show no appreciable staining of the syncytiotrophoblast. Antibody to SARS-CoV-2 nucleocapsid protein (GeneTex GTX135361, Irvine, CA). A, x15; B, x25; C, x30; D, x12.

Figure 10. Case 21. A,B. This placenta from a stillborn infant at 35 4/7 weeks gestation has intense positive immunohistochemical staining for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleocapsid protein in the syncytiotrophoblast as well as Hofbauer cells and villous endothelial cells. C. The endothelial cells of villous capillaries also showed positive staining in some villi. Two positive-staining endothelial cells are present in this vessel (arrows). Antibody to SARS-CoV-2 nucleocapsid protein (GeneTex GTX635686, Irvine, CA). A, x20; B, x25; C, x30.

Figure 11. Analysis of placentas for Hofbauer cell proliferation. A. Case 15 with double staining immunohistochemistry using antibody to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleocapsid protein (brown) and Ki67 (blue). Although the majority of proliferating cells are cytotrophoblast, some villous stromal cells, presumably Hofbauer cells, are proliferating. Antibodies to SARS-CoV-2 nucleocapsid protein and Ki67. x10. B. Case 10. Staining for Ki67 reveals almost all proliferating cells to be cytotrophoblast. Antibody to Ki67, x20. C. Third trimester placenta from a fetus with congenital Zika syndrome. Immunohistochemical double staining with Ki67 (brown) and CD68 (red) antibodies demonstrates prominent Hofbauer cell proliferation, a quite different appearance than Figures A and B. Antibodies to Ki67 and CD68, x40. Image courtesy of Jernej Mlakar, Dr. Med., Institute of Pathology, Ljubljana, Slovenia
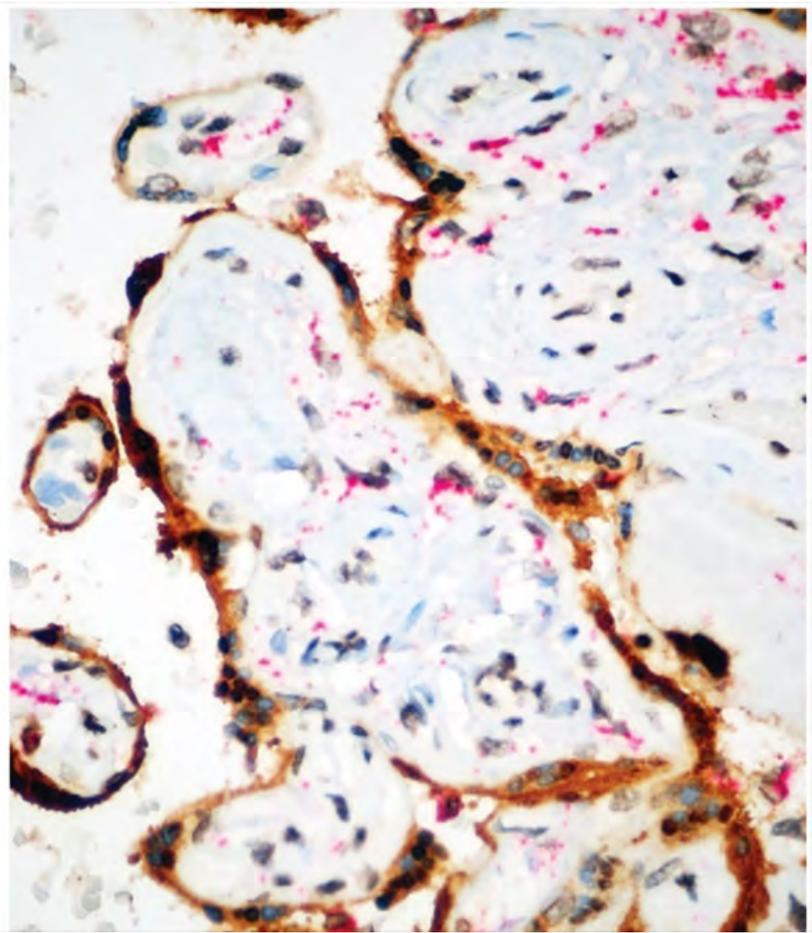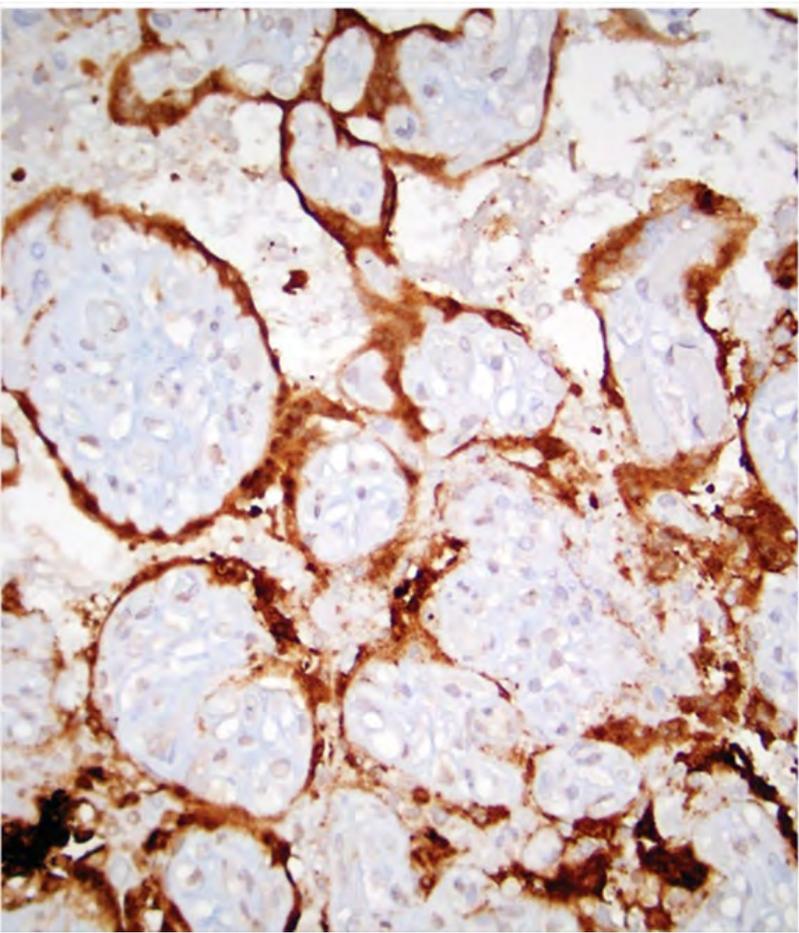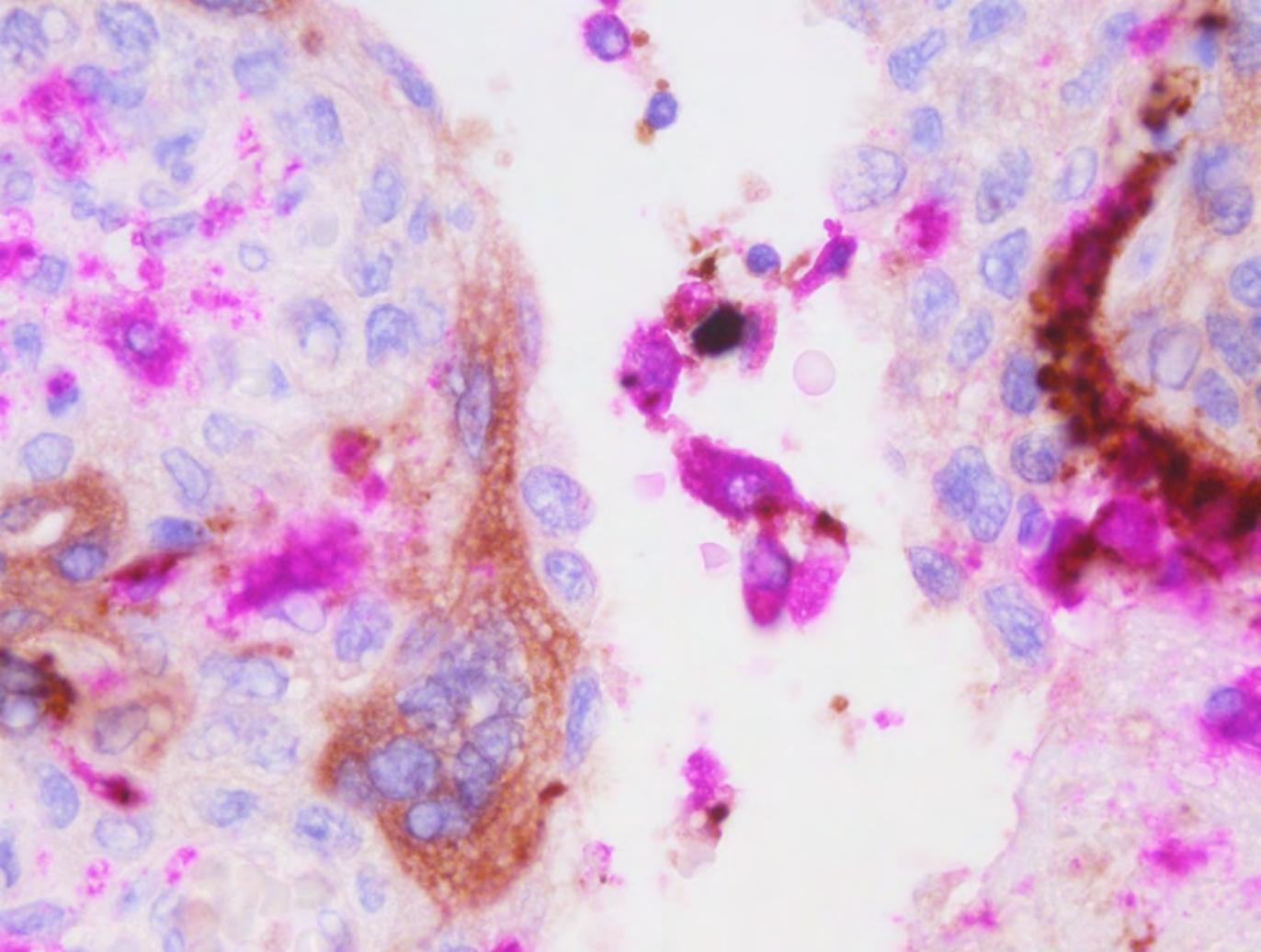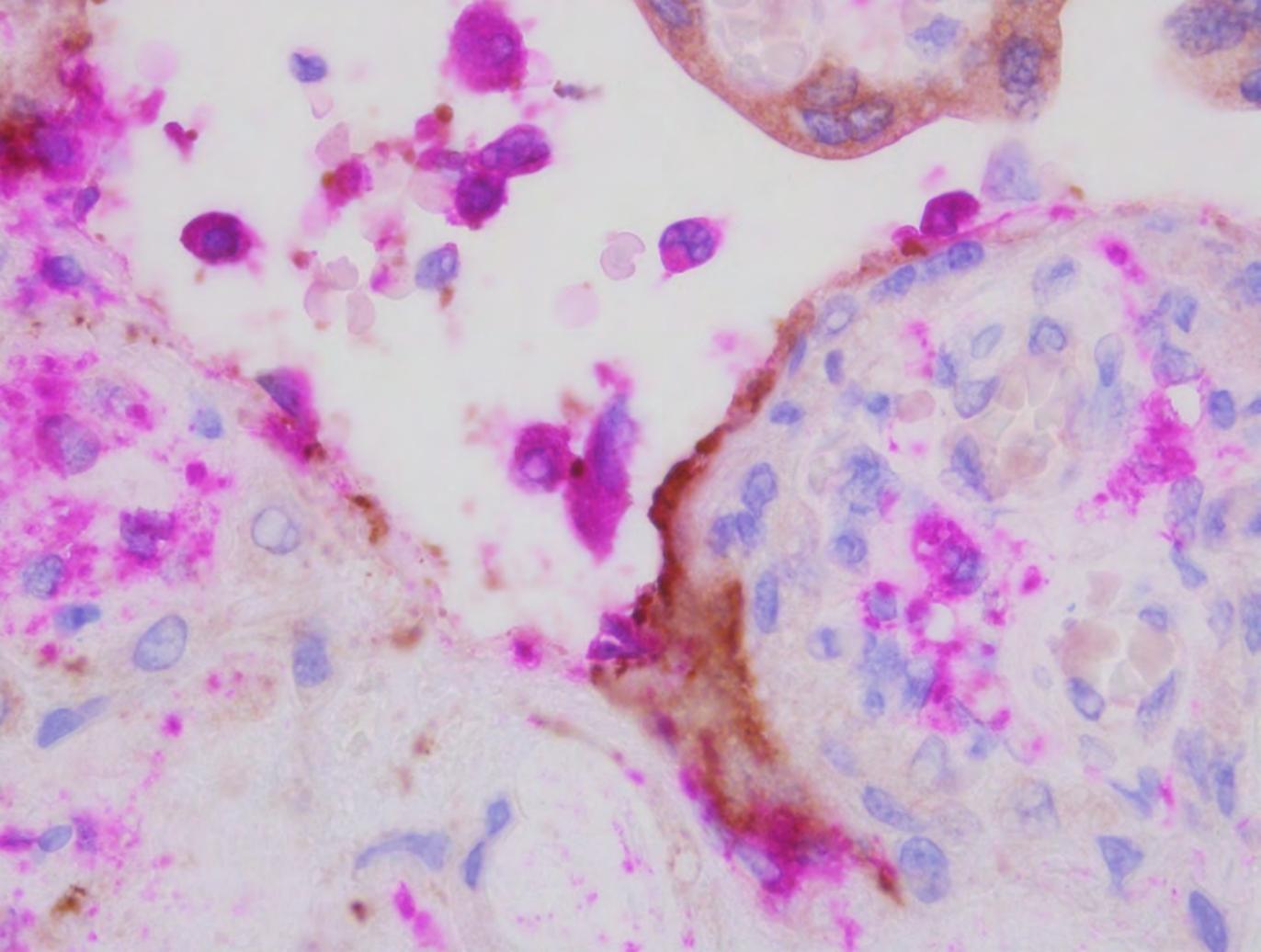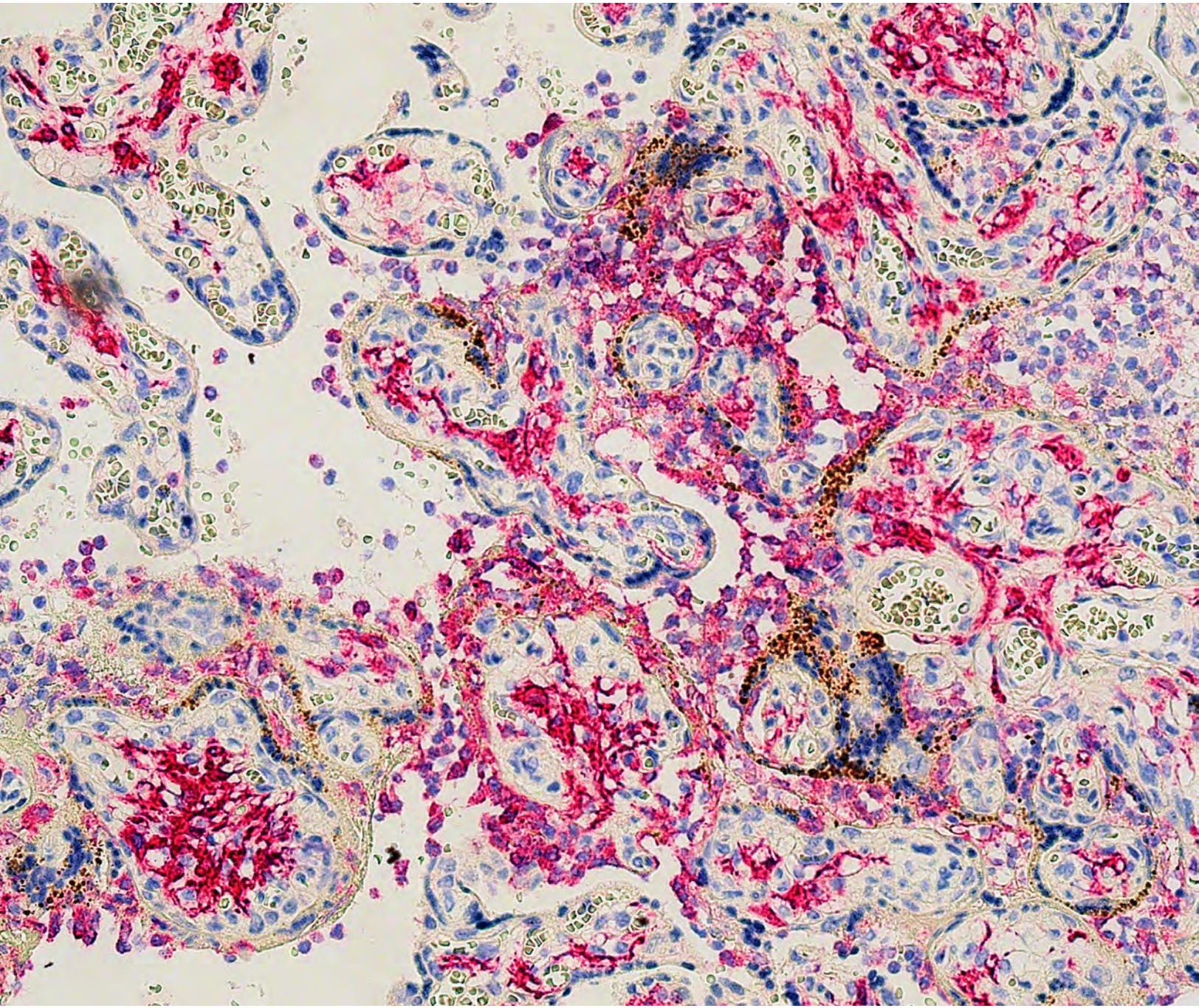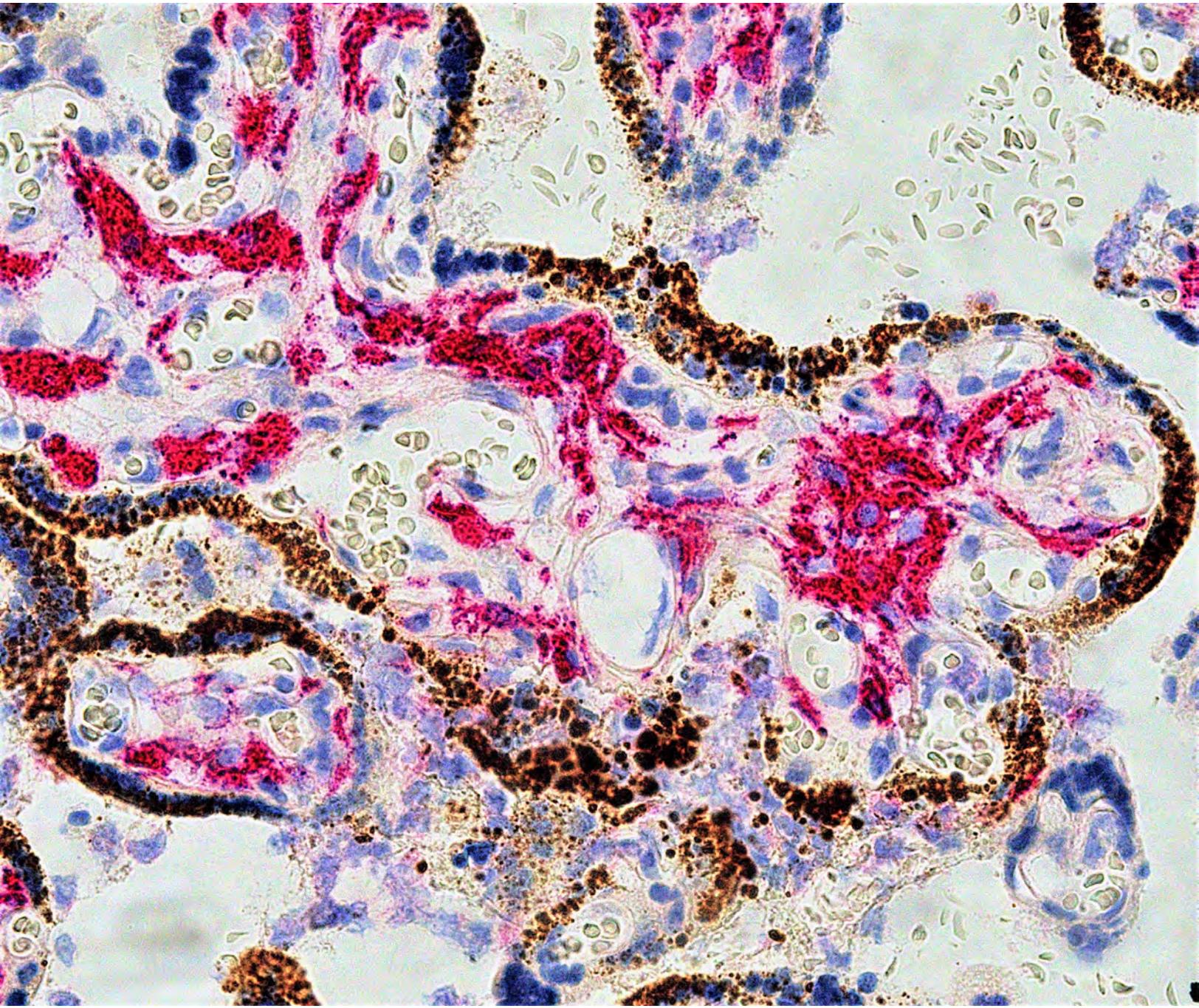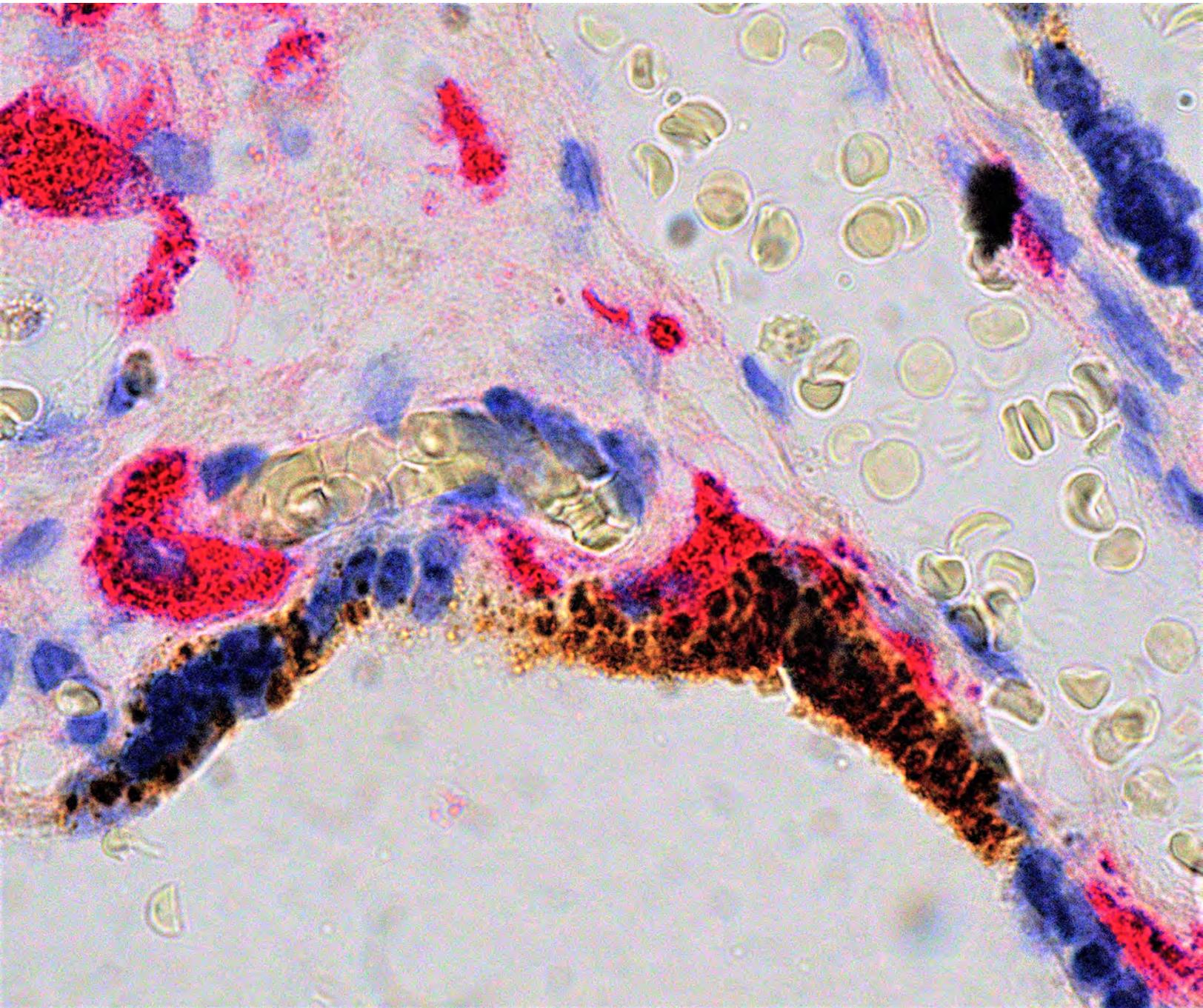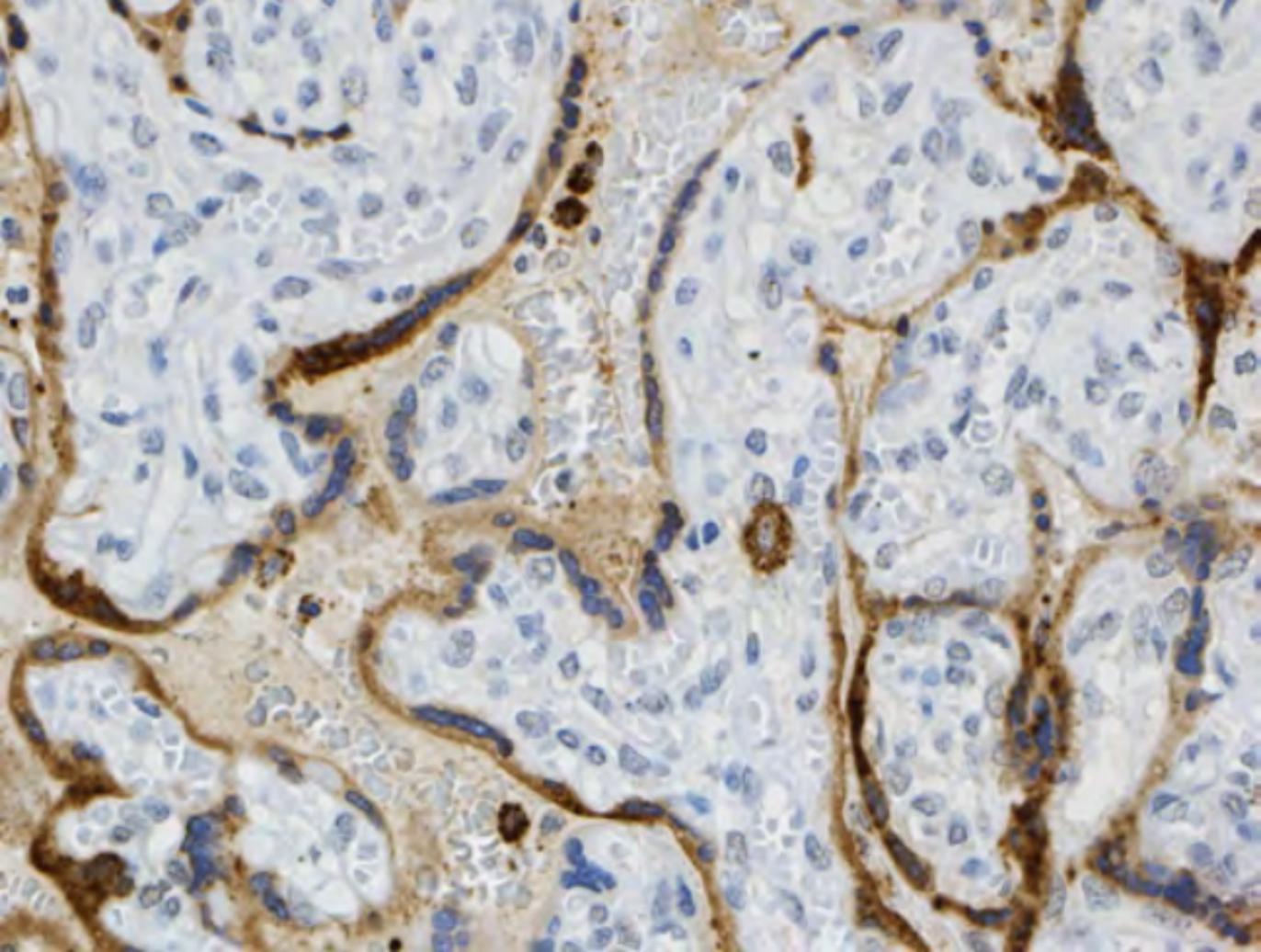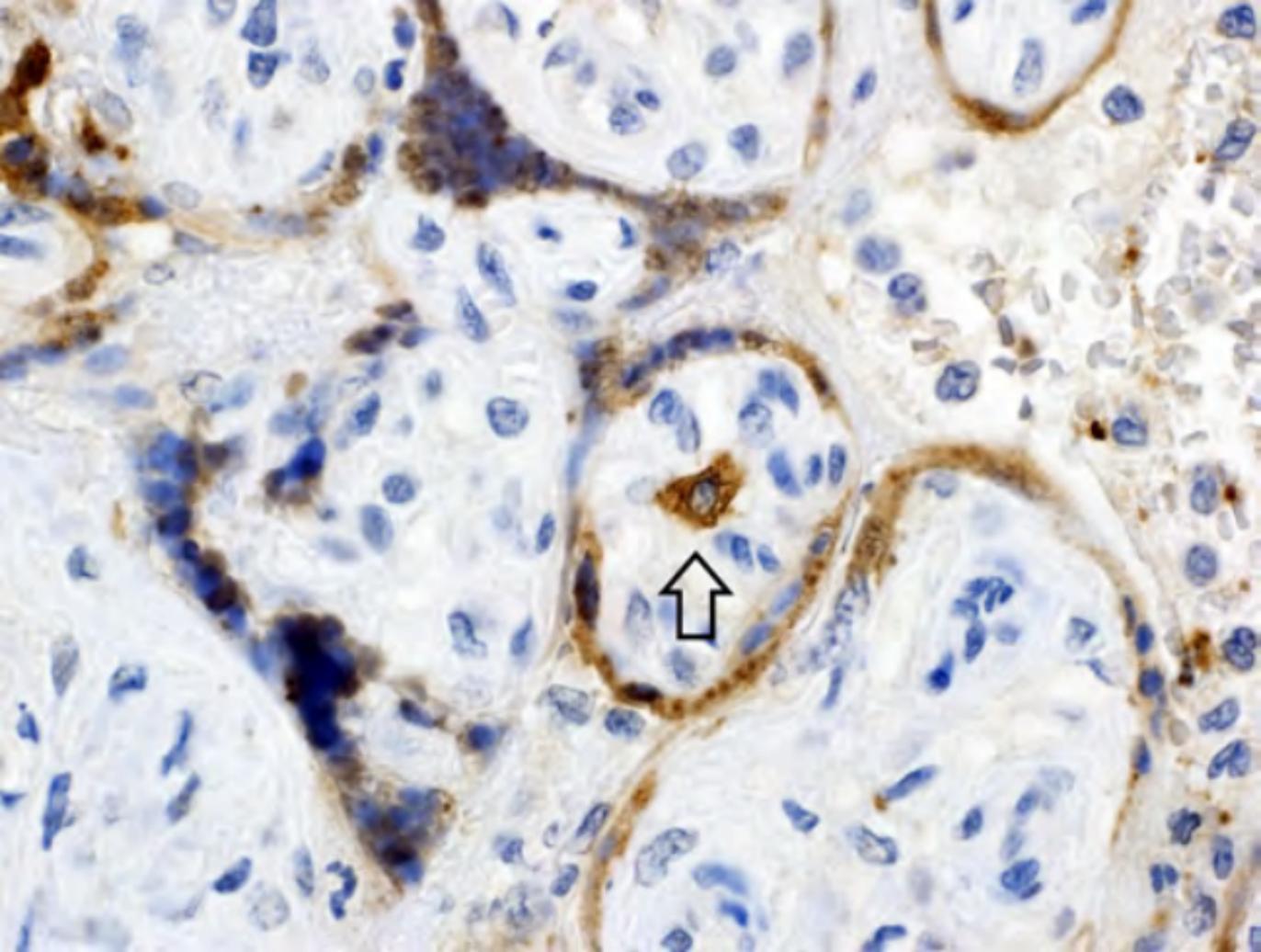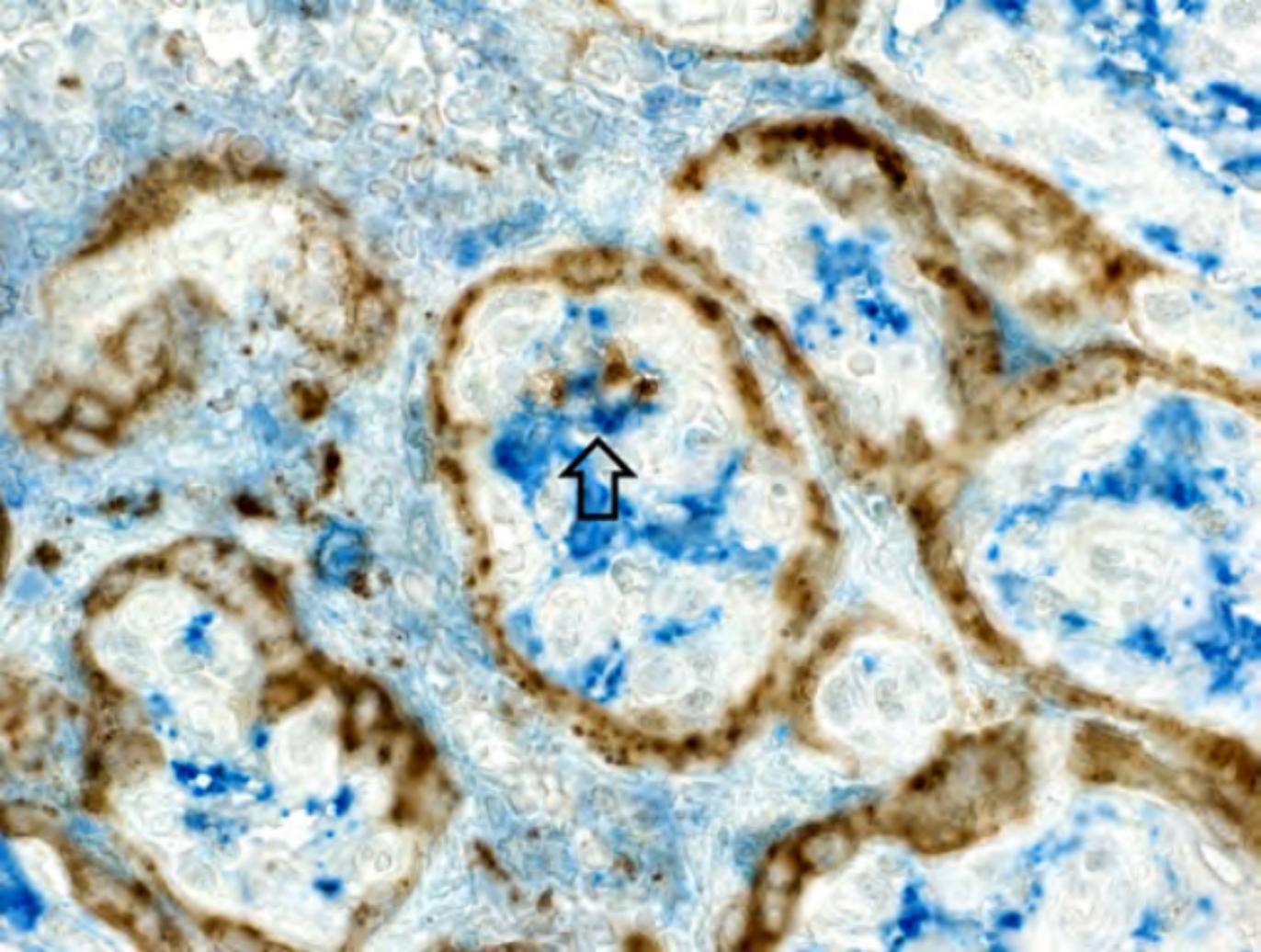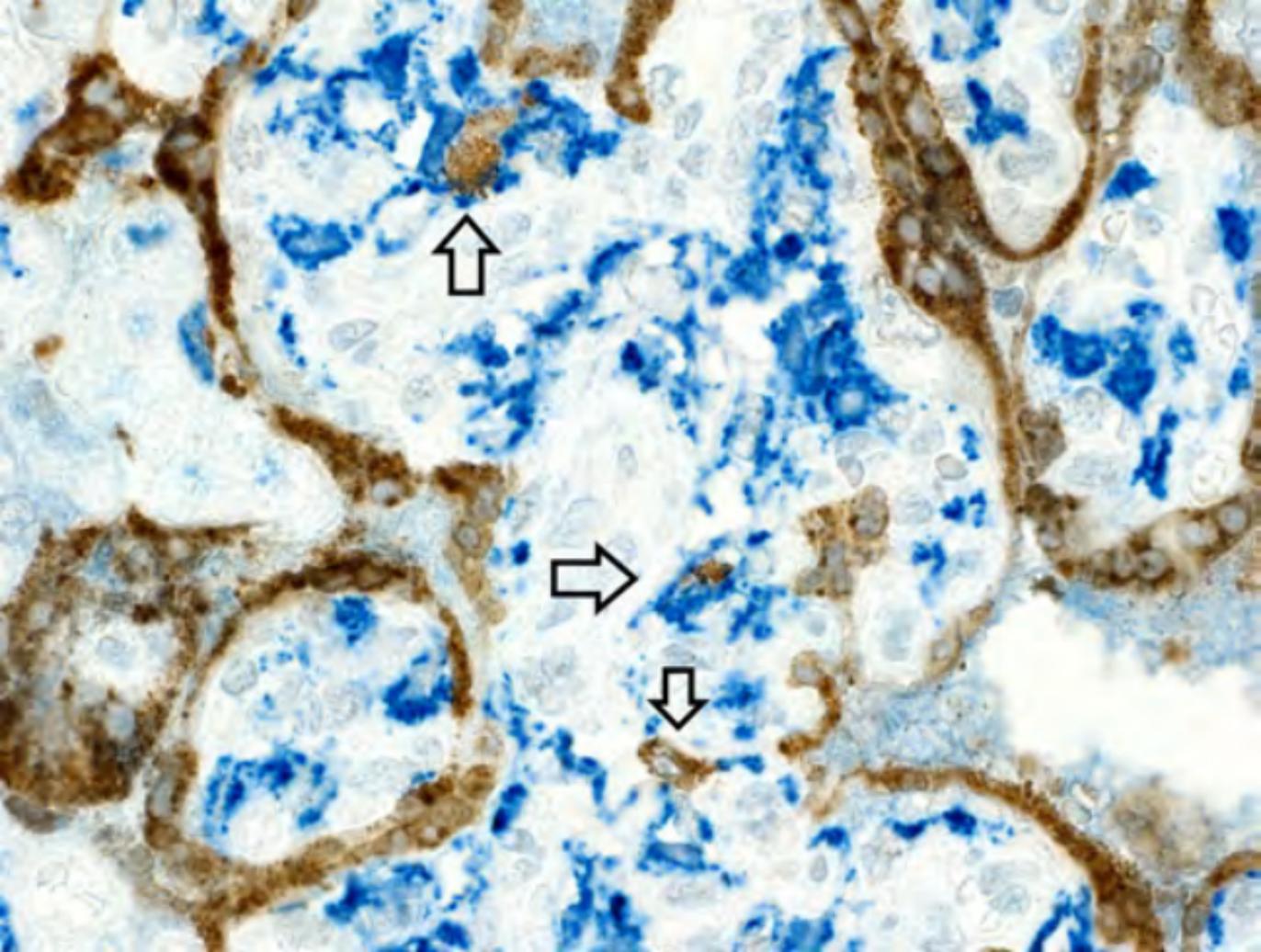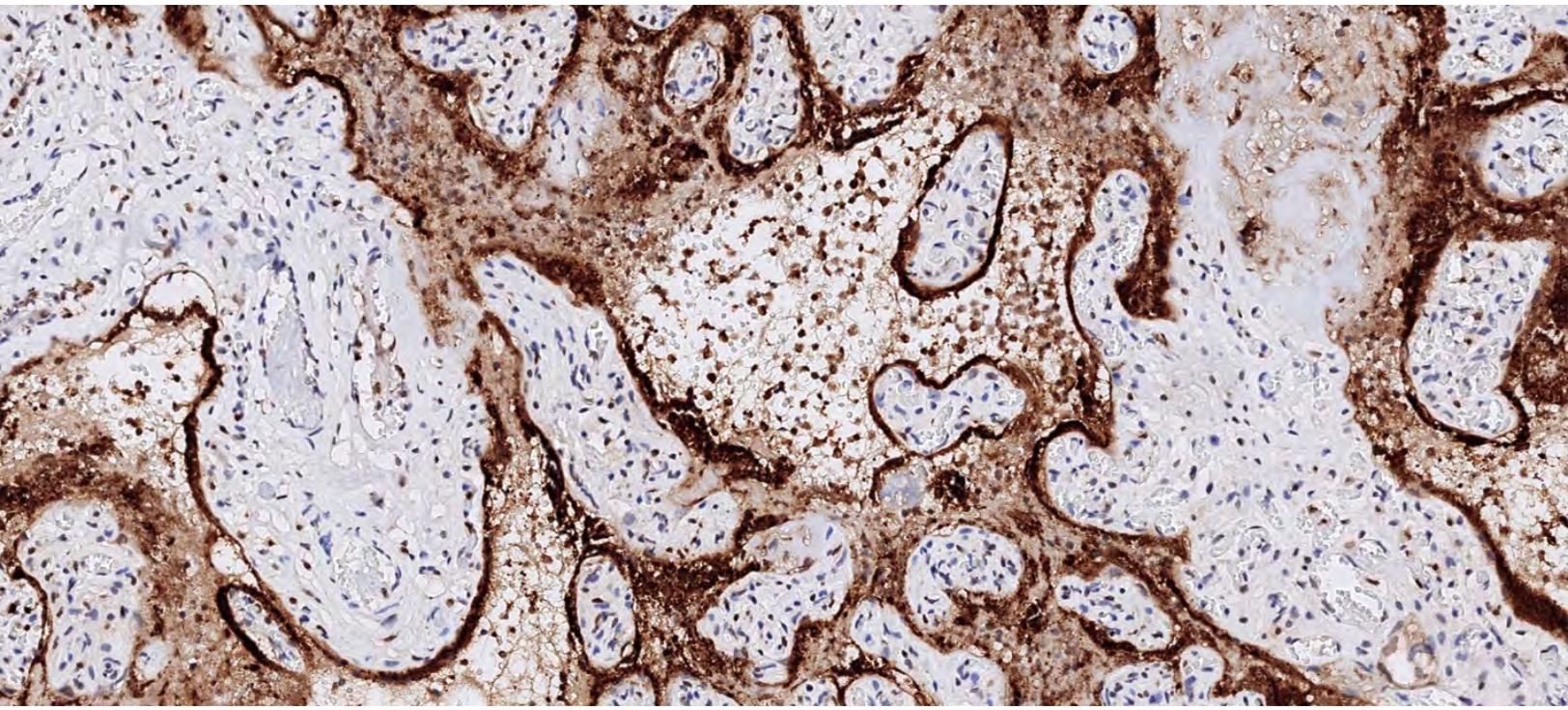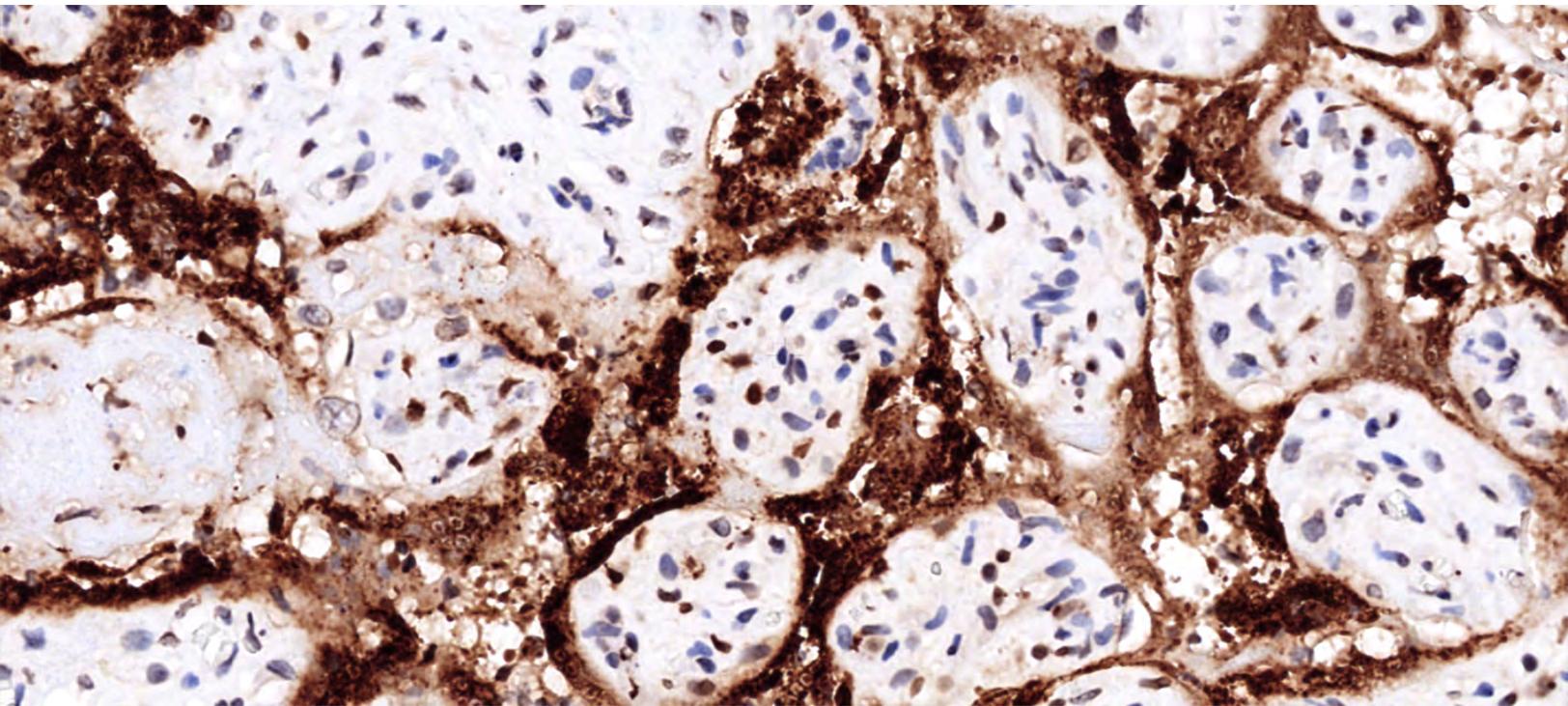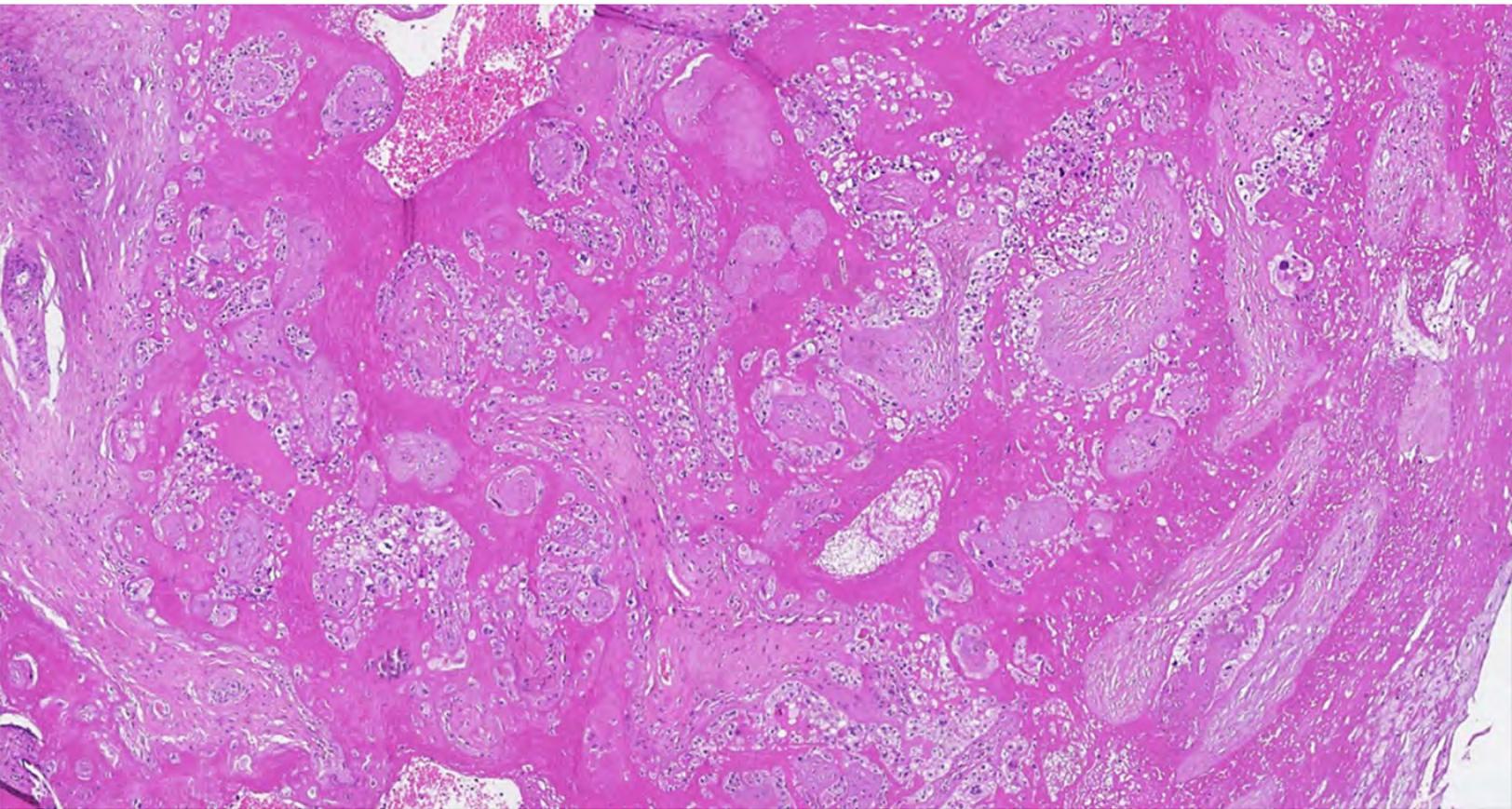