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 127 (2022) 73–76

Contents lists available at ScienceDirect

Placenta

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

Restriction of SARS-CoV-2 replication in the human placenta

Kazuhide Takada [a], Sayaka Shimodai-Yamada [b], Mayumi Suzuki [b], Quang Duy Trinh [a], Chika Takano [a], Kaori Kawakami [c], Mikiko Asai-Sato [c], Atsushi Komatsu [c], Aya Okahashi [d], Nobuhiko Nagano [d], Toshiya Misawa [e], Kyohei Yamaguchi [f], Tadaki Suzuki [g], Kei Kawana [c], Ichiro Morioka [d], Hideto Yamada [h], Satoshi Hayakawa [a], Hiroyuki Hao [b,**], Shihoko Komine-Aizawa [a,*]

[a] Division of Microbiology, Department of Pathology and Microbiology, Nihon University School of Medicine, Japan
[b] Division of Human Pathology, Department of Pathology and Microbiology Nihon University School of Medicine, Japan
[c] Department of Obstetrics and Gynecology, Nihon University School of Medicine, Japan
[d] Department of Pediatrics and Child Health, Nihon University School of Medicine, Japan
[e] Department of Obstetrics and Gynecology, Nagoya Ekisaikai Hospital, Japan
[f] Department of Obstetrics and Gynecology, National Hospital Organization, Mie Chuo Medical Center, Japan
[g] Department of Pathology, National Institute of Infectious Diseases, Japan
[h] Center for Recurrent Pregnancy Loss, Teine Keijinkai Hospital Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan

A B S T R A C T

Although SARS-CoV-2 can infect human placental tissue, vertical transmission is rare. Therefore, the placenta may function as a barrier to inhibit viral transmission to the foetus, though the mechanisms remain unclear. In this study, we confirmed the presence of the SARS-CoV-2 genome in human placental tissue by in situ hybridization with antisense probes targeting the spike protein; tissue staining was much lower when using sense probes for the spike protein. To the best of our knowledge, this is the first evidence directly indicating inefficient viral replication in the SARS-CoV-2-infected placenta. Additional studies are required to reveal the detailed mechanisms.

1. Introduction

Accumulative studies have reported that SARS-CoV-2 can infect placental tissue, especially the syncytiotrophoblast, during pregnancy. However, vertical transmission of the virus is unlikely to occur [1–5]. Therefore, the placental barrier might be effective in inhibiting viral transmission to the foetus, though the mechanisms are still unknown [6]. Inefficient viral infection and replication in the placenta have already been suggested by ex vivo and in vitro studies [7,8]. To date, however, compelling evidence of inefficient viral replication in the placenta of SARS-CoV-2-infected women is still lacking.

Some studies employed an in situ hybridization (ISH) technique to detect the SARS-CoV-2 genome in the placenta [5,8–20]. All these studies used “antisense” probes to detect the viral genome, which confirmed only the presence of SARS-CoV-2 in the tissue. Although other studies, such as [21], have also used the ISH technique, we could not find the details of the probes used. SARS-CoV-2 is a positive-sense, single-stranded RNA virus, and a negative-sense strand is generated during the replication process [4,22]. The negative-sense strand of SARS-CoV-2 can be detected by a “sense” probe [23]. We found only one study that used both antisense and sense probes to detect SARS-CoV-2 in the placenta [24]; however, it lacked detailed descriptions. To confirm the presence and replication of the SARS-CoV-2 genome, we performed ISH on serial sections of the placenta using antisense and sense probes.

2. Methods

2.1. Subjects

This study was approved by the ethical committees of the Nihon University School of Medicine (P20-22-0, and P20-29-0, and RK-200512-9), the Mie Chuo Medical Center (MCERB-202106), and the Nagoya Ekisaikai Hospital (2021–042). All patients were informed and signed an informed consent form. Nasopharyngeal swabs from the patients were used to diagnose SARS-CoV-2 infection, and 15 placental tissues were obtained from 13 mothers (two had twin pregnancies). Each

* Corresponding author. 30-1 Ohyaguchi-kamicho, Itabash-ku, Tokyo, Japan.
** Corresponding author. 30-1 Ohyaguchi-kamicho, Itabash-ku, Tokyo, Japan.
E-mail addresses: hao.hiroyuki@nihon-u.ac.jp (H. Hao), aizawa.shihoko@nihon-u.ac.jp (S. Komine-Aizawa).

https://doi.org/10.1016/j.placenta.2022.07.010
Received 31 January 2022; Received in revised form 13 June 2022; Accepted 14 July 2022
Available online 19 July 2022

