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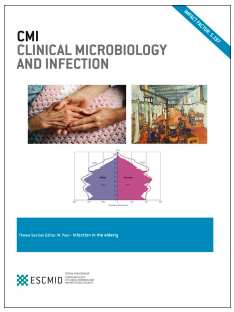

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SARS-CoV-2 ACE-Receptor detection in the placenta throughout pregnancy

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To the Editor,

Fetal risks for women exposed to SARS-CoV-2 and subsequent pregnancy outcomes remain uncertain (1). Recent reports have raised controversial concerns regarding the potential for vertical transmission by transplacental infection. It has been proposed that the occurrence of intrauterine transplacental SARS-CoV-2 among infected mother-infant dyads be based upon identification of SARS-CoV-2 in chorionic villous cells using immunohistochemistry or nucleic acid methods such as in situ hybridization (2). Hypothetically, two conditions are necessary for transplacental transmission to be possible: 1) the virus must reach the placenta; and 2) the receptor for the virus, angiotensin-converting enzyme 2 (ACE2), must be present in the placenta.

Regarding the first condition, we and others recently reported RT-PCR or RNAscope data supporting the presence of SARS-CoV-2 in placental tissue (3-6). To date, conflicting data exists regarding the second condition (4, 7, 8).

In order to investigate ACE2 expression in the placenta throughout pregnancy, we selected formalin-fixed placental tissues between 14 and 40 weeks gestation from 28 patients who delivered before the current COVID-19 outbreak (2 different cases for each 2 weeks gestation – i.e. 14, 16, 18, … 38, 40 weeks, annex), as well as placental tissues at 19 weeks gestation from a COVID-19 positive patient (figure 1). Kidney was used as positive (brush border of proximal renal tubules and podocytes of glomeruli) and negative (distal renal tubules and renal medulla) controls. Using a monoclonal anti-ACE2 antibody (Atlas antibodies, clone CL4035, dilution 1/1000), we demonstrated *in situ* expression of ACE2 at the maternal-fetal interface (⁄ 1 and annex), a prerequisite for transplacental transmission. We observed a strong and diffuse membranous staining of cytotrophoblast and syncytiotrophoblast cells of placental villi, as well as a membranous expression in extra-villous trophoblast. By testing placental tissues at various gestational ages in both COVID-19 positive and negative mothers, we

confirmed that ACE expression is present consistently throughout pregnancy regardless of COVID-19 status.

Our *in situ* analysis by specific immunohistochemistry and SARS-CoV-2 detection by RT-PCR indicate a possible placental infection by SARS-CoV2. Trophoblastic cells, which are in direct contact with the maternal blood in the intervillous space, show strong expression of ACE2 throughout pregnancy, supporting that SARS-CoV2 is able to infect the placenta via a receptor-mediated mechanism. SARS-CoV-2 can cross the placental barrier, as it has been demonstrated in a neonate born to a mother infected in the last trimester and presenting with neurological compromise (5). However, the rate of vertical transmission to the fetus warrants further investigation. By analogy to other pathogens (i.e. cytomegalovirus or toxoplasmosis), this may occur once sufficient time has elapsed for the virus to breach the placental barrier (6–8 weeks after infection).

SARS-CoV-2 infection of the maternal placental surface may induce acute or chronic placental insufficiency (3-5), or be responsible for subsequent miscarriage or fetal growth restriction as reported in 40% of MERS or SARS-1 maternal infections. In consequences, this findings could lead to increase protective measures for pregnant women, as well as a heightened level of concern/monitoring of pregnancies exposed to SARS-CoV-2.

Author Contributions:

Prof Baud and Dr Gengler had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conceptualization: Gengler, Dubruc, Baud

Acquisition, analysis, or interpretation of data: All authors

Writing – Original Draft: Gengler, Dubruc, Baud

Writing – Review & Editing: Gengler, Greub, Favre, de Laval, Baud

Administrative, technical, or material support: All authors

Supervision and Validation: Gengler, Baud

Conflict of Interest Disclosures:

Dr Greub reported having ongoing research agreements with Resistell and Becton-Dickinson and being medical advisor for Resistell; he also reported being developer of a card game on microbes and funding JeuPro, a start-up company that distributes the game Krobs. No other disclosures were reported.

