Alternative interpretation to the findings reported in visualization of severe acute respiratory syndrome coronavirus 2 invading the human placenta using electron microscopy

TO THE EDITORS: I am writing in response to the in-press article by Algarroba et al[1] describing a woman with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection tested using real-time polymerase chain reaction (RT-PCR). The authors examined the placenta by transmission electron microscopy to identify SARS-CoV-2 particles. They identified circular inclusions in the cytoplasm of several syncytiotrophoblasts that they concluded were SARS-CoV-2 virions.

The report of virus-like inclusions in syncytiotrophoblast is intriguing and thought-provoking. However, I respectfully offer an alternative interpretation of the data. The structures identified as SARS-CoV-2 virions look exactly like clathrin-coated pits or vesicles. Clathrin-coated vesicles are spherical structures employed by trophoblasts and other cell types to internalize cargos from the extracellular space.[2] Coated vesicles and coated pits derive their name from the external scaffold coat composed of clathrin triskelions that decorate the surface of the structure. In transmission electron micrographs in which tissue-thin sections are stained with uranyl acetate and lead citrate, coated vesicles have an electron-dense studded surface that appears identical to the "corona" comprising the spike protein that decorates all coronaviruses, including SARS-CoV-2 virions. It is this studded surface or corona that gives the genus *Betacoronaviridae* its characteristic morphology and name.

I propose that the structures identified by Algarroba et al[1] in their journal preproof paper are clathrin-coated vesicles and not SARS-CoV-2 particles. This conclusion is based on the following evidence: (1) the circular structures in the electron micrographs in the paper, identified as virions, have the size and shape of clathrin-coated vesicles found in nearly all eukaryotic cells[3]; (2) there is no evidence of virions bound to the apical surface of the syncytiotrophoblast (ACE2 or SARS-CoV-2 receptor)[4] as would be predicted in virus-infected cells; (3) U-shaped, corona-studded structures are apparent at the surface of the syncytiotrophoblast representing newly forming coated vesicles that have not yet pinched off (ie, endocytosed their cargo) (Figure); and (4) the neonate was determined to be virus-negative using RT-PCR.

To provide more convincing evidence of the presence of SARS-CoV-2 virions in the syncytiotrophoblast, the authors could have assessed the placenta for the presence of viral RNA using the same RT-PCR platform they used to test the mother for SARS-CoV-2 infection. In addition, the authors could have examined placentas of patients who tested negative for the presence of the virus as a control. If they failed to visualize any coated vesicular structures in placentas from patients without infection (control), it would have provided evidence that the observed structures in the trophoblasts of the woman with infection may have been SARS-CoV-2 particles but not definitive proof. Without such controls, it is premature to state with certainty that the structures reported in the paper are virions and not coated vesicles.

More definitive evidence to support SARS-CoV-2 infection of the trophoblast and therefore potential vertical transmission would be an experimental approach in which labeled recombinant spike (S) protein could bind to placental tissue sections and be visualized by high-resolution imaging methods. We are currently utilizing this technique to determine whether human trophoblasts bind and internalize virions as a means of vertical transmission. The importance of understanding the nature of vertical transmission necessitates that extensive controls be carried out using multidimensional approaches to address this clinically crucial question in an unambiguous manner. ■

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This communication has been published in the middle of the coronavirus disease 2019 pandemic and is available via expedited publication to assist patients and healthcare providers.

REFERENCES

1. Algarroba GN, Rekawek P, Vahanian SA, et al. Visualization of SARS-CoV-2 virus invading the human placenta using electron microscopy. Am J Obstet Gynecol 2020. [Epub ahead of print].
2. Harasaki K, Lubben NB, Harbour M, Taylor MJ, Robinson MS. Sorting of major cargo glycoproteins into clathrin-coated vesicles. Traffic 2005;6:1014–26.
3. McMahon HT, Boucrot E. Molecular mechanism and physiological functions of clathrin-mediated endocytosis. Nat Rev Mol Cell Biol 2011;12:517–33.

4. Li M, Chen L, Zhang J, Xiong C, Li X. The SARS-CoV-2 receptor ACE2 expression of maternal-fetal interface and fetal organs by single-cell transcriptome study. PLoS One 2020;15:e0230295.

