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.

Stability of severe acute respiratory syndrome coronavirus 2 RNA in placenta and fetal cells

TO THE EDITORS: We have read with great interest the systematic review and metaanalysis by Kotlyar and colleagues[1] reporting a pooled proportion of 3.2% for vertical transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The authors indicate the proportion of positive SARS-CoV-2 RNA testing in neonatal blood, urine, placental samples, and amniotic fluid, without considering the stability of viral RNA as a major limitation in the diagnosis of maternal-fetal transmission.

RNA is very susceptible to degradation, which occurs through hydrolysis and ribonuclease activity. Clinical samples are particularly vulnerable to RNA degradation by the action of host nucleases.[2] In the case of the diagnosis of vertical transmission of viruses, RNA is considerably less stable than DNA in the placental and fetal samples and requires more steps for detection at the laboratory level. A critical challenge for RNA preservation and detection in these samples is to prevent degradation by the nuclease during the sampling and purification processes.[3] The storage and transportation of clinical samples are also at risk of RNA hydrolysis, which represents a limitation for healthcare settings with a decentralized laboratory.[4] For example, the H5N1 RNA was undetectable if stored 24 hours at room temperature, whereas it remained detectable more than 40 days when stored in cold temperature, in RNA-safe buffer, or in dry pellet matrix, without exposure to high temperatures.[5] The same is true for Zika virus, which rapidly degrades if not stored in RNA lather, often becoming undetectable once frozen specimens are thawed.[6,7] These limitations have been well documented for reverse transcription−polymerase chain reaction (RT-PCR) assays in cases of congenital Zika virus infections.[8−10]

Conversely, genomic content from DNA viruses, such as Cytomegalovirus, is easily purified from whole blood or any other tissue or fluid (placenta, fetal liver and brain, amniotic fluid, urine, cerebrospinal fluid) and is less subjected to deterioration, increasing the sensitivity of PCR assays for the diagnosis of vertical transmissions.[11,12] When maternal infection occurs during the first trimester of pregnancy, DNA viruses are detectable throughout the pregnancy in fetal and placental tissues. This contrasts with RNA viruses (such as Zika virus or SARS-CoV-2), which are only transiently present and detectable.[9] Therefore, the absence of the detection of an RNA virus does not necessarily mean that the infection of the given tissue is absent. This issue should be mentioned in any study investigating the potential evidence of vertical transmission of SARS-CoV-2. ■

Léo Pomar, MSc
Materno-fetal and Obstetrics Research Unit
Department Woman-Mother-Child
Lausanne University Hospital
1011 Lausanne, Vaud, Switzerland

Karin Nielsen-Saines, MD, MPH
Division of Pediatric Infectious Diseases
David Geffen School of Medicine at UCLA
Los Angeles, CA

David Baud, MD, PhD
Materno-fetal and Obstetrics Research Unit
Department Woman-Mother-Child
Lausanne University Hospital
Lausanne, Vaud, Switzerland
david.baud@chuv.ch

The authors declare that they have no competing interests, and they attest to having met all authorship criteria.

REFERENCES

1. Kotlyar A, Grechukhina O, Chen A, et al. Vertical transmission of COVID-19: a systematic review and meta-analysis. Am J Obstet Gynecol 2020. [Epub ahead of print].
2. Nelson PT, Baldwin DA, Scearce LM, Oberholtzer JC, Tobias JW, Mourelatos Z. Microarray-based, high-throughput gene expression profiling of microRNAs. Nat Methods 2004;1:155–61.
3. Barnes MG, Tsoras M, Thompson SD, Martinez H, Iverson B, Nuñez R. Ambient temperature stabilization of purified RNA in GenTegra™ for use in Affymetrix Human Exon 1.0 ST arrays. BioTechniques 2010;48:468–9.
4. Martinez H, Beaudry G, Veer J, et al. Ambient temperature storage of RNA for use in a RT-qPCR test for the detection of GCC mRNA. Cancer Res 2011;70(Suppl8):819.
5. Relova D, Rios L, Acevedo AM, Coronado L, Perera CL, Pérez LJ. Impact of RNA degradation on viral diagnosis: an understated but essential step for the successful establishment of a diagnosis network. Vet Sci 2018;5:19.
6. Corman VM, Rasche A, Baronti C, et al. Assay optimization for molecular detection of Zika virus. Bull World Health Organ 2016;94:880–92.
7. Gorchakov R, Berry RM, Patel SM, El Sahly HM, Ronca SE, Murray KO. Optimizing PCR detection of Zika virus from various body fluids. Am J Trop Med Hyg 2019;100:427–33.
8. Pomar L, Vouga M, Lambert V, et al. Maternal-fetal transmission and adverse perinatal outcomes in pregnant women infected with Zika virus: prospective cohort study in French Guiana. BMJ 2018;363:k4431.
9. Schaub B, Vouga M, Najioullah F, et al. Analysis of blood from Zika virus-infected fetuses: a prospective case series. Lancet Infect Dis 2017;17:520–7.
10. Adebanjo T, Godfred-Cato S, Viens L, et al. Update: interim guidance for the diagnosis, evaluation, and management of infants with possible congenital Zika virus infection - United States, October 2017. MMWR Morb Mortal Wkly Rep 2017;66:1089–99.
11. Ross SA, Ahmed A, Palmer AL, et al. Urine collection method for the diagnosis of congenital cytomegalovirus infection. Pediatr Infect Dis J 2015;34:903–5.