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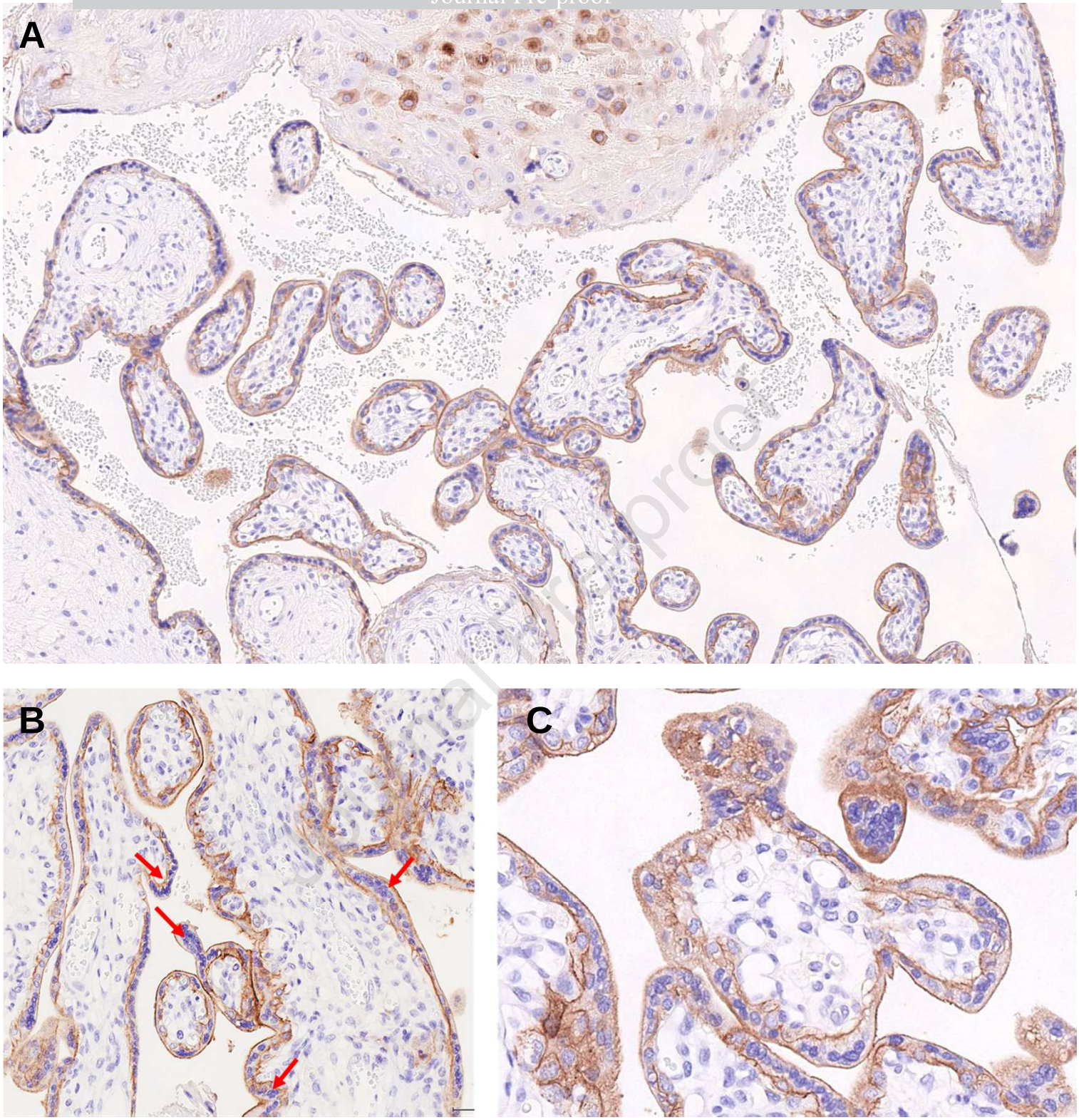
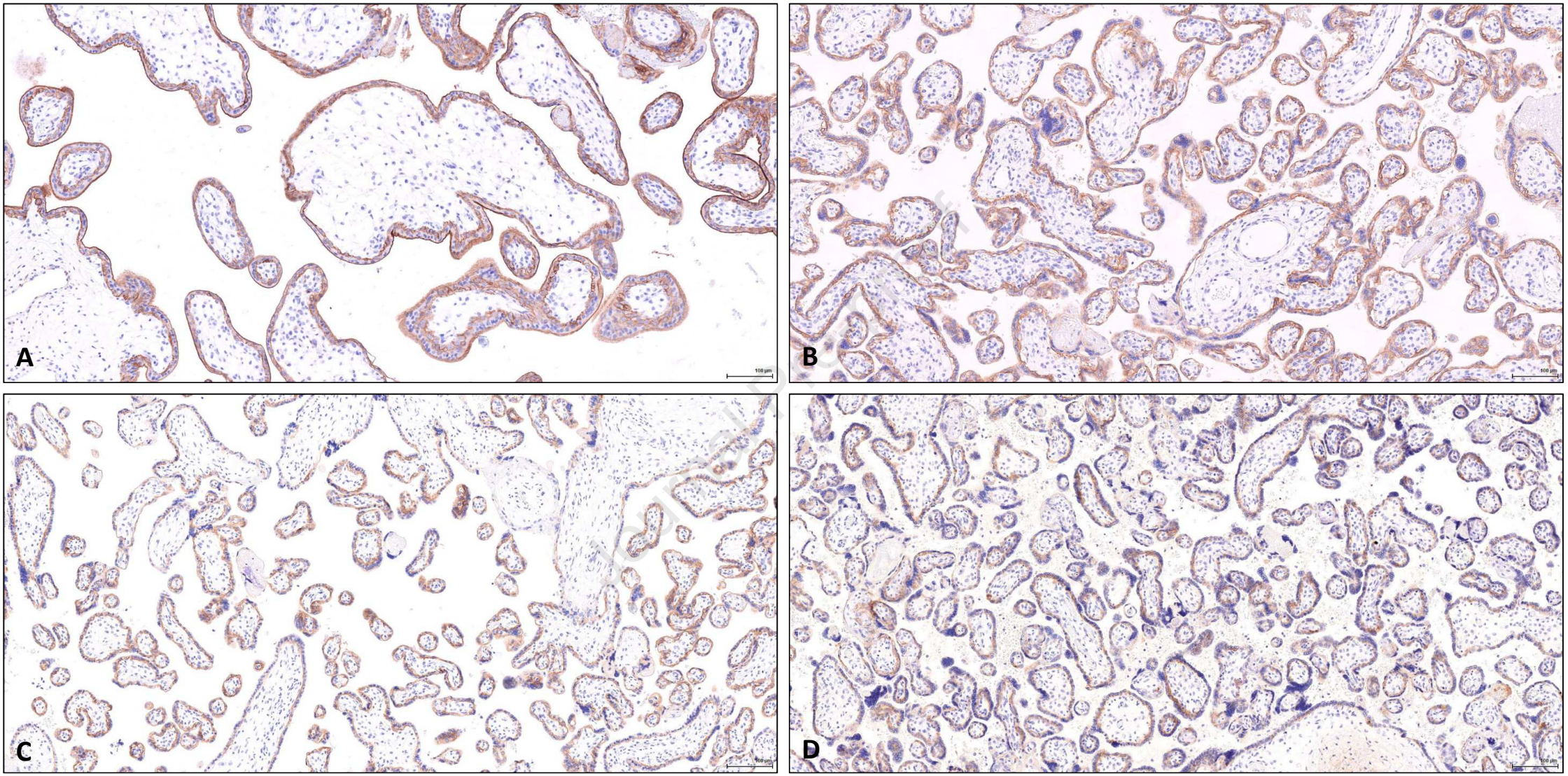