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REPLY

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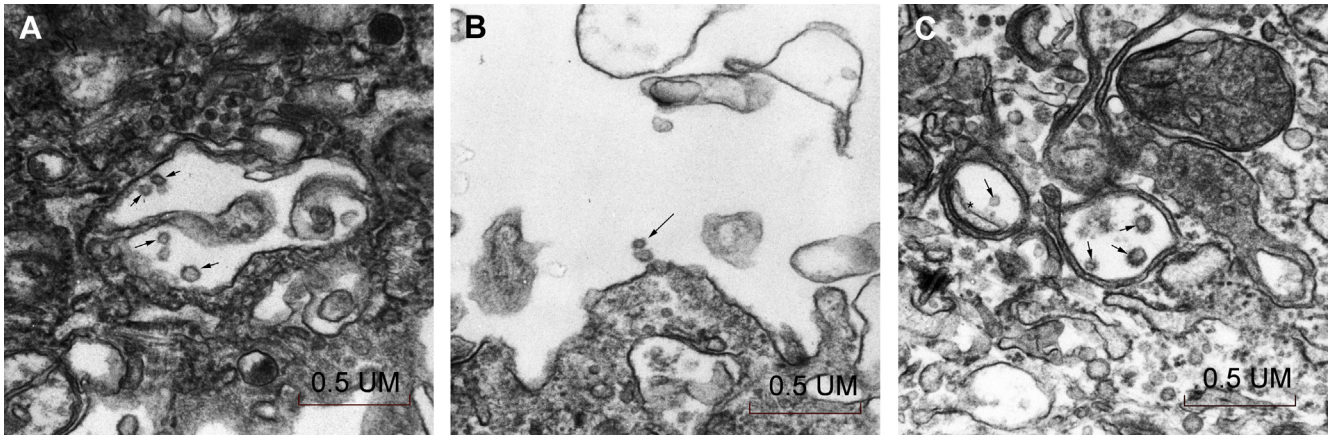
We firmly believe that the pictures of our published case report[1] represent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and not clathrin-coated vesicles for the following reasons:

1. Clathrin-coated vesicles are intracellular structures. Careful observation of the pictures shown in Figures 2 and 3 of our published case report[1] reveals that in addition to the single virion invading the syncytiotrophoblast—indicated by the arrow—there are several virions visible in the extracellular space at the right upper quadrant of the picture. We now provide additional evidence showing extracellular locations of the virions (Figure 1, A) including virions in the outer surface of the syncytiotrophoblast (Figure 1, B). Therefore, the possibility of clathrin-coated vesicles raised by Dr Kniss is extremely unlikely. Dr Kniss' claim also overlooks the possibility that the SARS-CoV-2 may utilize the clathrin-mediated endocytosis pathway for its entry to target cells.[2] One of Dr Kniss' criticisms is that the neonate tested negative for SARS-CoV-2 using real-time polymerase chain reaction (RT-PCR). However, the presence of the virus in the placenta is not equivalent to vertical transmission.
2. The morphology (spherical and occasionally pleomorphic particles) and size of the virions in our case are identical to those described by Goldsmith et al.[3] In the Goldsmith report, the mean diameter of the virions was 78 nm, and in our case, the mean diameter of the virions was 78.3 nm (n=10). Our case also exhibited the ultrastructural characteristics as described by Goldsmith et al[3] including virus-containing vesicles, double-membrane vesicles, and tubular structures in a virus-containing vesicle (Figure 1, C).
3. The size and morphology of our virions were identical to the pictures shown in Figures 4E, F, H, and I of the article by Hosier et al[4] who sequenced the SARS-CoV-2 thus providing molecular evidence of placental invasion by the SARS-CoV-2.
4. We used a control group of 5 placentas (coronavirus disease 2019 [COVID-19]-negative mothers and placentas; 3 different blocks from each placenta; total of 15 sections) and examined under electron micrograph for the presence of clathrin- or virion-like particles found in both intracellular and extracellular spaces. Two independent observers found 3 (intracellular) clathrin-coated vesicles in 2 placentas. Most importantly, none of the control placentas had clathrin- or virion-like structures found in both intracellular and extracellular locations, as in our case.
5. We performed immunohistochemical staining on paraffin-embedded slides from the placenta of our COVID-19—positive case, COVID-19—negative placentas, and nasopharyngeal aspirates from patients who tested positive and negative for SARS-CoV-2 using RT-PCR. We utilized an antibody for SARS-CoV-2 spike glycoprotein (Coronavirus ab272504; Abcam, Cambridge, MA). In our case, strong positive staining was seen in syncytiotrophoblasts of terminal villi and in stem villi, in underlying stromal cells, and in positive controls. No staining was identified in the negative controls (Figure 2).

FIGURE 1
Extracellular locations of virions



A, Extracellular locations of the virions. **B,** Virions at the outer surface of the syncytiotrophoblast near a microvillus. **C,** Virus-containing double-membrane vesicles with virions and tubular structure (*asterisk*). Virions shown by the *arrows*.

Algarroba. Reply. Am J Obstet Gynecol 2020.