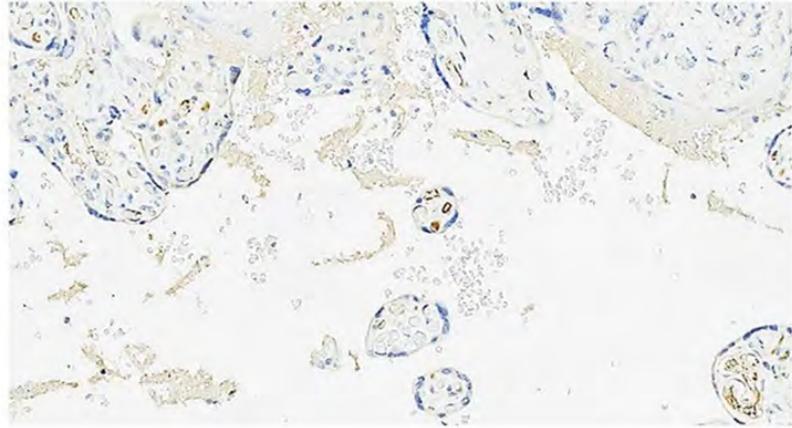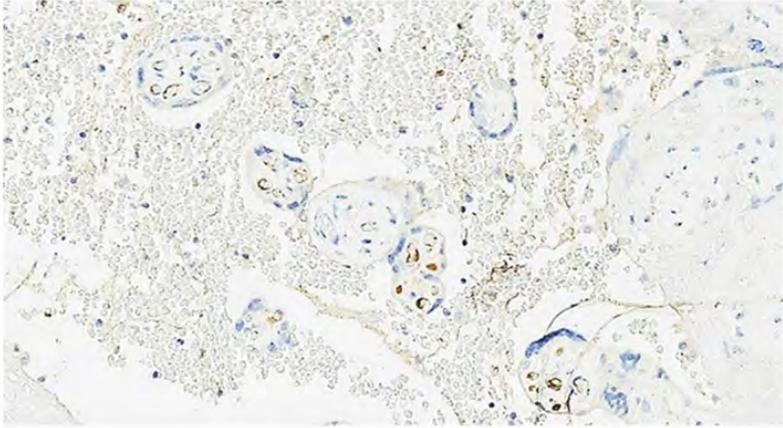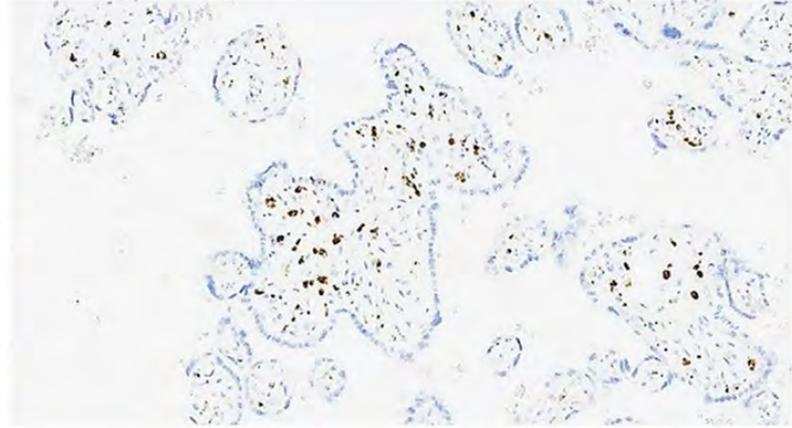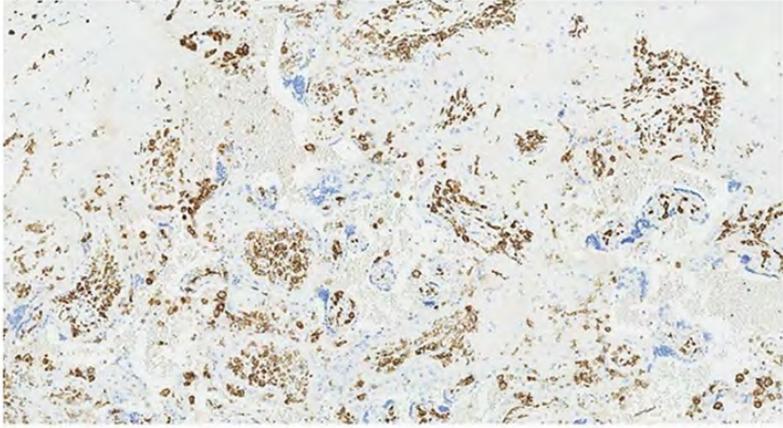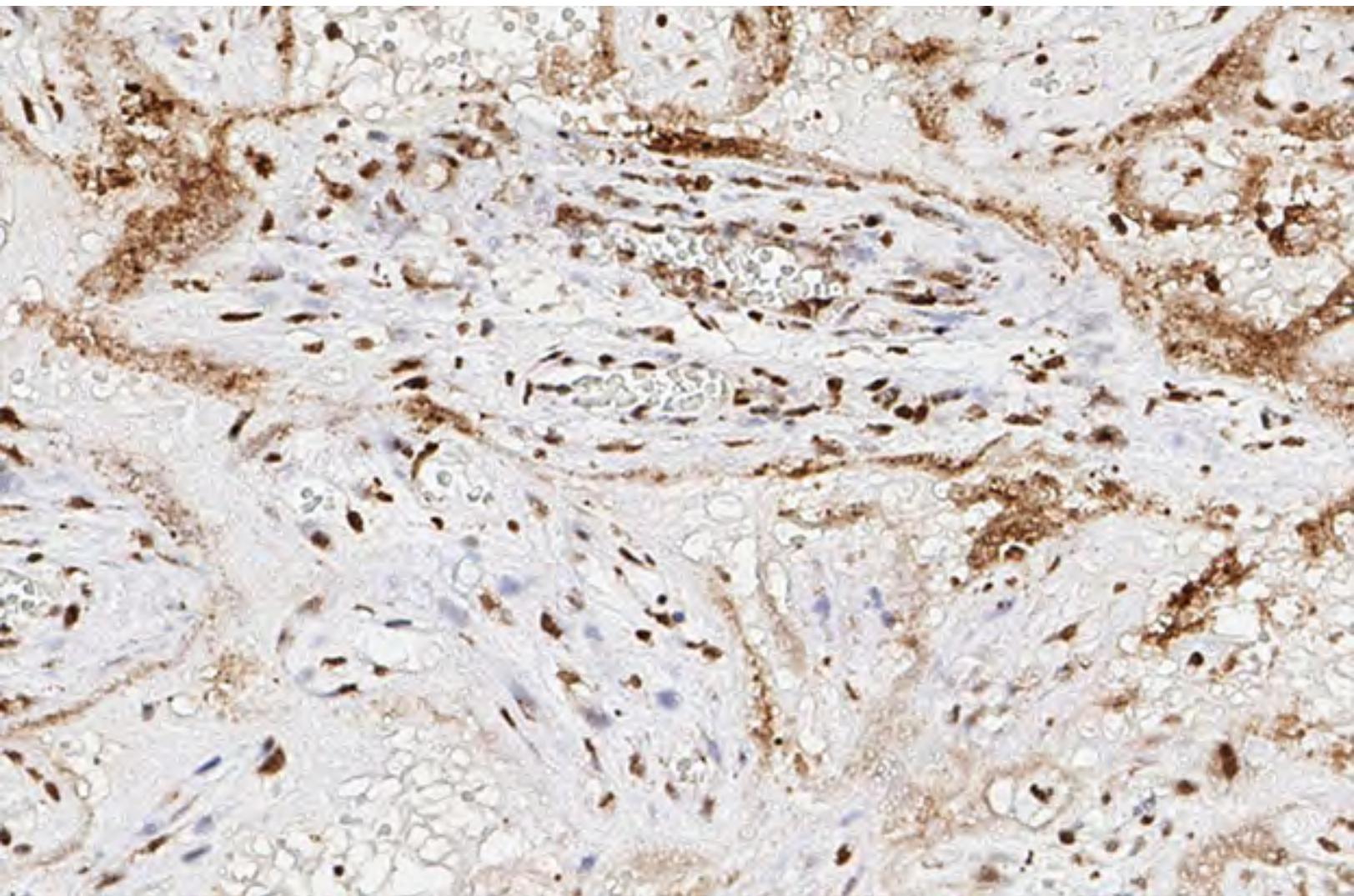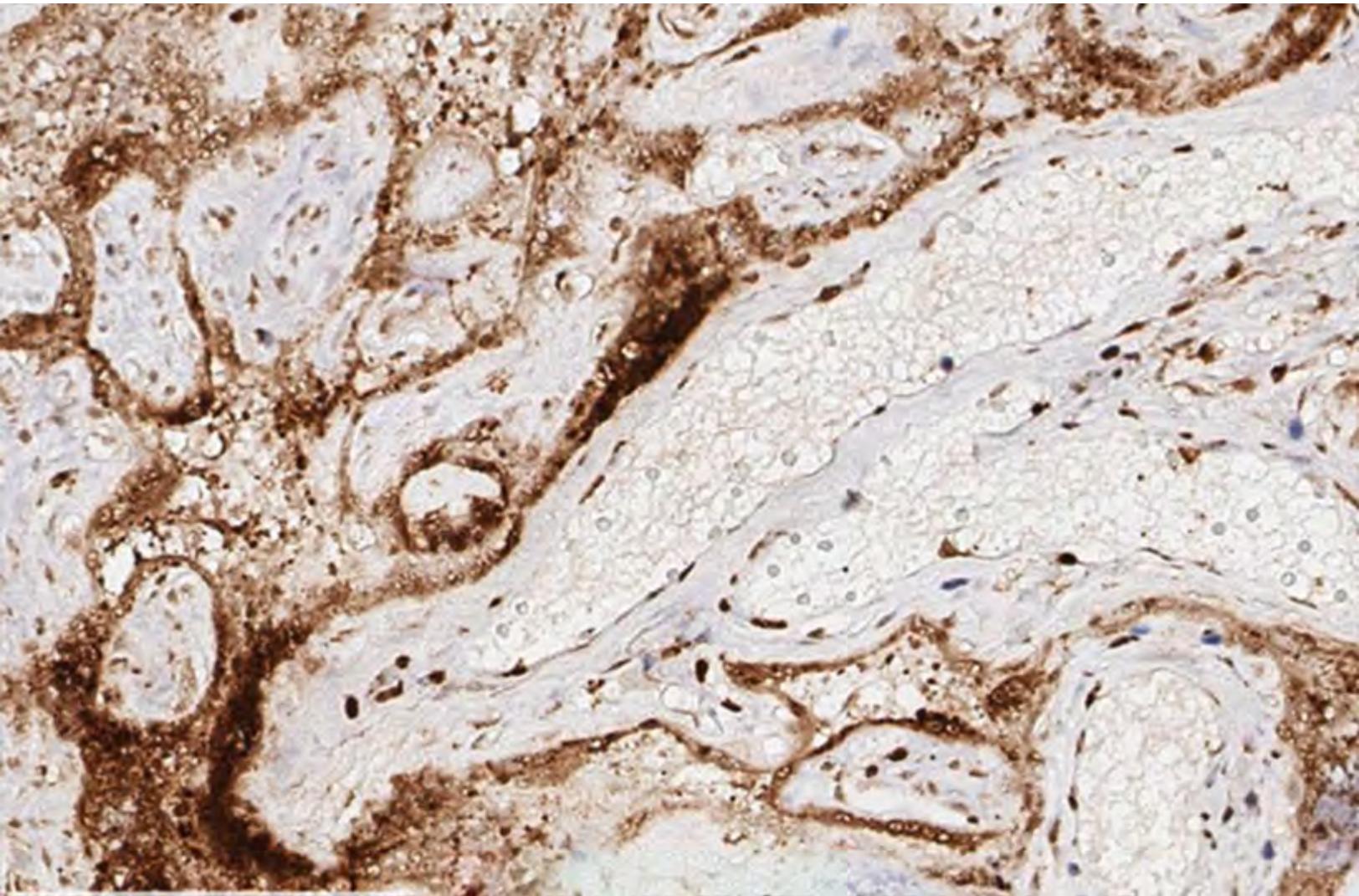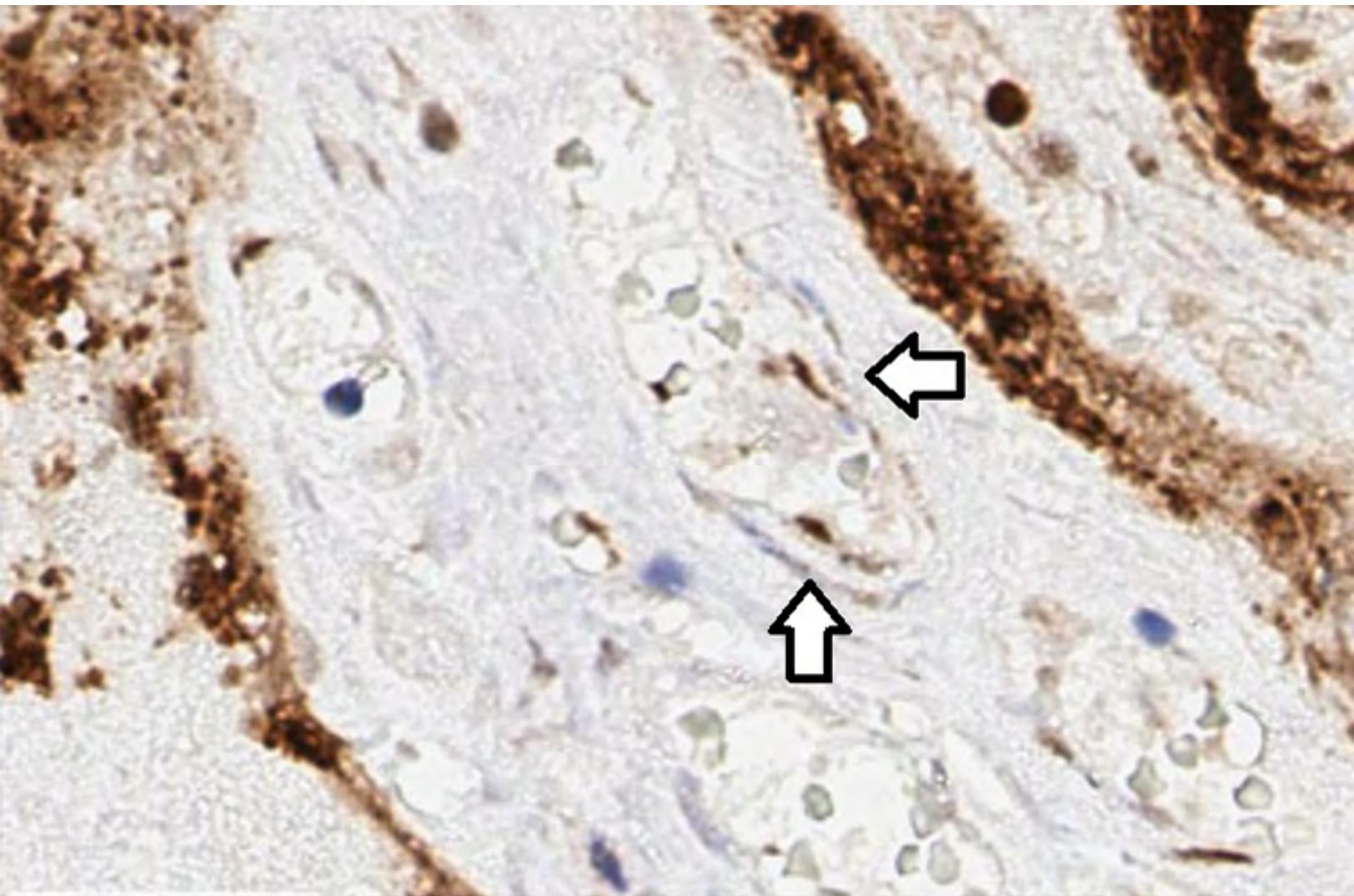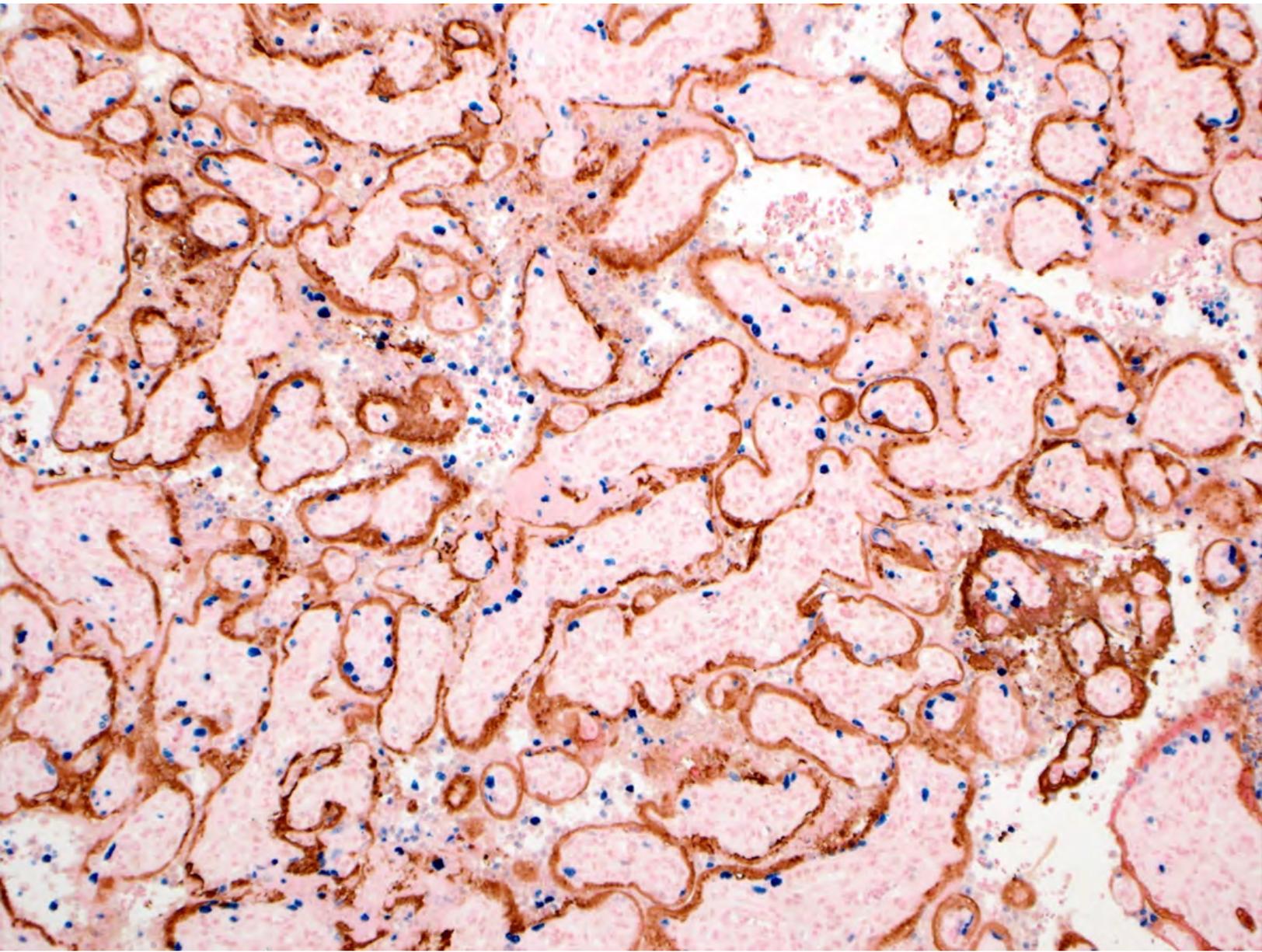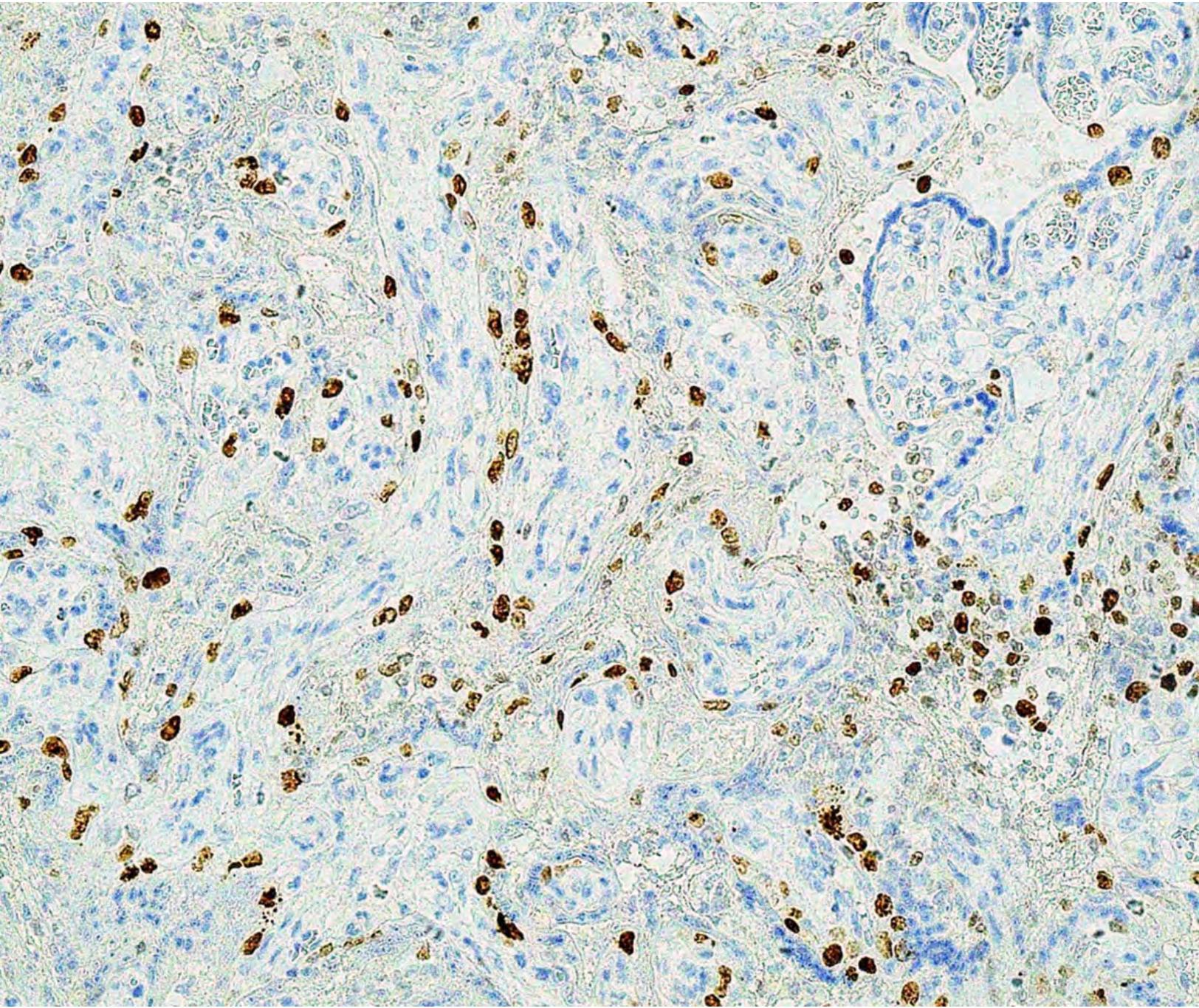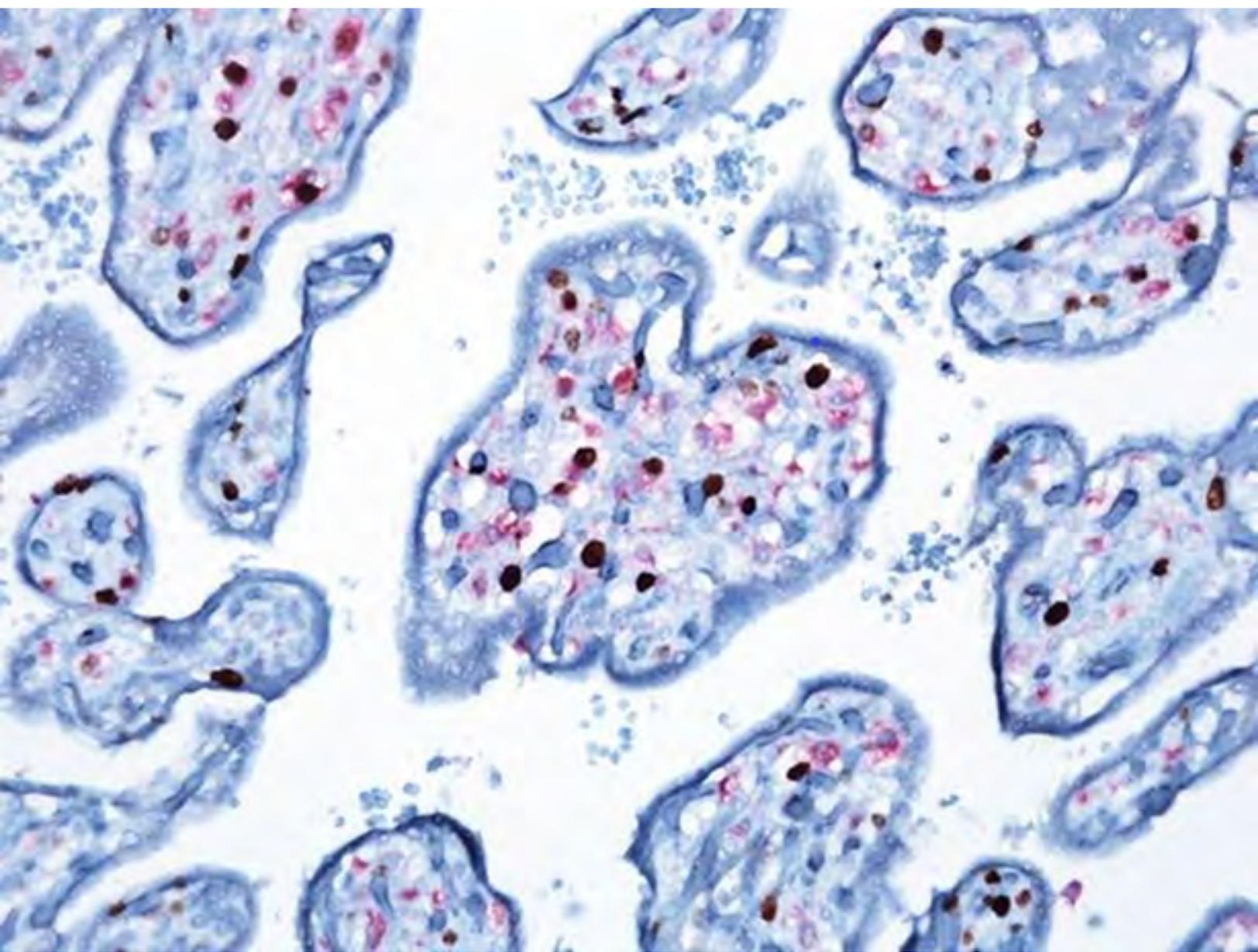
Table 1. Characteristics of 8 placentas from pregnant women with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in which the placenta was confirmed to be infected with the coronavirus, noting the status of Hofbauer cell infection.

| Case and Location (reference if published)	Case 1 New Haven, CT[24]	Case 2 New Haven, CT	Cases 3,4 Leuven, Belgium[30]	Case 5 New York, NY[34]	Case 6 Philadelphia, PA[32]	Case 7 Philadelphia, PA	Case 8 Philadelphia, PA
Maternal age (years)	35	40	30	33	32	24	26
Gestational age (weeks)	22	36	24	37	39 2/7	37 1/7	32 5/7
Liveborn or stillborn	Stillborn	Liveborn	Stillborn twins	Liveborn	Stillborn	Liveborn	Liveborn