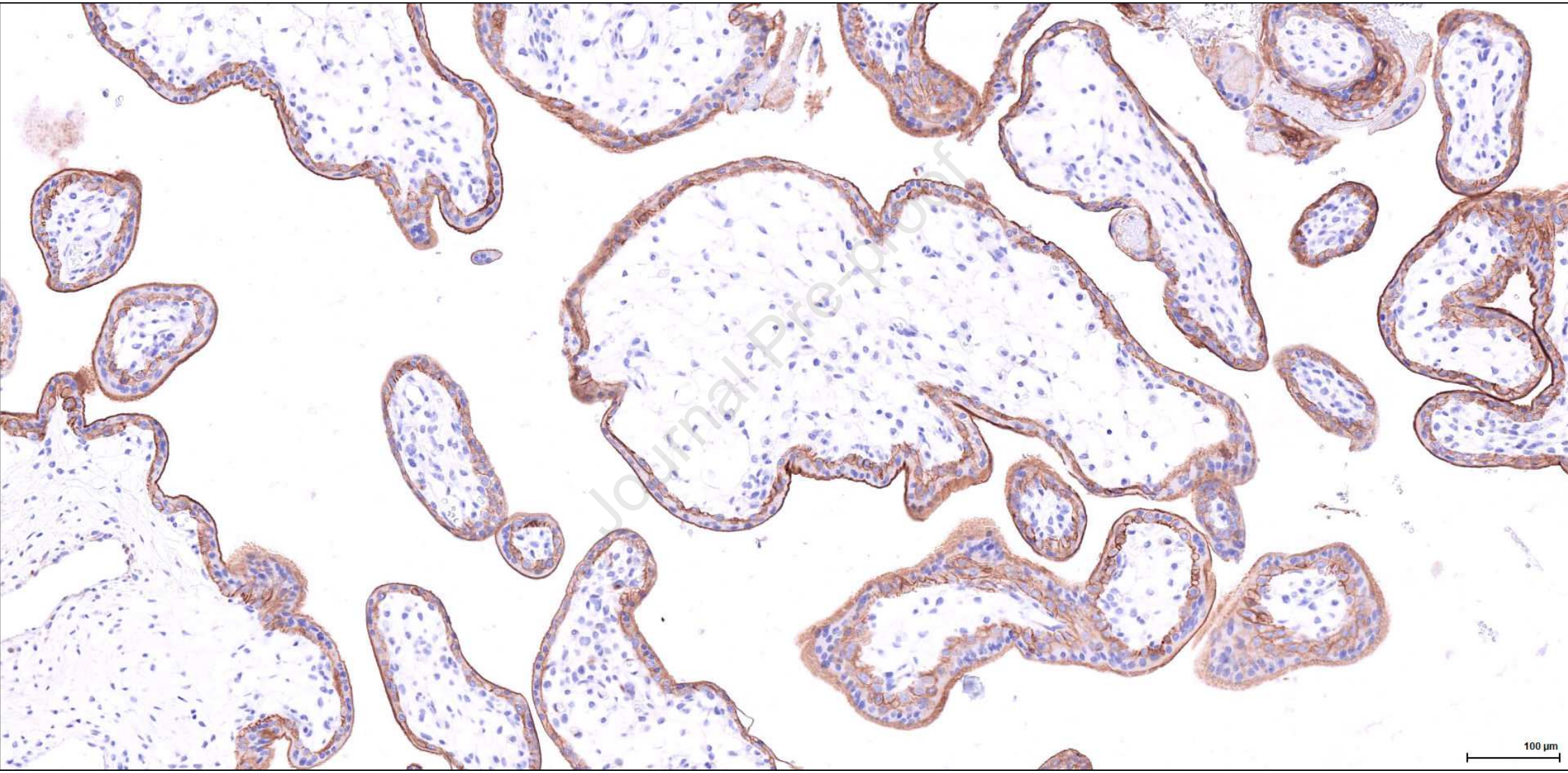


