Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Placenta 117 (2022) 187–193

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Placental SARS-CoV-2 distribution correlates with level of tissue oxygenation in COVID-19-associated necrotizing histiocytic intervillositis/perivillous fibrin deposition

Quanfu Mao [a], Sharon Chu [a], Svetlana Shapiro [a], Lawrence Young [a], Melissa Russo [b], Monique E. De Paepe [a,*]

[a] Department of Pathology, Women and Infants Hospital, and Department of Pathology and Laboratory Medicine, Alpert Medical School of Brown University, Providence, USA

[b] Department of Maternal-Fetal Medicine, Women and Infants Hospital, and Department of Obstetrics and Gynecology, Alpert Medical School of Brown University, Providence, USA

ARTICLE INFO

Keywords:
Angiotensin-converting enzyme 2
Coronavirus
Hypoxia

ABSTRACT

Introduction: Recent evidence supports the – rare – occurrence of vertical transplacental SARS-CoV-2 transmission. We previously determined that placental expression of angiotensin-converting enzyme 2 (ACE2), the SARS-CoV-2 receptor, and associated viral cell entry regulators is upregulated by hypoxia. In the present study, we utilized a clinically relevant model of SARS-CoV-2-associated chronic histiocytic intervillositis/massive perivillous fibrin deposition (CHIV/MPFVD) to test the hypothesis that placental hypoxia may facilitate placental SARS-CoV-2 infection.

Methods: We performed a comparative immunohistochemical and/or RNAscope in-situ hybridization analysis of carbonic anhydrase IX (CAIX, hypoxia marker), ACE2 and SARS-CoV-2 expression in free-floating versus fibrin-encased chorionic villi in a 20-weeks' gestation placenta with SARS-CoV-2-associated CHIV/MPVFD.

Results: The levels of CAIX and ACE2 immunoreactivity were significantly higher in trophoblastic cells of fibrin-encased villi than in those of free-floating villi, consistent with hypoxia-induced ACE2 upregulation. SARS-CoV-2 showed a similar preferential localization to trophoblastic cells of fibrin-encased villi.

Discussion: The localization of SARS-CoV-2 to hypoxic, fibrin-encased villi in this placenta with CHIV/MPVFD suggests placental infection and, therefore, transplacental SARS-CoV-2 transmission may be promoted by hypoxic conditions, mediated by ACE2 and similar hypoxia-sensitive viral cell entry mechanisms. Understanding of a causative link between placental hypoxia and SARS-CoV-2 transmittability may potentially lead to the development of alternative strategies for prevention of intrauterine COVID-19 transmission.

1. Introduction

The 2019 novel coronavirus, SARS-CoV-2, and the associated disease, COVID-19 [1–3], have affected more than 180 million persons worldwide and resulted in more than four million deaths as of July 2021 [World Health Organization situation report] [Johns Hopkins COVID-19 Case Tracker, https://coronavirus.jhu.edu/], creating an unrelenting public health threat. Like other coronaviruses linked to epidemics, such as severe acute respiratory syndrome-related coronavirus (SARS-CoV) [4] and Middle East respiratory syndrome coronavirus (MERS-CoV) [5], SARS-CoV-2 primarily targets the respiratory tract [6].

However, a growing number of suspected or confirmed intrauterine or intrapartum-acquired neonatal SARS-CoV-2 infections have been reported, suggesting intrauterine vertical transmission of SARS-CoV-2 from pregnant mother to placenta and/or fetus may be possible, albeit exceptional [7–15]. Among these, several recent reports have described the occurrence of placental SARS-CoV-2 infection in association with chronic histiocytic intervillositis (CHIV) with or without massive perivillous fibrin deposition (MPVFD) [10–13,16–20]. In the vast majority of reported CHIV cases in maternal COVID-19 infection, the virus was directly demonstrated within the syncytiotrophoblast by either immunohistochemistry or in-situ hybridization [11,13,16–19,21], supporting

* Corresponding author. Women and Infants Hospital, Dept. of Pathology, 101 Dudley Street, Providence, RI, 02905, USA.
E-mail address: mdepaepe@wihri.org (M.E. De Paepe).

https://doi.org/10.1016/j.placenta.2021.12.002
Received 2 August 2021; Received in revised form 12 October 2021; Accepted 1 December 2021
Available online 2 December 2021