Maternal rt-PCR for SARS-CoV-2	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Neonatal SARS-CoV-2 status	Negative in fetal parts; Positive qPCR in placenta	Negative	Positive amniotic fluid by rt-PCR; stillborns not tested	Negative NP swab rt-PCR	Negative NP swab by RT-PCR; Positive RT-PCR in placenta	Negative NP swab by RT-PCR; Positive RT-PCR in placenta	Unknown
Transplacental SARS-CoV-2 transmission to the fetus	Unknown	No	Possible	No	No	No	Unknown
Placental pathology	CHI; IF; TN	CHI; IF; TN	CHI; IF; TN	CHI; IF; TN	CHI; IF; TN; MVM	CHI; IF; TN; MVM	CHI; IF; TN
Trophoblast staining for SARS-CoV-2	+ IHC + ISH	+IHC	+IHC	+ IHC + ISH	+IHC +FISH	+IHC +FISH	+IHC +FISH
Hofbauer cell or villous stromal cell staining for SARS-CoV-2	None	None	None	None	None	None	None
Additional findings	None	None	None	None	None	None	None

CHI, chronic histiocytic intervillositis; IF, increased fibrin; TN, trophoblast necrosis; MVM, maternal vascular malperfusion; FVM, fetal vascular malperfusion; IVT, intervillous thrombi; VIL, villitis; IHC, immunohistochemistry; ISH, RNA in situ hybridization; FISH, fluorescence in situ hybridization; qPCR, quantitative