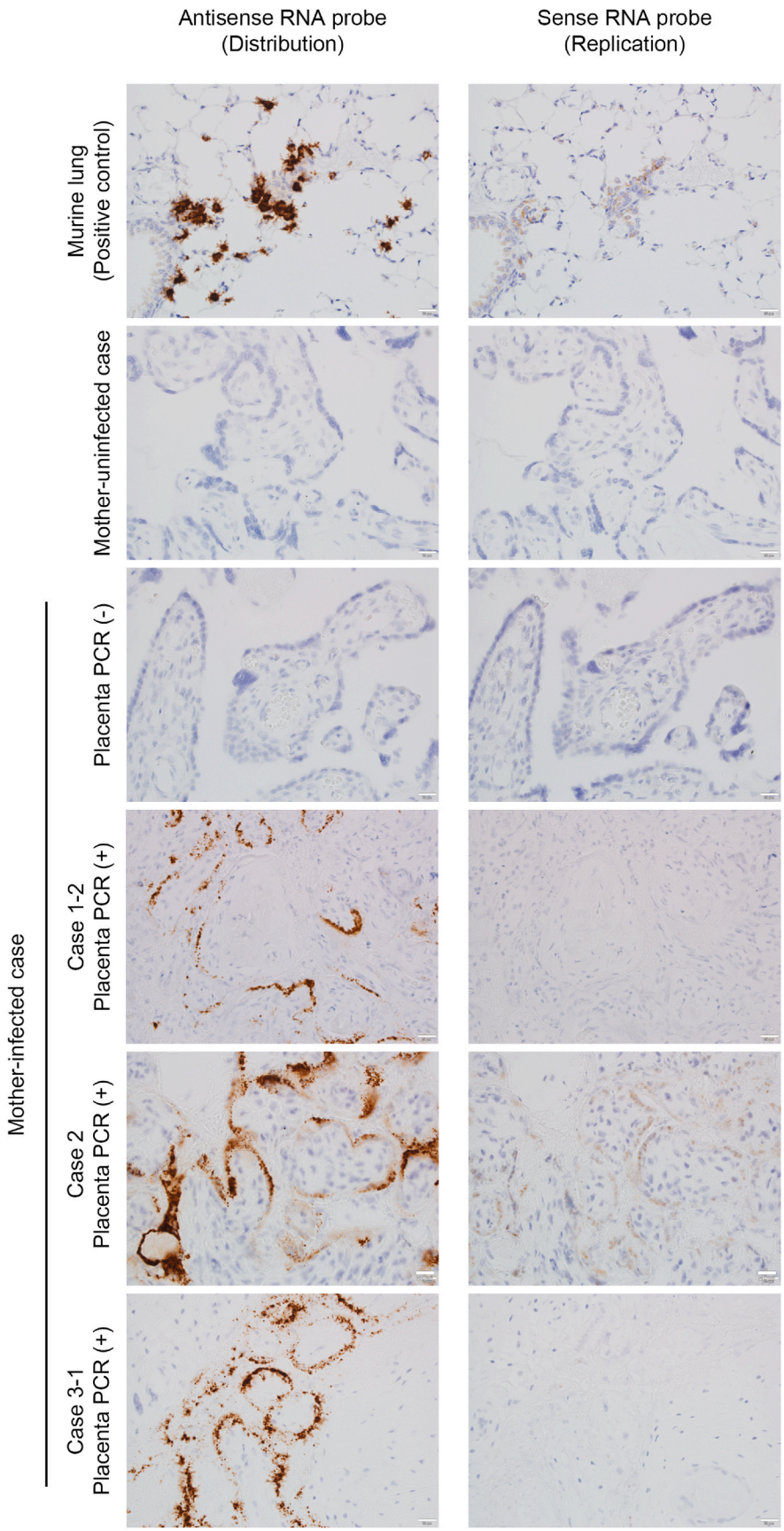

Fig. 1. In situ hybridizations of SARS-CoV-2 spike protein mRNA on serial sections of infected human placenta. Placental tissues were obtained from pregnant women with COVID-19. Images are representative of each case. Lung tissue of a SARS-CoV-2-infected mouse served as a positive control. Placentas obtained from uninfected pregnant women, as well as a PCR-negative placenta from a patient with COVID-19, were used as negative controls. An antisense probe for the positive-sense mRNA of the spike protein was used to detect the distribution of the virus. A sense probe for the negative-sense mRNA was used to detect the intermediate process of viral replication. Note that the reaction of the sense probe was very weak on the SARS-CoV-2-infected placenta compared with the reaction of the antisense probe on a serial section, indicating that viral replication was restricted in the placentas of cases 1 and 3. Magnification: 400x. Bars: 20 μM.