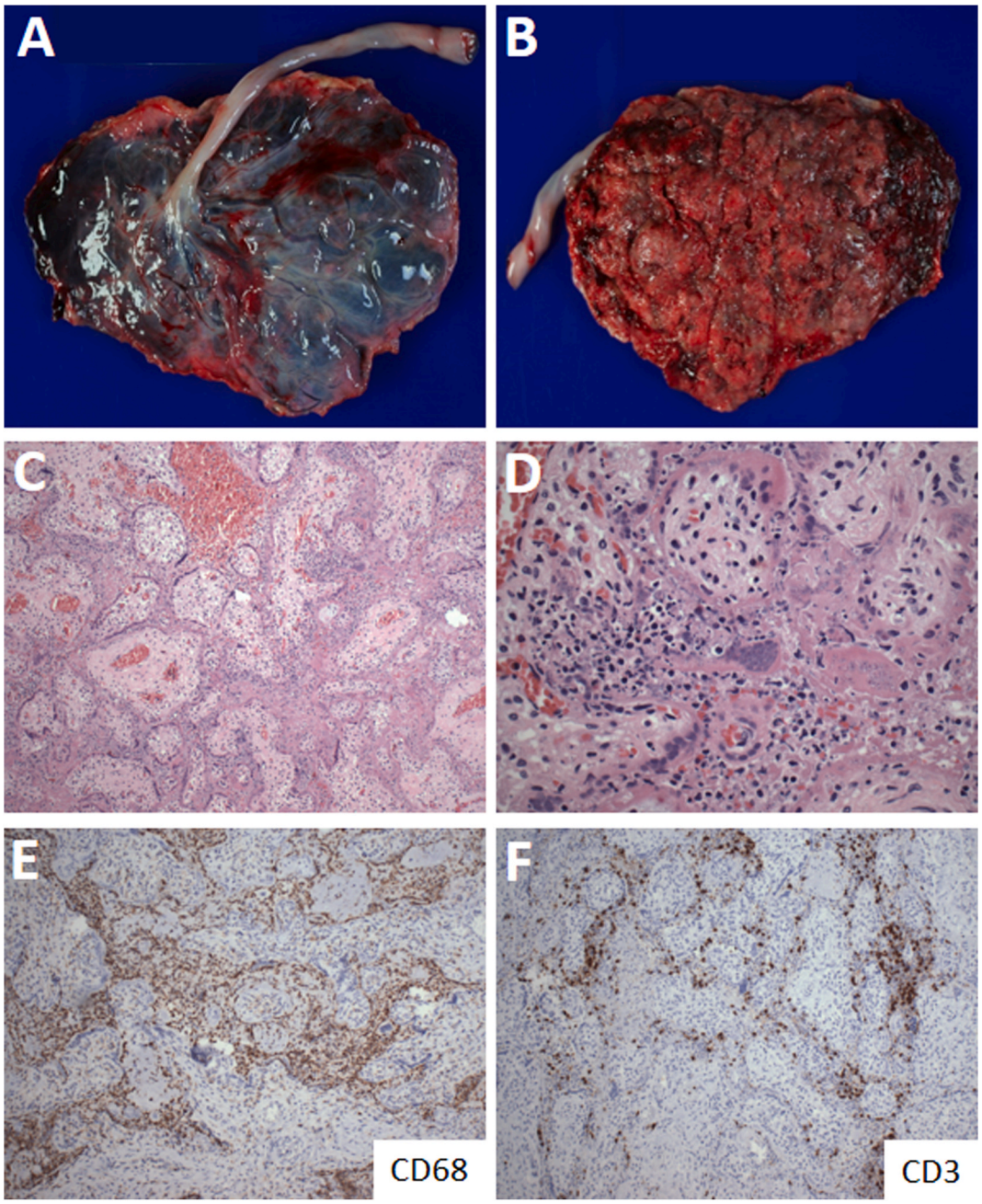

Fig. 1. Gross and microscopic appearance of the placenta. A-B. Fetal (A) and maternal (B) surface of the placenta, the latter demonstrating an irregular network of firm, tan to dark red fibrin plaques along the basal plate, characteristic of massive perivillous fibrin deposition. C-D. Representative micrographs demonstrating chorionic villi encased in fibrin, associated with abundant intervillous cellular infiltrates and extensive cytotrophoblast necrosis. E-F. Representative analysis of CD68-and CD3-immunoreactive mononuclear cells in the intervillous space. (C–D: hematoxylin-eosin stain; E–F: DAB-peroxidase system with hematoxylin counterstain, original magnification ×100 (C), X 200 (E–F), X 400 (D)). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the hypothesis that the placental inflammation may be related to the viral infection and may, in fact, represent a characteristic form of SARS-CoV-2 placentitis [16]. By analogy, CHIV has been linked to other infectious etiologies, such as malaria [22] and cytomegalovirus infection [23].

Schwartz et al. [24] recently reported the placental findings in 11 cases of confirmed intrauterine SARS-CoV-2 transmission, as defined by strict criteria [25]. The placental findings in the 6 liveborns and 5 stillborns or terminated fetuses with documented transplacental transmission arising from maternal SARS-CoV-2 infection were strikingly similar: all placentas displayed CHIV associated with syncytiotrophoblast necrosis and in all placentas the syncytiotrophoblast was positive for SARS-CoV-2 by immunohistochemistry, RNA in-situ hybridization, or both [24]. The common coexistence of CHIV/MPVFD with syncytiotrophoblast necrosis in liveborn and stillborn infants with *in utero* SARS-CoV-2 infection suggests a strong link between these placental findings and transplacental fetal SARS-CoV-2 infection and suggests the presence of CHIV/MPVFD may constitute an increased risk for transplacental intrauterine SARS-CoV-2 transmission.

Transmission of SARS-CoV-2 into and across the placenta critically depends on the availability and functionality of its viral entry