polymerase chain reaction; BAL, bronchoalveolar lavage fluid; RT-PCR, reverse transcription polymerase chain reaction; NP, nasopharyngeal; NC, SARS-CoV-2 nucleocapsid protein; VCE, villous capillary endothelium; CT, cytotrophoblast

Table 2. Characteristics of additional 7 placentas from pregnant women with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in which the placenta was confirmed to be infected with the coronavirus, noting the status of Hofbauer cell infection.

Case and Location (reference if published)	Case 9 Cork, Ireland[33]	Case 10 Bergamo, Italy[23]	Case 11 Bergamo, Italy[23]	Case 12 Cork, Ireland	Case 13 Cork, Ireland	Case 14 Cork, Ireland	Case 15 Brescia, Italy[26]
Maternal age (years)	26	35	27	35	28	28	29
Gestational age (weeks)	37	37+	35 1/7	24 3/7	33 6/7	20 3/7	37 5/7
Liveborn or stillborn	Liveborn	Liveborn	Liveborn	Stillborn	Stillborn	Stillborn	Liveborn
Maternal rt-PCR for SARS-CoV-2	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Neonatal SARS-CoV-2 status	Not tested as per protocol	Positive NP swab rt-PCR	Positive NP swab rt-PCR	Positive NP swab rt-PCR	Negative fetal tissue swab rt-PCR	Positive NP swab rt-PCR	Positive NP swab rt-PCR