Table 1
Results of RT-PCR and ISH for each case.

Case	RT-qPCR	ISH (anti-sense)	ISH (sense)
1–1	+	+	−
1–2	+	+	−
2	+	+	+
3–1	+	+	−
3–2	+	+	−

sample was obtained from the center of the placenta. The placental tissues were fixed with formalin and embedded in paraffin. The remaining unprocessed tissue samples were stored at −80 °C for RNA extraction. The presence of SARS-CoV-2 in the placental tissue was detected by reverse transcription–quantitative polymerase chain reaction (RT-qPCR) as described below.

2.2. Reverse transcription–quantitative polymerase chain reaction (RT-qPCR)

Viral RNA was extracted from the placental tissue by using ReliaPrep RNA Miniprep Systems (Promega, Madison, WI, United States) according to the manufacturer's instructions. The presence of SARS-CoV-2 was detected by RT–qPCR amplification of SARS-CoV-2 genes using primer/prove N2 (TaKaRa, Shiga, Japan) and One Step PrimeScript III RT–qPCR Mix (TaKaRa). Amplification was performed by a QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, United States) according to the manufacturer's instructions.

2.3. In situ hybridization (ISH)

Three PCR-positive samples (cases 1 to 3; two were twin pregnancies) were used for the ISH study. These patients delivered during the second trimester of pregnancy (8–15 days after the diagnosis of COVID-19). In case 1, the symptoms of COVID-19 were mild, but HELLP syndrome occurred just before the delivery. Cases 2 and 3 were asymptomatic.

We selected these three cases of COVID-19 with placental infection for ISH. ISH for the genome of the SARS-CoV-2 spike protein was performed using RNAscope Target Probe - V-nCoV2019-S (Cat No. 848561), RNAscope Target Probe - V-nCoV2019-S-sense (Cat No. 845701), and RNAscope 2.5 HD Reagent Kit-Brown (Cat No. 322300) (Advanced Cell Diagnostic, Hayward, CA, United States), according to the manufacturer's instructions. Six-micrometre sections of formalin-fixed, paraffin-embedded tissue were used. Sections were counterstained with haematoxylin. Lung tissue of a SARS-CoV-2-infected mouse served as a positive control. Tissues obtained from an uninfected case and a PCR-negative placenta from a patient with COVID-19 were used as negative controls.

3. Results

In the murine lung positive control, the reactions of the antisense and sense probes were detected at the same site on serial sections, although the reaction of the sense probe was weaker than that of the antisense probe (Fig. 1). The results obtained with the placental tissues showed that the reaction of the antisense probe was strong in the PCR-positive placental tissues, especially in the syncytiotrophoblast, but not evident in the decidua in all three independent cases. However, in cases 1 and 3, the reaction of the sense probe was very weak in serial sections from paraffin-embedded sections (Fig. 1). Neither the antisense nor the sense probe reacted with the tissues obtained from the uninfected case and with the PCR-negative placenta from the patient with COVID-19 (Fig. 1). The RT–qPCR and ISH results are summarized in Table 1.

4. Discussion

Here, we report that SARS-CoV-2-infected placental tissue was stained with an antisense probe targeting the spike genome of SARS-CoV-2. However, the reaction of the sense probe, proven to be the complementary strand of the coding sequence of the spike protein, was very weak against the consecutive sections from two independent cases (Table 1). In line with the data of a previous study [24], the result of case 2 indicated that SARS-CoV-2 replication could occur in the human placenta. However, the present results of both cases 1 and 3 indicated that the viral replication was restricted (Table 1). To address this difference, we are going to compare protein expression using mass spectrometry.

The lower rate of vertical transmission indicates the presence of a placental barrier to inhibit viral transmission to the foetus. Given that less than 20% of placental tissues are PCR positive [8], the placenta basically seems to be resistant to SARS-CoV-2 infection. In addition to this basic resistance of the placenta against viral infection, our results suggest that even if the syncytiotrophoblast is infected by the virus, the cells would be kept from producing the progeny virus (abortive infection).

In an early report, SARS-CoV-2 spike protein and ACE2 expression consistently localized in the syncytiotrophoblast [26], indicating that the viral replication process can start within the syncytiotrophoblast. In the human body, ACE2 expression was reported to be the highest in the small intestine, while the expression of the protein in the placenta is approximately 1/120 of that in the small intestine [27]. A recent study has shown that hypoxia-induced ACE2 upregulation in trophoblastic cells in fibrin-encased villi could be associated with SARS-CoV-2 infection [19]. In line with those of previous studies [1–5], our results showed that SARS-CoV-2 could infect the syncytiotrophoblast. However, unknown factors that restrict viral replication within the syncytiotrophoblast remain to be elucidated.