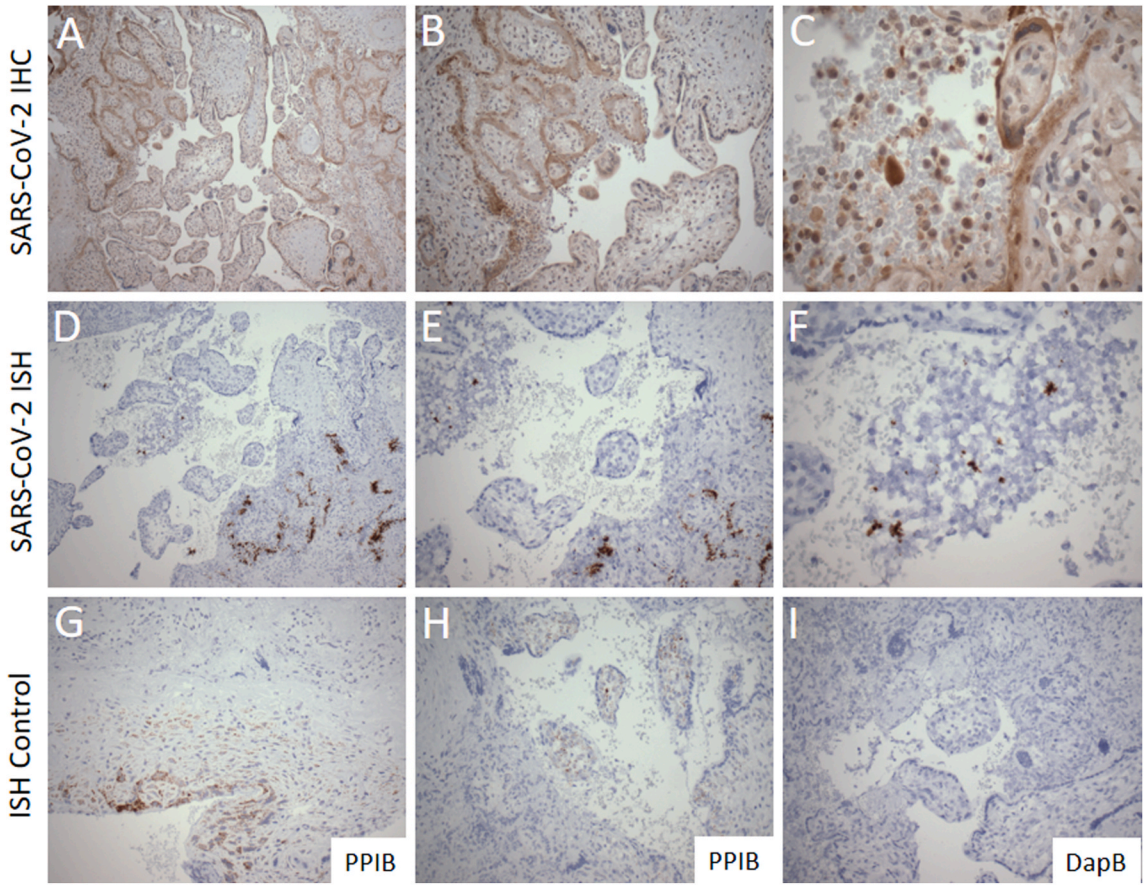

Fig. 2. Analysis of SARS-CoV-2 virus localization in the placenta. Representative immunohistochemical (A–C) and in-situ hybridization analysis (D–F) of localization of SARS-CoV-2 in the placenta demonstrating preferential localization of the virus to villi encased in fibrin with relative viral paucity in free-floating villi (A-B and D-E). Virus is also present in maternal nucleated cells in the intervillous space (C and F). In-situ hybridization analysis of the housekeeping gene PPIB (internal control) shows positive signal in decidual cells (G) and free-floating villi (H, right), but absence of signal in fibrin-encased villi (H, left). DapB (negative control) in-situ hybridization was negative throughout the placenta (I). (A–C: DAB-peroxidase system with hematoxylin counterstain) (Original magnification ×100 (A, D); X 2000 (B, E, G-I); X 400 (C, F)).

mechanisms in the placental syncytiotrophoblast layer. SARS-CoV-2 gains cellular entry by attachment of its surface-anchored spike protein (S) to the angiotensin-converting enzyme 2 (ACE2) receptor to trigger the initial attachment [26–28]. Further cell entry steps include activation of the virus internalization process by host cell proteases, in particular the cell surface protease, TMPRSS2 (transmembrane protease, serine 2), and lysosomal cathepsin proteases, fusion of viral and cellular membranes, and endocytosis [28–32].

In a previous study, centered on the twin anemia-polycythemia (TAPS) placenta as model of intertwin differential placental oxygenation, we demonstrated that placental expression of ACE2, localized mainly to (syncytio)trophoblastic cells, is upregulated in hypoxic conditions, associated with increased expression of the main cell entry regulators, TMPRSS2 and cathepsin B [33]. In the present study we tested the hypothesis that trophoblast hypoxia may facilitate placental SARS-CoV-2 transmission. To this end, we utilized a clinically relevant case of SARS-CoV-2-associated CHIV/MPVFD to determine the spatial correlation between placental tissue oxygenation, ACE2 expression and SARS-CoV-2 distribution *in vivo*.

2. Case report

2.1. Clinical history

A 25-year-old G5P2 patient at 20 weeks gestation presented to our institution for evaluation of cervical insufficiency. The patient reported decreased fetal movements over the preceding 24 h and intrauterine fetal demise was diagnosed in the Emergency Department. There was a history of prior fetal demise at 18 weeks gestation. Prenatal screening studies of the current pregnancy revealed low-risk cfDNA, normal alpha-fetoprotein (AFP) levels, and 46, XX karyotype. A COVID-19 test taken two days prior to the current admission was found to be positive. The patient was mildly symptomatic with sore throat and mild muscle aches, in the absence of associated respiratory symptoms. A stillborn was delivered vaginally following induction of labor.

The study was approved by the Institutional Review Board.

2.2. General placental and postmortem findings

Postmortem examination revealed a mildly macerated 327 g female stillborn whose postmortem foot and femur length measurements were consistent with the stated gestational age of 20 weeks. No dysmorphic features or congenital anomalies were identified. Postmortem bacterial cultures were negative. Whole genome chromosome SNP microarray analysis had a normal female result.

The placenta was small for gestational age (95 g; <10th %ile for 20 weeks gestation) [34] and showed an irregular pattern of fibrin deposition involving parenchyma and basal plate (Fig. 1A–B). Microscopic analysis of the placenta revealed diffuse intervillous fibrin deposition, associated with intervillous mononuclear cell infiltrates, composed of CD68-and CD3-immunoreactive histiocytes and T-lymphocytes, respectively (Fig. 1C–F). The massive perivillous fibrin deposition

