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.

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REPLY

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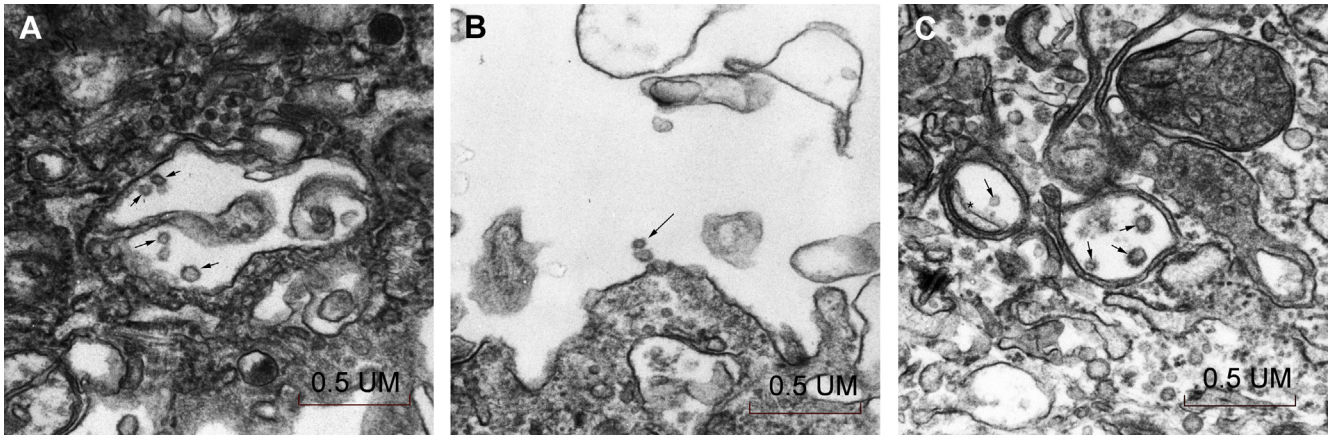
We firmly believe that the pictures of our published case report[1] represent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and not clathrin-coated vesicles for the following reasons:

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*.

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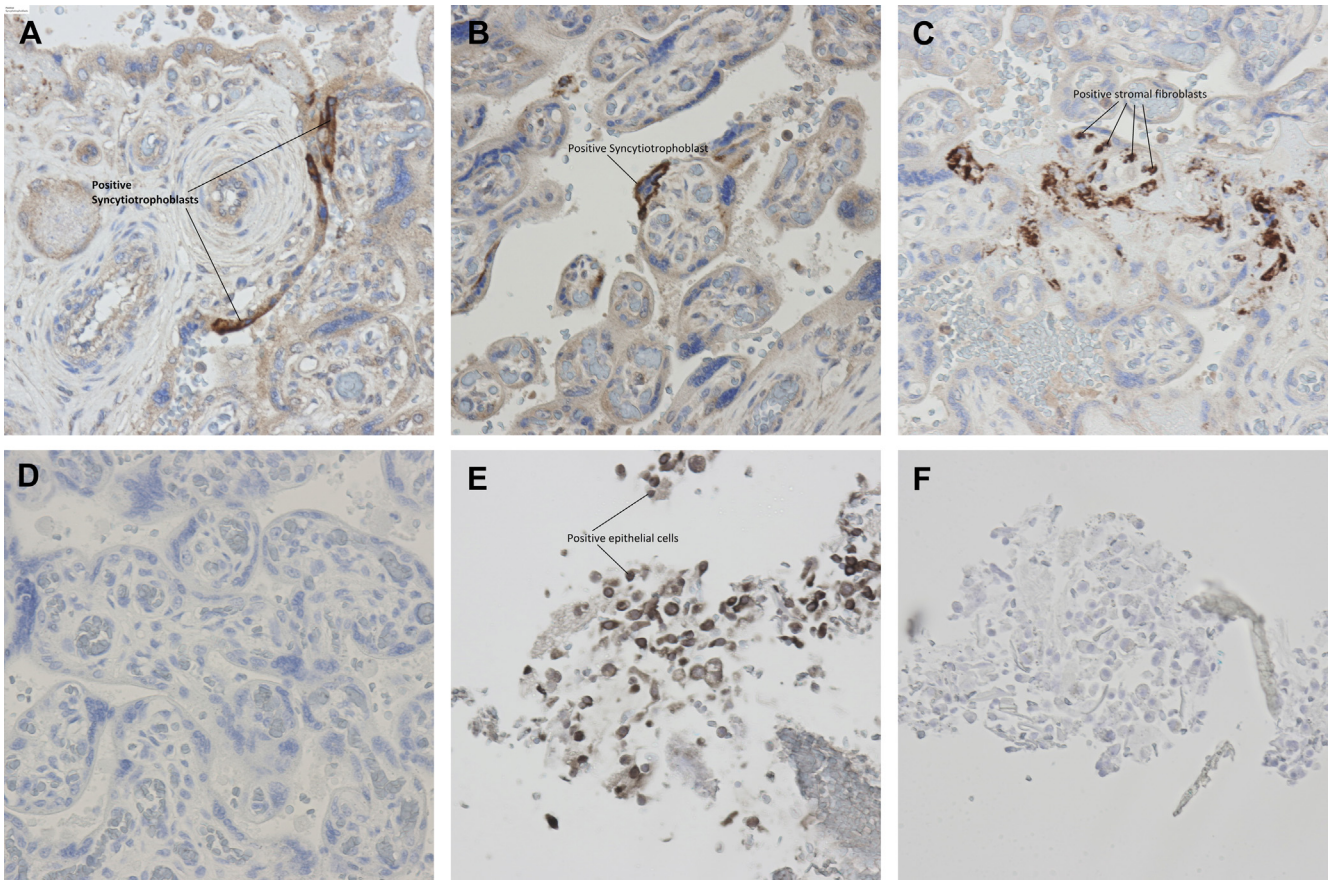
FIGURE 2
Immunohistochemical staining findings using with negative and positive controls