Figure 1:
Diffuse membranous staining of villous cytotrophoblast and syncytiotrophoblast cells (arrows) with monoclonal Anti-ACE2 antibody (clone CL4035), dilution 1/1000 in a COVID-19 positive mother, 19 weeks of amenorrhea.

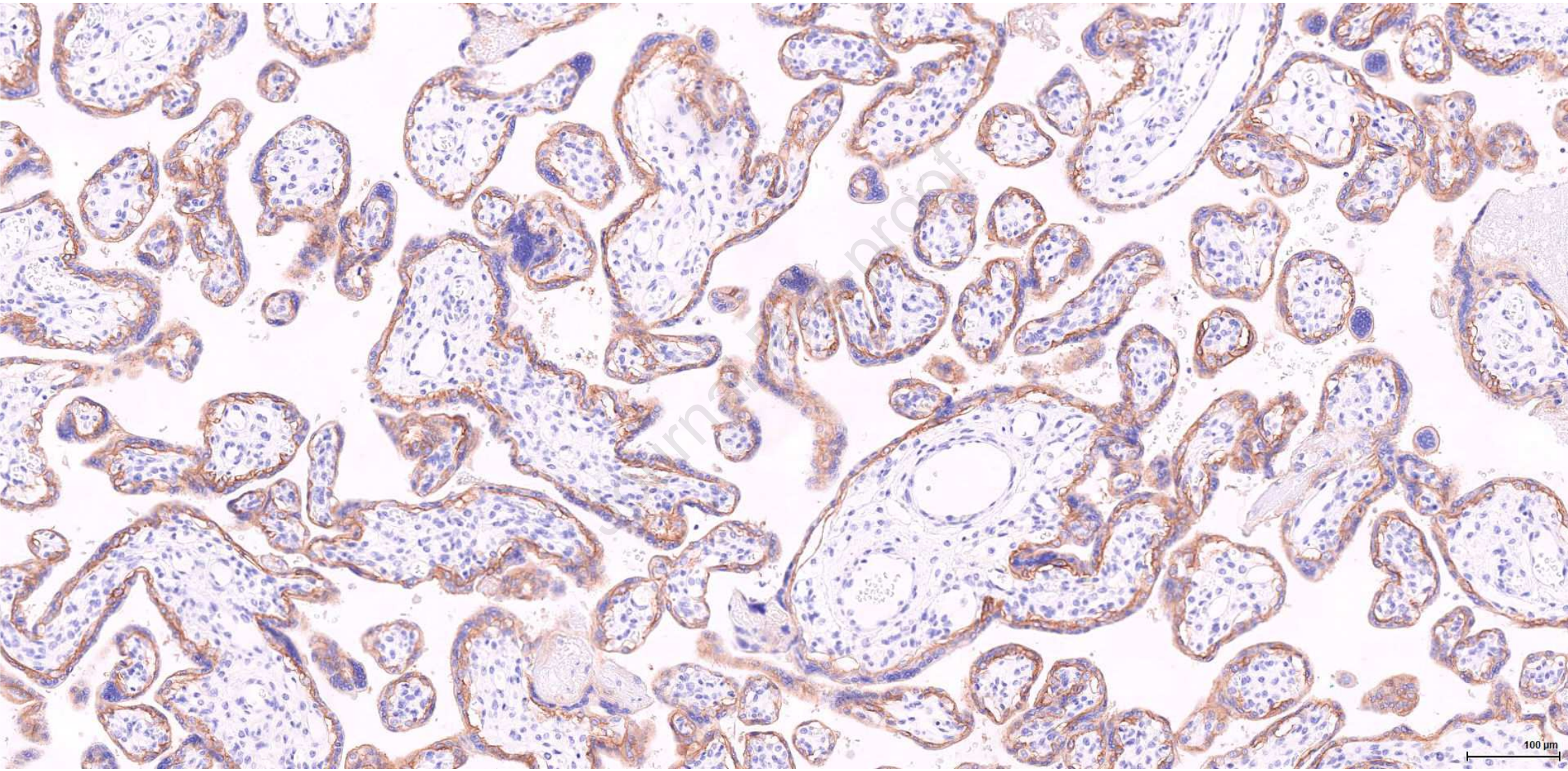


Annex: Diffuse membranous staining of villous cytotrophoblast cells with monoclonal Anti-ACE2 antibody (clone CL4035), dilution 1/1000, 10X at various gestational ages (16 (A), 22 (B), 31 (C) and 40 (D) weeks gestation) in control COVID-19 negative mothers.