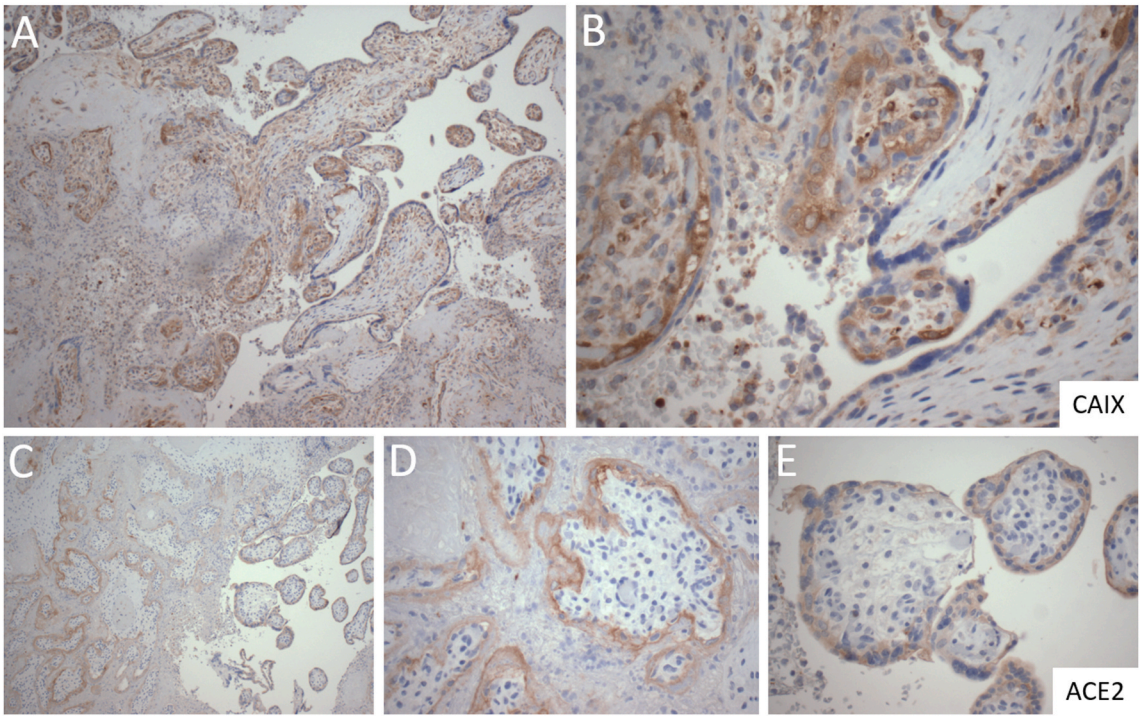

Fig. 3. Immunohistochemical analysis of CAIX and ACE2 protein expression in the placenta. Representative immunohistochemical analysis of expression of carbonic anhydrase IX (CAIX) (A–B) and angiotensin converting enzyme 2 (ACE2) (C–E) in the placenta. CAIX immunoreactivity, localized to villous trophoblastic and stromal cells, is more intense in fibrin-encased villi than in free-floating villi (A–B), consistent with relative tissue hypoxia. Similarly, protein expression of the SARS-CoV-2 receptor, ACE2, is more intense in fibrin-trapped villi than in free villi (C–E). (A–E: DAB-peroxidase system with hematoxylin counterstain, original magnification ×100 (A,C) and X 400 (B, D-E)).

(MPVFD) and chronic histiocytic intervillositis (CHIV) involved estimated 80% of the placental parenchyma, and were associated with extensive trophoblast necrosis (Fig. 1 D). The placenta further showed scattered subchorionic and intervillous hemorrhages, adherent blood clot, and focal plasma cell deciduitis. No viral cytopathic changes were detected. There was no evidence of CHIV or MPVFD in the placenta of the prior second-trimester pregnancy loss.

2.3. Analysis of SARS-CoV-2 distribution by immunohistochemistry (IHC) and in-situ hybridization (ISH)

SARS-CoV-2 protein and RNA were localized by immunohistochemical and RNAscope in-situ hybridization (ISH) analyses, respectively. For immunohistochemical staining, sections of placenta were routinely processed using a rabbit polyclonal antibody against SARS Nucleocapsid Protein (SARS-NP) (1:200, Novus biologicals, Littleton, CO). Controls for specificity consisted of omission of the primary antibody, which abolished all immunoreactivity. As shown in Fig. 2 A-B, SARS-CoV-2 protein distribution showed high regional variability, with a strong tendency to more intense SARS-CoV-2 immunoreactivity in villi encased in fibrin, compared to villi free-floating in the intervillous space. Within chorionic villi, SARS-NP was primarily expressed in the syncytiotrophoblast layer with scattered localization in cytotrophoblastic cells. Consistent with the known COVID-19-positive status of the patient, maternal cells in the intervillous space expressed strong SARS-NP immunoreactivity (Fig. 2 C). No expression was observed within chorionic plate or decidual tissues.

RNA in-situ hybridization was performed manually according to the manufacturer's instructions using RNAScope probes (Advanced Cell Diagnostics, (ACD), Newark, CA) directed against SARS-CoV2, targeting 21631–23303 base pairs (ACD #848561) [35,36]. The regional variability of SARS-CoV-2 viral distribution was even more pronounced in sections subjected to SARS-CoV-2 RNAscope in-situ hybridization where positive signal was virtually exclusively seen in villi contained by perivillous fibrin deposition, and conspicuously absent in free villi (Fig. 2D–E). SARS-CoV-2 infected maternal inflammatory cells in the intervillous space again served as internal positive staining controls (Fig. 2 F).

The housekeeping gene peptidylprolyl isomerase B (PPIB) was used as positive control probe to assess RNA integrity. Detection of PPIB RNA by RNAscope ISH was limited to decidual cells and to stromal and trophoblastic cells of free-floating villi (Fig. 2G–H). PPIB RNA signals were absent in villi encased in perivillous fibrin deposition suggestive of hypoxia/ischemia-induced RNA degradation of this housekeeping gene in these areas. The abundance of SARS-CoV-2 RNA in fibrin-encased villi, in sharp contrast to the lack of identifiable RNA levels of the PPIB housekeeping gene in these same areas, further underscores the likely presence of copious amounts of viral load in the – relatively degenerated – villi in areas of perivillous fibrin deposition. RNAscope analysis of expression of the bacterial gene diaminopimelate B (DapB), utilized as a negative control probe to assess non-specific background signals, was negative throughout the placenta (Fig. 2 I).

SARS-CoV-2 RNAscope in-situ hybridization of fetal lung, liver and gastrointestinal tract failed to produce positive signals (not shown). However, the PPIB positive control was similarly negative in these tissues, suggesting RNA degradation may have rendered the RNA scope technique unreliable for detection of viral presence in the autolyzed tissues of this stillborn, and false negative ISH results cannot be excluded.