12. Roberts TC, Buller RS, Gaudreault-Keener M, et al. Effects of storage temperature and time on qualitative and quantitative detection of cytomegalovirus in blood specimens by shell vial culture and PCR. J Clin Microbiol 1997;35:2224–8.

 http://dx.doi.org/10.1016/j.ajog.2020.09.003

REPLY

Check for updates

We thank Pomar and colleagues[1] for their interest in our article and the important point they raised in their letter. We acknowledge that RNA degrades more easily than DNA and that sampling, purification processes, storage, and transport conditions can influence RNA stability, which may impact the ability to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in placental and certain fetal samples using molecular assays. However, this seems to be much less of a concern when assessing the presence of SARS-CoV-2 RNA in neonatal nasopharyngeal (NP) specimens. Although our systematic review summarized the evidence of SARS-CoV-2 detection in various fetal and neonatal clinical sites, the vertical transmission rate estimate of 3.2% of SARS-CoV-2 infections occurring in the third trimester of pregnancy is based on our metaanalysis, which was focused specifically on neonatal NP swab testing, the gold standard to detect SARS-CoV-2 RNA, performed within 48 hours of birth.[2]

Established methods of viral NP specimen collection and transport seem to preserve discernible amounts of SARS-CoV-2 for an extended period. This exact question was recently addressed by Rogers et al.[3] In a prospective study, the authors assessed the effects of various media, temperatures, and storage times on the stability of SARS-CoV-2 RNA specimens obtained using NP swabs. Samples were defined as "positive" if their polymerase chain reaction (PCR) amplification cycle number (C_t) was <40 and "stable" if their mean C_t value did not increase by more than 3 amplification cycles of the initial C_t value. Importantly, all samples stored at room temperature over 7 days exhibited increasing C_t values over time but had C_t value variation <3, thus not impacting the qualitative interpretation of positive results. Such stability was maintained for all 5 media types tested. Similar stability levels were seen in refrigerated and frozen samples even after 14 days of storage in 5 different media.[3] RNA stability was assessed in this study for a much longer duration than is customary for routine clinical testing, further reducing the likelihood of clinical impact. These stability assessments complement previous studies evaluating the strong survival and persistence of the closely related SARS-CoV-1 in various different human specimens and environments, as it was shown to survive in serum and feces at infectious levels for at least 96 hours and in urine for at least 72 hours.[4]

The goal of our metaanalysis was to summarize existing data and arrive at preliminary estimates for the likelihood of vertical transmission of SARS-CoV-2 rather than exact viral transmission rates. Although we agree with the letter authors that viral RNA stability within placental and fetal specimens may lead to underestimation of viral detection by quantitative reverse transcription—polymerase chain reaction (qRT-PCR), the case for vertical transmission is all the more strengthened with the relative consistency of SARS-CoV-2 detection rates in all of the clinical specimen sources reported in our systematic review. Our analysis indicated that the rate of vertical transmission of 3.2% based on NP swabs is consistent with the neonatal anti—coronavirus disease 2019 (COVID-19) immunoglobulin M (IgM) serology positivity rate of 3.7%. If placental samples were more susceptible to nuclease-induced degradation, then we would have expected a lower positivity rate. In contrast, this rate was higher at 7.7%. Further meticulous research and large cohort studies are needed to establish the dynamics of SARS-CoV-2 infection in pregnancy and more accurately characterize vertical transmission rates. These should include consistent testing of multiple biologic samples immediately after delivery (cord blood, placental samples, amniotic fluid, urine, NP swab correlated with maternal samples) utilizing multiple methods to detect evidence of SARS-CoV-2 infection (RT-PCR, IgM serology, immunohistopathology, etc.). These efforts should be coupled with close monitoring of pregnant women with COVID-19 for fetal adverse outcomes and long-term neonatal sequelae. ■

Alexander Kotlyar, MD
Reshef Tal, MD, PhD
Section of Reproductive Endocrinology and Infertility
Department of Obstetrics, Gynecology, and Reproductive Sciences
Yale School of Medicine
Yale University
330 Cedar St.
New Haven, CT 06510
Alexander.kotlyar@yale.edu

The authors report no conflict of interest.

REFERENCES

1. Pomar L, Nielsen-Sanes K, Baud D. Stability of severe acute respiratory syndrome coronavirus 2 RNA in placenta and fetal cells. Am J Obstet Gynecol 2020;224:126–7.

2. Kotlyar A, Grechukhina O, Chen A, et al. Vertical transmission of COVID-19: a systematic review and mate-analysis. Am J Obstet Gynecol 2020. [Epub ahead of print].

3. Rogers AA, Baumann RE, Borillo GA, et al. Evaluation of transport media and specimen transport conditions for the detection of SARS-CoV-2 by use of real-time reverse transcription-PCR. J Clin Microbiol 2020;58:e00708–20.

4. Duan SM, Zhao XS, Wen RF, et al. Stability of SARS coronavirus in human specimens and environment and its sensitivity to heating and UV irradiation. Biomed Environ Sci 2003;16:246–55.

 http://dx.doi.org/10.1016/j.ajog.2020.09.004