Transplacental SARS-CoV-2 transmission to the fetus	Unknown	Yes	Yes	Yes	Not demonst-rated	Yes	Yes
Placental pathology	CHI; IF; TN	CHI; TN	CHI; TN	CHI, IF, TN	CHI, IF, TN, FVM	CHI, IF, TN	CHI; IF; TN; FVM
Trophoblast staining for SARS-CoV-2	+IHC	+IHC +ISH	+IHC +ISH	+IHC	+IHC	+IHC	+IHC +ISH
Hofbauer cell or villous stromal cell staining for SARS-CoV-2	None	None	None	None	None	None	Yes +IHC for NC
Additional findings	None	Hofbauer cell hyperplasia	None	None	None	None	Hofbauer cell hyperplasia

CHI indicates chronic histiocytic intervillositis; IF, increased fibrin; TN, trophoblast necrosis; MVM, maternal vascular malperfusion; FVM, fetal vascular malperfusion; IVT, intervillous thrombi; VIL, villitis; IHC, immunohistochemistry; ISH, RNA in situ hybridization; FISH, fluorescence in situ hybridization; qPCR, quantitative polymerase chain reaction; BAL, bronchoalveolar lavage fluid; RT-PCR, reverse transcription polymerase chain reaction; NP, nasopharyngeal; NC, SARS-CoV-2 nucleocapsid protein; VCE, villous capillary endothelium; CT, cytotrophoblast.