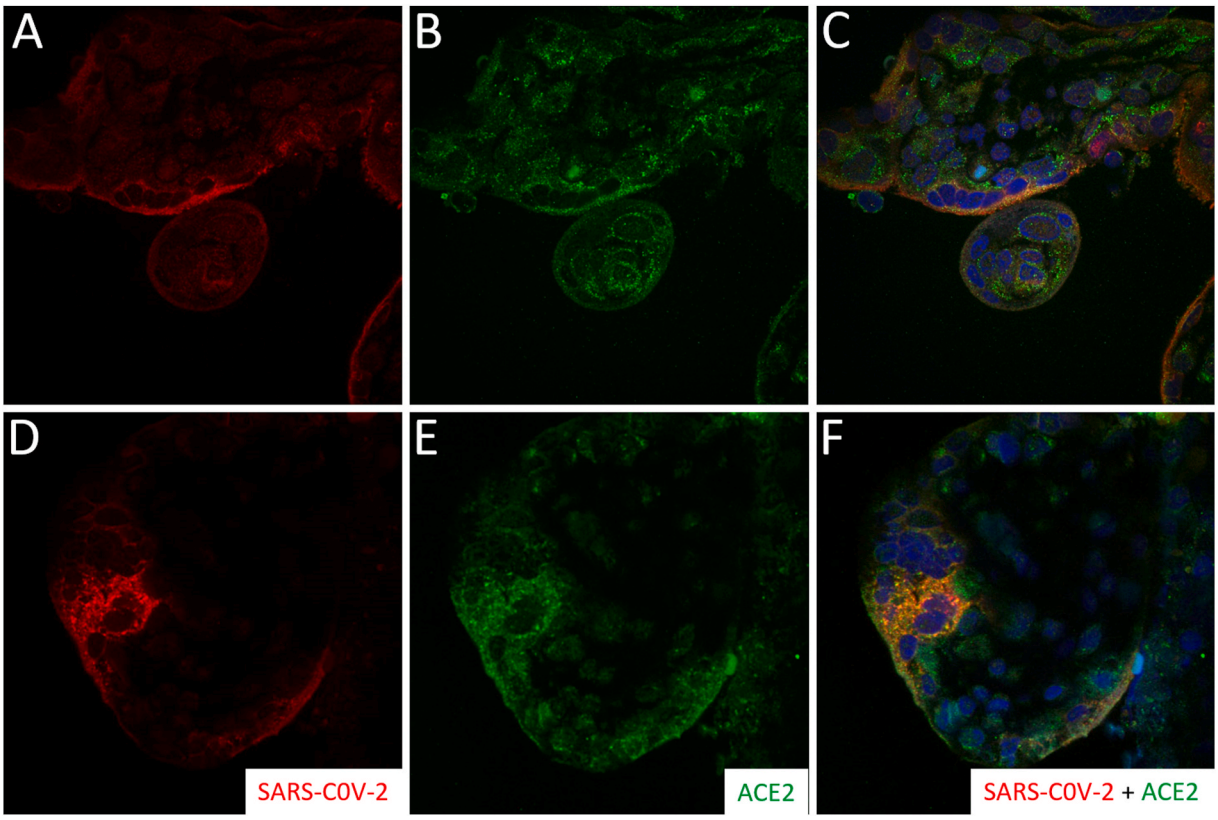

Fig. 4. Combined immunofluorescence analysis of placental colocalization of SARS-CoV-2 and ACE2. Confocal fluorescence microscopy of the placenta subjected to combined anti-ACE2 (green) and anti-SARS-CoV-2 (red) immunofluorescence, captured at the same settings. Yellow dots represent regions of colocalization of ACE2 and SARS-CoV-2 in trophoblastic cells. In selected fields, colocalization was confirmed quantitatively using Pearson's correlation coefficient analysis whereby a cutoff of $r^2 > 0.5$ was used to indicate positive colocalization. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.4. Analysis of tissue oxygenation

To determine whether the regional variation of SARS-CoV-2 localization (i.e. higher viral load in regions of perivillous fibrin deposition) correlated with similar regional variation in tissue oxygenation, placental oxygenation was estimated by analysis of expression of the endogenous hypoxia marker, carbonic anhydrase IX (CAIX), as described elsewhere [37,38]. The promoter region of the *CA9* gene contains a hypoxia-response element that is a transcriptional target of the hypoxia inducible factor-1 (HIF1) transcription factor. CAIX expression thus represents an indirect indicator of activation of the HIF-1 complex and can serve as endogenous cellular biomarker of chronic hypoxia in archival tissues [39,40]. Protein levels of CAIX, immunolocalized to villous cytotrophoblastic cells, villous stromal cells, and extravillous trophoblast, were consistently higher in villi trapped in perivillous fibrin compared with non-encased, free-floating villi, consistent with relative tissue hypoxia in areas of fibrin deposition (Fig. 3A–B).

2.5. Analysis of expression of ACE2, the SARS-CoV-2 receptor

To determine whether the observed regional variability of tissue oxygenation correlated with similar variability in availability of the SARS-CoV-2 cell entry axis, we studied protein expression of ACE2, the cellular receptor of SARS-CoV-2, by immunohistochemistry. As shown in Fig. 3 C-E, levels of ACE2 protein, immunolocalized to villous trophoblastic cells, were higher in hypoxic, fibrin-encased villi than in free villi.

Anatomic colocalization of SARS-CoV-2 and ACE2 was assessed further by double immunofluorescence studies. Tissue sections were incubated sequentially with polyclonal rabbit anti-SARS-CoV-2 antibody, Alexa Fluor 594-conjugated anti-rabbit IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), mouse monoclonal anti-ACE2 antibody (ThermoFisher Scientific (Invitrogen), Waltham, MA), biotinylated anti-mouse IgG (Vector Laboratories, Inc., Burlingame, CA) and Alexa Fluor 488-conjugated streptavidin (Jackson ImmunoResearch Laboratories, Inc.). Confocal images were acquired with a Nikon Ti-E spinning disk confocal microscope (Nikon Inc., Melville, NY) using diode lasers 405, 488, and 561, and processed as previously described [37]. Foci of colocalization (yellow fluorescence) of ACE2 (green fluorescence) and SARS-CoV-2 (red fluorescence) were readily observed in villous syncytiotrophoblastic cells (Fig. 4).

3. Discussion

In a prior study, we utilized the TAPS model to determine the effect of differential tissue oxygenation on the expression of ACE2 and ACE2–related SARS-CoV-2 cell entry regulators in the placenta [33]. In the present study we investigated whether hypoxia-induced ACE2 upregulation in trophoblastic cells effectively promotes SARS-CoV-2 infection of these cells. To this end, we took advantage of the regional variation in placental tissue oxygenation in SARS-CoV-2-associated chronic histiocytic intervillositis/massive perivillous fibrin deposition (CHIV/MPVFD) to determine the spatial correlation between placental tissue oxygenation and SARS-CoV-2 distribution. Using a combination of immunohistochemical/-fluorescence and RNA in-situ hybridization methods, we established that SARS-CoV-2 preferentially localizes to fibrin-encased, hypoxic trophoblastic cells.