In conclusion, to the best of our knowledge, this is the first evidence directly indicating inefficient viral replication in the SARS-CoV-2-infected placenta. Further studies are required to reveal the detailed mechanisms of the placental barrier against SARS-CoV-2 vertical transmission.

Declaration of competing interest

The authors have no conflict of interest.

Acknowledgements

This work was supported by the Ministry of Health, Labour and Welfare of Japan (Grant Number 20CA2033) (to H.Y), the Subcommittee on Perinatal Infection of the Committee on the Perinatal Period of Japan Society of Obstetrics and Gynecology (to H.Y), research funding from Akaeda Medical Research Foundation (to K.T.), and Nihon University School of Medicine Alumni Association 60th Anniversary Fund Research Grant (to K.T). We thank Mr. Toyoharu Jike (Medical Research Support Center, Nihon University School of Medicine) for technical assistance. We also thank American Journal Experts for their editorial assistance.

References

[1] C. Fenizia, M. Biasin, I. Cetin, P. Vergani, D. Mileto, A. Spinillo, M.R. Gismondo, F. Perotti, C. Callegari, A. Mancon, S. Cammarata, I. Beretta, M. Nebuloni, D. Trabattoni, M. Clerici, V. Savasi, Analysis of SARS-CoV-2 vertical transmission during pregnancy, Nat. Commun. 11 (1) (2020) 5128.

[2] D.A. Schwartz, An analysis of 38 pregnant women with COVID-19, their newborn infants, and maternal-fetal transmission of SARS-CoV-2: maternal coronavirus infections and pregnancy outcomes, Arch. Pathol. Lab Med. 144 (7) (2020) 799–805.

[3] K.F. Walker, K. O'Donoghue, N. Grace, J. Dorling, J.L. Comeau, W. Li, J. G. Thornton, Maternal transmission of SARS-COV-2 to the neonate, and possible