Table 3. Characteristics of additional 7 placentas from pregnant women with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in which the placenta was confirmed to be infected with the coronavirus, noting the status of Hofbauer cell infection.

Case and Location (reference if published)	Case 16 New York, NY[32]	Case 17 New York, NY	Case 18 Rotterdam, The Netherlands[35]	Case 19 Lund, Sweden[36]	Case 20 Milan, Italy[38]	Case 21 Miami, FL[37]	Case 22 Paris, France[29]
Maternal age (years)	38	46	30	27	38	31	23
Gestational age (weeks)	35 2/7	39+	31 4/7	34 4/7	37	35 4/7	35 2/7
Liveborn or stillborn	Stillborn	Liveborn	Liveborn	Liveborn	Liveborn	Stillborn	Liveborn
Maternal rt-PCR for SARS-CoV-2	Positive	Positive	Positive	Positive	+26 wks; Negative at 37 wks	Positive	Positive
Neonatal SARS-CoV-2 status	Positive NP swab rt-PCR; bronchial swab negative	Negative NP swab rt-PCR	Negative PCR from multiple neonatal sites; Positive qPCR in placenta	Positive NP swab rt-PCR	Negative NP swab rt-PCR	Not tested	Positive NP & rectal swabs rt-PCR; Positive blood & BAL fluid