The predilection of SARS-CoV-2 for hypoxic placental regions is likely mediated, at least in part, by hypoxia-induced upregulation of the viral receptor, ACE2, in trophoblastic cells. In line with the reported

effects of hypoxia on ACE2 expression in a wide range of tissues and organ systems [41–45], including our prior study in TAPS placentas [33], we determined that ACE2 expression was significantly upregulated in the hypoxic fibrin-trapped villi of CHIV/MPVFD placentas. In accordance with studies by others in SARS-CoV-2-positive placentas [46], both SARS-CoV-2 spike glycoprotein and ACE2 expression consistently localized primarily within the outer syncytiotrophoblast layer of the chorionic villi, a key physiologic interface between mother and fetus.

The striking spatial distribution pattern of SARS-CoV-2 in CHIV/PVFD placentas, with preferential localization of the virus in the syncytiotrophoblast of hypoxic, ACE2-overexpressing, fibrin-encased villi, suggests placental SARS-CoV-2 transmission may be facilitated by intrauterine hypoxia. Conversely, it is possible that SARS-CoV-2 infection itself may cause regional placental hypoxia by direct villous injury, leading to accumulation of perivillous fibrin and inflammation of the intervillous space.

Detection of the virus in placental villous tissue, as in the current case, indicates intrauterine transplacental transmission. This should be distinguished from intrauterine vertical transmission, defined by positive RT-PCR testing of neonates for the virus at or shortly after birth, early onset of symptoms in neonates, and elevated levels of specific IgM antibodies after delivery [25]. By these criteria our case represents an intrauterine transplacental transmission with undetermined intrauterine vertical transmission as autolysis rendered IHC and ISH studies in the fetal tissues unreliable.

In summary, this study in a SARS-CoV-2 associated case of CHIV/MPVFD offers further support for a causative relationship between SARS-CoV-2 intrauterine transmittability and hypoxia. Increased understanding of the biology of SARS-CoV-2 may contribute to further development of intervention strategies targeting viral cell entry mechanisms.

Declaration of competing interest

Quanfu Mao, Sharon Chu, Svetlana Shapiro, Lawrence Young, Melissa Russo, Monique E. De Paepe. The above authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

References

[1] China Novel Coronavirus I, Research T N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, X. Zhao, B. Huang, W. Shi, R. Lu, P. Niu, F. Zhan, X. Ma, D. Wang, W. Xu, G. Wu, G.F. Gao, W. Tan, A novel coronavirus from patients with pneumonia in China, 2019, N. Engl. J. Med. 382 (2020) 727–733.

[2] N. Chen, M. Zhou, X. Dong, J. Qu, F. Gong, Y. Han, Y. Qiu, J. Wang, Y. Liu, Y. Wei, J. Xia, T. Yu, X. Zhang, L. Zhang, Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study, Lancet 395 (2020) 507–513.

[3] C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Z. Cheng, T. Yu, J. Xia, Y. Wei, W. Wu, X. Xie, W. Yin, H. Li, M. Liu, Y. Xiao, H. Gao, L. Guo, J. Xie, G. Wang, R. Jiang, Z. Gao, Q. Jin, J. Wang, B. Cao, Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, Lancet 395 (2020) 497–506.

[4] T.G. Ksiazek, D. Erdman, C.S. Goldsmith, S.R. Zaki, T. Peret, S. Emery, S. Tong, C. Urbani, J.A. Comer, W. Lim, P.E. Rollin, S.F. Dowell, A.E. Ling, C.D. Humphrey, W.J. Shieh, J. Guarner, C.D. Paddock, P. Rota, B. Fields, J. DeRisi, J.Y. Yang, N. Cox, J.M. Hughes, J.W. LeDuc, W.J. Bellini, L.J. Anderson, S.W. Group, A novel coronavirus associated with severe acute respiratory syndrome, N. Engl. J. Med. 348 (2003) 1953–1966.

[5] A.M. Zaki, S. van Boheemen, T.M. Bestebroer, A.D. Osterhaus, R.A. Fouchier, Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia, N. Engl. J. Med. 367 (2012) 1814–1820.

[6] K.P. Patel, S.R. Vunnam, P.A. Patel, K.L. Krill, P.M. Korbitz, J.P. Gallagher, J. E. Suh, R.R. Vunnam, Transmission of SARS-CoV-2: an update of current literature, Eur. J. Clin. Microbiol. Infect. Dis. (2020).

[7] L. Zeng, S. Xia, W. Yuan, K. Yan, F. Xiao, J. Shao, W. Zhou, Neonatal early-onset infection with SARS-CoV-2 in 33 neonates born to mothers with COVID-19 in Wuhan, China, JAMA Pediatr. (2020).

[8] M. Zamaniyan, A. Ebadi, S. Aghajanpoor, Z. Rahmani, M. Haghshenas, S. Azizi, Preterm delivery, maternal death, and vertical transmission in a pregnant woman with COVID-19 infection, Prenat. Diagn. (2020).

[9] M.C. Alzamora, T. Paredes, D. Caceres, C.M. Webb, L.M. Valdez, M. La Rosa, Severe COVID-19 during pregnancy and possible vertical transmission, Am. J. Perinatol. 37 (2020) 861–865.

[10] M. Kirtsman, Y. Diambomba, S.M. Poutanen, A.K. Malinowski, E. Vlachodimitropoulou, W.T. Parks, L. Erdman, S.K. Morris, P.S. Shah, Probable congenital SARS-CoV-2 infection in a neonate born to a woman with active SARS-CoV-2 infection, CMAJ (Can. Med. Assoc. J.) 192 (2020) E647–E650.

[11] L. Patane, D. Morotti, M.R. Giunta, C. Sigismondi, M.G. Piccoli, L. Frigerio, G. Mangili, M. Arosio, G. Cornolti, Vertical transmission of COVID-19: SARS-CoV-2 RNA on the fetal side of the placenta in pregnancies with COVID-19 positive mothers and neonates at birth, Am. J. Obstet. Gynecol. MFM (2020) 100145.