routes for such transmission: a systematic review and critical analysis, BJOG An Int. J. Obstet. Gynaecol. 127 (11) (2020) 1324–1336.
[4] K.M. Moore, M.S. Suthar, Comprehensive analysis of COVID-19 during pregnancy, Biochem. Biophys. Res. Commun. 538 (2021) 180–186.
[5] 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. Patanè, 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 (5) (2021) 517–528.
[6] S. Komine-Aizawa, K. Takada, S. Hayakawa, Placental barrier against COVID-19, Placenta 99 (2020) 45–49.
[7] A.C. Tallarek, C. Urbschat, L. Fonseca Brito, S. Stanelle-Bertram, S. Krasemann, G. Frascaroli, K. Thiele, A. Wieczorek, N. Felber, M. Lütgehetmann, U.R. Markert, K. Hecher, W. Brune, F. Stahl, G. Gabriel, A. Diemert, P.C. Arck, Inefficient placental virus replication and absence of neonatal cell-specific immunity upon sars-CoV-2 infection during pregnancy, Front. Immunol. 12 (2021), 698578.
[8] A. Colson, C.L. Depoix, G. Dessilly, P. Baldin, O. Danhaive, C. Hubinont, P. Sonveaux, F. Debiève, Clinical and in vitro evidence against placenta infection at term by severe acute respiratory syndrome coronavirus 2, Am. J. Pathol. 191 (9) (2021) 1610–1623.
[9] L. Patanè, D. Morotti, M.R. Giunta, C. Sigismondi, M.G. Piccoli, L. Frigerio, G. Mangili, M. Arosio, G. Cornolti, Vertical transmission of coronavirus disease 2019: severe acute respiratory syndrome coronavirus 2 RNA on the fetal side of the placenta in pregnancies with coronavirus disease 2019-positive mothers and neonates at birth, Am. J. Obstet Gynecol MFM 2 (3) (2020), 100145.
[10] J.L. Hecht, B. Quade, V. Deshpande, M. Mino-Kenudson, D.T. Ting, N. Desai, B. Dygulska, T. Heyman, C. Salafia, D. Shen, S.V. Bates, D.J. Roberts, SARS-CoV-2 can infect the placenta and is not associated with specific placental histopathology: a series of 19 placentas from COVID-19-positive mothers, Mod. Pathol. 33 (11) (2020) 2092–2103.
[11] M.C. Smithgall, X. Liu-Jarin, D. Hamele-Bena, A. Cimic, M. Mourad, L. Debelenko, X. Chen, Third-trimester placentas of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-positive women: histomorphology, including viral immunohistochemistry and in-situ hybridization, Histopathology 77 (6) (2020) 994–999.
[12] M. Garrido-Pontnou, A. Navarro, J. Camacho, F. Crispi, M. Alguacil-Guillén, A. Moreno-Baró, J. Hernandez-Losa, M. Sesé, Y.C.S. Ramón, I. Garcia Ruíz, B. Serrano, P. Garcia-Aguilar, A. Suy, J.C. Ferreres, A. Nadal, Diffuse trophoblast damage is the hallmark of SARS-CoV-2-associated fetal demise, Mod. Pathol. 34 (9) (2021) 1704–1709.
[13] I. Alamar, M.H. Abu-Arja, T. Heyman, D.J. Roberts, N. Desai, P. Narula, B. Dygulska, A possible case of vertical transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a newborn with positive placental in situ hybridization of SARS-CoV-2 RNA, J. Pediatric Infect Dis. Soc. 9 (5) (2020) 636–639.
[14] J.C. Watkins, V.F. Torous, D.J. Roberts, Defining severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) placentitis: a report of 7 cases with confirmatory in situ hybridization, distinct histomorphologic features, and evidence of complement deposition, Arch. Pathol. Lab Med. 145 (11) (2021) 1341–1349.
[15] 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.
[16] J. Thomas, Y. Sun, L. Debelenko, Infrequent placental and fetal involvement in SARS-CoV-2 infection: pathology data from a large medical center, J. Dev. Biol. 9 (4) (2021).
[17] F. Facchetti, M. Bugatti, E. Drera, C. Tripodo, E. Sartori, V. Cancila, M. Papaccio, R. Castellani, S. Casola, M.B. Boniotti, P. Cavadini, A. Lavazza, SARS-CoV2 vertical transmission with adverse effects on the newborn revealed through integrated immunohistochemical, electron microscopy and molecular analyses of Placenta, EBioMedicine 59 (2020), 102951.
[18] A.G. Edlow, J.Z. Li, A.Y. Collier, C. Atyeo, K.E. James, A.A. Boatin, K.J. Gray, E. A. Bordt, L.L. Shook, L.M. Yonker, A. Fasano, K. Diouf, N. Croul, S. Devane, L. J. Yockey, R. Lima, J. Shui, J.D. Matute, P.H. Lerou, B.O. Akinwunmi, A. Schmidt, J. Feldman, B.M. Hauser, T.M. Caradonna, D. De la Flor, P. D'Avino, J. Regan, H. Corry, K. Coxen, J. Fajnzylber, D. Pepin, M.S. Seaman, D.H. Barouch, B. D. Walker, X.G. Yu, A.J. Kaimal, D.J. Roberts, G. Alter, Assessment of maternal and neonatal SARS-CoV-2 viral load, transplacental antibody transfer, and placental pathology in pregnancies during the COVID-19 pandemic, JAMA Netw. Open 3 (12) (2020), e2030455.
[19] Q. Mao, S. Chu, S. Shapiro, L. Young, M. Russo, M.E. De Paepe, Placental Sars-Cov-2 Distribution Correlates with Level of Tissue Oxygenation in Covid-19-Associated Necrotizing Histiocytic Intervillositis/perivillous Fibrin Deposition, 2021. Placenta.
[20] 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 (4) (2020), 100197.
[21] 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. Larsen, A.M. Gariepy, G. Aguilar, K. L. Fardelmann, M. Harigopal, H.S. Taylor, C.M. Pettker, A.L. Wyllie, C.D. 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. 130 (9) (2020) 4947–4953.
[22] A.G. Harrison, T. Lin, P. Wang, Mechanisms of SARS-CoV-2 transmission and pathogenesis, Trends Immunol. 41 (12) (2020) 1100–1115.
[23] 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 (12) (2020).
[24] T. Menter, K.D. Mertz, S. Jiang, H. Chen, C. Monod, A. Tzankov, S. Waldvogel, S. M. Schulzke, I. Hösli, E. Bruder, Placental pathology findings during and after SARS-CoV-2 infection: features of villitis and malperfusion, Pathobiology 88 (1) (2021) 69–77.
[26] 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.
[27] F. Hikmet, L. Méar, Å. Edvinsson, P. Micke, M. Uhlén, C. Lindskog, The protein expression profile of ACE2 in human tissues, Mol. Syst. Biol. 16 (7) (2020), e9610.