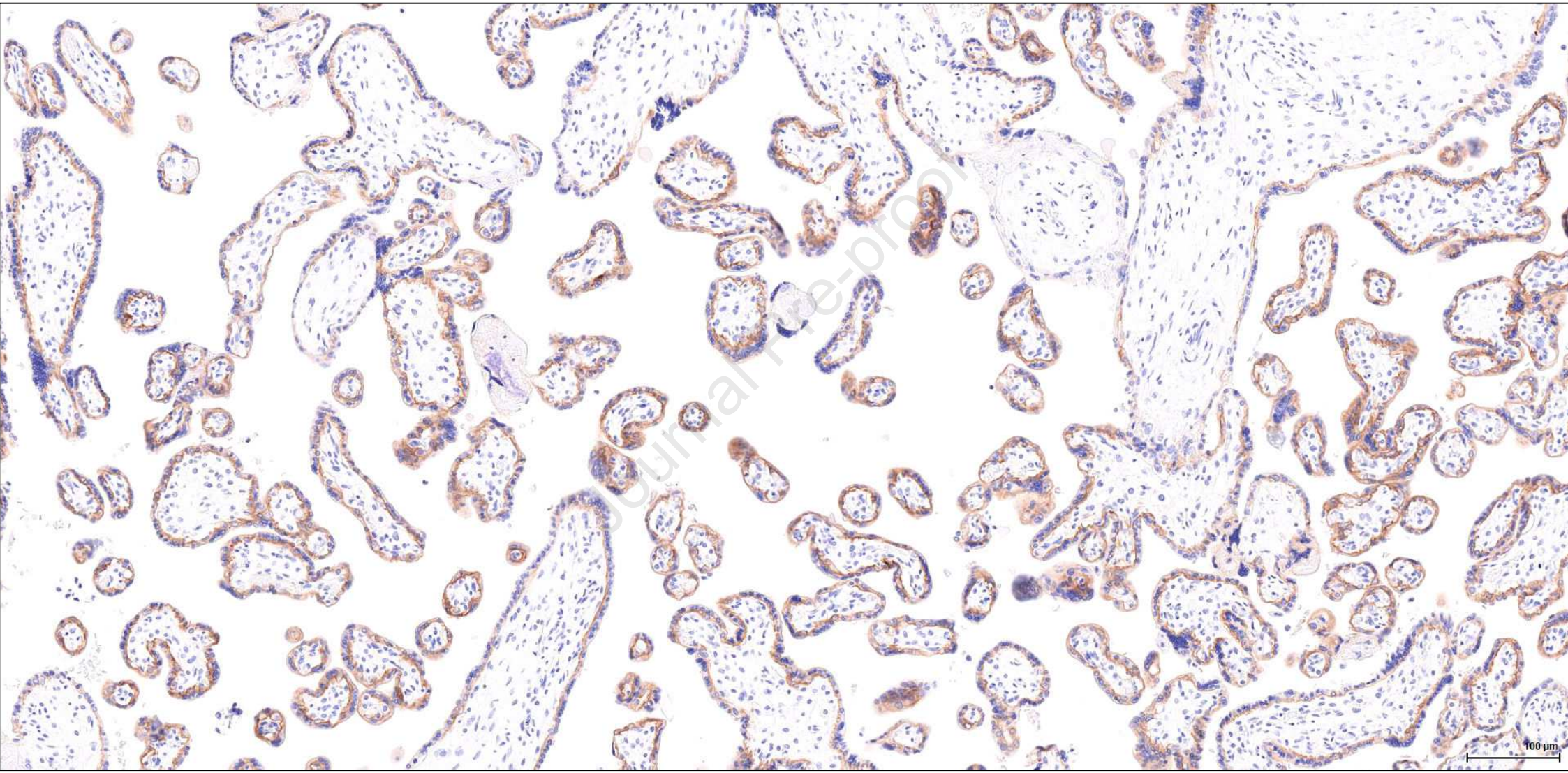
16 weeks



22 weeks



31 weeks



40 weeks