[12] J. Sisman, M.A. Jaleel, W. Moreno, V. Rajaram, R.R.J. Collins, R.C. Savani, D. Rakheja, A.S. Evans, Intrauterine transmission of sars-cov-2 infection in a preterm infant, Pediatr. Infect. Dis. J. (2020).

[13] B. Pulinx, D. Kieffer, I. Michiels, S. Petermans, D. Strybol, S. Delvaux, M. Baldewijns, M. Raymaekers, R. Cartuyvels, W. Maurissen, Vertical transmission of SARS-CoV-2 infection and preterm birth, Eur. J. Clin. Microbiol. Infect. Dis. (2020).

[14] A. Demirjian, C. Singh, M. Tebruegge, R. Herbert, N. Draz, M. Mirfenderesky, V. Jones, P. Hinstridge, R. Seneviratne, R. Myers, J. Ellis, S. Miah, C.I. Meadows, J. Kenny, A. Alonso, J. Handforth, S. Perkins, M. Butler, F. Blackburn, S. Douthwaite, D. Pillay, M. Chand, M. Zambon, Probable vertical transmission of SARS-CoV-2 infection, Pediatr. Infect. Dis. J. (2020).

[15] I. Barcelos, I.A.A. Penna, A.G. Soligo, Z.B. Costa, W.P. Martins, Vertical transmission of SARS-CoV-2: a systematic Review, Rev. Bras. Ginecol. Obstet. 43 (2021) 207–215.

[16] L. Linehan, K. O'Donoghue, S. Dineen, J. White, J.R. Higgins, B. Fitzgerald, SARS-CoV-2 placentitis: an uncommon complication of maternal COVID-19, Placenta 104 (2021) 261–266.

[17] A.J. Vivanti, C. Vauloup-Fellous, S. Prevot, V. Zupan, C. Suffee, J. Do Cao, A. Benachi, D. De Luca, Transplacental transmission of SARS-CoV-2 infection, Nat. Commun. 11 (2020) 3572.

[18] P. Zhang, C. Salafia, T. Heyman, C. Salafia, S. Lederman, B. Dygulska, Detection of severe acute respiratory syndrome coronavirus 2 in placentas with pathology and vertical transmission, Am. J. Obstet. Gynecol. MFM 2 (2020) 100197.

[19] H. Hosier, S.F. Farhadian, R.A. Morotti, U. Deshmukh, A. Lu-Culligan, K. H. Campbell, Y. Yasumoto, C.B. Vogels, A. Casanovas-Massana, P. Vijayakumar, B. Geng, C.D. Odio, J. Fournier, A.F. Brito, J.R. Fauver, F. Liu, T. Alpert, R. Tal, K. Szigeti-Buck, S. Perincheri, C.P. Larsen, A.M. Gariepy, G. Aguilar, K. L. Fardelmann, M. Harigopal, H.S. Taylor, C.M. Pettker, A.L. Wyllie, C.S. Dela Cruz, A.M. Ring, N.D. Grubaugh, A.I. Ko, T.L. Horvath, A. Iwasaki, U.M. Reddy, H. S. Lipkind, SARS-CoV-2 infection of the placenta, J. Clin. Invest. (2020).

[20] A.L. Hsu, M. Guan, E. Johannesen, A.J. Stephens, N. Khaleel, N. Kagan, B.C. Tuhlei, X.F. Wan, Placental SARS-CoV-2 in a pregnant woman with mild COVID-19 disease, J. Med. Virol. 93 (2021) 1038–1044.

[21] L. Debelenko, I. Katsyv, A.M. Chong, L. Peruyero, M. Szabolcs, A.C. Uhlemann, Trophoblast damage with acute and chronic intervillositis: disruption of the placental barrier by severe acute respiratory syndrome coronavirus 2, Hum. Pathol. 109 (2021) 69–79.

[22] J. Ordi, M.R. Ismail, P.J. Ventura, E. Kahigwa, R. Hirt, A. Cardesa, P.L. Alonso, C. Menendez, Massive chronic intervillositis of the placenta associated with malaria infection, Am. J. Surg. Pathol. 22 (1998) 1006–1011.

[23] M. Taweevisit, K. Sukpan, S. Siriaunkgul, P.S. Thorner, Chronic histiocytic intervillositis with cytomegalovirus placentitis in a case of hydrops fetalis, Fetal Pediatr. Pathol. 31 (2012) 394–400.

[24] D.A. Schwartz, M. Baldewijns, A. Benachi, M. Bugatti, R.R.J. Collins, D. De Luca, F. Facchetti, R.L. Linn, L. Marcelis, D. Morotti, R. Morotti, W.T. Parks, L. Patane, S. Prevot, B. Pulinx, V. Rajaram, D. Strybol, K. Thomas, A.J. Vivanti, Chronic histiocytic intervillositis with trophoblast necrosis is a risk factor Associated with placental infection from coronavirus disease 2019 (COVID-19) and intrauterine maternal-fetal severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission in live-born and stillborn infants, Arch. Pathol. Lab Med. 145 (2021) 517–528.

[25] D.A. Schwartz, D. Morotti, B. Beigi, F. Moshfegh, N. Zafaranloo, L. Patane, Confirming vertical fetal infection with coronavirus disease 2019: neonatal and pathology criteria for early onset and transplacental transmission of severe acute respiratory syndrome coronavirus 2 from infected pregnant mothers, Arch. Pathol. Lab Med. 144 (2020) 1451–1456.

[26] W. Li, M.J. Moore, N. Vasilieva, J. Sui, S.K. Wong, M.A. Berne, M. Somasundaran, J.L. Sullivan, K. Luzuriaga, T.C. Greenough, H. Choe, M. Farzan, Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus, Nature 426 (2003) 450–454.

[27] Z. Liu, X. Xiao, X. Wei, J. Li, J. Yang, H. Tan, J. Zhu, Q. Zhang, J. Wu, L. Liu, Composition and divergence of coronavirus spike proteins and host ACE2 receptors predict potential intermediate hosts of SARS-CoV-2, J. Med. Virol. 92 (2020) 595–601.