A and B, Positive placental syncytiotrophoblast staining. **C,** Positive placental fibroblast staining. **D,** COVID-19—negative placenta (negative) control. **E,** COVID-19—positive epithelial cell staining from nasopharyngeal aspirate from patient tested positive using RT-PCR. **F,** COVID-19—negative epithelial cell staining from nasopharyngeal aspirate from patient tested negative using RT-PCR.

COVID-19, coronavirus disease 2019; *RT-PCR*, real-time polymerase chain reaction.

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6. Lastly, we used immunogold labeling with the aforementioned antibody for SARS-CoV-2 spike glycoprotein. The tissue was embedded in LR White resin, sectioned, blocked, and exposed to the primary antibody. The sections were washed and reacted with a secondary antibody of goat anti-rabbit with 10 nm gold particles. The immunogold staining was positive for viral antigen and protein with clusters of gold particles with little background (Figure 3). ■

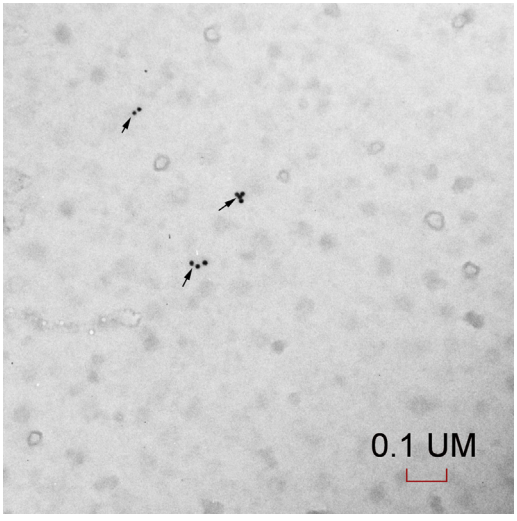
Gabriela N. Algarroba, MD
Patricia Rekawek, MD
Sevan A. Vahanian, MD
Department of Obstetrics and Gynecology
NYU Langone Health
NYU Winthrop Hospital
NYU Long Island School of Medicine
Mineola, NY

Poonam Khullar, MD
Department of Pathology
NYU Langone Health
NYU Winthrop Hospital
NYU Long Island School of Medicine
Mineola, NY

Thomas Palaia, MS
Morgan R. Peltier, PhD
Department of Foundations of Medicine
NYU Langone Health
NYU Winthrop Hospital
NYU Long Island School of Medicine
Mineola, NY

Martin R. Chavez, MD
Department of Obstetrics and Gynecology
NYU Langone Health
NYU Winthrop Hospital

FIGURE 3
Immunogold labeling findings



Clusters of 10 nm immunogold particles (*arrows*) cross-reacting with the SARS-CoV-2 spike glycoprotein antibody.

SARS-CoV-2, sever acute respiratory syndrome coronavirus 2.

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NYU Long Island School of Medicine
120 Mineola Blvd., Ste. 110
Mineola, NY 11501
martin.chavez@nyulangone.org

Anthony M. Vintzileos, MD
Department of Obstetrics and Gynecology
NYU Langone Health
NYU Winthrop Hospital
NYU Long Island School of Medicine
Mineola, NY

The authors report no conflict of interest.

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.

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