Transplacental SARS-CoV-2 transmission to the fetus	Unknown	No	No	Yes	No	Unknown	Yes
Placental pathology	CHI; IF; TN	CHI; IF; TN; IVT	CHI; IF; TN	CHI; IF; TN	CHI: IF; TN; VIL	CHI, IF, TN	CHI; IF; TN;
Trophoblast staining for SARS-CoV-2	+IHC	+IHC	+IHC +ISH	+IHC +ISH	Negative	+IHC	+IHC
Hofbauer cell or villous stromal cell staining for SARS-CoV-2	None	None	None	Yes +IHC for NC	Yes +IHC for NC	Yes +IHC for NC	None
Additional findings	None	None	None	None	+IHC for NC in VCE; Hofbauer cell hyperplasia	+IHC for NC in VCE	None

Abbreviations: CHI, chronic histiocytic intervillositis; IF, increased fibrin; TN, trophoblast necrosis; MVM, maternal vascular malperfusion; FVM, fetal vascular malperfusion; IVT, intervillous thrombi; VIL, villitis; IHC, immunohistochemistry; ISH, RNA in situ hybridization; FISH, fluorescence in situ hybridization; qPCR, quantitative polymerase chain reaction; BAL, bronchoalveolar lavage fluid; RT-PCR, reverse transcription polymerase chain reaction; NP, nasopharyngeal; NC, SARS-CoV-2 nucleocapsid protein; VCE, villous capillary endothelium; CT, cytotrophoblast.