[28] M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Kruger, T. Herrler, S. Erichsen, T. S. Schiergens, G. Herrler, N.H. Wu, A. Nitsche, M.A. Muller, C. Drosten, S. Pohlmann, SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor, Cell 181 (2020) 271–280 e8.

[29] Network HCALB W. Sungnak, N. Huang, C. Becavin, M. Berg, R. Queen, M. Litvinukova, C. Talavera-Lopez, H. Maatz, D. Reichart, F. Sampaziotis, K. B. Worlock, M. Yoshida, J.L. Barnes, SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes, Nat. Med. 26 (2020) 681–687.

[30] K.H. Stopsack, L.A. Mucci, E.S. Antonarakis, P.S. Nelson, P.W. Kantoff, TMPRSS2 and COVID-19: serendipity or opportunity for intervention? Cancer Discov. 10 (2020) 779–782.
[31] R. Lu, X. Zhao, J. Li, P. Niu, B. Yang, H. Wu, W. Wang, H. Song, B. Huang, N. Zhu, Y. Bi, X. Ma, F. Zhan, L. Wang, T. Hu, H. Zhou, Z. Hu, W. Zhou, L. Zhao, J. Chen, Y. Meng, J. Wang, Y. Lin, J. Yuan, Z. Xie, J. Ma, W.J. Liu, D. Wang, W. Xu, E. C. Holmes, G.F. Gao, G. Wu, W. Chen, W. Shi, W. Tan, Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding, Lancet 395 (2020) 565–574.
[32] A.C. Walls, Y.J. Park, M.A. Tortorici, A. Wall, A.T. McGuire, D. Veesler, Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein, Cell 181 (2020) 281–292, e6.
[33] Q. Mao, S. Chu, S. Shapiro, J.M. Bliss, M.E. De Paepe, Increased placental expression of angiotensin-converting enzyme 2, the receptor of SARS-CoV-2, associated with hypoxia in twin anemia-polycythemia sequence (TAPS), Placenta 105 (2021) 7–13.
[34] H. Pinar, C.J. Sung, C.E. Oyer, D.B. Singer, Reference values for singleton and twin placental weights, Pediatr. Pathol. Lab. Med. 16 (1996) 901–907.
[35] A. Best Rocha, E. Stroberg, L.M. Barton, E.J. Duval, S. Mukhopadhyay, N. Yarid, T. Caza, J.D. Wilson, D.J. Kenan, M. Kuperman, S.G. Sharma, C.P. Larsen, Detection of SARS-CoV-2 in formalin-fixed paraffin-embedded tissue sections using commercially available reagents, Lab. Invest. 100 (2020) 1485–1489.
[36] J. Liu, A.M. Babka, B.J. Kearney, S.R. Radoshitzky, J.H. Kuhn, X. Zeng, Molecular detection of SARS-CoV-2 in formalin-fixed, paraffin-embedded specimens, JCI Insight 5 (2020).
[37] Q. Mao, S. Chu, S. Shapiro, H. Yao, M.E. De Paepe, Discordant placental oxygenation and autophagy in twin anemia-polycythemia sequence (TAPS), Placenta 90 (2020) 9–17.
[38] S. Ravishankar, G. Bourjeily, G. Lambert-Messerlian, M. He, M.E. De Paepe, F. Gundogan, Evidence of placental hypoxia in maternal sleep disordered breathing, Pediatr. Dev. Pathol. 18 (2015) 380–386.
[39] C.C. Wykoff, N.J. Beasley, P.H. Watson, K.J. Turner, J. Pastorek, A. Sibtain, G. D. Wilson, H. Turley, K.L. Talks, P.H. Maxwell, C.W. Pugh, P.J. Ratcliffe, A. L. Harris, Hypoxia-inducible expression of tumor-associated carbonic anhydrases, Cancer Res. 60 (2000) 7075–7083.
[40] P. Swietach, A. Hulikova, R.D. Vaughan-Jones, A.L. Harris, New insights into the physiological role of carbonic anhydrase IX in tumour pH regulation, Oncogene 29 (2010) 6509–6521.
[41] L.M. Burrell, J. Risvanis, E. Kubota, R.G. Dean, P.S. MacDonald, S. Lu, C. Tikellis, S. L. Grant, R.A. Lew, A.I. Smith, M.E. Cooper, C.I. Johnston, Myocardial infarction increases ACE2 expression in rat and humans, Eur. Heart J. 26 (2005) 369–375, discussion 22-4.
[42] S. Joshi, H. Wollenzien, E. Leclerc, Y.P. Jarajapu, Hypoxic regulation of angiotensin-converting enzyme 2 and Mas receptor in human CD34(+) cells, J. Cell. Physiol. 234 (2019) 20420–20431.
[43] S. Joshi, N. Balasubramanian, G. Vasam, Y.P. Jarajapu, Angiotensin converting enzyme versus angiotensin converting enzyme-2 selectivity of MLN-4760 and DX600 in human and murine bone marrow-derived cells, Eur. J. Pharmacol. 774 (2016) 25–33.
[44] N.E. Clarke, N.D. Belyaev, D.W. Lambert, A.J. Turner, Epigenetic regulation of angiotensin-converting enzyme 2 (ACE2) by SIRT1 under conditions of cell energy stress, Clin. Sci. (Lond.) 126 (2014) 507–516.
[45] S.J. Delforce, Y. Wang, M.E. Van-Aalst, C. Corbisier de Meaultsart, B.J. Morris, F. Broughton-Pipkin, C.T. Roberts, E.R. Lumbers, K.G. Pringle, Effect of oxygen on the expression of renin-angiotensin system components in a human trophoblast cell line, Placenta 37 (2016) 1–6.
[46] E. Taglauer, Y. Benarroch, K. Rop, E. Barnett, V. Sabharwal, C. Yarrington, E. M. Wachman, Consistent localization of SARS-CoV-2 spike glycoprotein and ACE2 over TMPRSS2 predominance in placental villi of 15 COVID-19 positive maternal-fetal dyads, Placenta 100 (2020) 69–74.